Waters™

Application Note

Confidence in Your Calibrators: MassTrak™ Immunosuppressant Calibrator and Quality Control Sets for the LC-MS/MS Analysis of Cyclosporine, Everolimus, Sirolimus, and Tacrolimus

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

Abstract

The immunosuppressive drugs cyclosporine, everolimus, sirolimus and tacrolimus have historically been measured using immunoassay. However variable accuracy at low concentrations coupled with specificity issues due to cross-reactivity of antibodies with other components, such as metabolites, can cast doubt on results. This phenomenon is well documented in the literature. As such, many clinical laboratories increasingly analyse these drugs using liquid chromatography with tandem mass spectrometry (LC-MS/MS), for which they require reliable, reproducible calibrators, and quality control sets (QCs) for confidence in their results.

Waters™ MassTrak Immunosuppressant Calibrator and QC Sets (IVD) provide confidence in the accuracy and aid harmonization of results when using validated LC-MS/MS methods.

The MassTrak Immunosuppressant Calibrators and Quality Control Sets performance was demonstrated using the ACQUITY™ UPLC™ I-Class FL and Xevo™ TQ-S micro Triple Quadrupole Mass Spectrometer and an in-house developed LC-MS/MS methodology.

Benefits

- Guiding principles described in ISO17511 adhered to for value assignment
- Confidence in the accuracy of immunosuppressants and provides a path to laboratory method harmonization
- Lyophilized calibrators and QCs that reduce sample preparation time

Introduction

Routine analysis of immunosuppressant drugs in whole blood by immunoassay is highly automatable and affords analytical sensitivity, however issues relating to selectivity and multiplexing remain. As such, reliability of results, particularly at low concentrations can be a concern. Latterly, LC-MS/MS has become increasingly more-widely adopted for immunosuppressant analysis in order to overcome these known limitations.

LC-MS/MS methods in clinical laboratories are often based on laboratory developed tests (LDTs), validated to local regulatory guidelines. These guidelines are constantly evolving and there is increasing demand for all aspects of clinical methods to comply with these changing regulations. This includes the calibrator and QC materials used to generate and independently check the accuracy of the calibration within the method.

The Waters MassTrak Immunosuppressant Calibration and Quality Control Sets (IVD) (Figure 1) contains cyclosporine, everolimus, sirolimus, and tacrolimus in lyophilized whole blood that have been sourced to obtain the highest level of metrological traceability available. In order to demonstrate the quality of materials found in this product, we have shown the proof-of-concept performance of the materials using protein precipitation and separation and detection of the samples using the ACQUITY UPLC I-Class FL with Xevo TQ-S micro Triple Quadrupole Mass Spectrometer.

Figure 1. The Waters MassTrak Immunosuppressant Calibration and Quality Control sets.

Experimental

The Waters MassTrak Immunosuppressant Calibration and Quality Control Sets contain the following immunosuppressant drugs in lyophilized whole blood: cyclosporine, everolimus, sirolimus, and tacrolimus. Assigned concentrations for the calibration range and QCs are found in Table 1.

Immunosuppressant drug	Calibrator range (ng/mL)	Nominal QC concentrations (ng/mL)
Cyclosporine	25-1500	150, 400, 900
Everolimus	1-30	2, 8, 22
Sirolimus	1-30	2, 8, 22
Tacrolimus	1-30	2, 8, 22

Table 1. Concentration ranges of the MassTrak Immunosuppressant Calibrator and Quality Control Sets. The calibrators and QCs are reconstituted following the instructions for use (IFU), prior to sample preparation and analysis.

Sample Description:

Sample preparation was performed by using protein precipitation

Protein Precipitation:

To 50 µL of whole blood sample, 200 µL of 0.1 M aqueous zinc sulfate was added and mixed for five seconds. 500 µL of internal standard (ISTD) was added, followed by mixing for twenty seconds. Samples were then centrifuged for two minutes at 4696 g.

