Automated Extraction and Quantification of Immunosuppressants from Dry Blood Spots Using the Transcend DSX-1 System

Pragya Sharma[1], Richard Lahr[1], Alison Lightfoot[1], Jennifer Faber[1], Jingshu Guo[2], Loralie Langman[1], Paul J. Jannetto[1] and Anthony Maus[1]

[1]Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2]ThermoFisher Scientific, San Jose, CA

ABSTRACT

BACKGROUND

Therapeutic drug monitoring (TDM) of immunosuppressants in whole blood is critical for clinical follow-up of transplant patients to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse effects. Immunosuppressants are routinely quantified using LC-MS/MS in whole blood which translates to frequent phlebotomy visits for patients for the purpose of venous blood collection. Alternatively, dry blood spot (DBS) measurements have the potential to reduce the cost of sample collection and shipping, while simultaneously increasing the convenience of at home collection for patients.

METHODS

Calibrators and QC were made by spiking immunosuppressants (Cyclosporine A, Tacrolimus, Sirolimus and Everolimus) into bovine whole blood. Residual venous blood samples previously analyzed for Cyclosporine A, Tacrolimus, Sirolimus and/or Everolimus on validated LC-MS/MS methods were utilized for this study. Calibrators, QC, and patient samples were spotted (25 µL) on PerkinElmer 226 Bioanalysis RUO Card with Ahlstrom 226 grade paper. Immunosuppressants were extracted using a fully automated Thermo Scientific™ Transcend™ DSX-1 UHPLC system that performs internal standard addition, analyte extraction, 2-D LC matrix cleanup and analyte separation without any manual intervention. Sample analysis was performed on a Thermo Scientific™ TSQ Altis™ MD mass spectrometer and data was analyzed using TraceFinder™ LDT software.

RESULTS

Extraction, LC separation, and quantification of immunosuppressants using the Transcend™ DSX-1 system was accomplished using a 11.45-minute method. This method includes extensive wash and equilibration steps to minimize carryover. The intra-run imprecision of four different concentrations of QC for the four immunosuppressants measured was ±12%. The signal-to-noise ratio for all samples was greater than ten. Patient results from the Transcend™ DSX-1 were compared to established LC-MS/MS testing methods that utilized osmotic shock lysis by water followed by protein precipitation with methanolic zinc sulfate. Linear regression comparison indicated less than or equal to 10% bias and R^2 greater than 0.85 for all analytes tested.

CONCLUSIONS

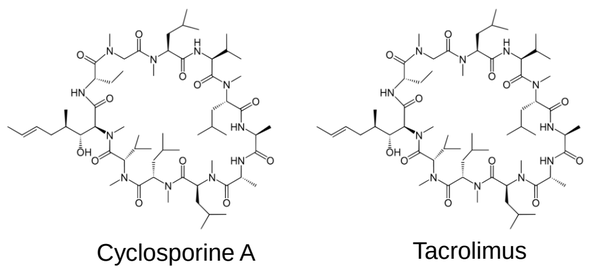
Preliminary studies suggest that the Transcend™ DSX-1 platform has the potential to provide a fully automated extraction of DBS for immunosuppressants. This sample matrix enables convenient sample collection for patients for therapeutic drug monitoring of immunosuppressants. Although our initial results are promising, additional studies are required to demonstrate if this platform can achieve the necessary analytical performance characteristics and determine if simultaneous venous whole blood collection and DBS of patients undergoing immunosuppressant therapy produce clinically equivalent results.

OBJECTIVE

To evaluate the analytical applicability of a DBS method for monitoring of immunosuppressants in venous blood samples using an automated extraction platform.

