Analysis of Drugs in Whole Blood by PaperSpray-FAIMS-MS/MS

Rae Ana Snyder, Cornelia Boeser, Neloni Wijeratne and Mary Blackburn, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134

ABSTRACT

Purpose: Reduce background interferences and increase signal-to-noise for PaperSpray analysis of drugs in whole blood using the Thermo Scientific™ FAIMS Pro™ interface.

Methods: 10 µL samples of blood spiked with varying levels of immunosuppressant drugs were spotted onto Thermo Scientific™ VeriSpray™ PaperSpray ion source sample plates and monitored on a Thermo Scientific™ TSQ Altis™ mass spectrometer. Calibration curves were generated for PaperSpray-FAIMS-MS/MS and PaperSpray-MS/MS methods.

Results: The FAIMS Pro interface improves linearity and detection limits for immunosuppressant drugs ionized with PaperSpray.

INTRODUCTION

Therapeutic drug research is important for a variety of clinical research. Common tools for drug research include immunoassays and HPLC, both of which have drawbacks. Immunoassays may suffer from antibody cross-reactivity and limited dynamic range and HPLC is often costly and requires highly trained technicians. Using PaperSpray technology, high specificity as well as high throughput can be achieved using small amounts of samples, while retaining ease-of-use.

PaperSpray is a rapid technique for analysis of compounds directly from unprocessed dried sample spots. Because no to minimal sample preparation is required, the technique is particularly beneficial for biological sample matrices, which normally require time-consuming and labor intensive sample clean up when analyzed by LC/MS. However, because of the lack of sample cleanup, PaperSpray-MS signals can have high chemical background, which can limit the signal-to-noise ratio (S/N), and compromise the LOQ and LOD of the method. Field Asymmetric Ion Mobility Spectrometry (FAIMS, also sometimes called DMS) is a type of ion mobility which enhances selectivity of an analytical method by adding an additional dimension of separation. It operates by applying an asymmetric waveform between a set of electrodes. Alternating between high-field and low-field portions of the waveform causes a drift in the motion of the ions through the carrier gas due to changes in mobility at high field strength. By applying an DC voltage offsetting this drift (compensation voltage, or CV) ions of particular differential mobility are transmitted through the electrodes to the mass spectrometer inlet, while background ions get neutralized on the electrodes.

By combining PaperSpray and FAIMS technology, background signal can be reduced, and signal-to-blank ratios enhanced. Here we demonstrate this principle by analyzing the immunosuppressive drugs Everolimus, Sirolimus, Tacrolimus, and Cyclosporin A using the new VeriSpray PaperSpray ion source, both with, and without the FAIMS Pro interface.

MATERIALS AND METHODS

Sample Preparation

EDTA blood samples were spiked with immunosuppressant drugs (Everolimus, Sirolimus, Tacrolimus, and Cyclosporin A from Sigma Aldrich) and corresponding internal standards (Everolimus-$^2H_4$, Sirolimus-$^2H_3$, Tacrolimus-$^{13}C,^2H_2$, and Cyclosporin A-$^2H_4$ from Sigma Aldrich) and equilibrated overnight. Blood samples were spotted in 10 µL aliquots onto the paper of VeriSpray sample plates and allowed to dry.

Test Methods

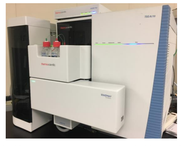
PaperSpray-MS/MS: Tip location of the paper on the VeriSpray sample plates was optimized relative to the MS inlet (4.5 mm from inlet). Methanol with 0.1% sodium acetate was applied to the spotted paper in the VeriSpray sample plates for electrospray generation (15 spray solvent dispensations of 150 µL applied with increasing, 1-10 second, delays). Optimized SRM transitions (see Table 1) were monitored on a TSQ Altis mass spectrometer for one minute while a time dependent source voltage was applied (2800 V from 0.1 to 0.9 minutes).

PaperSpray-FAIMS-MS/MS: For data collected with the FAIMS Pro interface, a dispersion voltage of -5000 V and 100˚C/100˚C inner/outer electrode temperatures were used and spray tip location was re-optimized relative to the FAIMS inlet (1.7 mm from inlet). Optimized compensation voltage (CV) parameters are listed in Table 1. All other parameters were the same as the PaperSpray-MS/MS method. Instrumental setup is shown in Figure 1.

