Quantitative Analysis of Immunosuppressants in Dried Blood Spots Using the TSQ Endura Triple Quadrupole MS for Research

Pavel Aronov[1], Katerina Sadilkova[2], Jane Dickerson[2], Marta Kozak[1]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Seattle Children's Hospital, Seattle, WA

Application Note 603

Key Words
Immunosuppressant drugs, dried blood spots, TSQ Endura

Goal
To develop a rapid, sensitive, selective, and cost effective LC-MS/MS research method to determine the concentrations of cyclosporine A, tacrolimus, and sirolimus in dried blood spots down to 3 mm size.

Introduction
Immunosuppressants (IMS) have narrow therapeutic margins and thus have to be monitored routinely. Dried blood spots (DBS) on paper become a desirable method of sample collection because they can be collected in the field and shipped for analysis with minimal transportation safety requirements. Normally, 8 mm dried blood spots are used; however, reducing their size to 3 mm offers advantages in both minimizing sample volume sevenfold and automating sample preparation because standard size office paper punchers can be used to cut the dried blood spots. Sample reduction inevitably leads to a need for sensitive LC-MS/MS assays. In this application note, IMS in dried blood spots were analyzed using the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.

Methods
Sample Preparation
A stock internal standard (IS) solution in acetonitrile was prepared by spiking ascomycin (AsC), sirolimus-d_3 (d_3-SrL), and cyclosporin D (CsD) to a final concentration of 6 ng/mL (AsC and CsD) and 30 ng/mL (d_3-SrL).

A working IS solution was obtained by mixing two parts of the stock IS solution and one part of 0.01 M zinc sulfate in water to a final concentration of AsC, CsD, and d_3-SrL of 4, 4, and 20 ng/mL, respectively. Working IS solution was stored at 4 °C for 3 months.

Discs were punched from the DBS cards with an 8 mm punch into 2 mL microcentrifuge tubes. Then, 150 µL of working IS solution containing 0.01 M $ZnSO_4$ was added, ensuring that the entire spot was completely saturated. Tubes were vortex mixed gently for 3 sec and centrifuged at 15,700 rcf for 3 min. The sample was then mixed for 20 min. The supernatant was immediately transferred to autosampler vials, further diluted sevenfold with 66% acentonitrile in water to emulate 3 mm DBS, and 20 µL were injected into the LC-MS/MS system.

Liquid Chromatography

System:	Thermo Scientific™ Dionex™ UltiMate™ HPG3400-RS pump, UltiMate WPS-3000 autosampler, UltiMate TDS-3000 column compartment
Column:	Proprietary
Mobile phase A:	10 mM ammonium formate/0.1% formic acid in water (Fisher Chemical™ brand)
Mobile phase B:	10 mM ammonium formate/0.1% formic acid in methanol (Fisher Chemical brand)
LC gradient:	Refer to Table 1

Table 1. Chromatographic gradient

	Retention time (min)	Flow (mL/min)	% B
1	0.00	0.500	30
2	0.25	0.500	30
3	0.50	0.500	100
4	1.50	0.500	100
5	1.51	0.750	30
6	2.00	0.750	30

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MS Method

MS analysis was performed on a TSQ Endura triple quadrupole mass spectrometer (Figure 1). The MS conditions were as follows:

Ionization:	Heated electrospray ionization (HESI)
Vaporizer temp:	400 °C
Capillary temp:	250 °C
Spray Voltage:	1000 V
Sheath gas:	45 AU
Auxiliary gas:	5 AU
Sweep gas:	1 AU
Data acquisition mode:	Selected-reaction monitoring (SRM)
Chrom filter peak width:	3 s
Collision gas pressure:	2 mTorr
Cycle time:	0.5 s
Q1 (FWMH):	0.7
Q3 (FWMH):	0.7
SRM parameters:	Refer to Table 2

