Application Note LCMS-105

Online Analysis of Immunosuppressants in Whole Blood with the EVOQ™ Tandem Quadrupole Mass Spectrometer

Abstract

This study demonstrates a robust, rapid and reliable research method to quantitate the immunosuppressants cyclosporine A, tacrolimus, sirolimus and everolimus in whole blood samples using the Bruker Advance™ UHPLC with OLE (online extraction) coupled to the EVOQ Elite™ tandem quadrupole MS. The integrated online extraction, together with a ClinMass® LC-MS/MS complete kit allows fast and easy sample cleanup. The analyses with an overall run time of only 3 min showed excellent linearity of calibration and accuracy as well as a very good intraday and interday precision.

Introduction

Immunosuppressants are used after organ transplantation to prevent allograft rejection by inhibition of the body's immune system. These drugs have narrow active concentration ranges and highly variable pharmacokinetics. To improve the scientific understanding of dosage requirements it is necessary to continue research on these compounds. Their quantitative analysis has been performed primarily using immunoassays. However, immunoassays can exhibit high levels of cross reactivity, particularly to metabolites and are therefore prone to error. Conversely, the superior specificity and selectivity of liquid chromatography

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coupled to tandem mass spectrometry (LC-MS/MS) has made it a preferred analytical technique of choice for the determination of immunosuppressants.
This application note describes a simple and quick online extraction method for the analysis of the immunosuppressants cyclosporine A, tacrolimus, sirolimus and everolimus (Figure 1) in whole blood using Bruker's Advance UHPLC system with integrated online extraction (OLE) coupled to the EVOQ tandem quadrupole LC-MS/MS system (Figure 2).

Chemical structure

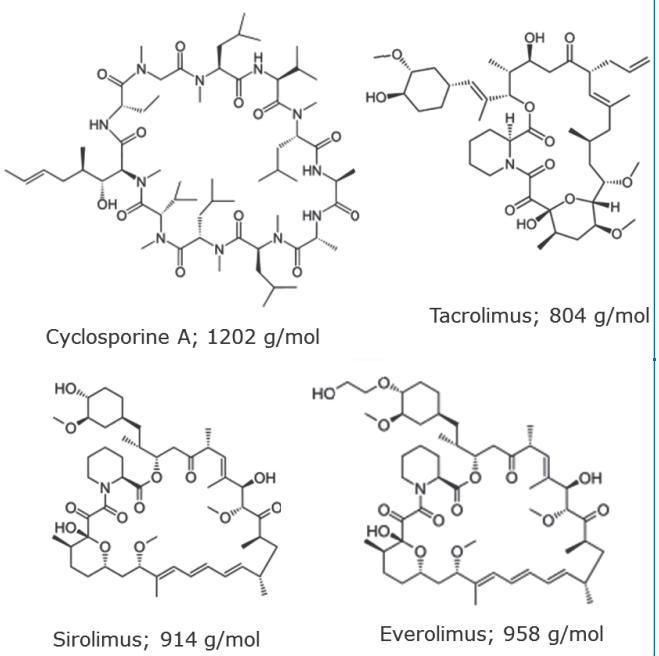

Figure 1: Chemical structure of the four immunosuppressants

Experimental

The analysis was performed using a ClinMass® LC-MS/MS complete kit (RECIPE Chemicals + Instruments GmbH, Munich, Germany) which contained the SPE buffer, mobile phase, autosampler washing solution, precipitation reagent, whole blood calibrators and quality control samples. The HPLC column, SPE column and an inline filter were also provided by RECIPE.

Sample Preparation

200 µL precipitation reagent and 20 µL internal standard solution containing the isotopic labelled analytes (see Table 1) were added to 100 µL of whole blood sample. After vortexing for 30 sec the samples were incubated for 5 min at room temperature. They were vortexed for another 10 sec and centrifuged for 5 min. After online extraction using the Bruker OLE system, the supernatant was analyzed by LC-MS/MS.

