A CLINICAL RESEARCH METHOD FOR THE ANALYSIS OF IMMUNOSUPPRESSANT DRUGS IN WHOLE BLOOD USING CAPITAINER™ B DEVICES

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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 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

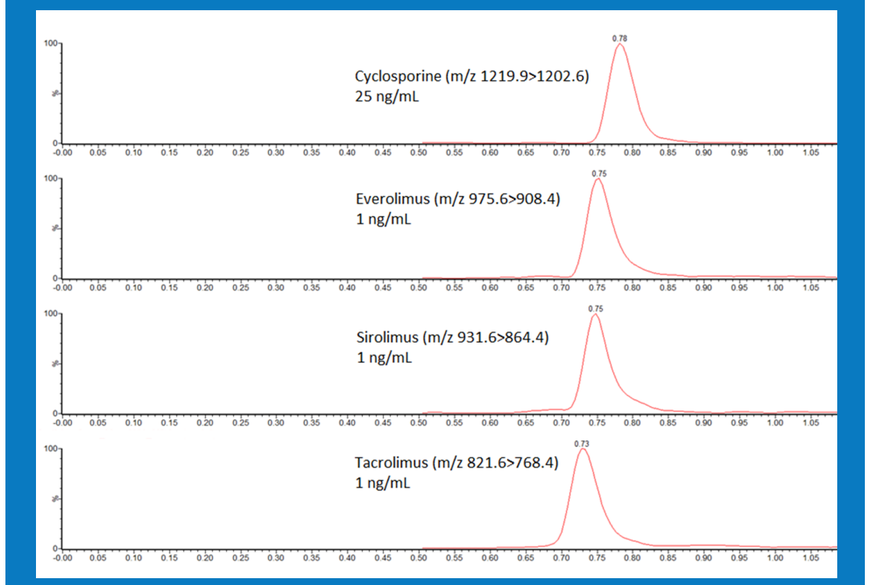
Materials and Sample Preparation

- MassTrak™ Immunosuppressant Calibrator and Control Sets were used (see poster 749 for more details).
- 30 µL of whole blood was pipetted onto the inlet of the Capitainer™ B device, which resulted in a 10 µL dried blood spot (DBS).
- Following overnight drying, the DBS 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 was added, vortex mixed and centrifuged.
- 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 and analyzed with a Xevo TQ Absolute Mass Spectrometer in ESI+,
- The run time is 1.5 minutes (approximately 2.2 minutes injection-to-injection).

Analytical sensitivity from a 10 µL DBS



Analytical sensitivity of a calibrator 1 sample containing 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus and tacrolimus

RESULTS

Five analytical runs were performed using this method.

- **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 precision and repeatability** was ≤7.6%CV (Figure 1) 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) except cyclosporine, four runs and n=20.

Figure 1. *Total precision and repeatability*

- **Mean Accuracy** (%bias) to whole blood External Quality Assurance samples was within ±6% of the LC-MS All Laboratory Trimmed Mean (ALTM, Table 1).

Analyte	Number of samples analyzed	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 1. *EQA accuracy summary*

Note: Waters, ACQUITY, UPLC, Xevo, MassTrak, and TruView are trademarks owned by Waters Technologies Corporation. Capitainer is a trademark owned by Capitainer AB.

CONCLUSION

- **Using Capitainer B microsampling devices and very small sample volumes (an initial 30µL whole blood sample resulted in a 10 µL dried blood spot), an in-house laboratory method was developed for cyclosporine, everolimus, sirolimus and tacrolimus.**
- **The method met validation goals for linearity, analytical sensitivity, precision and accuracy demonstrating the potential for the Xevo TQ Absolute Mass Spectrometer for clinical research using microsampling.**
- **MassTrak Immunosuppressant Calibrator and Quality Control Sets may be used, allowing significant time and resource savings.**

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