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Testing robustness: Immunosuppressant drugs in blood with a TSQ Quantis MS for clinical research

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Goal

Demonstrate the robustness of the Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer by analysis of immunosuppressant drugs in whole blood for clinical research.

Introduction

Mass spectrometry is a popular choice of technology in clinical research for the quantitation of immunosuppressant drugs in whole blood. A sensitive and robust instrument is required for reliable quantitation workflows. In this study, we demonstrate the TSQ Quantis triple quadrupole mass spectrometer for this application.

Experimental

Sample preparation

Calibrators and controls were obtained from RECIPE Chemicals + Instruments GmbH (Munich, Germany). Blank whole blood was obtained from BioreclamationIVT (New York, USA). Briefly, whole blood calibrators, controls, and 10 different lots of blank whole blood were processed by precipitation with $ZnSO_4$/methanol solution containing internal standards (cyclosporin D and ascomycin). Samples were vortexed for one minute, left to stand for 30 minutes in a refrigerator, and centrifuged at 13,000 rpm for 10 minutes. Supernatant was transferred to an autosampler vial, and 15 µL was injected onto the HPLC system. This processing method removes the majority of blood proteins but does not remove phospholipids.

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Liquid chromatography

Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Flex Binary HPLC system. The column used was a Thermo Scientific™ Hypersil GOLD™ C8 LC column (50 × 2.1 mm, 5 µm particle size) maintained at 80 °C. Mobile phases A and B consisted of 10 mM ammonium formate with 0.1% formic acid in Fisher Chemical™ Optima™ grade water and methanol, respectively. No divert valve was used. The total run time was 3 minutes.

Mass spectrometry

Compounds were detected on a TSQ Quantis triple quadrupole mass spectrometer equipped with a heated electrospray ionization source. The ion source was set to Medium (M) at position 2, with sweep gas set to 2 (arb units). All the compounds formed an ammoniated adduct that was used as the precursor ion. Two selected reaction monitoring (SRM) transitions were monitored for each analyte and one was monitored for the internal standards. Dioctyl phthalate and three endogenous phospholipids were also monitored as surrogates for instrument cleanliness.

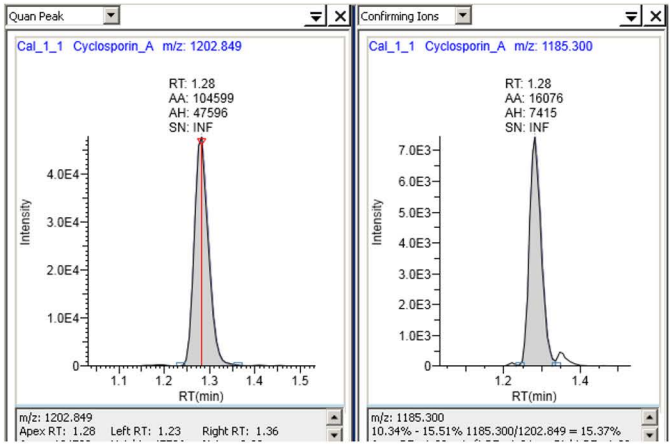
Test of robustness

The daily sequence of samples consisted of an initial set of eight calibrators. This was followed by repeated sets of five controls and 20 blank blood samples. A set of calibrators was inserted approximately halfway through the total sequence and again at the end. The total number of samples per sequence was approximately 300. The sequence was repeated for five consecutive days. Peak areas of the internal standards and calculated concentration of the mid-level control were monitored for stability.

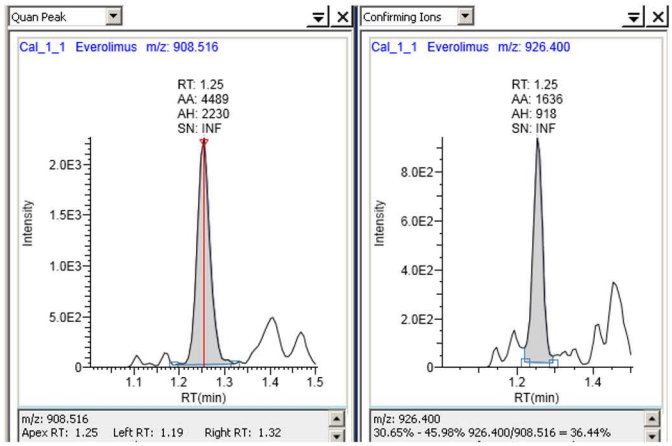
Data analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. Figure 1 shows representative chromatograms for analytes at their respective LOQs and internal standards.