EVOQ Tandem Quadrupole

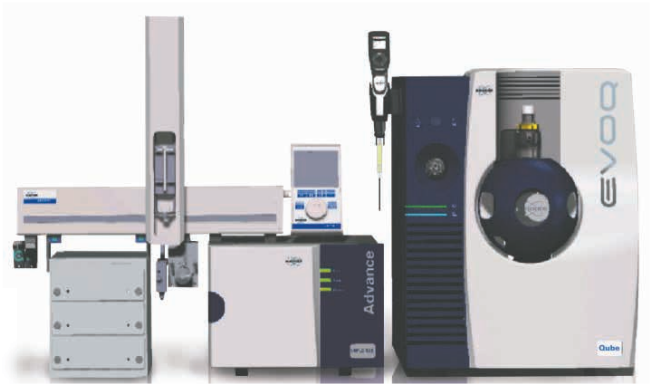

Figure 2: EVOQ™ tandem quadrupole MS with Advance™ UHPLC OLE

Instrumentation

Liquid chromatography

Instrument:	Bruker Advance UHPLC with OLE (Figure 3)	
Injection volume:	20 µL	
Column oven:	60°C	
Equilibration flow:	2 mL/min (21 sec)	
Loading flow:	2 mL/min (30 sec)	
Extraction time:	69 sec	
Flow rate:	0.00 – 0.80 min	500 µL/min
	0.80 – 0.85 min to	1000 µL/min
	0.85 – 1.05 min	1000 µL/min
	1.05 – 1.15 min to	500 µL/min
	1.15 – 2.00 min	500 µL/min
Total run time:	3 min	

Mass Spectrometry

Instrument:	EVOQ Elite tandem quadrupole mass spectrometer
Ion source:	VIP H-ESI positive, 4800 V
Probe gas:	40 units at 400°C
Cone gas:	30 units at 350°C
Nebulizing gas:	60 units
Active exhaust:	on
Collision gas:	Argon, 1.5 mTorr
MRM transitions:	see Table 1

Bruker Online Extraction

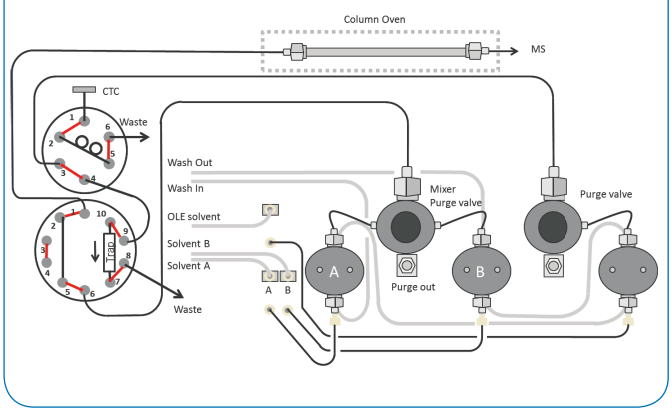

Figure 3: Bruker OLE (Online Extraction) pump configuration

Table 1: MRM transitions

Compound	Rt (min)	Precursor Ion	Product Ion 1	CID 1 (V)	Product Ion 2	CID 2 (V)
Tacrolimus	0.76	821.6	768.4	18	576.3	22
$^{13}C_1d_2$-Tacrolimus (IS)	0.76	824.6	771.4	18	579.3	22
Sirolimus	0.86	931.7	864.5	10	882.5	4
$^{13}C_1d_3$-Sirolimus (IS)	0.86	935.7	864.5	14	882.4	5
Everolimus	0.88	975.7	908.4	14	926.7	6
$^{13}C_2d_4$-Everolimus (IS)	0.88	981.7	914.5	14	932.6	6
Cyclosporine A	0.98	1220.0	1202.8	14	1184.8	24
d_{12}-Cyclosporine A (IS)	0.98	1232.1	1214.7	15	1196.8	21

Table 2: Results for cyclosporine A

Sample	Specified Value [µg/L]	Bias [%]	Intraday precision RSD [%]	Interday precision RSD [%]
Cal. 1	25.8	0.0	2.9	1.3
Cal. 2	49.0	0.9	2.0	1.9
Cal. 3	95.7	0.5	1.8	2.4
Cal. 4	181	-4.5	2.7	1.2
Cal. 5	439	-1.2	3.0	0.3
Cal. 6	1243	4.3	1.6	1.5
QC I	58.7	1.4	3.1	0.5
QC II	116	0.7	0.4	2.4
QC III	228	3.4	2.5	3.0