METHODS

IMMUNOSUPPRESSANTS



Cyclosporine A | Tacrolimus | Sirolimus | Everolimus

LC-MS/MS PARAMETERS

Analyte	Precursor (m/z)	Product (m/z)
Cyclosporine A	1220	1202.8
Cyclosporin A-[15N]11	1230.9	1213.9
Tacrolimus	821.9	768.5
FK-506-[13C]-D2	824.9	771.5
Sirolimus	931.7	864.5
Rapamycin D3	934.7	864.5
Everolimus	975.8	908.5
Everolimus-D4	979.8	912.5

Columns:
TurboFlow Cyclone™ 1 x 50 mm, HypersilGOLD™ aQ 2.1 x 30 mm

Mobile Phase A:
1mM Ammonium Formate in Water with 0.1% Formic Acid

Mobile Phase B:
1mM Ammonium Formate in Methanol with 0.1% Formic Acid

Mobile Phase C:
45/45/10: Isopropanol/Acetonitrile/Acetone

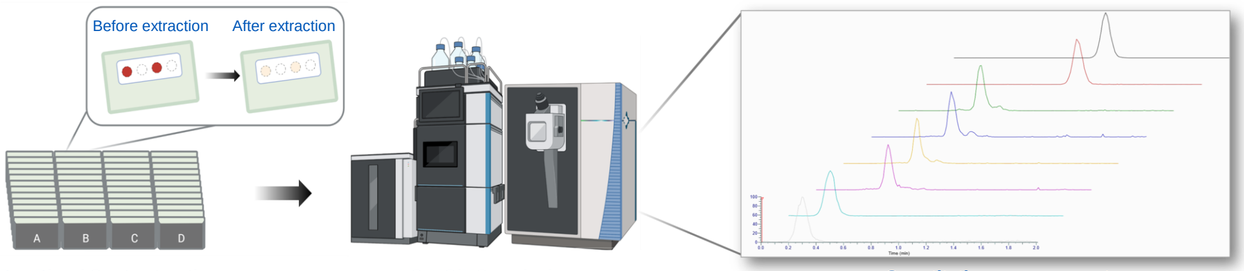
Wash 1: 0.1% Formic Acid in Water;

Wash 2: 0.1% Formic Acid in Acetonitrile;

Wash 3: 45/45/10: Isopropanol/Acetonitrile/Acetone

TurboFlow Column									Analytical Column			
Start	Time (min)	Flow Rate (mL/min)	Gradient	%A	%B	%C	Tee	Loop	Divert	Flow Rate (mL/min)	Gradient	%A	%B
0	0.8	1	Step	80	-	20	-----	out	Waste	0.4	Step	99	1
0.8	0.5	1	Step	60	40	-	-----	out	Waste	0.4	Step	99	1
1.3	0.1	0.15	Step	20	80	-	-----	out	Waste	0.4	Step	99	1
1.4	1	0.15	Step	20	80	-	T	in	Waste	0.4	Step	99	1
2.4	0.05	1.5	Step	-	-	100	-----	in	Det	0.4	Step	42	58
2.45	1.5	1.5	Step	-	-	100	-----	in	Det	0.4	Ramp	29.5	70.5
3.95	1.5	1.5	Step	20	80	-	-----	in	Det	0.4	Ramp	17	83
5.45	1	1.5	Step	20	80	-	-----	in	Det	0.4	Step	1	99
6.45	1	1.5	Step	100	-	-	-----	out	Waste	0.4	Ramp	99	1
7.45	4	1	Step	80	-	20	-----	out	Waste	0.4	Step	99	1