LC Conditions:

System:	ACQUITY UPLC I-Class with FL
Needle:	20 µL
Loop:	50 µL
Column:	ACQUITY UPLC HSS C_{18} SB Column; 1.8 µm, 2.1 x 30 mm (p/n: 186004117)

Column temp.:	55 °C
Sample temp.:	8 °C
Injection volume:	20 µL
Injection mode:	PLNO, with Load Ahead enabled
Mobile phase A:	Water + 2 mM ammonium acetate + 0.1% formic acid
Mobile phase B:	Methanol + 2 mM ammonium acetate + 0.1% formic acid
Weak wash:	Water:methanol 95:5 (v:v), 600 µL
Strong wash:	Water:methanol:acetonitrile:IPA 1:1:1:1 (v:v:v:v), 200 µL
Seal wash:	Water:methanol 80:20 (v:v)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.45	50	50	Initial
0.2	0.45	50	50	1
0.6	0.45	0	100	6
1.2	0.80	50	50	11

MS Conditions

MS system:	Xevo TQ-S micro Triple Quadrupole Mass Spectrometer
Ionization mode:	ESI+
Capillary voltage:	0.8 kV

MRM Parameters

Analyte	MRM	ID	Cone (V)	Collision (V)
Cyclosporine	1219.9>1202.8	Quantifier	35	18
	1219.9>1184.8	Qualifier	35	34
	1231.9>1214.8	ISTD	35	18
Everolimus	975.6>908.6	Quantifier	35	16
	975.6>926.6	Qualifier	35	10
	981.6>914.6	ISTD	35	16
Sirolimus	931.6>864.6	Quantifier	35	16
	931.6>882.6	Qualifier	35	10
	809.6>756.6	ISTD	35	20
Tacrolimus	821.6>768.6	Quantifier	35	20
	821.6>786.6	Qualifier	35	16
	809.6>756.6	ISTD	35	20

Method Events

Time (min)	Event	Action
0	Flow state	Waste
0.6	Flow state	LC
1.4	Flow state	Waste

Results and Discussion

The four immunosuppressive drugs were chromatographed using the ACQUITY UPLC 2.1 mm x 30 mm HSS C_{18} SB Column.

Figure 2 shows an example chromatogram of calibrator 1 (25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus).

