LC-MS/MS ANALYSIS OF IMMUNOSUPPRESSANT DRUGS IN WHOLE BLOOD USING THE XEVO TQ ABSOLUTE WITH CAPITAINER® B 50 DEVICES FOR CLINICAL RESEARCH

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INTRODUCTION

Traditional laboratory analysis of the immunosuppressant drugs cyclosporine, everolimus, sirolimus and tacrolimus is well-established in clinical research. However there remains a need for individuals to undergo an invasive, time-consuming and disruptive process under the supervision of trained staff in order to collect a sufficient volume of whole blood for laboratory analysis.

A reliable, remote sampling method may find utility in a clinical research setting. Here we describe the use of Capitainer® B 50 Devices to obtain analytically sensitive, precise and accurate data for cyclosporine, everolimus, sirolimus and tacrolimus analysis using small sample volumes for clinical research studies.

The Waters™ ACQUITY™ UPLC™ I-Class FL with Xevo™ TQ Absolute mass spectrometer was used to analyze these samples.

METHODS

Materials and Sample Preparation

- MassTrak Immunosuppressant Calibrator and Control Sets were used.
- 30 µL of whole blood was pipetted onto the inlet of the Capitainer® B 50 device, which resulted in a 10 µL dried blood spot.
- Following overnight drying, the dried blood spot was removed and placed in a 2mL microcentrifuge tube.
- 200 µL of internal standard (12.5 ng/mL $^{2}H_{12}$-cyclosporine, 1 ng/mL ascomycin, $^{13}C_{2}{}^{2}H_{4}$-everolimus and $^{2}H_{3}$-sirolimus in 10% methanol) was added, and the tube underwent mixing and sonication steps.
- Add 10 µL of 0.05M hydrochloric acid and 1 mL *tert*-methyl butyl ether, vortex mix and centirfuge.
- 850 µL of the top layer was transferred to a clean, TruView Total Recovery vial (p/n: 186005669CV), and dried under nitrogen at 40°C.
- Samples were reconstituted in 200 µL mobile phase A:mobile phase B 50:50 (v:v).

LC-MS/MS Parameters

- Using an ACQUITY UPLC I-Class FL System, samples were injected onto an ACQUITY UPLC HSS C_{18} SB Column, 1.8µm 2.1x30mm (p/n: 186004117), using a water/acetonitrile/ammonium fluoride gradient (Table 1) and analyzed with a Xevo TQ Absolute Mass Spectrometer (Figure 1) in ESI+, using MRM parameters described in Table 2.
- The run time is 1.5 minutes (approximately 2.2 minutes injection-to-injection).

Time [min]	Flow rate [mL/min]	A [%]	B [%]	Curve
Initial	0.8	50	50	Initial
0.2	0.8	50	50	1
0.6	0.8	0	100	6
1.2	0.8	50	50	11

Table 1. *LC gradient for analysis of the immunosuppressants using the ACQUITY UPLC I-Class FL System*

Analyte	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision Energy (V)
Cyclosporine $^{2}H_{12}$-Cyclosporine	1219.8 1231.8	1202.8 (1184.8) 1214.8	0.02 0.02	50 50	18 (34) 18
Everolimus $^{13}C_{2}{}^{2}H_{4}$-Everolimus	975.6 981.6	908.6 (926.6) 914.6	0.02 0.02	50 50	16 (12) 16
Sirolimus $^{2}H_{3}$-Sirolimus	931.6 934.6	864.5 (882.5) 864.5	0.02 0.02	50 50	16 (12) 16
Tacrolimus Ascomycin	821.5 809.5	768.5 (786.5) 756.5	0.02 0.02	50 50	20 (18) 22

Table 2. *MRM transitions and parameters of the immunosuppressants and internal standards (qualifier parameters in parentheses)*

Figure 1. *The Xevo TQ Absolute Mass Spectrometer*

RESULTS

Five analytical runs were performed using this method.

Chromatography and Analytical Sensitivity

- The analytical sensitivity of the developed method for the lowest calibrator (1 ng/mL for everolimus, sirolimus and tacrolimus; 25 ng/mL for cyclosporine) is shown in Figure 2, with S/N (PtP) > 10 for the four analytes over the five runs.

