

LC-MS/MS Analysis of Immunosuppressant Drugs in Whole Blood using the Xevo™ TQ Absolute with the Capitainer® B Device for Clinical Research

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Abstract

Here we describe a clinical research method using liquid-liquid extraction from a dried blood spot, itself collected from a small volume of pipetted human whole blood.

Benefits

- Analytical sensitivity from low volume samples using the Xevo TQ Absolute
- Fast analytical run time, with simultaneous analysis of four analytes, afforded by the analytical selectivity of the chromatography and mass detection
- Small sample volume with a Capitainer B device provided good precision and accuracy

Introduction

Deprez *et al* have previously demonstrated an accurate, reproducible LC-MS/MS method for the analysis of cyclosporine, everolimus, sirolimus, and tacrolimus from a small volume of whole blood, by pipetting onto the inlet of a Capitainer B Device in order to generate a dried blood spot of known volume.¹

Here we describe a clinical research method using liquid-liquid extraction from a dried blood spot, itself collected from a small volume of pipetted human whole blood. Chromatographic elution using a Waters™ ACQUITY™ UPLC™ HSS C₁₈ SB Column on a Waters ACQUITY UPLC I-Class PLUS was completed within 1.5 minutes. Simultaneous analysis of all four analytes, followed by detection on a Xevo TQ Absolute Mass Spectrometer (Figure 1) was achieved with a time of 2.2 minutes from injection-to-injection.



Figure 1. The Waters Xevo TQ Absolute Mass Spectrometer.

Experimental

Sample Preparation

Whole blood MassTrak Immunosuppressant Calibrators and Quality Control Sets containing cyclosporine, everolimus, sirolimus and tacrolimus were used. Respectively, $^2\text{H}_{12}$ -cyclosporine, $^{13}\text{C}_2^2\text{H}_4$ -everolimus, $^2\text{H}_3$ -sirolimus, and ascomycin were used as internal standards.

The calibrator and QC concentrations (at low, medium, and high concentrations) are detailed in Table 1.

Analyte	Calibrator range (ng/mL)	QC concentrations (ng/mL)
Cyclosporine	25–1500	150, 400, 900
Everolimus	1–30	2, 8, 22
Sirolimus	1–30	2, 8, 22
Tacrolimus	1–30	2, 8, 22

Table 1. Calibrator and QC concentrations.

Sample Extraction

Pipette 30 μL sample onto the inlet of the device, and check that a viable, 10 μL , dried blood spot is formed, then allow to dry overnight. Transfer the dried blood spot to a microcentrifuge tube, add 200 μL working internal standard, then mix on a multi-tube vortex mixer (2500 r.p.m. for 30 minutes), sonicate (20 minutes), and a final vortex mix (2500 r.p.m. for 10 minutes). Add 10 μL of 0.05M hydrochloric acid and 1 mL *tert*-methyl butyl ether. Mix on a multi-tube vortex mixer (2500 r.p.m. for three minutes) and centrifuge at 25155 g for two minutes. Transfer 850 μL of the upper layer to a total recovery vial (p/n: [186005669CV < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186005669cv-truview-lcms-certified-clear-glass-12-x-32-mm-screw-neck-total-r.html>](https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186005669cv-truview-lcms-certified-clear-glass-12-x-32-mm-screw-neck-total-r.html)) and evaporate to dryness under a stream of nitrogen at 40 °C. Reconstitute in 200 μL of mobile phase A:mobile phase B 50:50 (v:v).

