thermoscientific

TECHNICAL NOTE 65206

Tomorrow's quantitation: increased robustness for quantitation of immunosuppressant drugs in blood with the TSQ Fortis MS for clinical research

Authors

Neloni Wijeratne, Claudia Martins, Kristine Van Natta, Xiaolei Xie, Thermo Fisher Scientific, San Jose, CA

Debadeep Bhattacharyya, Thermo Fisher Scientific, Boston, MA

Keywords

Immunosuppressant drugs, LC-MS/MS, tacrolimus, sirolimus, everolimus, cyclosporin A, triple quadrupole MS, TSQ Fortis MS, quantitation workflow, TraceFinder software, clinical research, robustness

Goal

Development of a robust, reliable, and reproducible workflow solution for the analysis and quantitation of immunosuppressant drugs in blood for clinical research using triple quadrupole mass spectrometry coupled with ultra-high performing liquid chromatography.

Application benefits

- Development of a robust workflow for the analysis and quantitation of immunosuppressant drugs in blood with an ultra-high performing liquid chromatograph (UHPLC) and Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer (QqQ)
- Leveraging enhanced performance of a robust QqQ with the sensitivity needed to develop a robust, reliable, reproducible quantitative workflow

Introduction

Starting with cyclosporine A, several immunosuppressant drugs have been developed and introduced, including the macrolides tacrolimus, sirolimus, and everolimus (Figure 1).

