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Application Data Sheet

LC-MS
Liquid Chromatograph Mass Spectrometer

Measurement of immunosuppressants, Tacrolimus, Sirolimus, Everollmus and Cyclosporine A from whole blood using on-line SPE and LCMS-8030

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Introduction

Immunosuppressants are an important class of compounds which are commonly used by transplant recipients to avoid organ rejection. In addition, they are used for the treatment of immune-mediated diseases or disorders of the immune system and non-autoimmune inflammatory reactions such as heavy allergic asthma. The therapeutic concentration range of these compounds, typically narrow, requires careful monitoring from whole blood to ensure the correct patient dosage.
In these experiments on-line separation of whole blood was performed, analyzing the immunosuppressant compounds tacrolimus, sirolimus, everolimus and cyclosporine A (CSA) by liquid chromatography tandem mass spectrometry LC-MS/MS.

Materials and methods

The LCMS-8030 triple quadrupole mass spectrometer was coupled to a Nexera UHPLC system equipped with a 6-port 2-way switching valve (load/inject). Four immunosuppressant compounds (tacrolimus, sirolimus, everolimus, CSA) were optimized, each with two MRM transitions to quantify and qualify compound measurement (Table 1).
Chemical standards, control samples, SPE column, analytical column and mobile phase solvents were taken from a commercially available test kit for therapeutic drug monitoring (TDM) ClinMass® Complete Kit for Immunosuppressants in Whole Blood, MS1000 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992 München, Germany). For all compounds the $[M+NH_4]^+$ ion was measured and used as the precursor ion (positive electrospray ionization).

Analytical Conditions

UHPLC: Nexera UHPLC
0.1 mL/min 50% pumps A&B
0.5 mL/min pump C.
LC time program: RECIPE kit, MS1000.
Injection volume: 50 µL
Column temperature: 60° C
Mass spectrometer: LCMS-8030
Source conditions: Desolvation Line: 220 ° C
Heat Block: 400 ° C
Nebulizer Gas: 3 L/min
Drying Gas: 15 L/min
Interface voltage: 2.5 kV
Dwell time: 10 msec
Pause time: 3 msec
Ionization: Electrospray ionization (ESI), positive mode.
Scan Type: Multiple-reaction-monitoring mode (MRM).

Results

The combination of on-line SPE coupled to an LCMS-8030 enabled development of a rapid and effective method from whole blood. The implementation of the RECIPE test kit for immunosuppressants proved simple to work with, containing standard compounds for MRM optimization and pre-selected columns for fast separation (Figure 1).

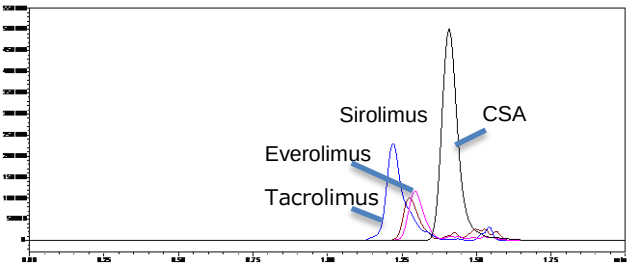

Fig. 1 LC-MS separation of tacrolimus, sirolimus, everolimus and CSA in under two minutes by on-line SPE.

Table 1 Tacrolimus, sirolimus, everolimus and CSA, MRM transitions, retention times (RT). T/I = target or internal standard.

Compound		Formula	MRM1	MRM2	RT
Tacrolimus	T	C44H69NO12	821>768	821>786	1.22
Sirolimus	T	C51H79NO13	931>864	931>846	1.28
Everolimus	T	C53H83NO14	975>908	975>858	1.29
Cyclosporine A	T	C62H111N11O12	1220>120	1220>1184	1.40
Ascomycin	I	C43H69NO12	809>756	809>774	1.21
D4 Everolimus	I	C53H79D4NO14	979>912	979>894	1.29
Cyclosporine D	I	C62H97D14N11O1	1234>121	1234>1199	1.44

Calibration curves of the four compounds showed good sensitivity and linearity using RECIPE kit samples (Figure 2). These were measured over a variety of concentration ranges specific to clinically relevant therapeutic dosing levels (Table 2).

