

**Young Scientist Scientific Issues Series, Short Summary:
Validation of the MassTrak Immunosuppressant XE* Kit for TDM of Everolimus**

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Everolimus Background

The mTOR (mammalian target of rapamycin) inhibitor, everolimus, is an immunosuppressive (IMS) drug used to prevent episodes of acute rejection and to achieve long-term graft survival in transplant recipients. Novartis market everolimus under the trade names Zortress® in the US (approved for renal transplant recipients, 2010) and in many other countries as Certican® (approved for renal and cardiac transplant recipients, 2003).¹ Everolimus is a candidate for therapeutic drug monitoring (TDM) due to its wide intra- and inter-individual pharmacokinetic variability, narrow therapeutic index, potential for complex drug-drug interactions, drug toxicities, and long-term adherence issues.²

Everolimus Bioanalytics

The International Proficiency Testing Scheme (IPTS) (www.bioanalytics.co.uk) highlights that 63% and 26% of participating centres use hyphenated liquid chromatography (LC-MS(/MS)/HPLC-UV) and immunoassay methods for the TDM of everolimus, respectively (Figure 1).

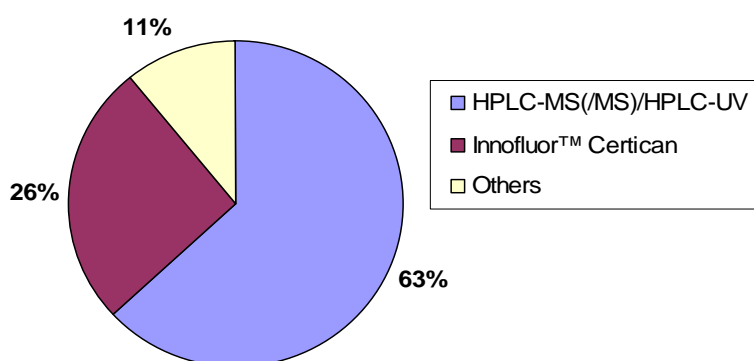


Figure 1: Everolimus IPTS, Distribution E57, November 2010.

This reflects the trend of the increasing use of LC-MS(/MS) for TDM in the clinical laboratory.³⁻⁵ LC-MS(/MS) is considered the gold standard technique for the TDM of everolimus as a result of its improved specificity and sensitivity. Despite common misconceptions, LC-MS(/MS) is prone to interferences⁶⁻⁷ and it is essential to ensure the methods are well validated. The IPTS is a valuable resource allowing us to assess new assays, monitor the development of new and existing assays, assess if they are fit for purpose and to educate participants. Data from the IPTS often highlights the lack of standardised protocols for the preparation of in-house calibrators as one of the main reasons for result inaccuracies for LC-MS(/MS).

Verification and Validation Studies

Waters are performing a multi-centre study to validate the MassTrak Immunosuppressant XE* Kit for use with LC-MS/MS to quantify everolimus in renal and cardiac transplant recipients. The verification and validation studies performed at the Analytical Unit, St George's - University of London included; 5-Day Precision, Linearity, Recovery

Performance, Dilution Accuracy, Interference Testing (anticoagulants and hematocrit) and Method Comparison. This short article will focus on presenting the data from the method comparison study.

MassTrak Immunosuppressant XE* vs Comparator Method

The MassTrak Immunosuppressant XE* testing was performed on the Waters ACQUITY TQD LC-MS/MS System (Figure 2) and the methodology carried out as specified in the Waters MassTrak Immunosuppressant XE* Kit Directions for Use.

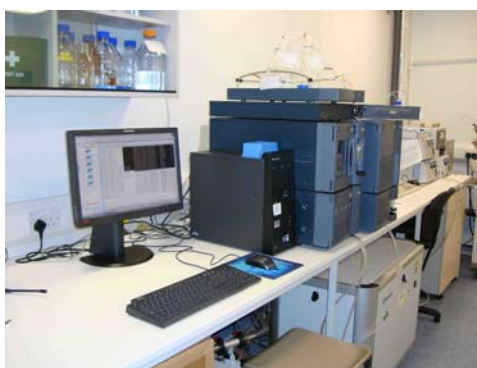


Figure 2: Waters ACQUITY TQD LC-MS/MS System

The Collaborator's in-house validated method (the Comparator method) was performed on an Agilent 1100 Series HPLC system coupled to an AB Sciex API4000 Mass Spectrometer. Both systems utilised reversed phase LC systems coupled to tandem quadrupole mass spectrometers with an electrospray (ESI) interface. The MassTrak Immunosuppressant XE* sample preparation involved a protein precipitation step followed by LC-MS/MS analysis with a total run time of 1.5mins (Figure 3A). The Comparator method utilised a protein precipitation method followed by liquid-liquid extraction prior to LC-MS/MS analysis with a total run time of 5.5mins (Figure 3B). Both methods employed labelled everolimus ($^{13}\text{C}_2\text{D}_4$ -Everolimus) as the internal standard (available to IATDMCT members, www.iatdmct.org). The nominal calibration range covered was 1-30ng/mL (QCs: 2, 8 & 22ng/mL) and 1-50ng/mL (QCs: 3, 15 & 30ng/mL) for the MassTrak Immunosuppressant XE* and Comparator methods, respectively.