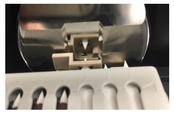
Table 1. Compound optimized parameters for PaperSpray-FAIMS-MS/MS analysis

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	FAIMS CV (V)
Tacrolimus	826.471	616.387	34.91	106	-21.0
Tacrolimus-$^{13}C,^2H_2$	829.487	619.417	35.45	113	-21.0
Sirolimus	936.544	409.292	54.45	138	-17.0
Sirolimus-$^2H_3$	939.565	409.375	53.15	168	-17.0
Everolimus	980.570	389.292	55.00	163	-26.0
Everolimus-$^2H_4$	984.599	393.321	54.45	161	-26.0
Cyclosporin A	1224.831	1112.917	55.00	223	-15.3
Cyclosporin A-$^2H_4$	1228.859	1112.774	55.00	153	-15.3

Figure 1. Instrumental setup for PaperSpray-FAIMS-MS/MS analysis



TSQ Altis mass spectrometer mounted with FAIMS Pro interface and VeriSpray source



The entrance plate of the FAIMS Pro interface with an installed VeriSpray sample plate

Data Analysis

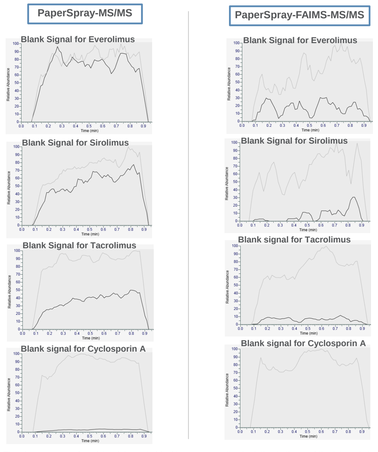
Thermo Scientific™ TraceFinder™ was used for integration of the signal from the analytes and internal standards. Thermo Scientific™ FreeStyle™ was used to plot chronograms. NumPy and Matplotlib were used to perform weighted least squares (where standard deviations were used for weighting ($n \geq 7$)) for calibration curve fits to the average response factor (average area ratio of the analyte to the internal standard), and to plot the calibration curves. Detection limits were obtained using the conventional method documented in CLSI EP17-A2.

RESULTS

Background signal reduction with the FAIMS Pro interface

Chronograms of whole blood matrix blanks spiked with isotopically labeled internal standards were taken using PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods. Representatives are shown in Figure 2.

Figure 2. Chronograms of the matrix blank normalized to the maximal signal of the internal standard



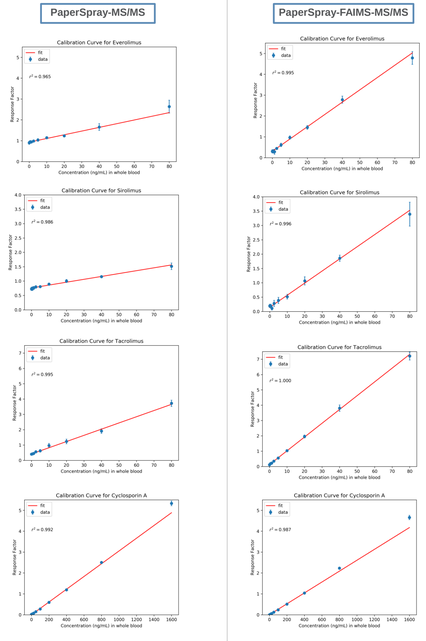
The chronograms of analytes (shown in black) were normalized relative to those of the internal standards (shown in light grey).

The FAIMS Pro interface reduced background signal relative to the internal standard by ~67 % for Everolimus, ~74 % for Sirolimus, ~72 % for Tacrolimus, and ~100 % for Cyclosporin A, allowing for increased selectivity of the PaperSpray method.

Improved sensitivity and linearity with the FAIMS Pro interface

Calibration curves were generated for each immunosuppressant drug using the PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods with 9 standard concentrations ranging from 0-80 ng/mL for Everolimus, Sirolimus and Tacrolimus and 0-1600 ng/mL for Cyclosporin A and are shown in Figure 3. The slopes of the calibration curves for Everolimus, Sirolimus and Tacrolimus increased when using the FAIMS Pro interface, indicating the improved sensitivity of the PaperSpray-FAIMS-MS/MS method for these immunosuppressant drugs.

Figure 3. Calibration curves for the quantitation of immunosuppressant drugs with the PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods



Weighted least squares regression was used to fit the calibration curves and is shown in red on each plot. Average response factors and their standard deviations are shown in blue.

Compared to the PaperSpray-MS/MS method, the PaperSpray-FAIMS-MS/MS method resulted in calibration curves with increased linearity for Everolimus (r^2 increased from 0.965 to 0.995) and Sirolimus (r^2 increased from 0.986 to 0.996) and similar linearity for Tacrolimus (0.995 without FAIMS and 1.000 with FAIMS) and Cyclosporin A (0.992 without FAIMS and 0.987 with FAIMS).