A



B



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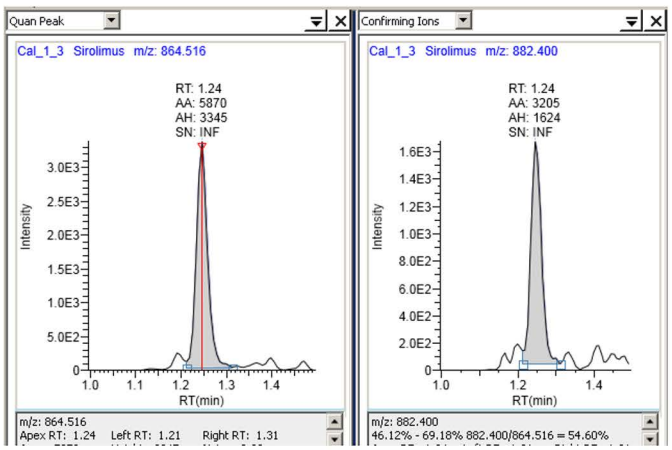

Figure 1A, 1B, and 1C. Chromatograms for lowest calibration standard showing ion ratio confirmation.

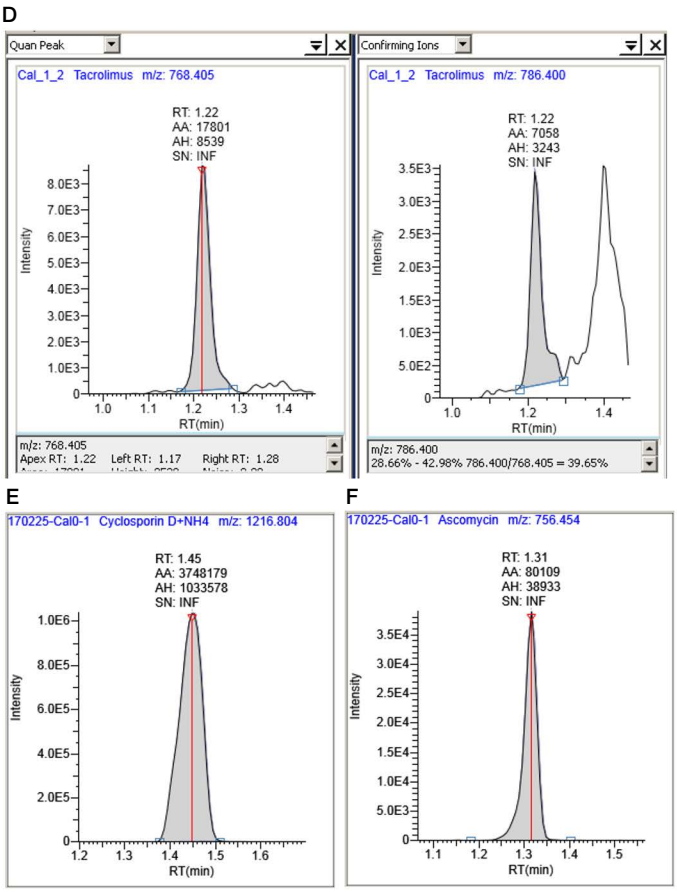

Figure 1D, 1E, and 1F. Chromatograms for lowest calibration standard showing ion ratio confirmation along with internal standards.

Results and discussion

Linearity

All compounds were linear over their calibration ranges of approximately 2–60 ng/mL for tacrolimus, sirolimus, and everolimus, and 25–1800 ng/mL for cyclosporin A. Figure 2 shows representative calibration curves for all compounds. All calibrators back-calculated to within 20% of theoretical values over the five days of testing.

