Application Note: 487b

Quantitative Analysis of Immunosuppressant Drugs in Whole Blood Using High Throughput LC-MS/MS

Marta Kozak[1], Raimund Ruzicka[2], Vani Bodepudi[2], Jeff Zonderman[3], [1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Thermo Fisher Scientific, Fremont, CA, USA, [3]Thermo Fisher Scientific, Franklin, MA, USA

Key Words

- TSQ Quantum Ultra
- Transcend LX-2 System
- Clinical Research
- Multiplexing

Introduction

Immunosuppressant drugs inhibit the body's immune system and are used in organ transplant patients to prevent organ rejection. Liquid chromatography-mass spectrometry (LC-MS/MS) is a widely-accepted technique for the determination of immunosuppressant drugs in whole blood by research laboratories.

This application note describes a fast, sensitive, reliable, and accurate LC-MS/MS quantitative method for use by research laboratories for the simultaneous analysis of tacrolimus, sirolimus, everolimus and cyclosporin A in whole blood.

Experimental Conditions

Sample Preparation

A protein precipitation solution was prepared by mixing MeOH containing internal standards (Ascomycin and Cyclosporin D) with $ZnSO_4$ solution. Blood samples were processed by adding precipitation solution. The mixture was vortexed and centrifuged. Supernatant was injected into the LC-MS/MS system.

HPLC

HPLC analysis was performed using Thermo Scientific Transcend LX-2 advanced multiplexing system. Samples were injected into a Thermo Scientific Javelin C18 guard column at 80 °C and analyzed with a 2-minute gradient method.

Mass Spectrometry

MS analysis was performed using a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source in selective reaction monitoring (SRM) data acquisition mode. Optimized SRM parameters for all of the analytes and internal standards are shown in Table 1.

Table 1: Optimized SRM parameters

Compound	Parent Ion	Fragment Ion	Collision Energy	Tube Lens Offset
Tacrolimus	821.4	768.3	18	190
Sirolimus	931.6	864.5	15	190
Everolimus	975.7	908.4	16	190
Ascomycin	809.4	756.4	18	190
Cyclosporin A	1219.9	1202.9	17	190
Cyclosporin D	1234.0	1216.9	17	190

Results and Discussion

Figure 1 displays the representative limits of quantitation (LOQ) chromatograms for tacrolimus, sirolimus, everolimus, cyclosporin A, and the internal standards. As shown in Tables 2 and 3, the intra- and inter-day variability were excellent. For each analyte, intra-day variability was determined by processing and analyzing 5 replicates of each QC sample. Inter-day variability was determined with 5 replicates of each QC sample in 3 different batches. The method tested negatively for all interferences and cross-reactivity. No ion suppression or enhancement was observed.

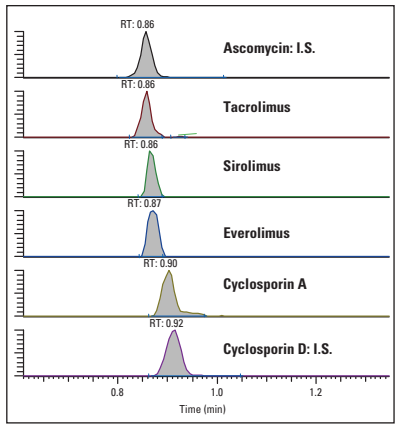

Figure 1: Chromatogram of lowest calibration standard

Assay performance summary

Target Analytes	Tacrolimus, Sirolimus, Everolimus and Cyclosporin A
Matrix	Whole blood
LOQ	1 ng/mL (Tacrolimus, Sirolimus, Everolimus) 10 ng/mL (Cyclosporin A)
Assay Linearity	1-50 ng/mL (Tacrolimus, Sirolimus, Everolimus) 10-2000 ng/mL (Cyclosporin A)
Analysis Time	2.0 min; 1.0 min with column multiplexing

Table 2: Intra-day variability (%RSD)

Analyte	QC1	QC2	QC3	QC4	QC5
Tacrolimus	6.8	4.6	4.9	-	-
Sirolimus	6.7	6.1	3.9	-	-
Everolimus	8.6	5.1	4.5	-	-
Cyclosporin A	5.5	4.6	3.4	3.4	3.5

Table 3: Inter-day variability (%RSD)

Analyte	QC1	QC2	QC3	QC4	QC5
Tacrolimus	4.2	4.1	1.7	-	-
Sirolimus	4.4	7.0	7.5	-	-
Everolimus	7.5	2.3	6.8	-	-
Cyclosporin A	1.8	2.0	2.4	1.7	4.7

Conclusion

A fast, sensitive, reliable and accurate method was developed for the quantification of tacrolimus, sirolimus, everolimus and cyclosporin A in whole blood by research laboratories. The use of column multiplexing technology allows for a 1 min analytical method, which enhances sample throughput.

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