Quantitation of Immunosuppressant Drugs in Blood Utilizing a Triple Quadrupole Mass Spectrometer

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Application Note 628

Key Words
Immunosuppressant, tacrolimus, sirolimus, everolimus, cyclosporine A, TSQ Endura, TraceFinder, ClinSpec, clinical research

Goal

Evaluate the Thermo Scientific™ TSQ Endura™ triple-stage quadrupole mass spectrometer for the analysis of immunosuppressant drugs in whole blood for clinical research.

Introduction

Mass spectrometry is increasingly used in clinical research to quantitate immunosuppressant drugs in whole blood because it can offer higher sensitivity and selectivity versus other analysis techniques. For quick analysis, a sensitive and robust instrument is required. Here, the suitability of the TSQ Endura triple-stage quadrupole mass spectrometer for this method is demonstrated.

Methods

Sample Preparation

Briefly, whole blood samples were processed by precipitation with a zinc sulfate/methanol solution containing internal standards. Samples were shaken for 30 minutes at room temperature and centrifuged at 13,000 rpm for 10 minutes. Supernatant was transferred to an autosampler vial, and 50 µL were injected into the HPLC system.

Liquid Chromatography

Chromatographic analysis was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with OAS autosampler. The column used was a Thermo Scientific™ Hypersil GOLD™ C18 Javelin™ guard column (10 x 2.1 mm, 5 µm particle size, P/N 25005-012106) maintained at 80 °C. Mobile phases A and B consisted of 10 mM ammonium formate with 0.1% formic acid in water and methanol (both Fisher Scientific™ Optima™ grade), respectively. Mobile phase C was acetonitrile/1-propanol/acetone (45:45:10 v/v/v)(Fisher Chemical brand). The total run time was 2 minutes.

Figure 1. UltiMate 3000 RSLC HPLC pump and UltiMate 3000 OAS autosampler with TSQ Endura MS.

Mass Spectrometry

Compounds were detected on a TSQ Endura triple quadrupole mass spectrometer equipped with a Thermo Scientific™ EZ Max NG source and atmospheric pressure chemical ionization (APCI) sprayer (Figure 1). All of the compounds formed an ammoniated adduct. One selected-reaction monitoring (SRM) transition was monitored for each compound.

Method Evaluation

Method evaluation consisted of analyzing replicates of quality controls along with a calibration curve on multiple days.

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Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. Figure 2 shows representative chromatograms for analytes at their respective limits of quantitation (LOQs) and internal standards.

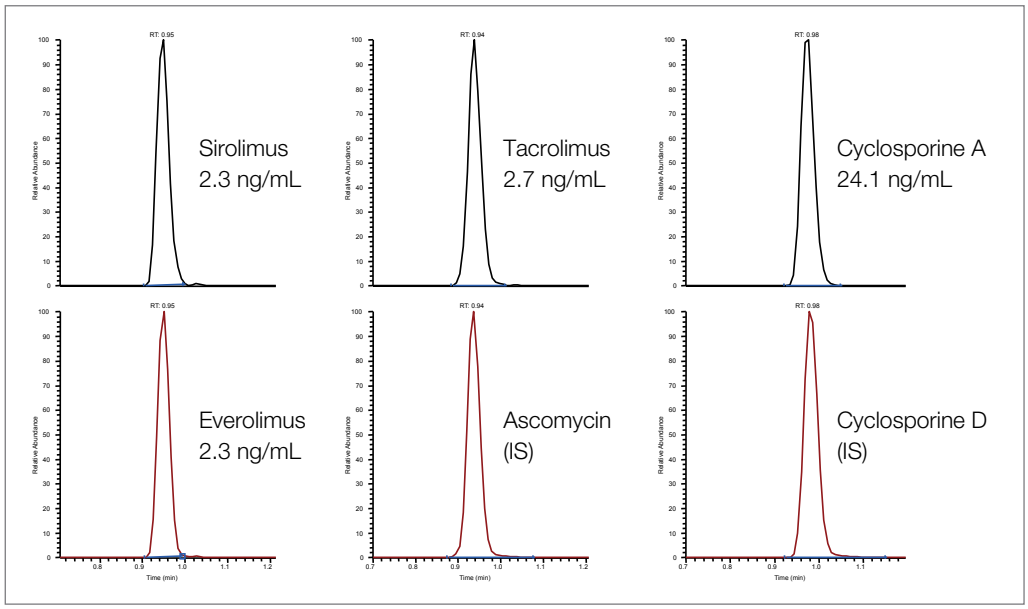


Figure 2. Chromatogram of lowest calibration standard showing analytes and internal standards.

Results

Linearity

All compounds were linear over the calibration ranges of approximately 2 to 50 ng/mL for tacrolimus, sirolimus and everolimus and 25 to 800 ng/mL for cyclosporine A. Figures 3 through 6 show representative calibration curves for all compounds. Standards back-calculated to within 9% for tacrolimus, 7% for sirolimus, 7% for everolimus, and 8% for cyclosporine A.

