

## ACQUITY™ UPLC™ I-Class/Xevo™ TQ Absolute IVD System: Analytical Performance for Immunosuppressive Agents

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This is an Application Brief and does not contain a detailed Experimental section.

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

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### Abstract

The Waters™ ACQUITY™ UPLC™ I-Class/Xevo™ TQ Absolute 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/Xevo TQ Absolute IVD System for the analysis of cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood.

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### Experimental

The ACQUITY UPLC I-Class/Xevo TQ Absolute IVD System was controlled by MassLynx™ IVD (v4.2) and the data processed using the TargetLynx™ 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, acetonitrile, and centrifuged.

## LC Conditions

Column:	ACQUITY UPLC HSS C <sub>18</sub> SB 1.8 µm, 2.1 mm x 30 mm,
Mobile phase A:	2 mM Ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.4mL/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 0.6 minutes at 0.6 mL/min

## MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.75 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

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## Results and Discussion

Performance characteristics of cyclosporine, everolimus, sirolimus, and tacrolimus on the ACQUITY UPLC I-Class/Xevo TQ Absolute 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)	Mean S/N (PtP) at LLOQ	Total precision	Repeatability	EQA mean bias
Cyclosporine	25–1500	25.2	503	≤2.6%	≤2.6%	+1.9%
Everolimus	1–30	1.1	33.1	≤5.4%	≤3.5%	+1.7%
Sirolimus	1–30	1.0	36.4	≤4.1%	≤2.7%	-5.6%
Tacrolimus	1–30	1.1	49.5	≤4.0%	≤3.0%	+2.1%

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*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)  $> 10$ . 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) values ( $n=27$  for cyclosporine,  $n=25$  for everolimus,  $n=28$  for sirolimus, and  $n=27$  for tacrolimus).*

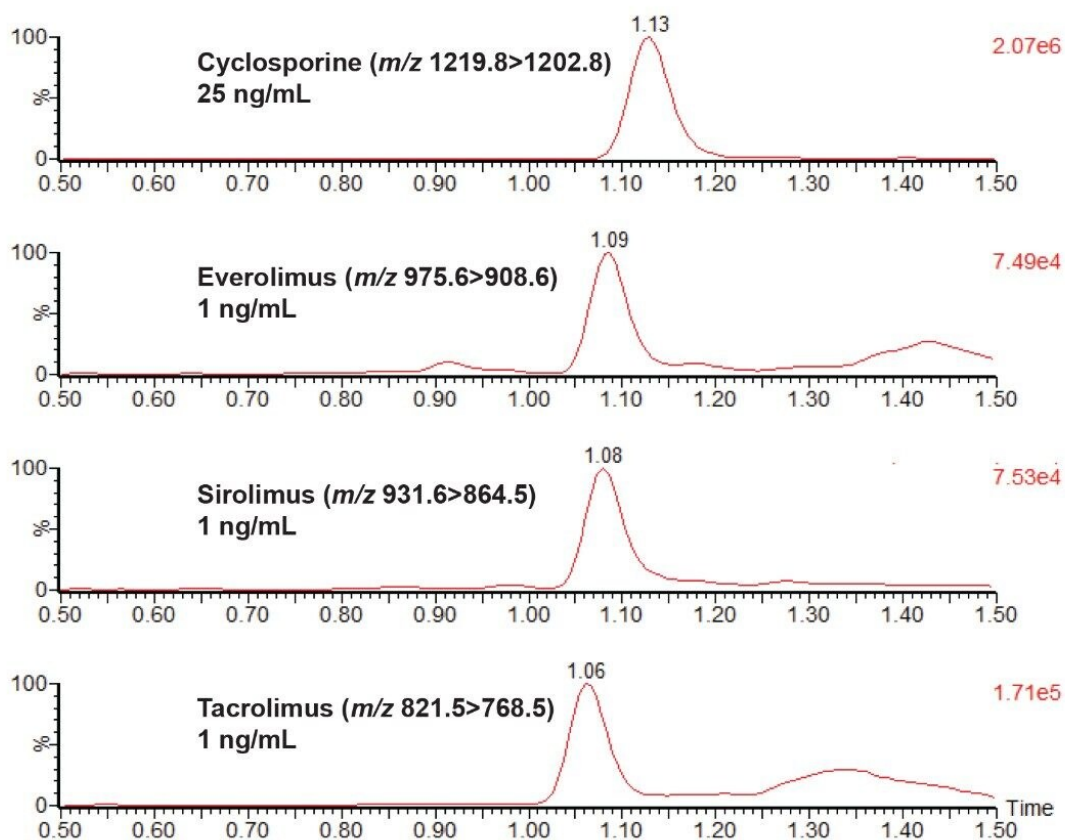


Figure 1. Chromatogram showing the analysis of 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus and tacrolimus using the ACQUITY UPLC I-Class/Xevo TQ Absolute IVD System.

## Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ Absolute IVD System has demonstrated the capability to deliver analytical sensitivity and precision for the analysis of cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood.

## 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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