

Extraction of a Range of Immunosuppressants from Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

This application note describes the extraction of sirolimus, tacrolimus, everolimus, cyclosporin A from whole blood samples using supported liquid extraction prior to LC-MS/MS analysis.

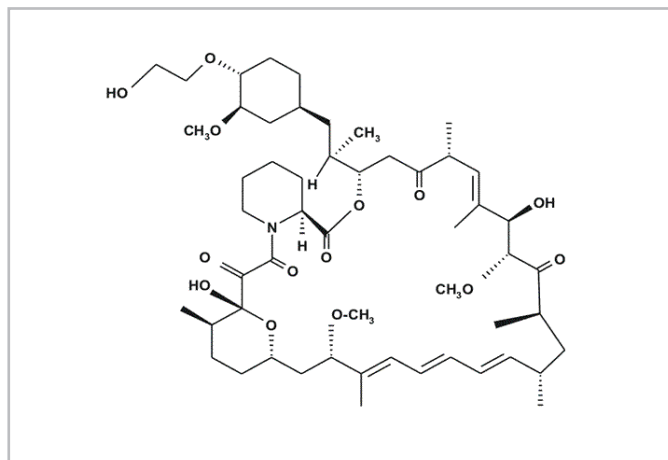


Figure 1. Structure of Everolimus

Introduction

Immunosuppressants inhibit or prevent the activity of the immune system used to prevent rejection of transplanted organs and treat autoimmune diseases. Due to the risks of immunodeficiency, those under treatment must undergo constant therapeutic drug monitoring (TDM) requiring reliable and robust analytical techniques for quantification of these drugs. Current methodologies use immunoassay techniques to measure immunosuppressant levels in patients which are expensive, time consuming and susceptible to issues with cross reactivity. The sample prep method in this application note offers an alternative approach to immunosuppressant analysis with LC-MS/MS giving more reliable and robust recoveries with no cross-reactivity and cost saving opportunities. Analyte recoveries range from 60–97% with RSDs all below 10%.

ISOLUTE® SLE+ Supported Liquid Extraction plates offer an efficient alternative to traditional liquid liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

Analytes

Sirolimus, Tacrolimus, Everolimus, Cyclosporin A.

Sample Preparation Procedure

- Configuration:** ISOLUTE SLE+ 400 μ L Supported Liquid Extraction Plate part number 820-0400-P01
- Sample Pre-treatment** In a 2 mL Eppendorf centrifuge tube, pipette whole blood (50 μ L). Add HPLC water (250 μ L) and vortex for 30 seconds. Centrifuge at 12,000 RPM for 10 minutes.
- Sample Loading:** Load the supernatant (275 μ L) onto the plate and apply a pulse of vacuum or positive pressure for 10 seconds. Allow the sample to adsorb for 5 minutes.
- Analyte Extraction:** Apply ethyl acetate (600 μ L) and allow to flow under gravity for 5 minutes. Apply a further aliquot of ethyl acetate (600 μ L) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.
- Post-extraction:** Evaporate the extract to dryness (30 $^{\circ}$ C). Reconstitute in water:acetonitrile (100 μ L, 25:75, v/v).

HPLC Conditions

Instrument:	Waters Acquity UPLC fitted with 20 µL loop
Column:	Waters UPLC BEH C18 (50 mm x 1.7 µm x 2.1 mm id)
Sample Temperature:	10 °C
Column Temperature:	60 °C
Injection Volume:	10 µL (partial loop)
Weak Needle Wash:	Water/acetonitrile (90:10, v/v)
Strong needle wash:	Water/acetonitrile (10:90, v/v)
Mobile phase:	A= 2 mM Ammonium Formate (aq) with 0.1% formic acid B= 2 mM Ammonium Formate (MeOH) with 0.1% formic acid

Gradient:

Time (min)	%A	%B	Curve
0	25	75	-
0.2	0	100	11
0.8	25	75	11
2.8	25	75	-

MS Conditions

Instrument:	Premier XE triple quadrupole mass spectrometer equipped with an electrospray interface for mass analysis.
Desolvation temperature:	450 °C
Ion source temperature:	150 °C

Table 1. Positive ions acquired in the multiple reaction monitoring (MRM) mode

Analyte	Transition	Cone voltage (V)	Collision energy (V)
Tacrolimus (Quant)	826.5 - 616.4	65	35
Tacrolimus (Qual)	826.5 - 505.4	65	39
Sirolimus (Quant)	936.5 - 409.4	65	50
Sirolimus (Qual)	936.5 - 209.2	65	60
Everolimus (Quant)	980.6 - 389.4	65	58
Everolimus (Qual)	980.6 - 453.4	65	51
Cyclosporin A (Quant)	1202.9 - 425.5	65	50
Cyclosporin A (Qual)	1202.9 - 298.4	65	57

Results

This SLE+ protocol demonstrates analyte recoveries ranges from 60-97% as shown in **Figure 2**. RSDs were all below 10% for all analytes. **Figure 3** shows the extracted ion chromatograms for the full range of Immunosuppressants.