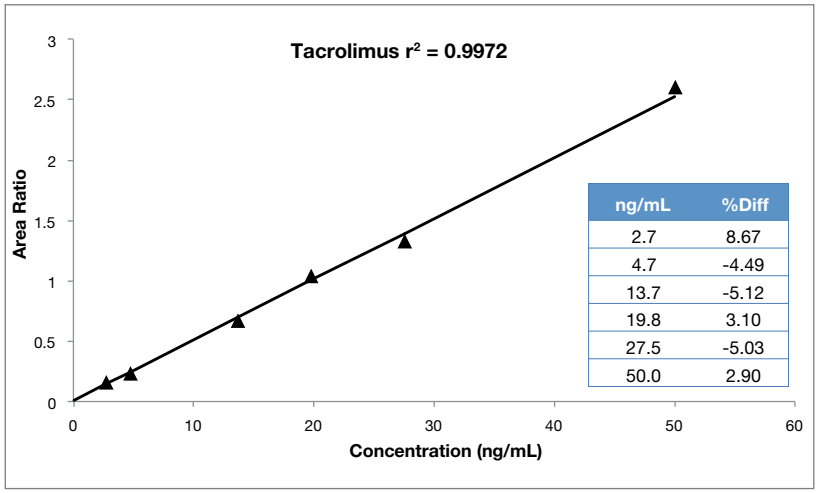
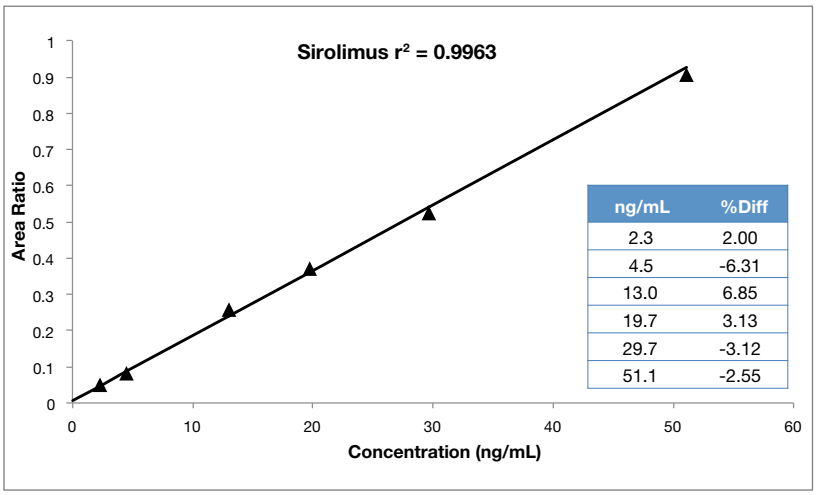
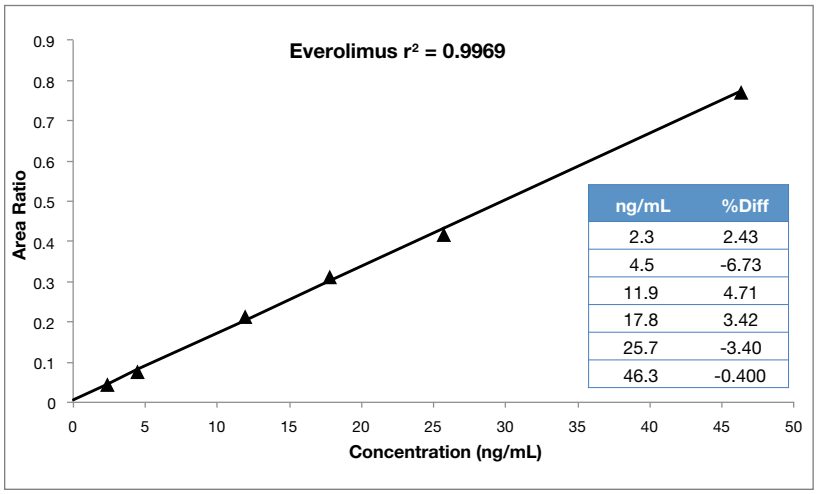


Figure 3. Calibration curve for tacrolimus.