UPLC Conditions

System:	ACQUITY UPLC I-Class PLUS with FL
Needle:	20 µL
Loop:	50 µL
Column:	ACQUITY UPLC HSS C ₁₈ SB Column; 1.8 µm, 2.1 x 30 mm (p/n: 186004117)
Column temperature:	55 °C
Sample temperature:	10 °C
Injection volume:	20 µL
Injection mode:	Partial Loop, with Load Ahead disabled
Mobile phase A:	Water + 0.05 mM ammonium fluoride
Mobile phase B:	Methanol + 0.05 mM ammonium fluoride
Weak wash:	Water:methanol 95:5 (v:v), 600 µL
Strong wash:	Water:methanol:acetonitrile:IPA 25:25:25:25 (v:v:v:v), 200 µL
Seal wash:	Water:methanol 80:20 (v:v)
Run time:	1.5 minutes (2.2 minutes)

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.80	50	50	Initial
0.2	0.80	50	50	1
0.6	0.80	0	100	6
1.2	0.80	50	50	11

Table 2. Gradient for immunosuppressants chromatographic elution.

MS Conditions

System:	Xevo TQ Absolute
Resolution:	MS1 (0.7 FWHM) MS2 (0.7 FWHM)
Acquisition mode:	Multiple Reaction Monitoring (MRM) (see Table 3 for details)
Polarity:	ESI positive ionization (ESI+)
Capillary:	1.0 kV
Source temperature:	120 °C
Desolvation temperature:	400 °C
Inter-scan delay:	0.003 seconds
Inter-channel delay:	0.02 seconds

Data Management

MassLynx™ v4.2 with TargetLynx™ Application Manager.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)	Dwell time (s)
Cyclosporine (Quantifier)	1219.8	1202.8	50	18	0.020
Cyclosporine (Qualifier)	1219.8	1184.8	50	34	0.020
² H ₁₂ -Cyclosporine (Internal standard)	1231.8	1214.8	50	18	0.020
Everolimus (Quantifier)	975.6	908.6	50	16	0.020
Everolimus (Qualifier)	975.6	926.6	50	12	0.020
¹³ C ₂ ² H ₄ -Everolimus (Internal standard)	981.6	914.6	50	16	0.020
Sirolimus (Quantifier)	931.6	864.5	50	16	0.020
Sirolimus (Qualifier)	931.6	882.5	50	12	0.020
² H ₃ -Sirolimus (Internal standard)	934.6	864.5	50	16	0.020
Tacrolimus (Quantifier)	821.5	768.5	50	20	0.020
Tacrolimus (Qualifier)	821.5	786.5	50	18	0.020
Ascomycin (Internal standard)	809.5	756.5	50	22	0.020

Table 3. Guideline MRM parameters for analytes and internal standards used in this study.

Results and Discussion

Five analytical runs were performed using this method.

Figure 2 shows example chromatograms of calibrator 1 (25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus).