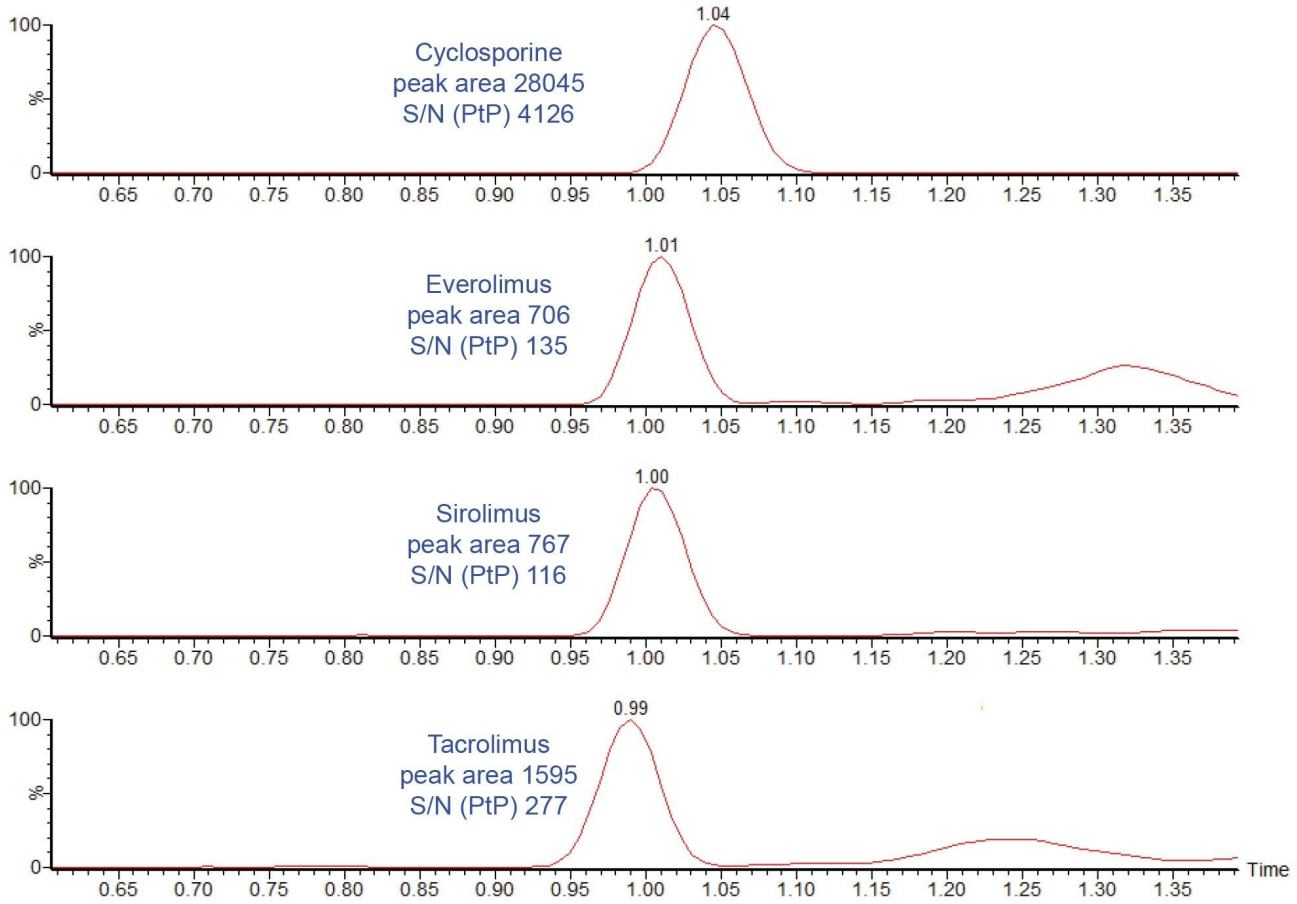
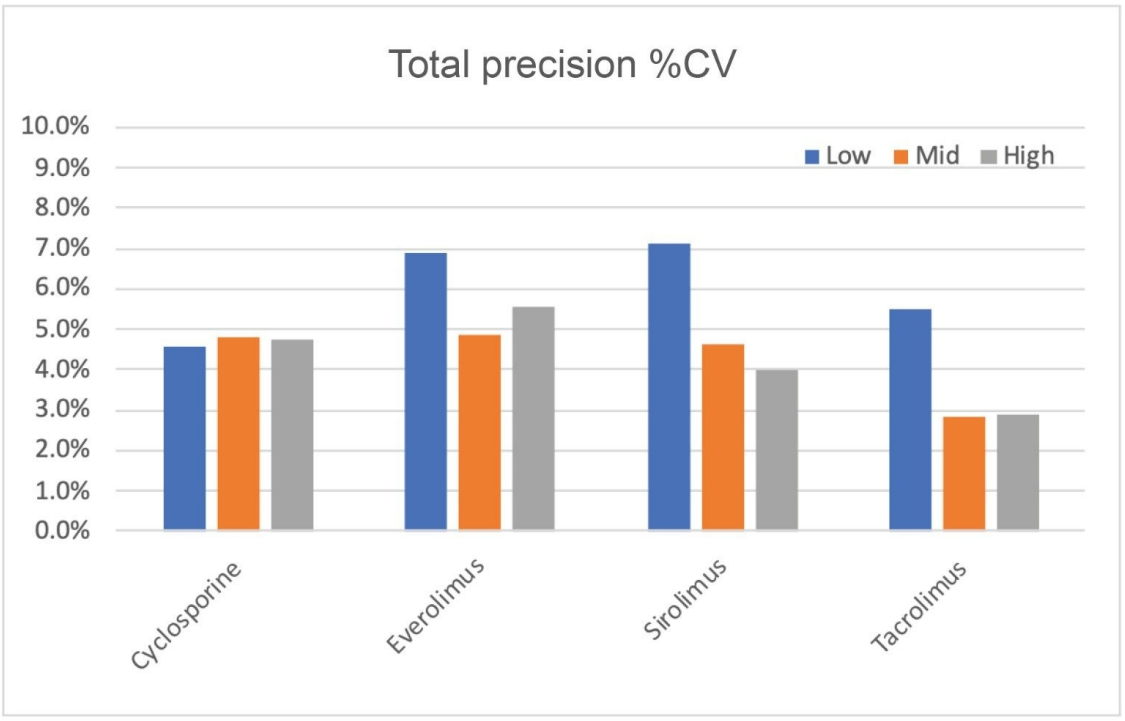


Figure 2. Performance characteristics of the extracted C1 calibrator from the MassTrak Immunosuppressant Calibrator Set analyzed using the ACQUITY UPLC I-Class/Xevo TQ-S micro System. Linearity of the calibration ranges was demonstrated with mean r^2 values for the calibration lines ≥0.9988 across the four immunosuppressant drugs. Analytical sensitivity of the method was determined through the signal:noise (S/N) evaluation of the low calibration (C1) standard for the immunosuppressant drugs. The S/N (PtP) was ≥10 at each of calibrator 1 concentrations across several analytical runs. This is summarized in Table 2 and an example of the S/N at the low calibrator can also be seen in Figure 2.

