

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

Waters™

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INTRODUCTION

The immunosuppressive drugs cyclosporine, everolimus, sirolimus and tacrolimus have historically been measured using immunoassay.

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.

As such, many clinical laboratories increasingly analyze these drugs using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

THE MASSTRAK SOLUTION

The Waters MassTrak™ Immunosuppressant Calibrator and Quality Control Sets* contain pre-weighed, metrological traceable, ready to use (following reconstitution) cyclosporine, everolimus, sirolimus and tacrolimus in lyophilized whole blood.

The benefits of the sets include:

- **Metrological Traceability** for compliance with ISO 15189
- **Lot-to-lot consistency** for improvements in longitudinal performance of immunosuppressant LC-MS/MS methods
- **Confidence** in laboratory results

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

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 and repeatability was determined by extracting and quantifying five replicates of tri-level QC material on one occasion per day over five consecutive days (n=25). 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).

Have confidence in your calibrators



Waters MassTrak Calibrator and Quality Control Sets

CONCLUSION

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

Methods

- 50µL whole blood samples were pipetted into a 96 well plate. 200µL of 0.1M aqueous zinc sulfate was added and mixed. Subsequently, 500µL of internal standard was added, mixed and the plate was centrifuged for two minutes at 4969g.
- Samples were injected onto a 2.1 x 30mm Waters ACQUITY™ UPLC™ HSS C₁₈ SB Column and chromatographed using a Waters ACQUITY UPLC™ I-Class FL System and a water/methanol/ammonium acetate/formic acid gradient (1.9 minutes injection-to-injection).
- Ammoniated analytes were detected in positive ESI, using MRM mode on a Waters Xevo™ TQ-S micro Mass Spectrometer.

RESULTS

Chromatography, Analytical Sensitivity and Linearity

- For each of the four immunosuppressant drugs; cyclosporine, everolimus, sirolimus and tacrolimus the following was obtained:
 - separation by mass was achieved
 - analytical sensitivity for calibrator 1 of S/N (PtP) > 10
 - linearity of the calibration ranges with mean r² values for the calibration curves >0.999

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 1).

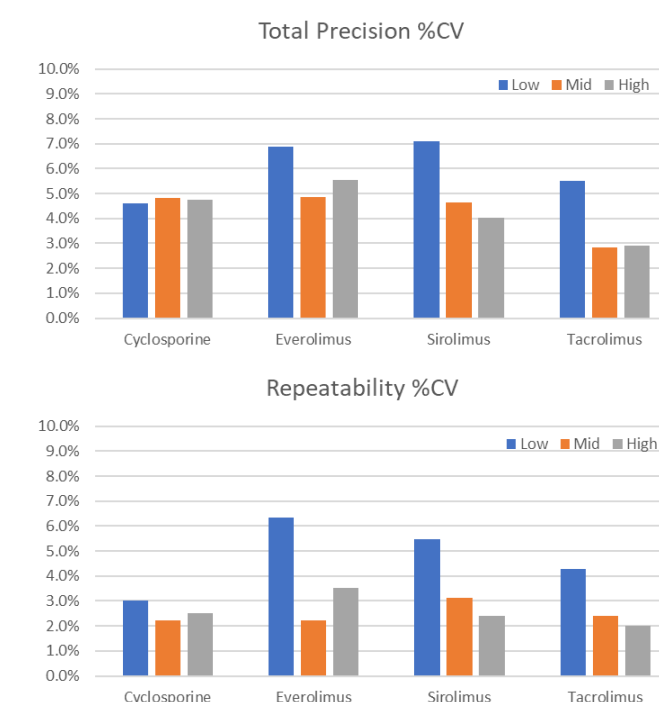


Figure 1. Total precision and repeatability of the four analytes in the MassTrak Immunosuppressant Quality Control Set

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

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