ThermoFisher SCIENTIFIC

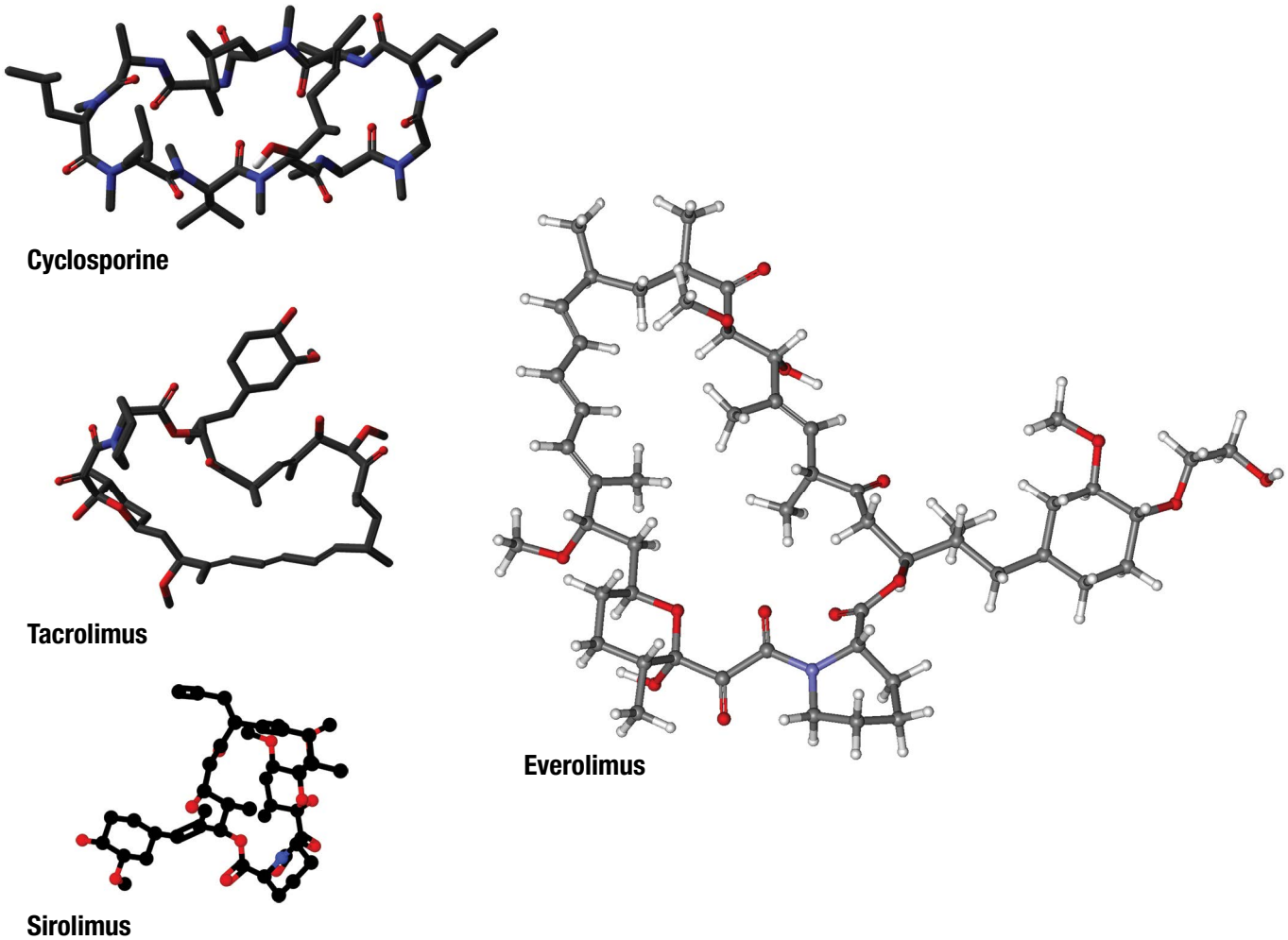

Figure 1. Molecular structures of the four immunosuppressants used in this study

While several analytical techniques are available for determination and quantitation of analytes in complex matrices, most of them lack sensitivity or selectivity, both of which are critical for determination and quantitation of immunosuppressants in blood.

Liquid chromatography (LC) coupled to triple quadrupole mass spectrometery (QqQ) has gained widespread popularity and is considered the analytical platform of choice for analysis of immunosuppressants. Since these drugs are required to be monitored in whole blood, there is an additional layer of complexity and challenge in sample preparation. In this study, we developed a robust, reliable, reproducible method for the determination and quantitation of immunosuppressant drugs in blood for clinical research with LC-MS/MS using a Thermo Scientific™ Vanquish™ Flex UHPLC system, a Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ 4.1 software.

Experimental

Sample preparation

Calibrators and controls were obtained from RECIPE Chemicals and 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 1 min, left to stand for 30 min in a refrigerator, and centrifuged at 13,000 rpm for 10 min. Supernatant was transferred to an autosampler vial and was injected onto the HPLC system.

Liquid chromatography

LC analysis was performed on a Vanquish Flex Binary UHPLC system. The column used was a Thermo Scientific™ Hypersil GOLD™ C8 LC column (50 × 2.1 mm, 5 µm particle size, P/N 25205-01002), maintained at 75 °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. The total UHPLC gradient was 3 minutes (Table 1). For each of the 2077 injections, 15 µL of sample was used.

Table 1. UHPLC gradient information at 0.8 mL/min

Time (min)	%B Concentration (mM)
0.00	30.0
0.25	30.0
0.75	95.0
1.90	95.0
2.00	30.0
3.00	30.0

Mass spectrometry

The TSQ Fortis triple quadrupole mass spectrometer was used for this analysis. All compounds for this study were analyzed in positive heated electrospray ionization mode (HESI). The experimental conditions were optimized with a static spray voltage, a cycle time of 0.4 s, and both Q1 and Q3 resolutions maintained at 0.7 Da FWHM. The source parameters and SRM table along with other critical MS features for all the target analytes are listed in Tables 2 and 3, respectively.

Table 2. Source parameters for analysis of immunosuppressants on the TSQ Fortis triple quadrupole mass spectrometer

Ion Source Parameter	Value
Spray Voltage	Static
Positive Ion	3000 V
Sheath Gas	50 Arb
Aux Gas	10 Arb
Sweep Gas	2 Arb
Ion Transfer Tube Temperature	300 °C
Vaporizer Temperature	325 °C
CID Gas	2 mTorr

Individual standards were infused into the mass spectrometer to determine optimum tube lens settings and collision energies for the product ions.