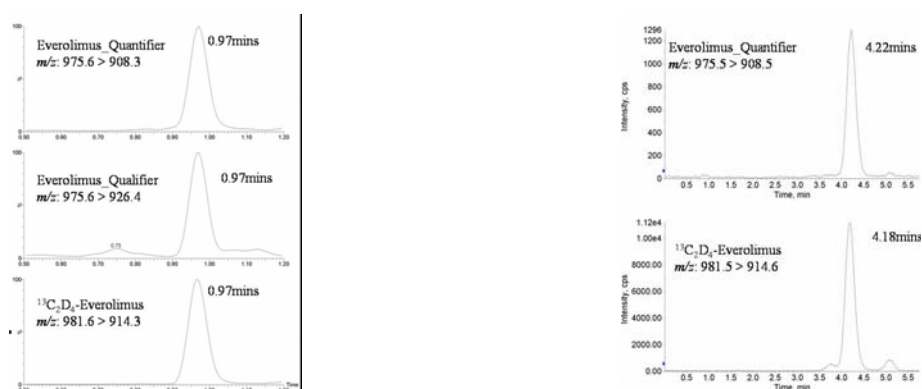


Figure 3: Example Chromatograms from renal transplant recipients receiving everolimus.
(A) MassTrak XE: 5.0ng/mL (B) Comparator: 5.2ng/mL.

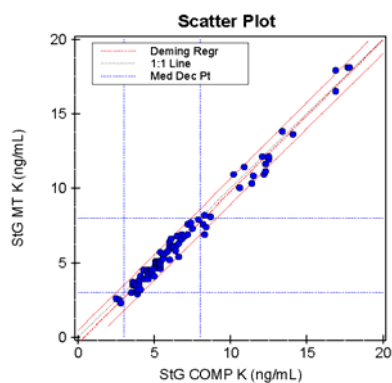
Method Comparison Testing Protocol

Individual patient samples from 50 renal and 50 cardiac transplant recipients were tested. Eight samples were tested in duplicate per run per day by both the MassTrak Immunosuppressant XE* and comparator methods. Both assays were performed within 2 hours of each other.

Method Comparison Results and Discussion

Following analysis of the patient samples by both the MassTrak Immunosuppressant XE* and Comparator method, Deming Regression was performed and the statistical analysis of results is shown for both the renal (Table 1, Figure 4A) and cardiac (Table 1, Figure 4B) data.

(A)



(B)

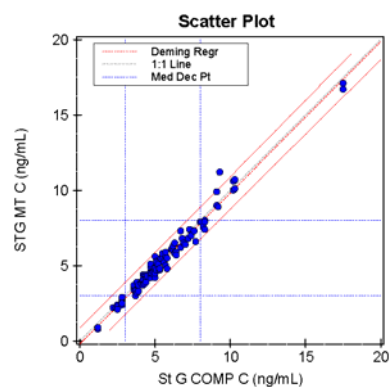


Figure 4: Deming Regression individual result scatter plots (A) renal (B) cardiac.

The acceptance criteria were that the predicated bias at the two medical decision points for everolimus, 3ng/mL and 8ng/mL, must be within $\pm 15\%$ for both graft types. From Table 1 and Figure 5 it is evident that for both the renal (3ng/mL: -13.3%, 8ng/mL: -3.8%) and cardiac (3ng/mL: -6.7%, 8ng/mL: -2.5%) data that this acceptance criterion was met.

Data Type	Renal Data	Cardiac Data
Regression method	Deming	Deming
Linear regression line	$y=1.017x-0.41$	$y=1.003x-0.21$
Number of results used	98 (49 samples in duplicate)	100 (50 samples in duplicate)
Concentration range (X)	2.5 to 17.8ng/mL	1.2 to 17.5ng/mL
Correlation coefficient (R)	0.9924	0.9887
Bias calculated at 3ng/mL $\pm 95\%$ CI	-13.3% (-16.7% to -6.7%)	-6.7% (-10.0% to -3.3%)
Bias calculated at 8ng/mL $\pm 95\%$ CI	-3.8% (-5% to -2.5%)	-2.5% (-3.8% to -1.25%)

Table 1: Method comparison results following statistical analysis.

Conclusion

From the IPTS data it is evident that standardisation for the preparation of calibrator material for LC-MS(/MS) is essential. The study results are a positive step in achieving calibration harmonization for LC-MS(/MS) analysis of IMS in clinical laboratories. The results for the validation of the Waters MassTrak Immunosuppressant XE* Kit for the quantification of everolimus for use with LC-MS/MS shows the potential of these devices.

*NOTE: The MassTrak Immunosuppressant XE Kit is not available for commercial distribution in any market.

References

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