Table 2. SRM transitions

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Ascomycin	809.75	756.4	21	203
Tacrolimus	821.6	768.45	20	187
Sirolimus	931.85	864.5	17	191
Sirolimus-d3	934.85	864.5	17	191
Cyclosporin A	1220	1202.8	17	224
Cyclosporin D	1234	1216.85	17	200

Results and Discussion

All data were acquired and processed with Thermo Scientific™ TraceFinder™ software version 3.1. The high selectivity of SRM detection using the TSQ Endura triple quadrupole mass spectrometer makes it possible the use of rapid chromatographic separation (2 min) on a short, 10 mm column achieving chromatographic peaks with excellent shape (Figure 1). Internal calibration curves were built for each analyte (Figures 2–4). QC and donor samples were analyzed in triplicate resulting with good correlation between spiked and measured results (Table 3).

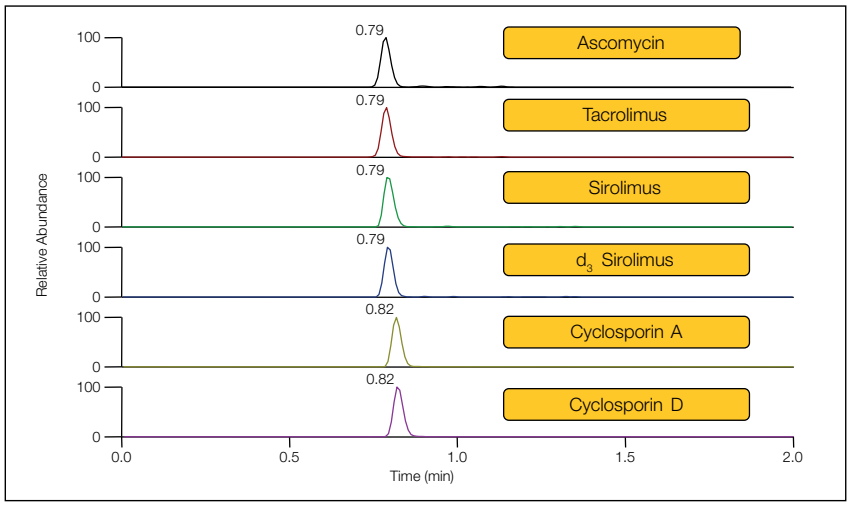

Figure 1. Chromatography

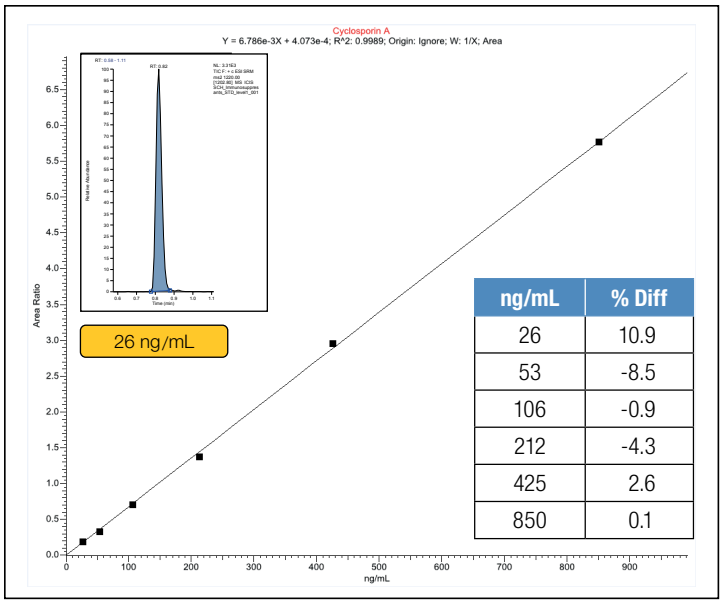

Figure 2. Cyclosporin A