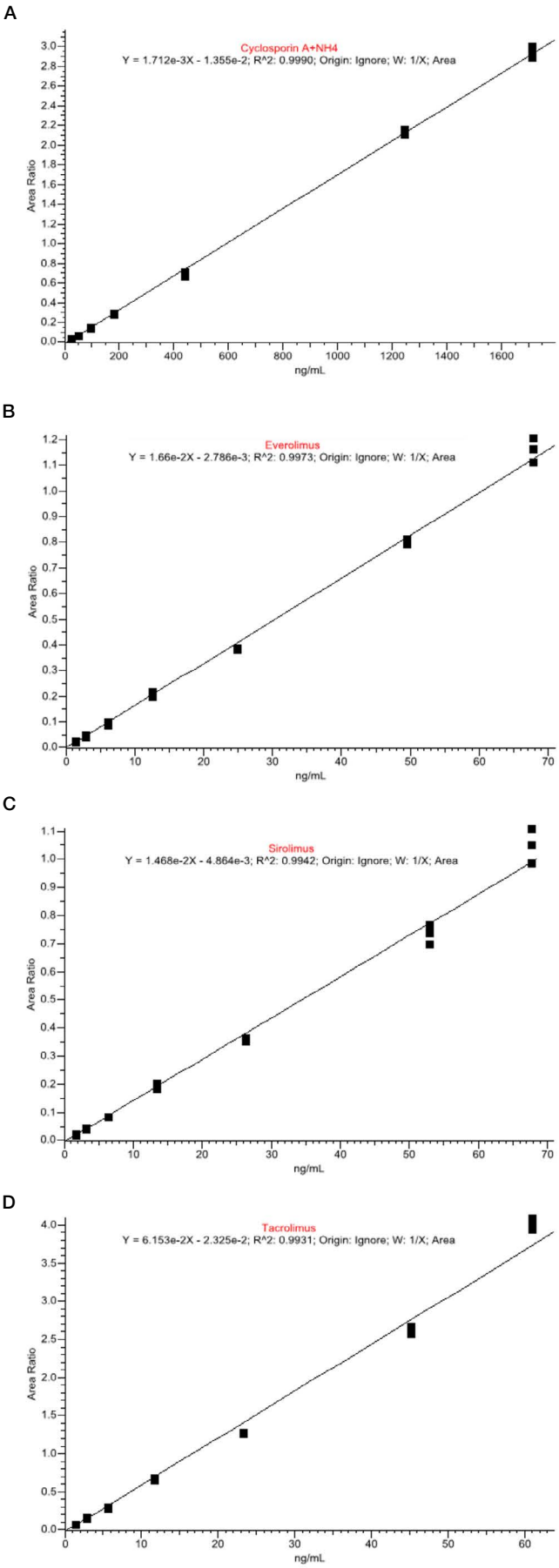

Figure 2. Representative calibration curves.

Robustness

Peak areas for cyclosporin D and ascomycin, the two internal standards used in this study, showed precisions of 4% and 8%, respectively, over the five days and over 1500 injections (Figure 3). Calculated concentration precisions for Control III for cyclosporin A, everolimus, sirolimus, and tacrolimus were 2.36%, 4.22%, 4.08%, and 3.57%, respectively, over the five days (n=60 injections each) (Figure 4). Phospholipids showed no buildup over the course of the study.

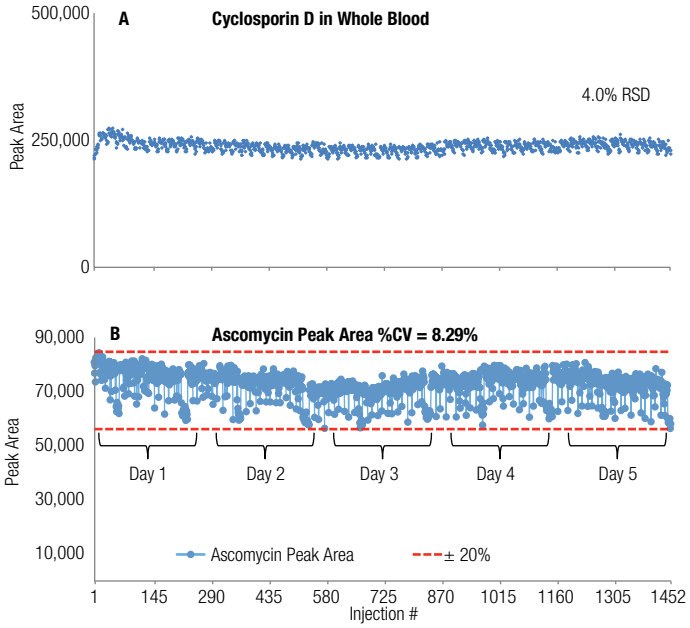

Figure 3. Raw peak area reproducibility across 5 days at approximately 300 injections per day from different batches of whole blood for (A) cyclosporin D internal standard and (B) ascomycin internal standard.

