thermoscientific

Direct Quantitation of Five Immunosuppressant Drugs in Volume-Controlled Dried Whole Blood Spots by a Fully Automated DSM-LC-MS System

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ABSTRACT

Goal: Demonstrate a complete and fully automated workflow for dried blood spot analysis of five commonly used therapeutic immunosuppressant drugs.

Methods: The analytical method was developed on the Thermo Scientific™ Transcend™ DSX-1 system consisting of a dried matrix spot module coupled with Thermo Scientific™ TurboFlow™ technology and a triple quadrupole mass spectrometer (Figure 1).

Results: High-throughput 5-minute quantification of target analytes in dried blood spots was achieved to satisfy different cut-off needs in clinical settings.

Figure 1. A fully automated Transcend DSX-1 system was paired with a TSQ Altis Plus mass spectrometer

Dried Spot Module | Transcend UHPLC system | TSQ Altis Plus

INTRODUCTION

Therapeutic drug monitoring of immunosuppressants drugs is vital for recipients of organ transplants to ensure concentrations are high enough to prevent transplant rejection, but low enough to avoid intoxication.

Liquid chromatography-mass spectrometry (LC-MS) is increasingly used in clinical research to quantify immunosuppressant drugs in whole blood as it can offer higher sensitivity and selectivity than other analytical techniques. LC-MS systems may be coupled with upstream dried spot modules within one high-throughput, integrated and online workflow, allowing the extraction of matrices from pre-collected sample cards collected in a quick and minimally invasive manner.

In this poster, such a workflow will be utilized to demonstrate the quantitation of five commonly monitored therapeutic immunosuppressants; cyclosporin A, everolimus, mycophenolic acid, sirolimus and tacrolimus.

MATERIALS AND METHODS

Calibration and QC samples were prepared to varying concentrations in whole blood. 10 µL of each level of calibrant was then spotted in triplicate on HemaXis DMS cards via a HemaXis DB-10 collection device. Immunosuppressants and standards were then extracted by an automated Transcend DSX-1 system via flow-through desorption with a heated clamp (Table 1).

The 2-dimensional TurboFlow technology allowed interference removal in the extracted samples prior to analytical separation. An integrated software, Aria MX, controlled each step of sample desorption and separation. Analyte quantitation was performed by a Thermo Scientific™ TSQ Altis™ Plus triple-stage quadrupole mass spectrometer (Tables 2/3), and the data was analyzed using Thermo Scientific™ TraceFinder™ 5.1 general quantitation software.

Table 1. Liquid chromatography conditions

	TurboFlow Column						Analytical Column				
Time (min)	**Flow Rate (mL/min)**	**% A**	**% B**	**%C**	**Tee**	**Loop**	**Divert**	**Flow Rate (mL/min)**	**Gradient**	**%A**	**%B**
0.00	2.0	100	-	-	====	out	Waste	0.5	Step	70	30
0.10	0.1	100	-	-	====	out	Waste	0.5	Step	70	30
0.20	2.0	100	-	-	====	out	Waste	0.5	Step	70	30
0.60	0.1	-	100	-	====	out	Waste	0.5	Step	70	30
0.68	0.1	-	100	-	T	in	Det	0.4	Step	70	30
1.68	2.0	-	-	100	====	in	Det	0.5	Step	70	30
1.93	2.0	-		100	====	in	Det	0.5	Ramp	30	70
2.18	1.5	100	-	-	====	out	Det	0.5	Ramp	25	75
2.43	2.0	-	-	100	====	in	Det	0.5	Ramp	20	80
2.68	1.0	100	-	-	====	out	Det	0.5	Ramp	15	85
2.93	1.0	-	100	-	====	in	Det	0.5	Ramp	5	95
3.43	1.0	100	-	-	====	out	Det	0.5	Step	5	95
3.93	1.0	100	-	-	====	out	Det	0.5	Step	70	30
5.00	1.0	100	-	-	====	out	Det	0.5	Step	70	30
Clamp Washes	Wash 1: 0.1% formic acid in water Wash 2: 0.1% formic acid in acetonitrile Wash 3: Isopropanol/acetonitrile/acetone, 2/2/1 (v/v/v)										
Mobile Phases	A: 0.1% formic acid, 10% acetone in water B: 10 mM ammonium formate, 0.05% formic acid in methanol C: Isopropanol/acetonitrile/acetone, 2/2/1 (v/v/v)						A: 10 mM ammonium formate, 0.05% formic acid in water B: 10 mM ammonium formate, 0.05% formic acid in methanol				
Columns	Cyclone-P TurboFlow column, 50 x 0.5 mm at room temperature						Hypersil Gold C8, 50 x 2.1 mm, 3 µm, 70 ° C				