Software

Data acquisition and processing were conducted using TraceFinder software version 4.1.

Table 3. Optimized mass spectrometer transitions for the immunosuppressant drugs in blood with retention time of 1.5 min, retention time window of 3 min, and positive polarity for each sample. All precursor ions are monitored as ammoniated adducts.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	Tube Lens (V)
Ascomycin	809.52	756.46	20.65	150
Tacrolimus	821.52	768.47	20.65	151
Tacrolimus	821.52	786.55	17.01	151
Sirolimus	931.59	864.54	16.79	157
Sirolimus	931.59	882.54	11.59	157
Everolimus	975.62	908.55	17.32	160
Everolimus	975.62	926.61	12.73	160
Cyclosporin-A	1219.88	1184.82	32.29	184
Cyclosporin-A	1219.88	1202.84	17.28	184
Cyclosporin-D-A	1233.74	1216.88	18.30	186

Results and discussion

Test of robustness

The daily sequence of samples comprised 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 350. The sequence was repeated for six consecutive days. Peak areas of the internal standards and calculated concentration of the mid-level control were monitored for stability. Consistent performance was demonstrated by monitoring the calculated concentration of the analytes reported under strict acceptance criteria concerning accuracy and precision. In addition, after 1750 consecutive injections, the ion transfer tube was cleaned to demonstrate consistent performance after user level maintenance (Figures 2, 4, and 5).

Peak areas of the two internal standards (cyclosporine D and ascomycin) showed remarkable precision over the duration of six days for 2077 injections. Calculated precisions for cyclosporin A, everolimus, sirolimus, and tacrolimus were 4%, 6%, 6%, and 5%, respectively (Figure 2).

Linearity

All analytes exhibited a high degree of linearity over their calibration ranges of approximately 2–60 µg/L (except cyclosporin with a range of 26–1700 µg/L), throughout the duration (6 days) of analysis (Figure 3). All calibrators back-calculated to within 20% of theoretical values over the six days of testing. Figure 4 highlights the excellent quantitative performance throughout the 6 day operation window. Consistent quantitative performance is observed before and after user level maintenance.