Table 2 Calibration curve range measured for tacrolimus, sirolimus, everolimus and CSA.

Compound	Concentration (µg/L)
Tacrolimus	1.34-23.5
Sirolimus	1.33-23.9
Everolimus	1.33-23.9
Cyclosporine A	24.9-1264

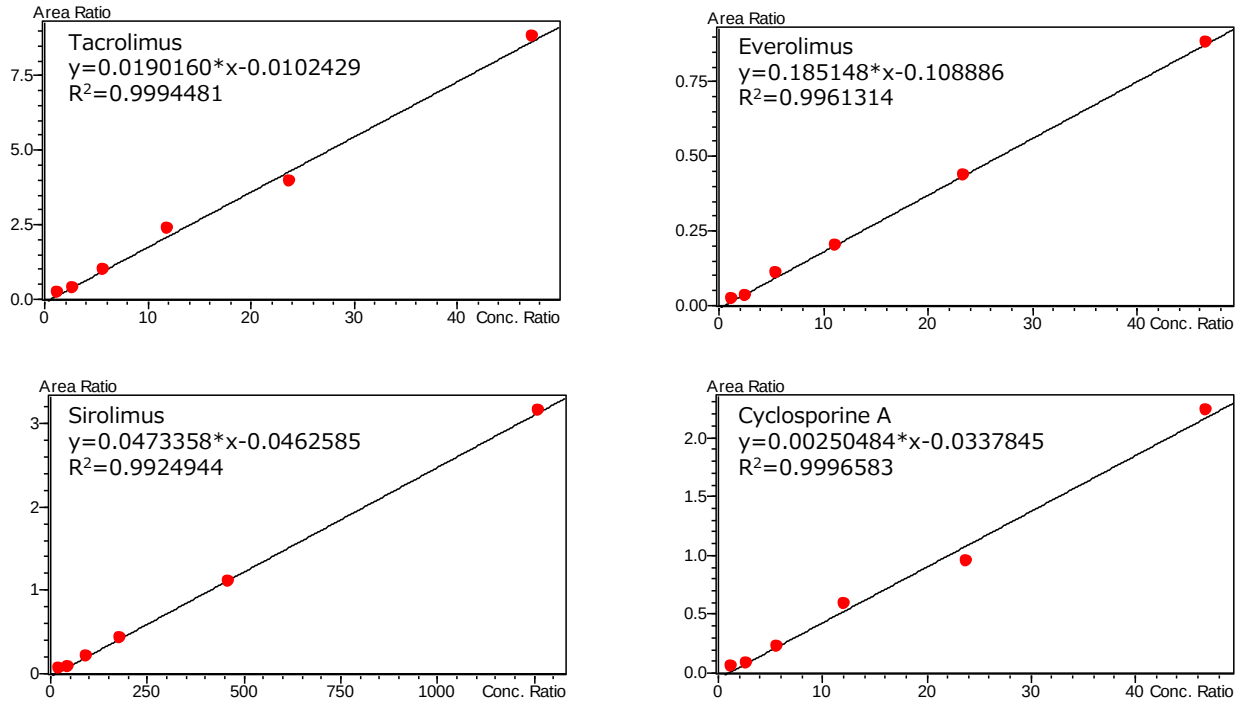

Fig. 2 Calibration curves for tacrolimus, sirolimus, everolimus and CSA.

Conclusion

The LCMS-8030 in combination with the Nexera UHPLC with on-line SPE showed good sensitivity and suitability for measurement and quantitative determination of immunosuppressant compounds directly from whole blood.

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