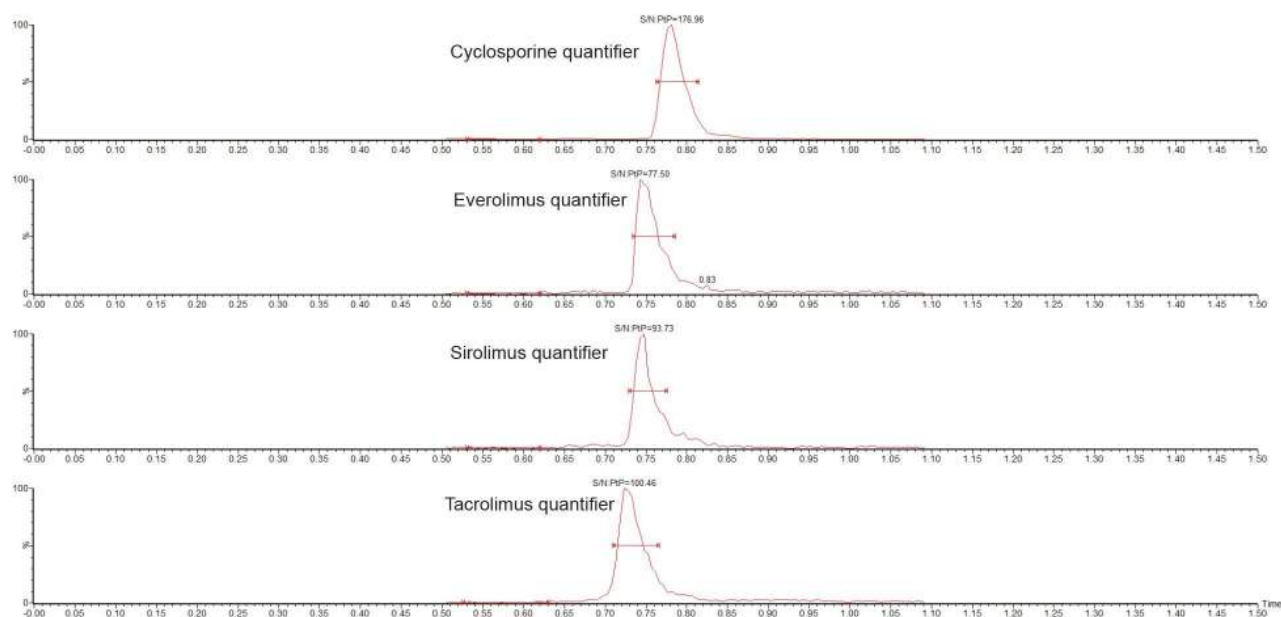


Figure 2. Chromatograms showing the analysis of 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus and tacrolimus using the ACQUITY UPLC I-Class PLUS FL/Xevo TQ Absolute System.

Analytical sensitivity of the lowest calibrators was demonstrated with S/N (PtP) > 10:1 across the five analytical runs (Table 4).

Analyte	Concentration (ng/mL)	Mean S/N (PtP)
Cyclosporine	25.2	216
Everolimus	1.1	76
Sirolimus	1.0	63
Tacrolimus	1.1	128

Table 4. Analytical sensitivity summary.

No system carryover was observed in each run following analysis of a calibrator 6 sample with a blank sample for cyclosporine, everolimus, sirolimus, and tacrolimus.

The calibration curves over five analytical runs were linear with $r^2 \geq 0.99$ for each of the immunosuppressants over their calibration ranges (presented in Table 1).

Total precision was determined by extracting and quantifying five replicates of three concentrations of whole blood pools over five separate days (n=25). The exception was for cyclosporine, for which there were four separate days (n=20). Repeatability was assessed by analysing five replicates at each QC level. Figure 3 presents results of these experiments, where total precision and repeatability at the three concentrations assessed was $\leq 7.6\%$ RSD.

