Application Note: 518

Simultaneous Quantitative Analysis of Four Immunosuppressive Drugs Using High Resolution Accurate Mass LC-MS

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Key Words

- Exactive
- Accela U-HPLC
- Therapeutic Drug Monitoring
- Clinical Research

Introduction

Immunosuppressive drugs have been quantitatively analyzed by selected reaction monitoring (SRM) analysis using tandem mass spectrometry for over 10 years in the clinical research setting. High resolution accurate mass (HRAM) mass spectrometry offers the same quantitative performance characteristics with the added benefit of significantly faster method development. The HRAM method development time depends only on the sample preparation and chromatography conditions. In addition, mass analysis methods can be established rapidly because there is no requirement to tune SRM transitions, collision energies, or transfer lens voltages.

Goal

In this preliminary evaluation a set of calibrators, clinical samples, and QCs are investigated with the analysis of multiple replicates over the course of 7 days. The current in-house validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) method data is directly compared against the use of HRAM LC-MS data.

Experimental Conditions

Sample Preparation

Commercial calibration standards in frozen stabilized whole blood were sourced from Chromsystems (München, Germany). Commercial quality control material in stabilized whole blood was sourced from More Diagnostics (Los Osos, CA, USA). All calibrators, QCs, and whole blood samples were extracted using a plate-based solid phase extraction (SPE) procedure.

HPLC

Chromatographic separation was accomplished using a Thermo Scientific Accela U-HPLC system. A Thermo Scientific AQUASIL C18 column (150 x 2.1 mm, 5 µm) heated to 50 °C, was used with an isocratic gradient of 90% MeCN + ammonium acetate (2 mM). For each sample, 20 µL was injected.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific Exactive high performance benchtop mass spectrometer powered by Orbitrap™ technology. Atmospheric pressure chemical ionization (APCI) was used to generate the $[M+NH_3]^+$ ions for tacrolimus, sirolimus, and everolimus, and the $[M+H]^+$ ions for cyclosporin, as well as two internal standards: ascomycin (for cyclosporin and tacrolimus) and desmethoxyrapamycin (for sirolimus and everolimus).

The Exactive™ mass spectrometer was set to scan at 50 K resolution over the range *m/z* 700 – 1300 and was calibrated once at the start of the 7-day analysis. Data acquisition and analysis were carried out with Thermo Scientific LCQUAN software.

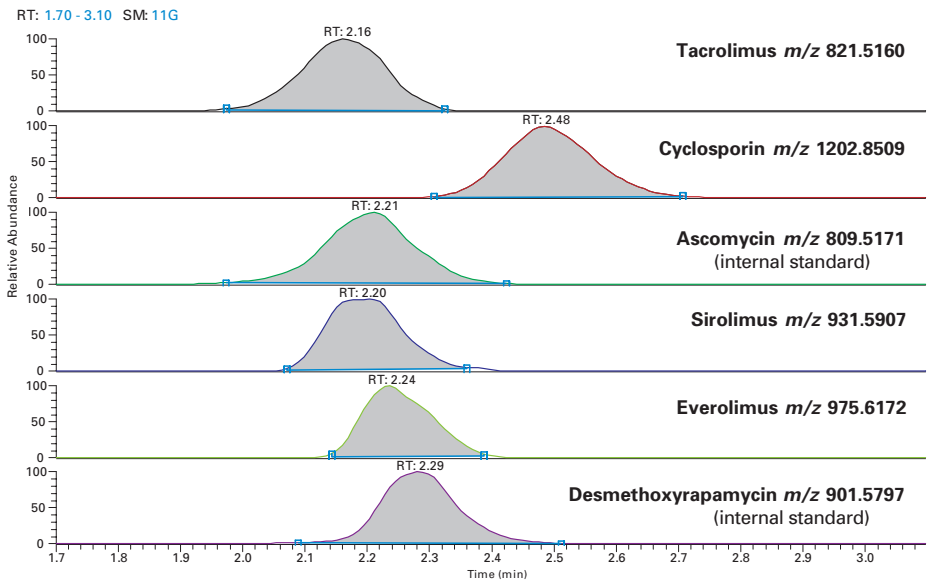

Figure 1. XIC of lowest calibration standard

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Results and Discussion

An accurate mass extracted ion chromatogram of the lowest calibration standard for each compound is presented in Figure 1. An example calibration line for each of the analytes is presented in Figure 2 A, B, C and D.

Inter-assay variability was determined by processing 30 replicates of each quality control over multiple batches. The precision data for inter-assay validation are presented in Table 1. The limit of quantitation (LOQ) has been set at 1 ng/mL for each analyte, and the highest CVs obtained at this concentration were 10.2%. The lower limit of quantitation (LLOQ) has not yet been fully investigated. Although cyclosporin, which also has the largest concentration range, achieved CVs of 12.5% at 0.3 ng/mL.