Analyte	Calibrator range (ng/mL)	Mean r^2	Mean S/N PtP at Cal 1
Cyclosporine	25.2–1474.7	0.9996	4525
Everolimus	1.1–31.8	0.9990	77
Sirolimus	1.0–26.5	0.9988	95
Tacrolimus	1.1–31.5	0.9994	149

Table 2. Summary of calibration linearity and analytical sensitivity performance of the immunosuppressant drugs in the MassTrak Immunosuppressant Calibrator Set.

Total precision and repeatability were determined by extracting and quantifying five replicates of three level QC materials per day over five separate days (n=25). Low, mid, and high concentrations were 154.6, 391.6, and 888.2 ng/mL for cyclosporine; 2.2, 8.4, and 22.6 ng/mL for everolimus; 1.9, 7.3, and 19.4 ng/mL for sirolimus and 2.2, 8.4, and 22.8 ng/mL for tacrolimus. Total precision and repeatability were determined to be ≤7.1% CV across all immunosuppressant drugs at the three QC concentrations (Figure 3).



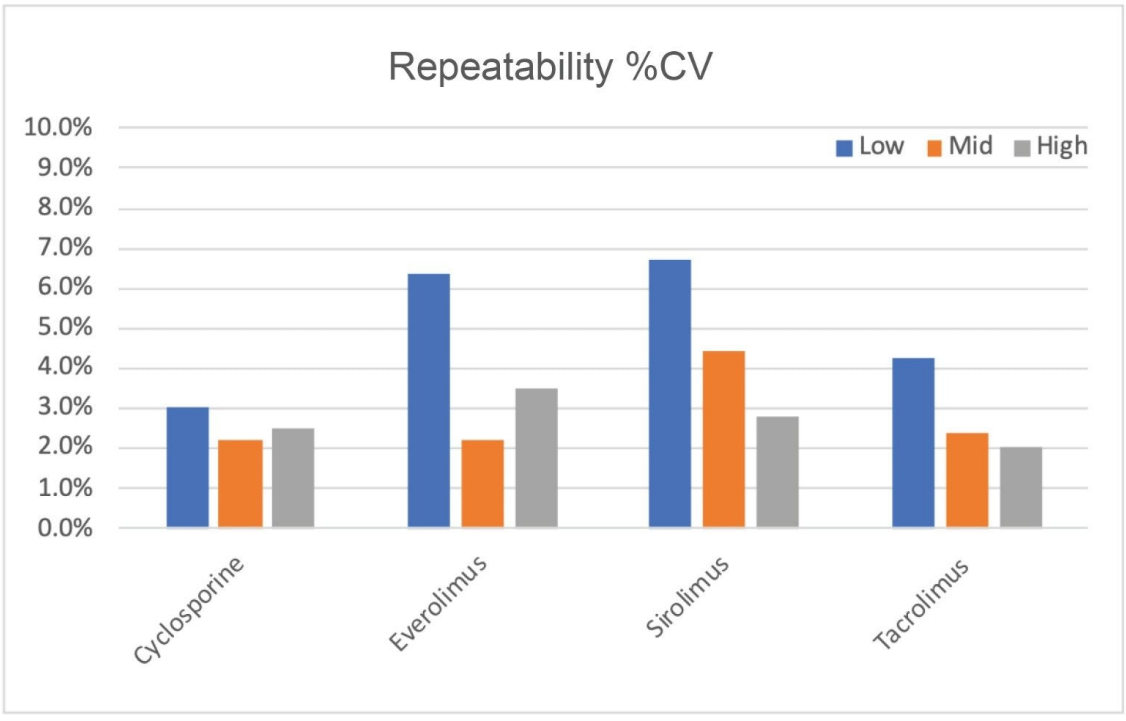


Figure 3. Total precision and repeatability for the analysis of the four immunosuppressant drugs in the MassTrak

Immunosuppressant Quality Control Set.

In addition, the accuracy of the QCs was evaluated in comparison to the calibrators over the five analytical; runs. The mean accuracy for the QCs across the four immunosuppressant drugs ranged from 94.5–103.6% (Table 3).

	QC Accuracy		
Analyte	**Q1**	**Q2**	**Q3**
Cyclosporine	99.6%	101.0%	102.7%
Everolimus	100.0%	103.6%	103.1%
Sirolimus	100.0%	94.5%	95.4%
Tacrolimus	100.0%	101.2%	103.1%

Table 3. Accuracy of the Waters MassTrak Immunosuppressant Quality Control set analyzed in replicates of five at three concentrations over five analytical runs.