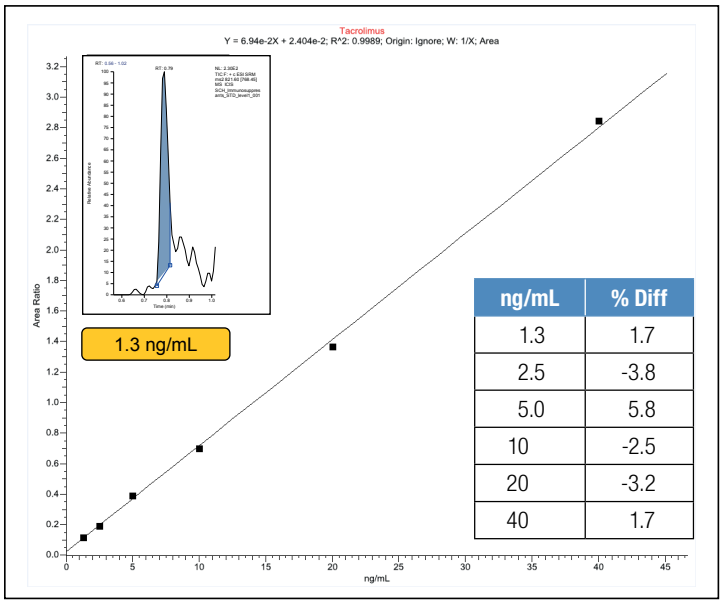

Figure 3. Tacrolimus

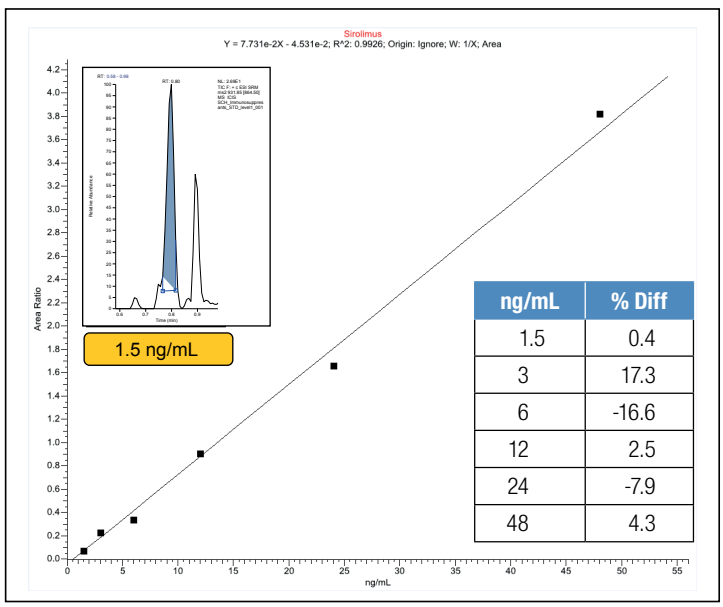

Figure 4. Sirolimus

Table 3. QC and unknowns

	Tacrolimus		Sirolimus		Cyclosporin A	
	Spiked (ng/mL)	Measured [average ± st dev] (ng/mL)	Spiked (ng/mL)	Measured [average ± st dev] (ng/mL)	Spiked (ng/mL)	Measured [average ± st dev] (ng/mL)
Low Control	6.0	6.2 ± 0.2	3.6	3.2 ± 0.3	76	74 ± 2
Mid Control	12	13.1 ± 0.5	9.7	11.0 ± 0.4	199	175 ± 3
High Control	22	21.2 ± 2.8	17.4	17.6 ± 4.2	311	276 ± 9
Subject 1	10	11.0 ± 1.0	5.3	6.2 ± 0.5	0	< LOQ
Subject 2	4.2	4.9 ± 0.4	0	< LOQ	0	< LOQ
Subject 3	0	< LOQ	2.1	3.0 ± 0.9	59	57 ± 3

Conclusion

A high-throughput, cost-efficient research method was developed for the precise and accurate measurement of immunosuppressant drugs in dried blood spots using a TSQ Endura triple quadrupole mass spectrometer. This method met analytical laboratory precision and accuracy criteria for 3 mm dried blood spots.

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