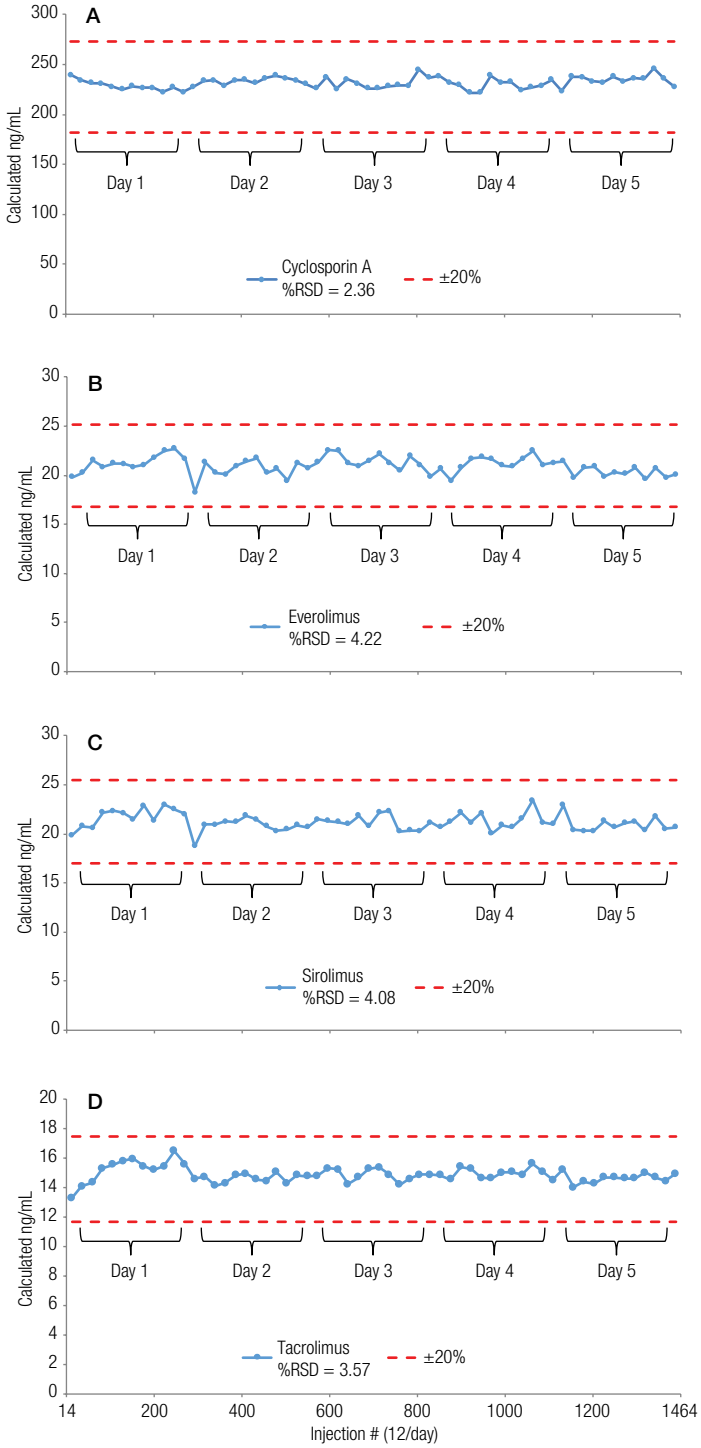

Figure 4. Quality control precision for (A) cyclosporin A, (B) everolimus, (C) sirolimus, and (D) tacrolimus, during 5-day robustness testing. Twelve QCs per day were injected throughout each day's run of approximately 300 samples.

Conclusion

- The TSQ Quantis triple quadrupole MS demonstrated robust, reliable, and consistent performance for more than 1500 samples over five days with no need for maintenance.
- Calibrators and controls maintain precision and accuracy demonstrating reliability.
- The quantitative workflow exhibited reproducible results on a sensitive and robust platform suitable for analysis of immunosuppressant drugs in blood for clinical research.

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