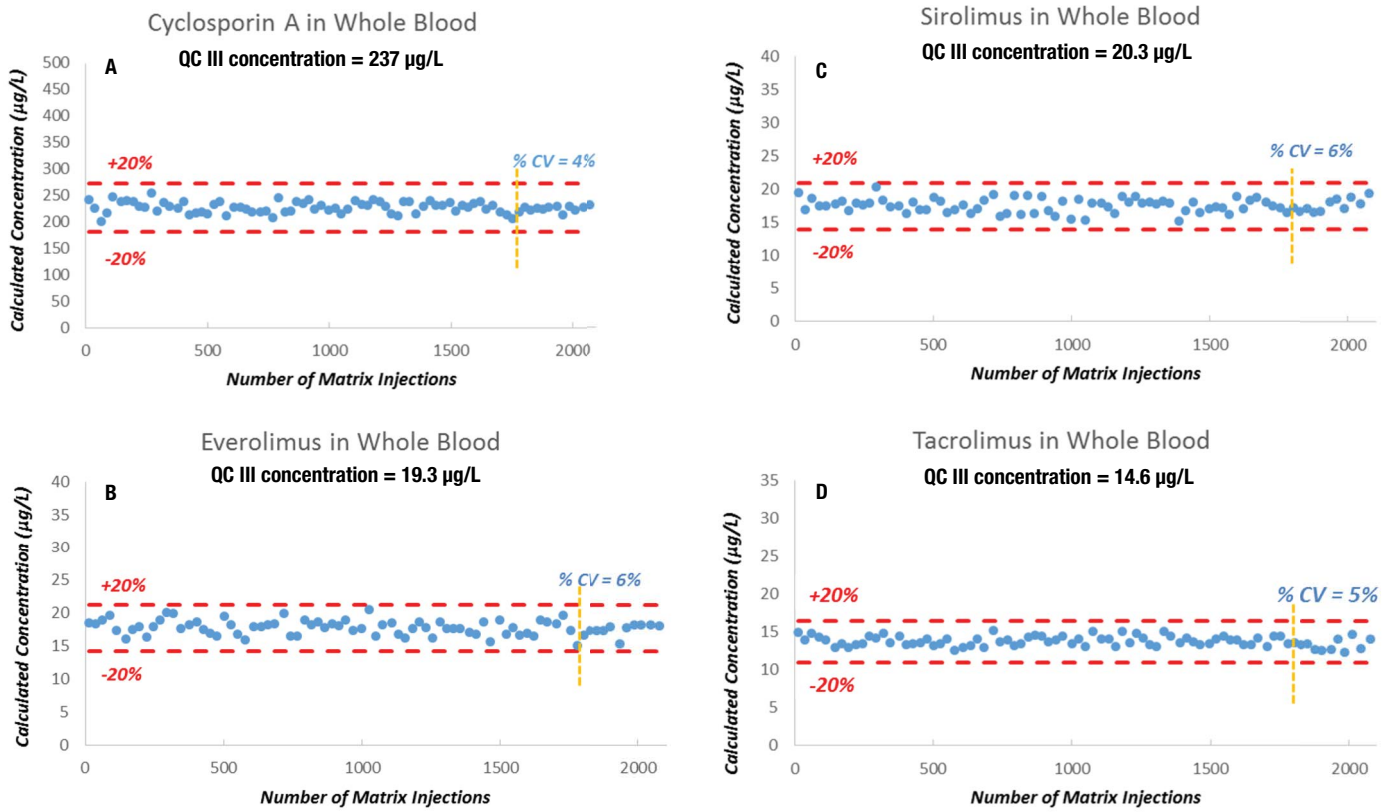

Figure 2. Quality control precision for (A) cyclosporin A, (B) everolimus, (C) sirolimus, and (D) tacrolimus in six days over 2077 injections. Thirteen mid-level QCs per day were injected throughout each day's run of approximately 350 samples. The yellow marks represent the period in which the ion transfer tube was cleaned. This cleaning operation takes place without venting the system.