Accuracy was assessed for the four immunosuppressant drugs through the analysis of EQA samples from UK NEQAS. The data obtained was compared to the mass spectrometry method mean for the samples and Altman-Bland agreement was performed on the data sets. Altman-Bland agreement for cyclosporine, everolimus, sirolimus and tacrolimus provided a mean method bias within ±7.4%, demonstrating excellent agreement with the EQA method values for the immunosuppressant drugs (Figures 4a-d).

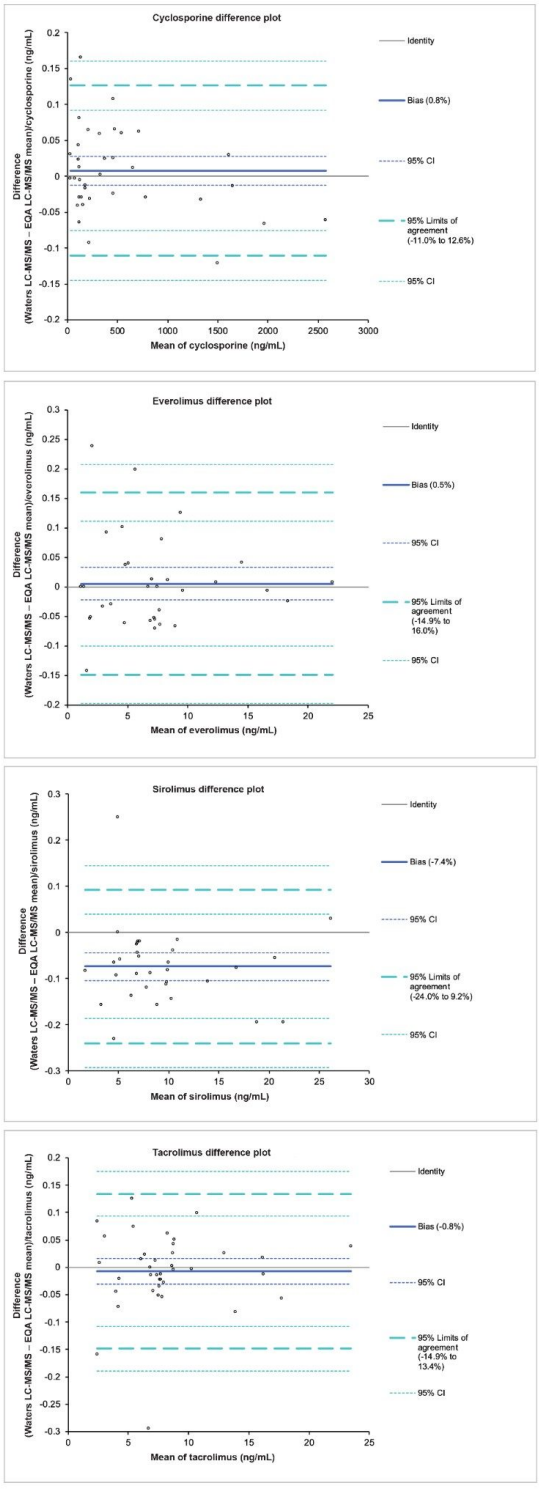


Figure 4. Altman-Bland agreement comparing the Waters LC-MS/MS method to the EQA scheme MS method

mean for (a) cyclosporine (b) everolimus (c) sirolimus (d) tacrolimus.

Conclusion

Through this proof of performance evaluation, it has been demonstrated the MassTrak Immunosuppressant Calibrator and Quality Control Sets (IVD) can provide precise and accurate quantification of the four immunosuppressive drugs in whole blood.

The ACQUITY UPLC I-Class FL with Xevo TQ-S micro Triple Quadrupole Mass Spectrometer is able to provide sufficient analytical sensitivity to analyse lowest required concentrations using only 50 µL sample volume. Excellent levels of precision across the calibration range have been demonstrated with total precision and reproducibility ≤7.1% CV. In addition, the accuracy of the QC set was established with accuracies ranging from 94.5–103.6%. An indication of metrological traceability through agreement to EQA samples was also shown, with the method providing excellent agreement to EQA samples, with mean method bias ±7.4% compared to method mean values from the schemes.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>

MassTrak Immunosuppressant Calibrator, Quality Control and Internal Standard Sets

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

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