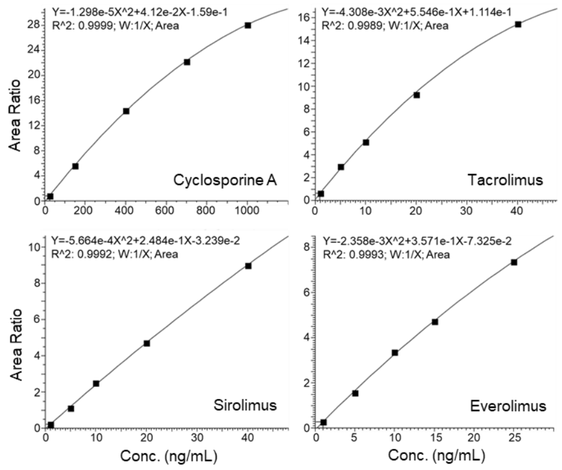
AUTOMATED WORKFLOW



Dry Blood Spot extraction | LC-MS/MS analysis | Quantitative measurements

RESULTS

CALIBRATION



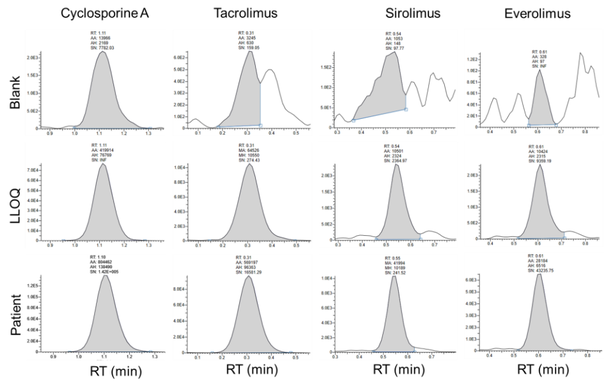
INTRA/ INTER-DAY IMPRECISION

Four different levels of QC were measured in five batches in replicates of three per batch.

- Intra-day: All analytes were within acceptable limit
- Inter-day: All analytes except Cyclosporine A meet the acceptance criteria.

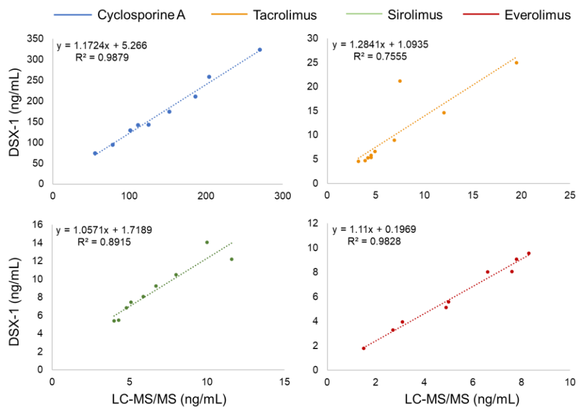
Analyte	Cyclosporine A %CV	Tacrolimus %CV	Sirolimus %CV	Everolimus %CV
Intra-day				
LLOQ	4.6	6.9	4.7	5.2
Low	5.4	5.3	6.1	4.8
Medium	4.3	3.6	5.1	6.2
High	9.4	2.4	5.5	4.3
Inter-day				
LLOQ	20.7	10.4	9.5	8.4
Low	23.2	8.6	8.1	7
Medium	24.1	4.1	7.5	8.3
High	30.8	6.3	9.1	6.5

CHROMATOGRAPHY

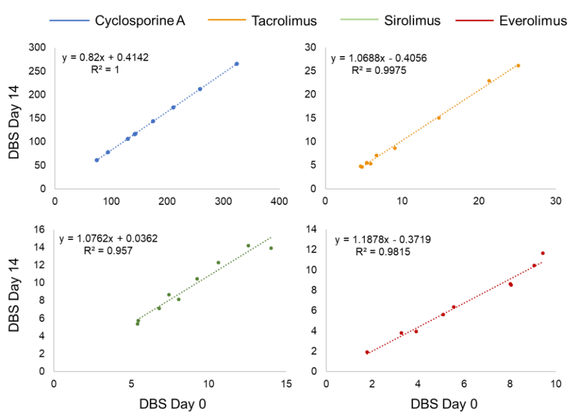


SAMPLE ACCURACY

Patient results were compared with the established LC-MS-MS method.



AUTHENTIC SAMPLE STABILITY



CONCLUSIONS

- A working and reproducible TurboFlow and analytical method for measurement of immunosuppressants was developed.
- The validated method proved authentic patient sample stability for 14 days at room temperature. This supports DBS method as a convenient mode for at home sample collection and mailing without interfering with clinical interpretation during clinical follow-up of patients.
- Transcend™ DSX-1 shows potential to provide fully automated extraction of DBS for immunosuppressants, whereas additional studies are required to further evaluate the technology in clinical settings using simultaneous venous blood collection and spotting.

REFERENCES

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4. Mayo Clinic Laboratories, test catalog, specimenID 35143, 35144, 35145, and 35146.