

CONFIDENCE IN YOUR CALIBRATORS: ASSESSMENT OF MASSTRAK™ IMMUNOSUPPRESSANT CALIBRATOR AND CONTROL SETS

Waters™

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INTRODUCTION

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 analyze 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. The Waters™ MassTrak™ Immunosuppressant Calibrator and QC Sets (IVD) contain metrologically traceable materials, aiding laboratories in their compliance to ISO 15189, and provide confidence in the accuracy and harmonization of results when using validated LC-MS/MS methods. The performance of the MassTrak Immunosuppressant Calibrator and Quality Control (QC) Sets (IVD) was evaluated using an in-house developed LC-MS/MS method.

THE MASSTRAK SOLUTION

MassTrak Immunosuppressant Calibrator and Quality Control Sets

- The MassTrak Immunosuppressant Calibrator and Quality Control Sets* contain cyclosporine, everolimus, sirolimus and tacrolimus in lyophilized whole blood (Figure 1).



Figure 1. The MassTrak Immunosuppressant Calibrator and Quality Control Sets

Features

- The MassTrak Immunosuppressant Calibrator and QC Sets are pre-weighed, ready to use (following reconstitution) materials that provide a number of benefits, including:
 - Metrological Traceability for compliance with ISO 15189
 - Lot-to-lot consistency for improvements in longitudinal performance of immunosuppressant LC-MS/MS methods
- These materials also provide significant savings in time and resource, as shown in Table 1.

Calibrator Preparation Steps	MassTrak Immunosuppressant Calibrator Set
Source drug standards	✓
Source matrix	✓
Gravimetric preparation of calibrators	✓
Stability evaluation	✓
Calibrator accuracy evaluation	✓
Lot-to-lot evaluation	✓
Measurement of uncertainty	✓

Table 1. The MassTrak Immunosuppressant Calibrator Set improves laboratory efficiency, saving time and resource compared to the preparation of in-house calibrators

***The MassTrak Immunosuppressant Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability please contact your local representative**

METHODS

Materials

- The MassTrak Immunosuppressant Calibrator and Quality Control Sets were used for calibrator and QC materials in an LC-MS/MS method developed in-house.
- Total precision was determined by extracting and quantifying five replicates of tri-level QC material on one occasion per day over five consecutive days (n=25). Repeatability was determined by analyzing five replicates at each QC level.
- QC accuracy was also assessed during these experiments relative to the certified concentrations.
- Analytical method bias for cyclosporine, everolimus, sirolimus and tacrolimus was determined using EQA samples obtained from LGC (Bury, UK).

Methods

- 50µL whole blood samples were aliquotted into a 2 mL, 96 well sample collection plate.
- 200µL of 0.1M aqueous zinc sulfate was added and mixed for five seconds. Subsequently, 500µL of internal standard was added, followed by mixing for twenty seconds. Finally, the plate was centrifuged for two minutes at 4969g.
- Using a Waters ACQUITY™ UPLC™ I-Class FL System, samples were injected onto a 2.1 x 30mm Waters ACQUITY UPLC HSS C₁₈ SB Column, using a water/methanol/ammonium acetate/formic acid gradient and analyzed with a Waters Xevo™ TQ-S micro detector in positive ESI, using Multiple Reaction Monitoring.
- Ammoniated adducts were analyzed, with quantifier, qualifier and internal standards for each immunosuppressant drug.
- The analysis time per sample was approximately 1.9 minutes injection to injection, using the Load Ahead function.

RESULTS

Chromatography, Analytical Sensitivity and Linearity

- Cyclosporine, everolimus, sirolimus and tacrolimus were eluted using the HSS C₁₈ SB column and separated by mass.
- The analytical sensitivity of the developed method for the lowest calibrator is shown in Figure 2, with S/N (PtP) > 10 for the four immunosuppressant drugs.
- Linearity of the calibration ranges was demonstrated with mean r² values for the calibration lines >0.999 across the four immunosuppressant drugs.