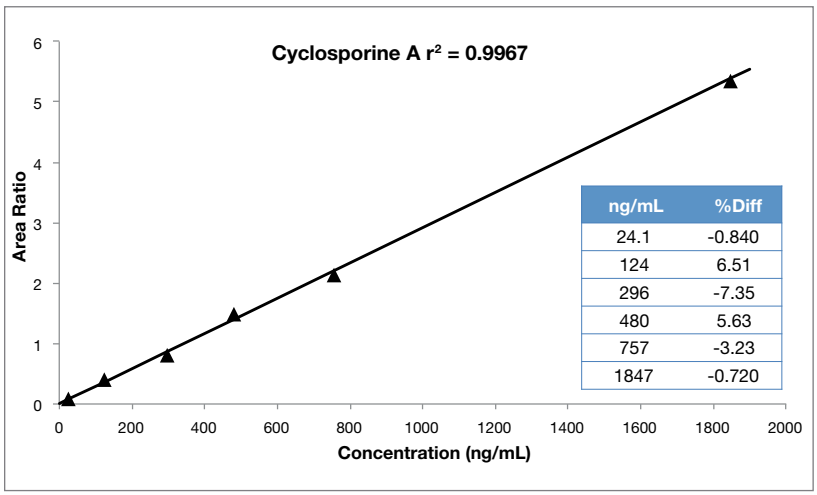
ng/mL	%Diff
2.3	2.00
4.5	-6.31
13.0	6.85
19.7	3.13
29.7	-3.12
51.1	-2.55

Figure 4. Calibration curve for sirolimus.



ng/mL	%Diff
2.3	2.43
4.5	-6.73
11.9	4.71
17.8	3.42
25.7	-3.40
46.3	-0.400

Figure 5. Calibration curve for everolimus.



ng/mL	%Diff
24.1	-0.840
124	6.51
296	-7.35
480	5.63
757	-3.23
1847	-0.720

Figure 6. Calibration curves for cyclosporine A.

Quality Controls

Quality control samples analyzed in this study showed good recovery and reproducibility. Table 1 shows statistics for quality controls analyzed in this study. There were no everolimus quality control samples analyzed in this study. Precisions were within 1.16% for tacrolimus, 2.98% for sirolimus, and 1.77% for cyclosporine A. Figure 7 shows representative chromatograms for compounds analyzed from donor samples.

Conclusion

- The method shows good linearity across required calibration ranges.
- Controls indicate good method precision and robustness.
- The TSQ Endura MS provides reproducible results on a robust platform suitable for analysis of immunosuppressant drugs for clinical research.

Table 1. Quality control concentrations and precision.

Immunosuppressant	QC1 ng/mL (%RSD)	QC2 ng/mL (%RSD)	QC3 ng/mL (%RSD)
Tacrolimus	4.5 (1.2)	15 (1.0)	9.0 (0.82)
Sirolimus	5.0 (3.0)	17 (2.7)	9.1 (2.2)
Cyclosporine A	83.2 (1.8)	184 (1.2)	353 (1.3)

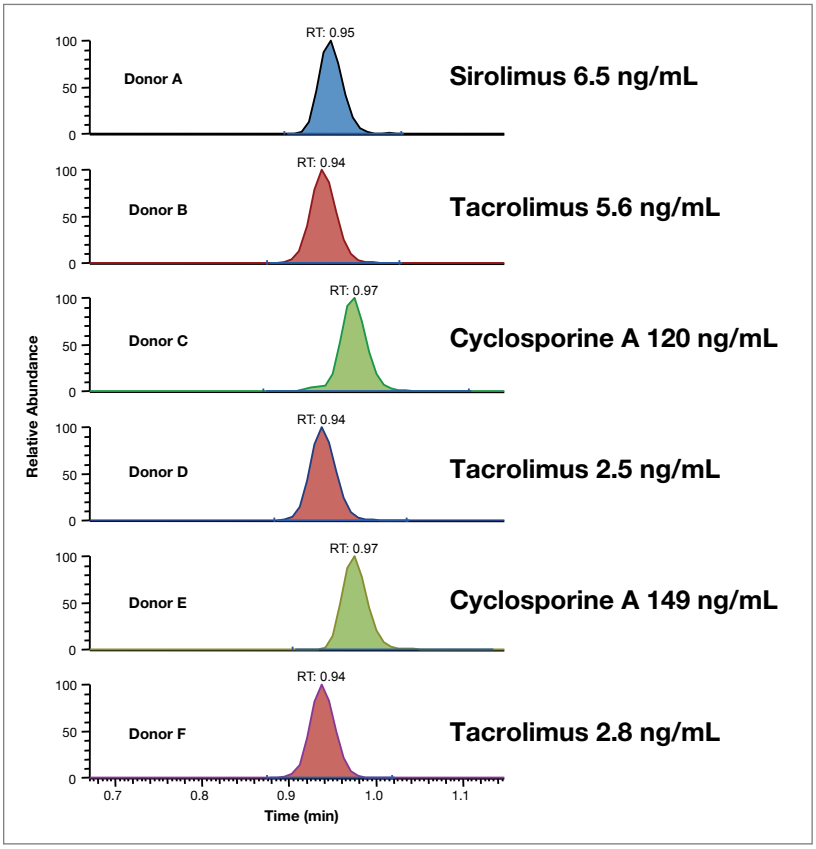


Figure 7. Examples of six different donor samples.

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