

## 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.

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### 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.

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### 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 <sub>18</sub> 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 (+)

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## 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%         |

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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  $S/N (PtP) > 10$  and  $\%RSD \leq 20\%$ .  $\%RSD$  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).*

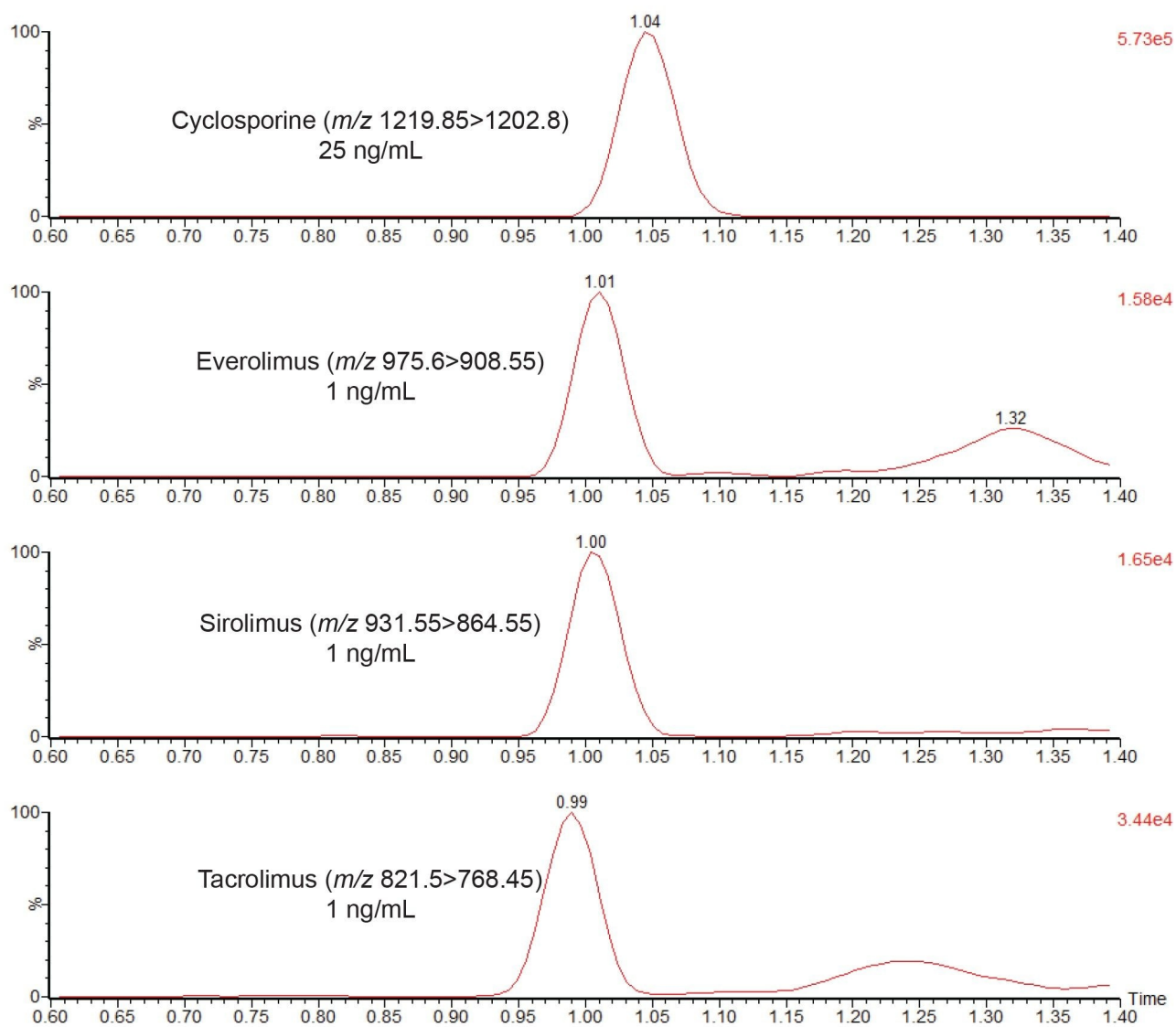


Figure 1. Chromatogram showing the analysis of 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus using the ACQUITY UPLC I-Class SM-FL/ Xevo TQ-S micro IVD System.

## 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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