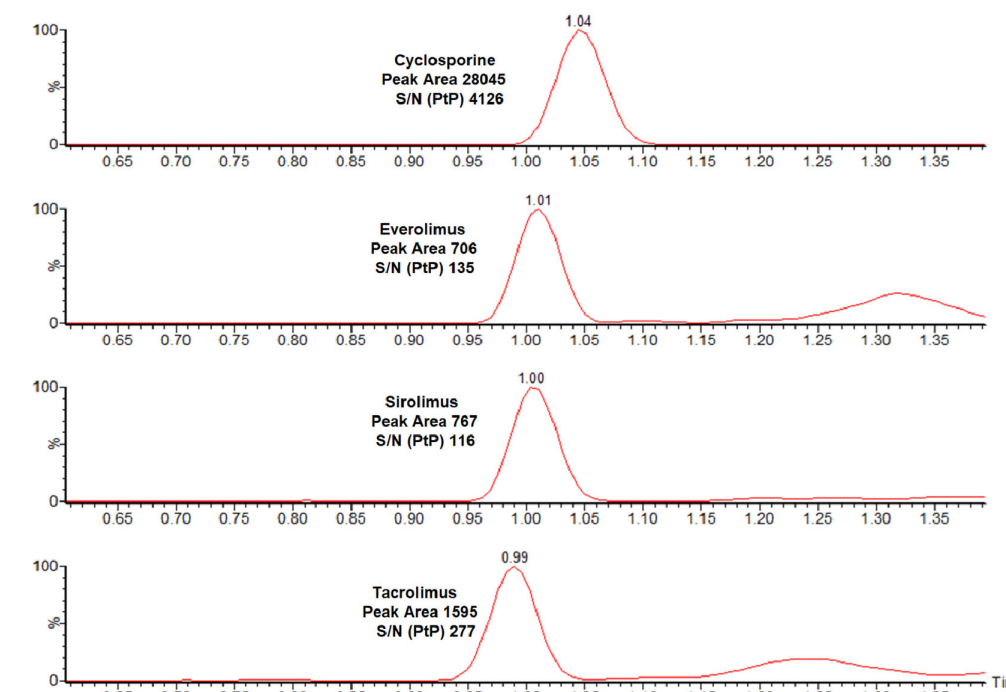


Figure 2. Analytical sensitivity of the lowest calibrator in the MassTrak Immunosuppressant Calibrator Set

Total Precision and Repeatability

- Total precision and repeatability of the MassTrak Immunosuppressant QC Sets using the developed LC-MS/MS method were ≤7.1% CV over five analytical runs (Figure 3).

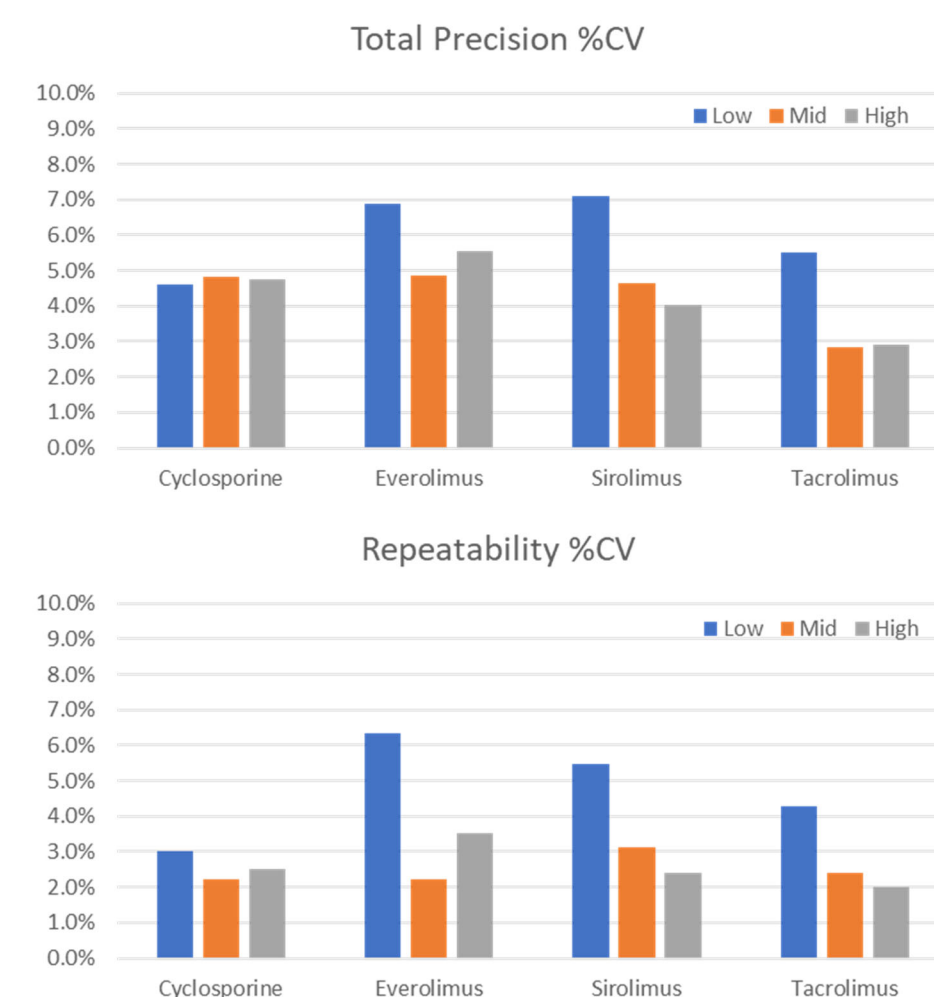


Figure 3. Total precision and repeatability of the 4 immunosuppressant drugs in the MassTrak Immunosuppressant Quality Control Set

QC Accuracy and EQA Agreement

- QC accuracy for the MassTrak Immunosuppressant QC Set relative to certified concentrations across the three concentrations ranged from 94.5 – 103.6% (Table 2).

Analyte	QC Accuracy		
	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 2. Accuracy of the MassTrak Immunosuppressant Quality Control set (5 replicates, 3 concentrations, 5 runs; n=25)

- Mean method bias for External Quality Assessment (EQA) samples was within ±7.4% for cyclosporine, everolimus, sirolimus and tacrolimus.

CONCLUSION

- The MassTrak Immunosuppressant Calibrator and QC Sets (IVD) contain metrologically traceable calibrators, aiding laboratories in their compliance to ISO 15189
- Significant savings in time and resource can be made by using pre-made calibrator and QC materials
- MassTrak Immunosuppressant Calibrators are reproducible and accurate across manufacturing lots (refer to application note 720007583) and cover the relevant reference ranges for each immunosuppressant drug
- Excellent reproducibility and accuracy of the MassTrak Immunosuppressant Quality Control Set and EQA samples were demonstrated with an in-house LC-MS/MS method

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