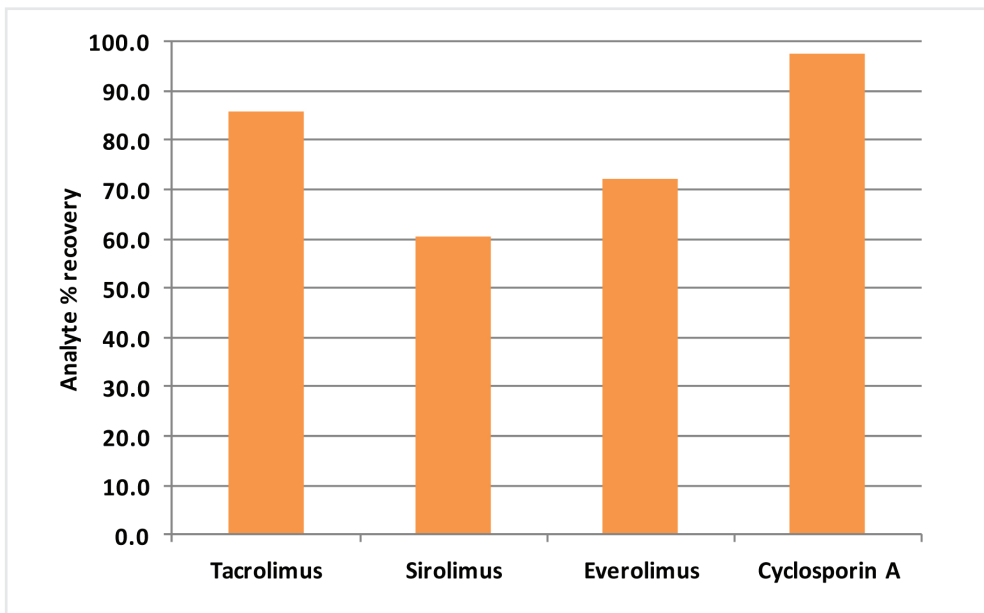


Figure 2. Typical analyte % recoveries for extracted immunosuppressants (n=7) using the ISOLUTE® SLE+ protocol.

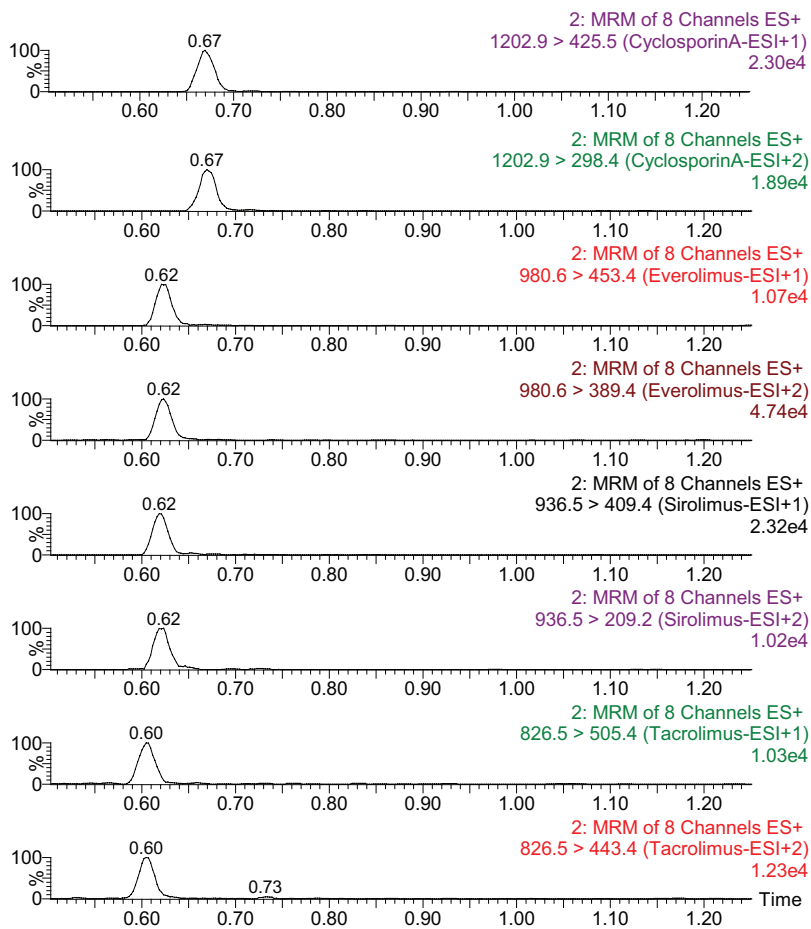


Figure 3. Extracted ion chromatograms for a range of Immunosuppressants at 80 ng/mL.

Acknowledgements

Biotage would like to thank Brian Keevil and his team in the Biochemistry Department at South Manchester University Hospital for their assistance in evaluating this application note.

Ordering Information

Part Number	Description	Quantity
820-0400-P01	ISOLUTE® SLE+ 400 Supported Liquid Extraction Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold 96 Position	1
121-9600	Biotage® VacMaster™-96 Sample Processing Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1

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