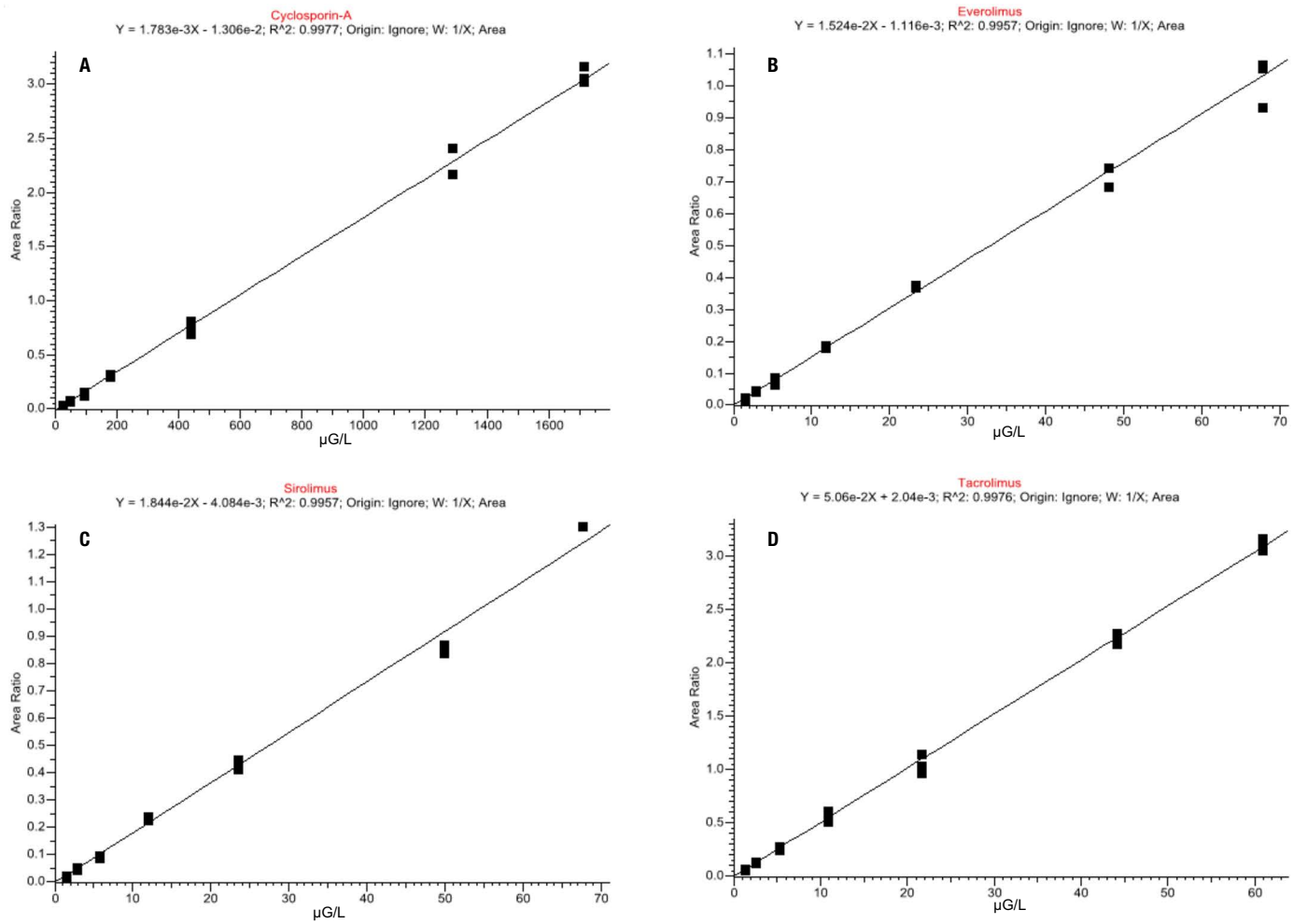

Figure 3. Calibration curve of (A) cyclosporin A, (B) everolimus, (C) sirolimus, and (D) tacrolimus observed on the sixth day of analysis

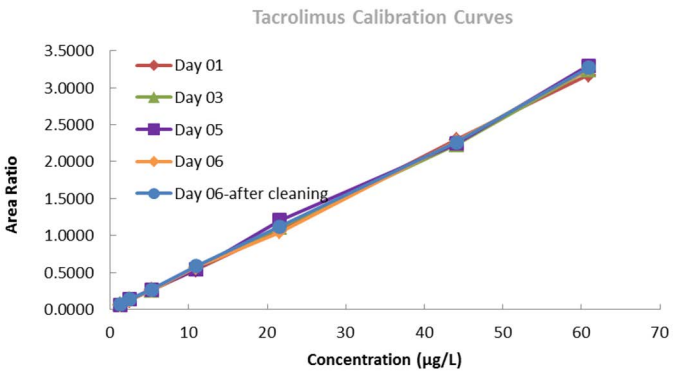

Figure 4. Overlapping calibration curves for tacrolimus obtained during the 6-day experiment (before and after cleaning of ion transfer tube)

Figure 5 shows representative chromatograms for all the four analytes on the sixth day of testing, before and after cleaning of the ion transfer tube. Similar responses have been obtained before and after the user-level maintenance, demonstrating the robustness of the system.

