Analysis of Immunosuppressant Drugs directly from Whole Blood using PaperSpray Technology

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ABSTRACT

Purpose: Minimizing sample preparation for the analysis of immunosuppressant drugs using PaperSpray technology for clinical research.

Methods: Cyclosporin A, Everolimus and Tacrolimus were analyzed using a 2-minute method directly from dried blood spots on Thermo Scientific™ VeriSpray™ PaperSpray ion source sample plates.

Results: Very good linearity and precision were achieved for all compounds. Cyclosporin A and Tacrolimus meet the clinical research range in whole blood. Everolimus exhibits high background signal which currently limits the LOQ. Strategies to further reduce interfering signals are currently in development.

INTRODUCTION

Immunosuppressant drugs are of significant interest in the clinical community. Various analysis methods have been published, the majority of them requiring a protein crash followed by LC/MS analysis [Ref 1]. LC/MS run times for this assay can be relatively short, e.g., 4.5 minutes and under [Ref 2]. However, extensive sample preparation and clean-up are necessary for LC injections, increasing the duration of the workflow.

PaperSpray technology is a rapid analysis technology specifically suitable for clinical research samples. Quick sample turnaround times of 2 minutes or less make it very competitive compared to traditional LC/MS-based techniques. Minimal sample preparation is required for analysis of dried urine or blood spots from a piece of triangular shaped paper. A rewet solvent is applied directly onto the dried sample spot to extract analytes. Next, a spray solvent is dispensed onto the paper, and a high voltage is applied to the paper to facilitate spray and ion formation.

The new VeriSpray PaperSpray ion source system uses PaperSpray technology to make clinical research workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 1, left). The VeriSpray plate loader holds up to 10 VeriSpray sample plates (Figure 1, right). Each VeriSpray sample plate contains 24 paper strips (12 on each side, A and B). Through Thermo Scientific™ Xcalibur™ software sequence setup, the full magazine can be run in an automated way.

Here we demonstrate a PaperSpray method for the analysis of immunosuppressant drugs from a single whole blood spot for research purposes.

Figure 1. VeriSpray ion source with VeriSpray plate loader (left) and VeriSpray sample plate (right).

MATERIALS AND METHODS

Sample Preparation

Human blood samples were spiked with the respective drugs and incubated at 4 C over night. On the next day, isotopically labelled internal standards were added and a volume of 10 uL was spotted on VeriSpray sample plates.

Method and Data Analysis

The spray solvent and inlet capillary temperature were optimized (see results section). Data were acquired for 2 minutes per sample, and 4 replicates of each calibrator and QC level were measured. Compounds were detected on a Thermo Scientific™ TSQ Quantis™ Triple Quadrupole mass spectrometer. Four transitions were monitored per compound, with a cycle time of 1.5 seconds and a collision gas pressure of 2.5 mTorr. The spray voltage was 3.6 kV, applied from 0.05 to 1.95 min and the distance paper tip to inlet was approximately 5.5 mm. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

METHOD OPTIMIZATION

Method of spiking the internal standard

Four different internal standard solutions were made which resulted in the same final internal standard concentration in blood, but used different spiking volumes. The following ratios (blood / IS solution) were spiked: IS solution 1: 5:1, IS solution 2: 1:1, IS solution 3: 1:2, IS solution 4: 1:3. %RSD values were obtained for the absolute areas and the response ratio (analyte / internal standard), and are summarized in Table 1. Because IS solution 3 gave the best %RSD values for both, absolute areas and area ratios, it was chosen for further method optimization.

Table 1. %RSD of the absolute area and response ratio (analyte/internal standard) obtained across 3 replicates per condition.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	400 ng/mL		20 ng/mL		20 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
IS solution 1	29.6	10.9	13.6	1.8	20.5	5.5
IS solution 2	31.5	2.5	15.6	5.4	33.3	7.6
IS solution 3	34.8	3.1	9.6	4.2	24.5	2.2
IS solution 4	54.3	12.4	31.8	9.1	35.4	3.3

Solvent system optimization

Table 2. %RSD of the response ratio (analyte/internal standard) and %RSD of the absolute areas obtained across 3 replicates per condition. Each solvent contains 0.1% sodium acetate.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	400 ng/mL		20 ng/mL		20 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
100% methanol	34.9	1.2	21.3	6.3	32.4	7.6
80% meth., 20% chlorof.	33.8	6.3	24.5	8.1	29.5	9.9
60% meth., 40% chlorof.	29.4	3.4	26.4	1.9	26.5	3.4

Best results were obtained for a solvent composition of 60% methanol, 40% chloroform, and 0.1% sodium acetate (see Table 2).

Inlet capillary temperature

For optimization of the inlet capillary temperature (Table 3) IS solution 3 was used and the spray solvent was 60% methanol, 40% chloroform and 0.1% sodium acetate.

