Application Note: 513

Validated LC-MS/MS Method for the Analysis of Immunosuppressant Drugs in Whole Blood Using the RECIPE ClinMass® Complete Kit

Samuele Scurati[1], Anke Trebstein[2], Lea Bonnington[2], Norbert Dirsch[2], Glenn Damkroeger[3], Miriam Drayss[3]; [1]Thermo Fisher Scientific, Rodano, Italy; [2]RECIPE Chemicals + Instruments GmbH, Munich, Germany; [3]Thermo Fisher Scientific, Dreieich, Germany

For Research Use Only. Not for use in diagnostic procedures.

Key Words

- TSQ Vantage
- Clinical Research
- Therapeutic Drugs

Introduction

Immunosuppressant drugs inhibit the immune system and are used in organ transplant patients to prevent organ rejection. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a widely accepted technique for the determination of immunosuppressant drugs in whole blood by clinical research laboratories. Tools providing reagents for sample extraction, calibrators, and QCs for analysis of these molecules are useful in facilitating analysis and increasing throughput.

Goal

To set up and validate an LC-MS/MS method for the analysis of Tacrolimus, Sirolimus, Everolimus, and Cyclosporin A in whole blood for clinical research laboratories by using the RECIPE ClinMass® Complete Kit with the Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Experimental

This method has been developed using the RECIPE ClinMass® Complete Kit for the determination of immunosuppressants in whole blood according to the instruction manual.

Sample preparation

In a sample preparation vial, 200 µL of precipitation reagent, 20 µL of internal standard, and 100 µL of whole blood sample were combined. The sample was mixed for 30 seconds and incubated at ambient temperature for 5 minutes. The sample was mixed again for 10 seconds and centrifuged. Then, 50 µL of the supernatant was injected into the LC-MS/MS system.

HPLC

High performance liquid chromatography (HPLC) analysis was performed online by use of a 6-port, 3-channel, automatic switching valve and two Thermo Scientific Accela HPLC pumps working in isocratic mode. The sample was injected onto the solid phase extraction (SPE) column (with the switching valve in the "load" position), which extracted the analytes selectively from the sample matrix. The matrix components passed the SPE column widely unhindered and were eluted to waste. Meanwhile, the analytical column was re-equilibrated from the previous injection cycle. When the automatic switching valve switched to the "inject" position, the extracted analytes were eluted from the SPE column in backflush mode and transferred to the analytical column. After elution of the analytes, the automatic switching valve returned to the "load" position. Both columns (SPE and analytical) were re-equilibrated for the next injection. The effective run time was two minutes.

MS

Mass spectrometry analysis was performed using a TSQ Vantage™ triple stage quadrupole mass spectrometer equipped with a heated electrospray ionization source (H-ESI II). The parameters are summarized in Table 1. MS analysis was performed in positive selected reaction monitoring (SRM) data acquisition mode. SRM parameters for all of the analytes and internal standards are shown in Table 2.

Table 1. Optimized ion source parameters

Ion Source	H-ESI II, positive
Resolution Q1 and Q3	0.7 amu
Spray Voltage	3500 V
Vaporizer Temp	300 °C
Sheath Gas Pressure	40
Ion Sweep Gas Pressure	2.0
Aux Gas Pressure	15
Capillary Temp	200 °C
Declustering Voltage	-2 V
Collision Pressure	1.5 mTorr

Table 2. SRM parameters used for the analysis

Compound	Precursor Ion	Product Ion	Scan Time [msec]	Collision Energy
Tacrolimus	821.6	768.4	50	18
Ascomycin	809.5	756.6	50	18
Sirolimus	931.7	864.6	75	15
Everolimus	975.7	908.8	75	16
d_4-Everolimus	979.7	912.6	75	16
Cyclosporin A	1220.0	1203.3	50	17
Cyclosporin D	1234.0	1217.0	50	17

Thermo SCIENTIFIC

Results and Discussion

Figure 1 displays the representative lower limit of quantification (LLOQ) chromatograms for Tacrolimus, Sirolimus, Everolimus, Cyclosporin A, and the internal standards.