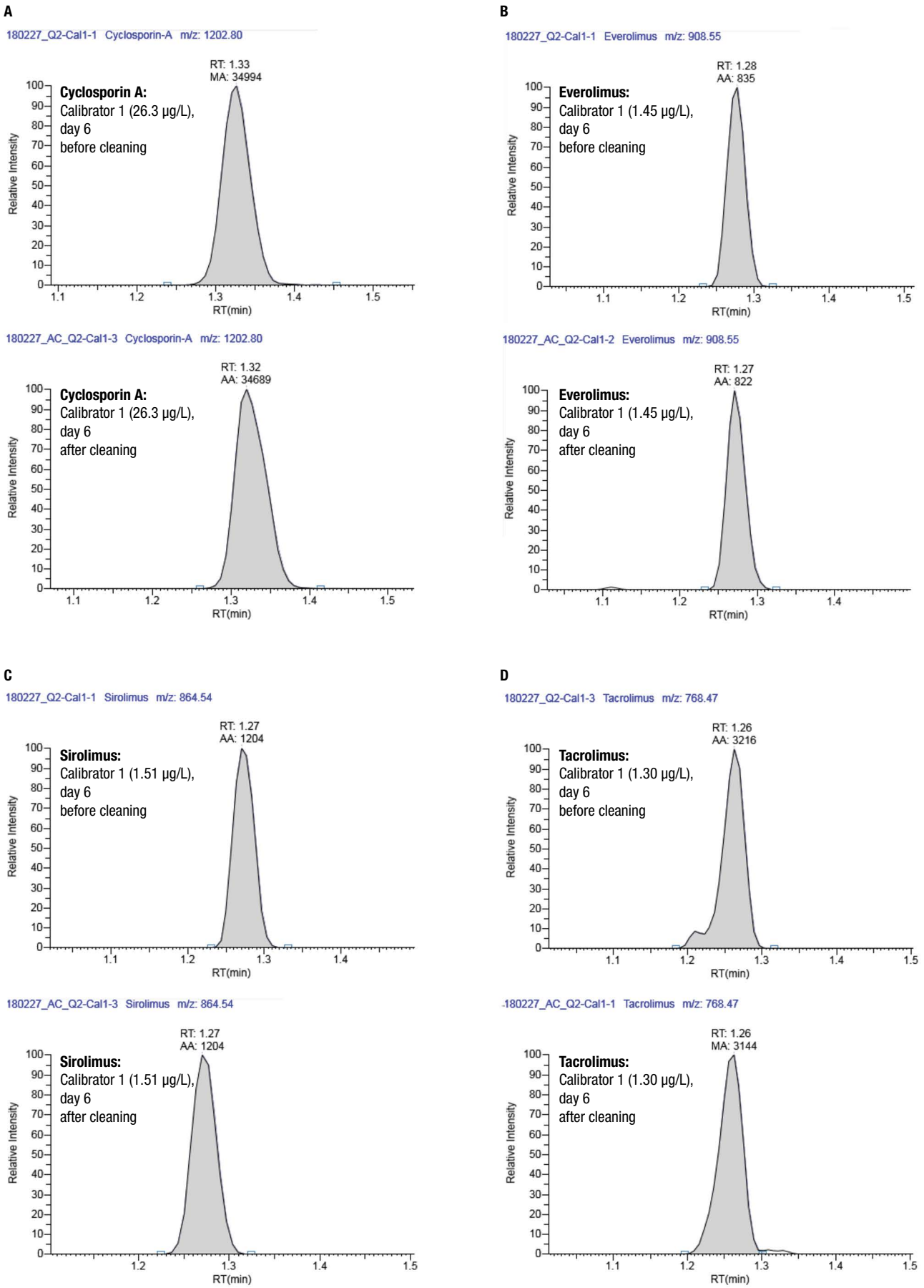

Figure 5. Chromatograms of (A) cyclosporin A, (B) everolimus, (C) sirolimus, and (D) tacrolimus observed on the sixth day of analysis, before and after cleaning of the ion transfer tube

thermo scientific

Conclusions

In this study, a simple, robust, reproducible method with the required sensitivity was developed for the analysis of four commonly used immunosuppressants obtained from whole blood samples for clinical research. Every analytical laboratory quantifying immunosuppressants in biological matrices require high robustness, sensitivity, and the ability to address a large volume of samples. The quantitation workflow detailed in this technical note highlights the unprecedented robustness, reliability, and reproducibility with sensitivity that can be obtained with Vanquish Flex Binary UHPLC and TSQ Fortis triple quadrupole mass spectrometer.

For Research Use Only. Not for use in diagnostic procedures.

Find out more at **thermofisher.com/Fortis**

ThermoFisher SCIENTIFIC