Table 3. %RSD of the response ratio (analyte/internal standard) and %RSD of the absolute areas obtained across 3 replicates per condition.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	250 ng/mL		12.5 ng/mL		12.5 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
325 C	50.0	1.5	39.4	11.2	39.3	1.5
350 C	36.2	5.9	19.3	4.7	28.0	4.2
375 C	14.2	2.7	28.7	9.4	11.9	4.5
400 C	35.3	6.1	22.1	4.6	32.4	9.5

Because Everolimus is the most challenging compound to detect using this method, the condition that produced the best result for Everolimus (400 C) was chosen for the final method.

RESULTS

Cyclosporin A and Tacrolimus

The final experiment was carried out using the following optimized conditions:

- Ratio (Blood / IS solution): 1:2
- Solvent: 60% methanol, 40% chloroform, 0.1% sodium acetate in rewet and spray solvent
- Inlet capillary temperature: 400 C

The resulting calibration curves for Cyclosporin A and Tacrolimus are shown in Figures 2 and 3.

Figure 2. Calibration curve for Cyclosporin A

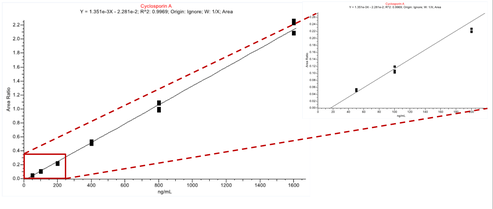
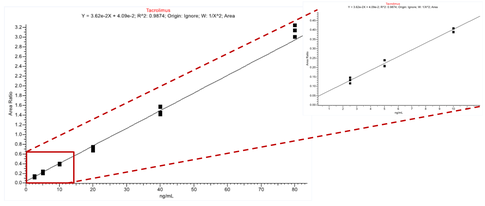


Figure 3. Calibration curve for Tacrolimus



For both, Cyclosporin A and Tacrolimus, precision and accuracy values meet the LOQ requirement (lower than 15% and 20% respectively) at all calibrator and QC levels, including the lowest calibrator level (see Tables 4 and 5). Resulting LOQs are:

Cyclosporin A: 50 ng/mL

Tacrolimus: 2.5 ng/mL

Table 4. Precision and accuracy of calibrator samples.

Level	Cyclosporin A			Tacrolimus		
	c (ng/mL)	%RSD	% Difference	c (ng/mL)	%RSD	% Difference
1	50	4.1	< 13.6	2.5	9.8	< 17.8
2	100	6.8	< 7.8	5	8.0	< 9.5
3	200	2.1	< 10.8	10	2.9	< 4.0
4	400	3.4	< 4.0	20	4.7	< 12.4
5	800	5.6	< 7.5	40	5.4	< 5.6
6	1600	4.3	< 5.8	80	3.6	< 10.8

Table 5. Precision and accuracy of QC samples.

Level	Cyclosporin A			Tacrolimus		
	c (ng/mL)	%RSD	% Difference	c (ng/mL)	%RSD	% Difference
1	160	1.9	- 4.9	8	3.1	- 9.8
			- 7.5			- 1.7
			- 5.6			- 6.8
			- 8.4			- 4.9
2	250	3.7	- 10.1	12.5	1.7	- 4.7
			- 2.7			- 1.2
			- 8.1			- 5.0
			- 5.8			- 2.3
3	500	0.8	- 11.7	25	3.1	1.2
			- 11.5			- 1.4
			- 10.9			- 6.3
			- 10.3			- 1.3

Everolimus

The resulting calibration curve and precision values for Everolimus are shown below. Good linearity and precision was achieved (see Figure 4 and Table 6). However, because of significant contribution of background signal, the accuracy was not sufficient to meet clinical research criteria.

Figure 4. Calibration curve for Everolimus.

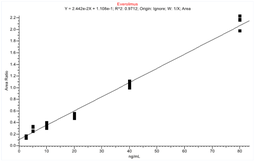


Table 6. Precision of calibrator and QC samples.

Level	Everolimus	
	c (ng/mL)	%RSD
Cal 1	2.5	11.6
Cal 2	5	14.9
Cal 3	10	13.2
Cal 4	20	6.8
Cal 5	40	4.6
Cal 6	80	5.2
QC 1	8	7.6
QC 2	12.5	5.3
QC 3	25	5.9

In order to meet clinical research criteria for Everolimus, as well as Sirolimus, the background signal coming from paper needs to be further reduced. Strategies to achieve this are currently in development.

CONCLUSIONS

- PaperSpray minimizes the need for sample preparation. Mixing internal standard with the sample, spotting samples on the sample plates and drying them are the only required steps.
- Quantification of Cyclosporin A and Tacrolimus is easily achievable in the required measurement range, with the necessary precision and accuracy.
- Further cleanup of the paper and sample plates is under investigation to reduce background signals for Everolimus.

REFERENCES

1. F.I. Al-Jenoobi et al., *Austin Chromatogr.* **2016**, 3(1), 1039.
2. A. Buchwald et al., *BMC Clin. Pharmacol.* **2012**, 12: 2.

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TRADEMARKS/LICENSING

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