Table 3: Results for tacrolimus

Sample	Specified Value [µg/L]	Bias [%]	Intraday precision RSD [%]	Interday precision RSD [%]
Cal. 1	1.37	-1.2	2.8	0.1
Cal. 2	2.86	0.8	2.5	0.7
Cal. 3	5.66	1.5	1.4	0.4
Cal. 4	11.7	5.5	1.8	1.0
Cal. 5	23.2	-0.5	2.0	0.1
Cal. 6	45.1	-6.2	3.6	0.9
QC I	3.71	-1.2	2.8	1.6
QC II	7.66	0.8	2.5	0.8
QC III	15.2	1.5	1.4	0.1

Table 4: Results for sirolimus

Sample	Specified Value [µg/L]	Bias [%]	Intraday precision RSD [%]	Interday precision RSD [%]
Cal. 1	1.62	2.0	5.1	1.8
Cal. 2	3.21	-1.3	5.7	2.2
Cal. 3	6.43	-5.5	4.9	3.8
Cal. 4	13.4	-2.3	3.2	1.2
Cal. 5	26.3	3.5	3.7	2.4
Cal. 6	52.9	3.6	2.3	2.1
QC I	4.18	-3.2	8.6	4.3
QC II	12.6	1.2	3.9	1.1
QC III	21.0	3.0	2.7	2.5

Table 5: Results for everolimus

Sample	Specified Value [µg/L]	Bias [%]	Intraday precision RSD [%]	Interday precision RSD [%]
Cal. 1	1.45	1.6	4.2	1.8
Cal. 2	2.90	-2.8	7.4	3.3
Cal. 3	6.01	-2.0	5.1	2.4
Cal. 4	12.6	1.1	5.5	1.3
Cal. 5	24.9	1.5	6.3	1.4
Cal. 6	49.4	0.5	3.9	1.8
QC I	3.89	-2.6	3.0	0.9
QC II	12.3	2.3	4.1	1.7
QC III	20.3	1.6	4.3	1.9

Results and Discussion

The integrated OLE system allowed interlacing of online extraction and chromatographic separation so that every 3 min a new sample could be analyzed (see Figure 4). Calibration curves including six whole blood calibrators showed excellent linearity ($r^2 \geq 0.997$). Replicate injections (n = 4) of the calibrators and three whole blood quality controls (QC) demonstrated good intraday precision with RSD 0.4 – 8.6% for all analytes. Interday precision on three days showed high robustness with RSD of means < 4.5%. Accuracy of calibrators and QCs was excellent with bias < ± 6.5%. Detailed results are shown in Tables 2 – 5.

Conclusion

The Bruker Advance™ UHPLC with OLE coupled to the EVOQ Elite™ tandem quadrupole MS together with a ClinMass® LC-MS/MS complete kit provides a quick and easy research method to quantitate four important immunosuppressants in whole blood samples. The run time of only 3 min including online extraction enables high throughput. Linearity of calibration, intraday and interday precision as well as accuracy were excellent showing the suitability of this workflow for the rapid quantitation of immunosuppressant drugs.

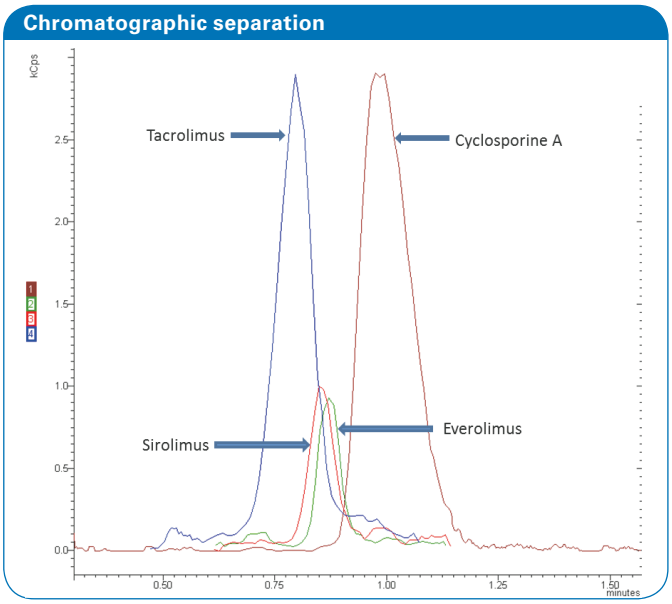

Figure 4: Chromatogram of calibrator 1

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