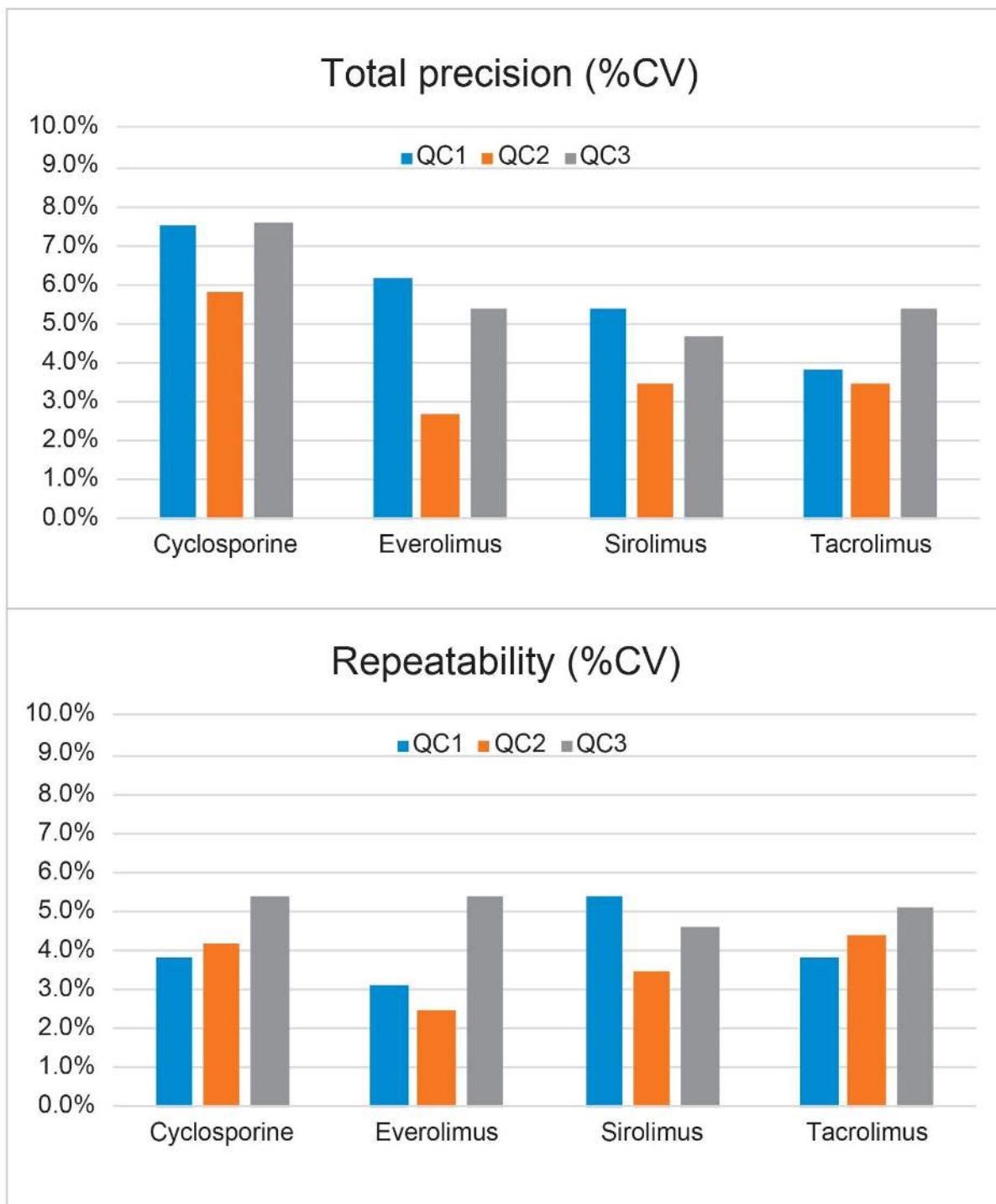


Figure 3 . Total precision and repeatability performance.

LGC (Bury, UK) whole blood External Quality Assurance samples were sourced and analysed to assess method accuracy. A summary is presented in Table 5.

Analyte	Number of samples analyzed (n)	Range (ng/mL)	Mean %bias from scheme LC-MS ALTM
Cyclosporine	20	31.0–1814.1	6.0
Everolimus	20	0–23.2	2.9
Sirolimus	25	1.9–22.9	-5.0
Tacrolimus	25	1.6–27.0	5.6

Table 5. Accuracy summary (Note: ALTM is the all-laboratory trimmed mean).

Conclusion

Simultaneous analysis of the immunosuppressant drugs cyclosporine, everolimus, sirolimus and tacrolimus can be achieved in a single analysis of a little over two minutes (injection-to-injection), using a very small whole blood volume together with a Capitainer B device to generate a dried blood spot.

The performance characteristics of the method indicate good analytical sensitivity, total precision, and repeatability ($\leq 7.6\%$ RSD) across all analytes and concentrations tested.

Finally, good agreement was obtained when analysing External Quality Assurance samples, providing confidence in the accuracy of the method.

Acknowledgements

Capitainer are thanked for the provision of Capitainer B devices for this study.

¹Deprez S, Van Uytendange K and Stove CP. Liquid chromatography-tandem mass spectrometry for therapeutic drug monitoring of immunosuppressants and creatinine from a single dried blood spot using the Capitainer qDBS device. *Analytica Chimica Acta*. 1242 (2023) 340797.

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720007893, March 2023

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