A total of 360 clinical research samples were analyzed by the HRAM method. The results were compared to the current LC-MS/MS method. Analysis of the clinical specimens by both HRAM LC-MS and LC-MS/MS demonstrate good correlation for cyclosporin, tacrolimus, and sirolimus across the required therapeutic range. No clinical research specimens were available for the method comparison of everolimus.

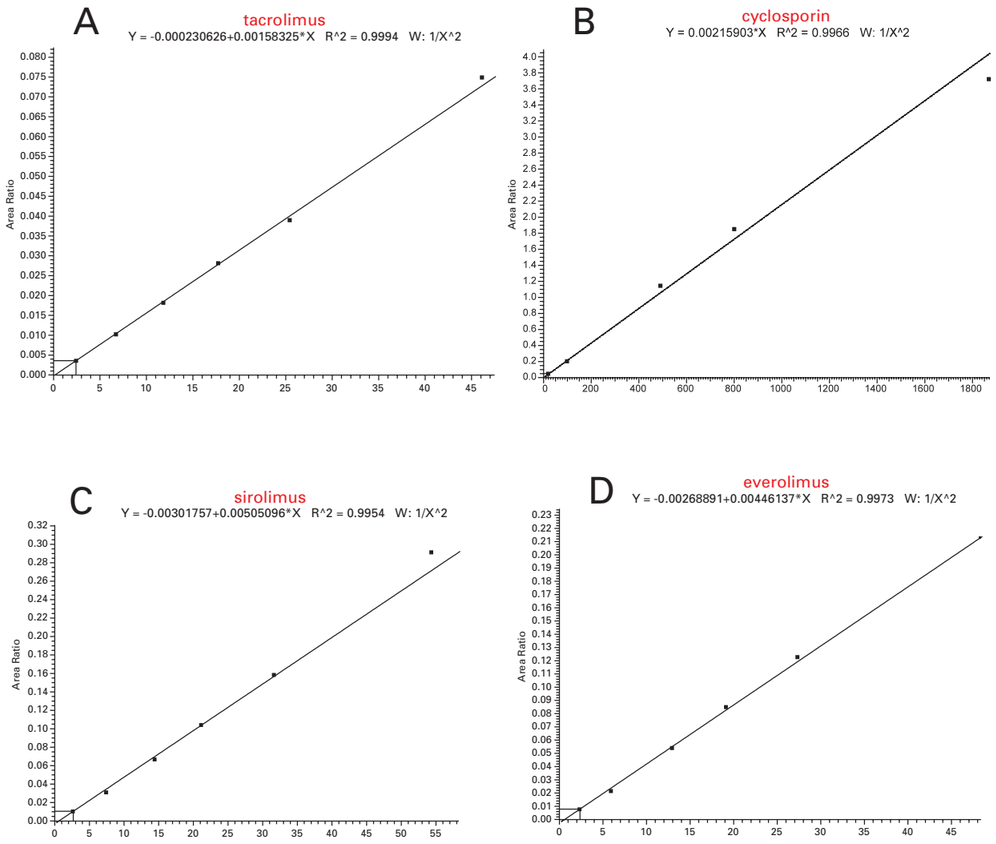

Figure 2. Calibration curves for (A) tacrolimus, (B) cyclosporin, (C) sirolimus, and (D) everolimus

Table 1. Method variability for each analyte

Analyte		Control 1	Control 2	Control 3	Control 4
Tacrolimus	Mean (ng/mL)	1.6	7.4	10.8	20.2
	%CV	12.8	4.2	4.2	2.9
Sirolimus	Mean (ng/mL)	3.3	14.4	25.3	41.6
	%CV	12.1	5.6	6.5	6.6
Everolimus	Mean (ng/mL)	2.9	13.9	24.2	41.8
	%CV	9.4	3.7	4.4	5.5
Cyclosporin	Mean (ng/mL)	83	176	362	787
	%CV	8.1	11.1	7.2	4.7

Conclusion

The HRAM analysis using the Exactive mass spectrometer demonstrates SRM comparable specificity, dynamic range, LOQ and precision in whole blood matrix. There is good correlation between SRM and HRAM results for the immunosuppressant drugs monitored.

The precision of HRAM LC-MS analysis meets current consensus guidelines and has acceptable performance to be used as a candidate clinical research method following further evaluation. All the method development time for this application was associated with the sample preparation and chromatography conditions. The mass analysis method was established in less than 5 minutes since there is no requirement to tune SRM transitions, collision energies or transfer lens voltages.

Acknowledgement

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