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Application Note

ACQUITY UPLC I-Class SM-FL/Xevo TQ-S micro IVD System: Analytical Performance for Immunosuppressive Agents

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For *in vitro* diagnostic use. Not available in all countries.

Abstract

The Waters ACQUITY™ UPLC™ I-Class SM-FL/Xevo™ TQ-S micro IVD System enables the quantification of organic compounds in human biological liquid matrices.

This document describes a test of the analytical performance of the ACQUITY UPLC I-Class SM-FL/Xevo TQ-S micro IVD System for the simultaneous analysis of cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood.

Experimental

The ACQUITY UPLC I-Class SM-FL/Xevo TQ-S micro IVD System was controlled by MassLynx™ Software (v4.2) and the data processed using the TargetLynx™ XS Application Manager. Whole blood Calibrators and Quality

Controls were processed using the following conditions:

Sample Preparation Conditions

50 µL sample was processed with zinc sulfate and acetonitrile, then centrifuged.

LC Conditions

Sample Manager:	SM-FL (PLNO, Load Ahead enabled)
Solvent Manager:	BSM
Column:	ACQUITY UPLC HSS C_{18} SB 1.8 µm, 2.1 mm x 30 mm,
Mobile Phase A:	2mM Ammonium acetate + 0.1% formic acid in water
Mobile Phase B:	2mM Ammonium acetate + 0.1% formic acid in methanol
Flow Rate:	0.45 mL/min
Gradient:	50% B for 0.2 minutes, 50–100% B over 0.4 minutes, hold 100% B for 0.6 minutes, equilibrate with 50% B for 0.3 minutes at 0.8 mL/min

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.75FWHM)
Acquisition Mode:	MRM

Polarity:	ESI (+)

Results and Discussion

Performance characteristics of cyclosporine, everolimus, sirolimus, and tacrolimus on the ACQUITY UPLC I-Class SM-FL/Xevo TQ-S micro IVD System are shown in Table 1. Analytical sensitivity of the chromatographic separation is illustrated in Figure 1.

Compound	Range (ng/mL)	LLOQ (ng/mL)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Cyclosporine	25–1500	15	7.8	≤4.8%	≤3.0%	+1.0%
Everolimus	1–30	0.8	16.7	≤6.9%	≤6.3%	+0.9%
Sirolimus	1–30	1.0	11.2	≤9.3%	≤6.5%	-6.9%
Tacrolimus	1–30	1.0	6.3	≤5.5%	≤4.3%	-0.5%

Table 1. Performance characteristics of cyclosporine, everolimus, sirolimus, and tacrolimus. Range defined by linear fit where r^2 >0.995. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %RSD at LLOQ determined through analytical sensitivity experiments performed over five occasions (n=50). Total precision and repeatability of QCs performed over five occasions in whole blood (n=25). EQA mean bias determined by comparison of obtained values to the LC-MS all laboratories trimmed mean (LC-MS ALTM) value (n=39 for cyclosporine and tacrolimus, n=35 for everolimus, and n=33 for sirolimus).

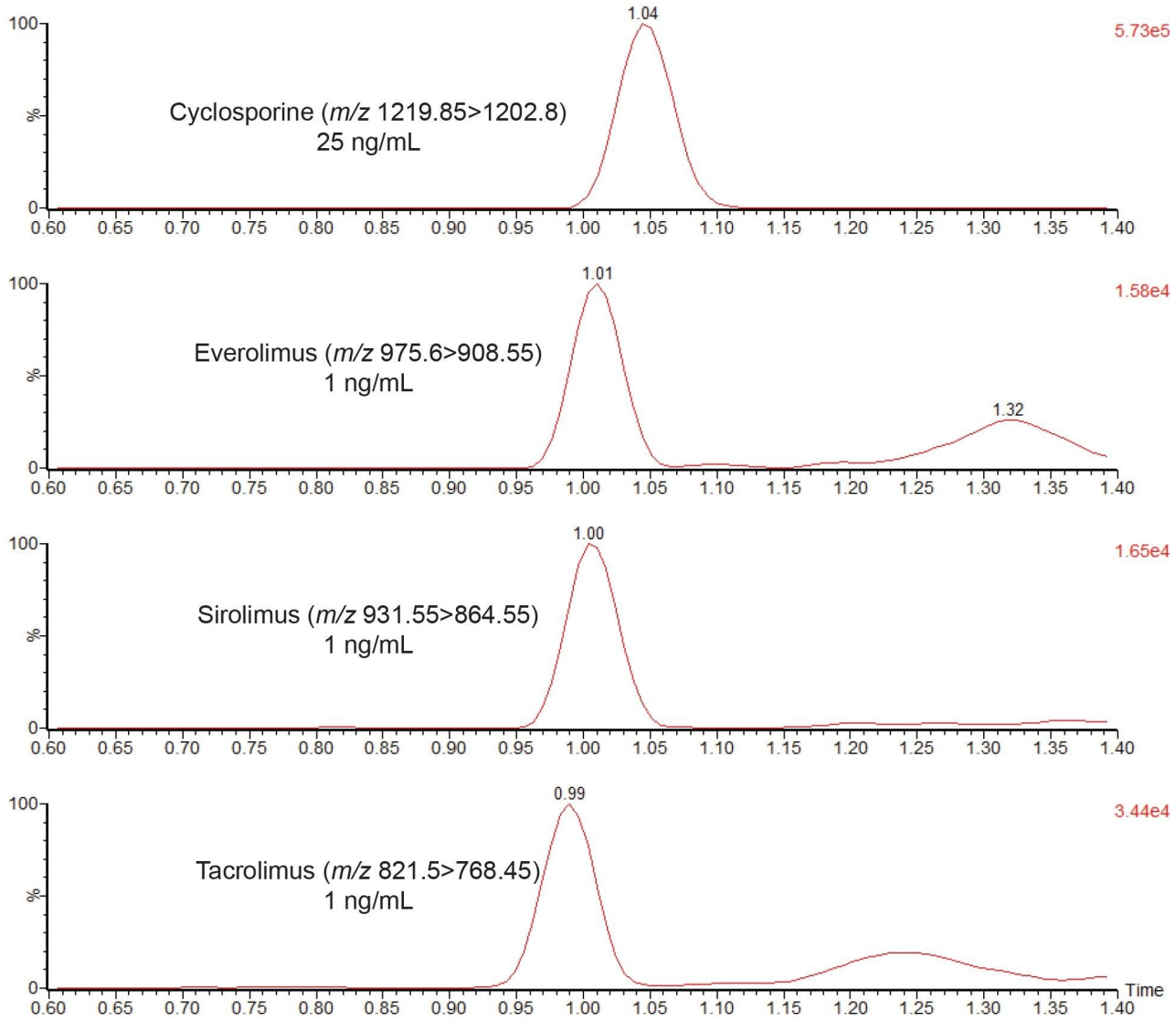

Figure 1. Chromatogram showing the analysis of 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus using the ACQUITY UPLC I-Class SM-FL/ Xevo TQ-S micro IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class SM-FL/Xevo TQ-S micro IVD System has demonstrated the capability to

analyze cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood samples simultaneously while delivering analytical sensitivity and precision.

Disclaimer

The analytical performance data presented here is for illustrative purposes only. Waters does not recommend or suggest analysis of the analytes described herein. These data are intended solely to demonstrate the performance capabilities of the system for analytes representative of those commonly analyzed using liquid chromatography and tandem mass spectrometry. Performance in an individual laboratory may differ due to a number of factors, including laboratory methods, materials used, intra-operator technique, and system conditions. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the analytes in this analysis.

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