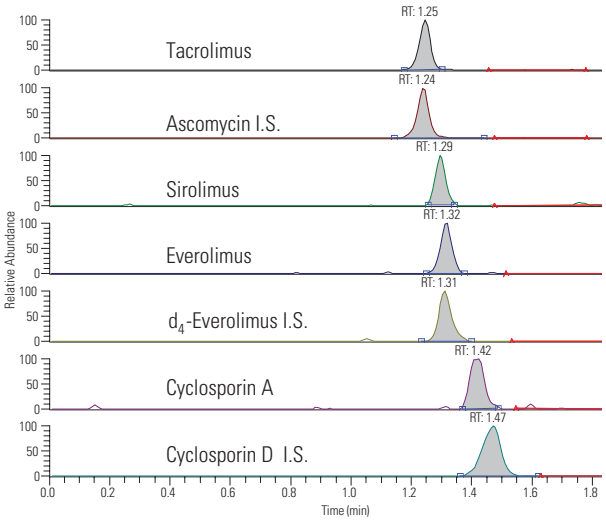

Figure 1: Chromatograms of the lowest calibration standard

In Table 3, the LLOQ and the linearity range for each analyte are reported and compared to the therapeutic range.

As shown in Tables 4 and 5, the intra- and inter-day variabilities were excellent as well as accurate. For each analyte, intra-day variability and accuracy were determined by performing two different extractions of each QC sample and analyzing them two times. Inter-day variability and accuracy were determined by repeating the intra-day procedure on three different days. Sample extractions were performed by different people.

Conclusion

A fast and reliable LC-MS/MS method for the quantification of Tacrolimus, Sirolimus, Everolimus, and Cyclosporin A in whole blood was validated using the RECIPE ClinMass® Complete Kit.

This method fulfills accuracy, precision, and dynamic range requirements of a routine method for clinical research.

Table 3. Summary of assay performance and therapeutic range

	Therapeutic Range [ng/mL]	LOQ [ng/mL]	Linearity Range [ng/mL]	I.S.
Tacrolimus	2 - 15	0.13	1.3 - 46.7	Ascomycin
Sirolimus	5 - 15	0.13	1.3 - 46.9	d_4-Everolimus
Everolimus	6 - 8	0.13	1.3 - 47.4	d_4-Everolimus
Cyclosporin A	100 - 350	24.90	24.90 - 1264.0	Cyclosporin D

Table 4. Intra-day variability (%RSD) and accuracy

	QC 1			QC 2			QC 3		
	Value	%RSD	%Accuracy	Value	%RSD	%Accuracy	Value	%RSD	%Accuracy
Tacrolimus	3.28	6.7	90.1	6.67	2.9	96.3	13.3	5.5	99.4
Sirolimus	3.64	2.7	81.7	11.20	3.8	93.6	18.9	5.2	101.8
Everolimus	3.34	7.2	90.1	10.60	7.1	97.4	18.2	7.2	101.5
Cyclosporin A	62.50	11.4	101.7	258.00	6.2	102.9	1341.0	2.8	94.6

Table 5. Inter-day variability (%RSD) and accuracy

	QC 1			QC 2			QC 3		
	Value	%RSD	%Accuracy	Value	%RSD	%Accuracy	Value	%RSD	%Accuracy
Tacrolimus	3.28	4.7	92.5	6.67	2.1	97.4	13.3	3.3	99.4
Sirolimus	3.64	8.4	89.6	11.20	4.6	95.7	18.9	5.1	102.8
Everolimus	3.34	7.6	96.7	10.60	5.1	96.5	18.2	4.7	100.9
Cyclosporin A	62.50	15.6	103.4	258.00	6.7	99.0	1341.0	12.0	102.9

www.thermoscientific.com

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840
Australia
+61 3 9757 4300
Austria
+43 1 333 50 34 0
Belgium
+32 53 73 42 41
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 50 34 0
Finland/Norway/Sweden
+46 8 556 468 00
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 561 688 8700
Middle East
+43 1 333 50 34 0
Netherlands
+31 76 579 55 55
New Zealand
+64 9 980 6700
Russia/CIS
+43 1 333 50 34 0
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Switzerland
+41 61 716 77 00
UK
+44 1442 233555
USA
+1 800 532 4752

ISO 9001 REGISTERED COMPANY
Thermo Fisher Scientific, San Jose, CA USA is ISO Certified.
AN63349_E 12/10S