Table 2. Mass spectrometer source conditions

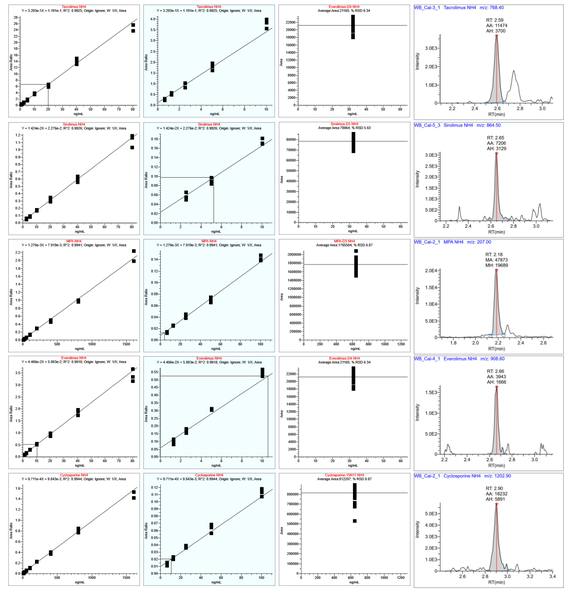
Polarity	(+)	Spray Voltage	4000
Sheath Gas (Arb)	50	Dwell Time (ms)	15
Aux Gas (Arb)	10	Q1 Resolution (FWHM)	0.7
Sweep Gas (Arb)	0	Q3 Resolution (FWHM)	0.7
Ion Transfer Tube Temp. (ºC)	400	Source Fragmentation	0
Vaporizer Temp. (ºC)	300	CID Gas (mTorr)	1.5

Table 3. Selected reaction monitoring transitions for each target analyte

Analytes	Precursor		Quantifier		Qualifier	
	m/z	RF Lens (V)	*m/z*	CE (V)	*m/z*	CE (V)
Mycophenolic acid	338.2	80	207.1	23	275.1	20
Tacrolimus	821.5	175	768.4	21	786.4	17
Sirolimus	931.5	173	864.5	17	882.4	12
Everolimus	975.6	176	908.6	16	926.4	12
Cyclosporin A	1219.9	226	1202.9	17	1184.8	32

RESULTS

Figure 2. Full calibration curves and the lower limit region for each target analyte (left), internal standard responses (center) and extracted ion chromatograms corresponding to LOQs (right)



Five common therapeutically monitored immunosuppressant drugs were quantified from blood spotted onto DBS cards using a rapid, online and fully automated workflow. This workflow resulted in a highly sensitive 5-minute method including analyte extraction to MS detection and column re-equilibration (Figure 2). Analyte carryover was minimized by using a C8 analytical column and rapid aqueous/organic washing steps. Calibration curves were fitted using a weighting factor of 1/x with requirements of $R^2 > 0.99$, |RSD| and |CV| < 15%. Calibrants were used to determine the limits of detection (LODs) and quantitation (LOQs - Table 4).

Table 4. Retention times, limits of detection and quantitation, and calibration validation statistics corresponding to the LOQ

Analytes	t_R (minutes)	LOD	ULOQ	LOQ	R^2	Variability at LOQ		
						%RSD	%CV	Ave. %Diff
Mycophenolic acid	2.19	6.25	1600	12.5	0.9941	3.11	2.16	12.80
Tacrolimus	2.60	0.310	80	1.25	0.9925	8.67	6.80	5.30
Sirolimus	2.65	1.25	80	5.00	0.9926	9.84	7.36	-0.66
Everolimus	2.67	0.625	80	2.50	0.9918	10.28	6.81	3.68
Cyclosporin A	2.91	6.25	1600	12.5	0.9944	12.58	6.85	-2.92

Innovative technologies

- **Volume-Controlled Spots** • Precise Sampling
- **HotCap** • Heated Extraction
- **FTD (Flow-Through Desorption)** • Direct Analyte Desorption and Extraction
- **IVC (Intelligent Vision Camera)** • Spot Recognition, Sample Traceability, Chain of Custody
- **AIS (Automated IS Addition)** • Precise IS Addition
- **96 DBS Cards Capacity**
- **TurboFlow** • Online Sample Cleanup
- **Aria MX Software** • Integrated Software Control

DMS analysis has numerous advantages, such as quick and easy sampling, efficient sample transfer and storage and good analyte stability. Traditional downsides to DMS analysis, such as labor and resource-intensive manual disc-punching and offline sample clean-up, have been avoided by the use of desorption and TurboFlow clean-up to provide a quick, robust, and fully automated online platform. In addition, the workflow securely maintains chain-of-custody for each dried spot throughout, with images provided from before and after each extraction.

CONCLUSIONS

Five common therapeutically monitored immunosuppressant drugs were quantified from dried blood spots using a rapid, online and fully automated workflow. The highlighted method utilized a fully automated Transcend DSX-1 system for quick and online analyte extraction, separation and detection.

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TRADEMARKS/LICENSING

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