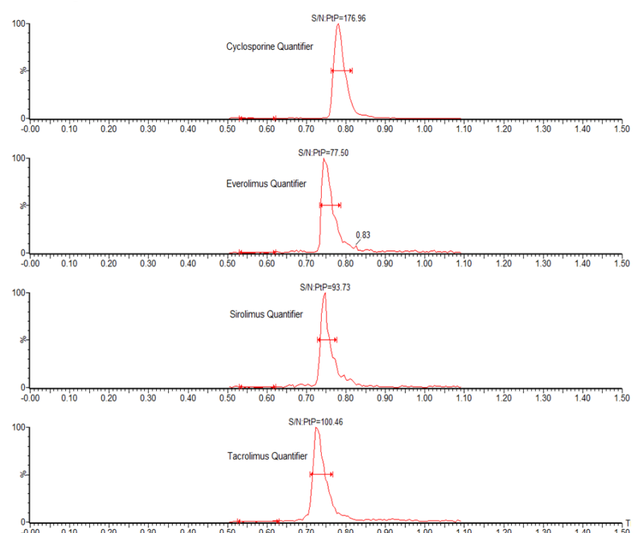

Figure 2. *Representative chromatograms of the lowest immunosuppressant calibrator, demonstrating analytical sensitivity*

Linearity and Precision

- Linearity of the calibration ranges (1-30 ng/mL for everolimus, sirolimus and tacrolimus; 25-1500 ng/mL for cyclosporine) was demonstrated with mean r^2 values for the calibration lines >0.99 over five analytical runs.
- Total reproducibility and repeatability across the immunosuppressants at the QC three concentrations (2, 8 and 22 ng/mL for all analytes except cyclosporine, which were 150, 400 and 900 ng/mL), with five* replicates over five analytical runs (n = 25*) was ≤7.6%CV (Figure 3).

  *except cyclosporine, four runs and n=20

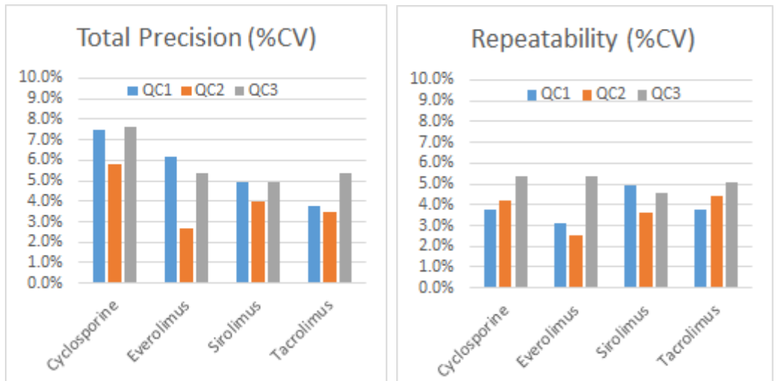

Figure 3. *Total reproducibility and repeatability*

Accuracy

- LGC (Bury, UK) whole blood External Quality Assurance samples were sourced and analyzed to assess method accuracy. A summary is presented in Table 3.
- All individual samples met the criteria of the scheme, with mean bias ≤8.7%.

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	-8.7
Tacrolimus	25	1.6-27.0	+5.6

Table 3. *EQA accuracy summary (note: ALTM is the all-laboratory trimmed mean)*

CONCLUSION

- **Capitainer B 50 devices prepared dried blood spots from a low initial whole blood volume, enabling simultaneous analysis of the immunosuppressant drugs cyclosporine, everolimus, sirolimus and tacrolimus in 2.2 minutes (injection-to-injection).**
- **The performance characteristics of the method indicate good analytical sensitivity, total precision and repeatability (≤7.6%CV) across all analytes and concentrations tested.**
- **Finally, good agreement was obtained when analyzing External Quality Assurance samples, providing confidence in the accuracy of the method and sample collection devices.**

ACKNOWLEDGEMENT

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