Improved accuracy with the FAIMS Pro interface

The absolute percent differences (% Diff) between expected and calculated concentrations improved for Everolimus, Sirolimus and Tacrolimus when the FAIMS Pro interface was employed (Table 2 shows representative data) while those of Cyclosporin A were comparable for the two methods. Lower levels showed greater accuracy improvement when using FAIMS with the accuracy of Level B (10 ng/mL) improving from 19.9 % to 12.5 % for Everolimus, from 32.4 % to 12.2 % for Sirolimus, and from 36.2 % to 1.4 % for Tacrolimus and the accuracy of Level A (2.5 ng/mL) improving from 30.9 % to 2.5 % for Everolimus, from 37.9 % to 30.1 % for Sirolimus, and from 38.4 % to 0.9 % for Tacrolimus.

Table 2. A comparison of accuracy, precision and signal-to-blank for Everolimus using PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods

	PaperSpray-MS/MS			PaperSpray-FAIMS-MS/MS		
Everolimus	**% Diff**	**% CV**	**S/B**	**% Diff**	**% CV**	**S/B**
Level A (2.5 ng/mL)	30.9	4.3	1.0	2.5	11.7	1.5
Level B (10 ng/mL)	19.9	4.2	1.1	12.5	8.3	3.2
Level C (80 ng/mL)	20.3	11.8	1.8	5.1	6.5	15.7
Sirolimus	**% Diff**	**% CV**	**S/B**	**% Diff**	**% CV**	**S/B**
Level A (2.5 ng/mL)	37.9	5.9	1.1	30.1	39.0	1.4
Level B (10 ng/mL)	32.4	4.3	1.2	12.2	16.3	2.7
Level C (80 ng/mL)	5.9	7.7	2.1	4.0	12.3	17.5
Tacrolimus	**% Diff**	**% CV**	**S/B**	**% Diff**	**% CV**	**S/B**
Level A (2.5 ng/mL)	38.4	11.4	1.1	0.9	3.8	3.0
Level B (10 ng/mL)	36.2	14.2	1.6	1.4	4.3	9.1
Level C (80 ng/mL)	2.1	5.6	4.8	1.6	3.6	63.3
Cyclosporin A	**% Diff**	**% CV**	**S/B**	**% Diff**	**% CV**	**S/B**
Level A (50 ng/mL)	3.4	2.8	6.0	7.8	5.3	2714.8
Level B (200 ng/mL)	2.8	2.3	24.3	2.6	1.2	11470.3
Level C (1600 ng/mL)	9.2	2.1	219.4	11.6	2.2	105112.3

% Diff is the absolute percent difference between calculated and standard amounts. % CV is the percent coefficient of variance for the response factor. S/B is the signal-to-blank ratio, which was calculated with response factors of the level indicated and the matrix blank.

Comparable precision with the FAIMS Pro interface

Response factors obtained from the PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods had comparable percent coefficients of variance (% CV) for Everolimus, Tacrolimus and Cyclosporin A, which ranged from 1.2 % to 14.2 % for Levels A, B and C (Table 2). The % CV of the response factors increased for Sirolimus when using the PaperSpray-FAIMS-MS/MS method, but remained less than 20 % for standard concentrations above 5 ng/mL.

Significant signal-to-blank improvement with the FAIMS Pro interface

Improvement in the signal-to-blank ratio was observed across the standard levels for all four immunosuppressant drugs. For Level B, there was a ~3x improvement for Everolimus, ~2x improvement for Sirolimus, ~3x improvement for Tacrolimus, and a ~450x improvement for Cyclosporin A. For Level C, there was a ~8x improvement for Everolimus and Sirolimus, a ~13x improvement for Tacrolimus and a ~480x improvement for Cyclosporin A.

Improved detection and quantitation limits with the FAIMS Pro interface

Limits of detection (LOD) were calculated using the conventional method documented in CLSI EP17-A2 for the PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods and are tabulated in Table 3. Calculated limits of detection improved for all immunosuppressant drugs when using the FAIMS Pro interface with a decrease in LOD values from 11.7 to 4.6 ng/mL for Everolimus, from 16.6 to 6.1 ng/mL for Sirolimus, from 4.3 to 0.8 ng/mL for Tacrolimus, and from 25.4 to 1.7 ng/mL for Cyclosporin A.

Limits of quantitation (LOQ) are listed in Table 3 and were determined based on a set of accuracy and precision criteria that included having less than 20 % absolute difference between expected and calculated concentrations, less than 20 % coefficient of variance and being greater than or equal to the calculated LOD values. Limits of quantitation improved for all immunosuppressant drugs when using the FAIMS Pro interface with a lowering of the LOQ from 20 to 5 ng/mL for Everolimus, from 40 to 10 ng/mL for Sirolimus, from 20 to 1.25 ng/mL for Tacrolimus and from 25 to 12.5 ng/mL for Cyclosporin A.

Table 3. Limits of detection and quantitation for the analysis of immunosuppressant drugs with PaperSpray-MS/MS and PaperSpray-FAIMS-MS/MS methods

	PaperSpray-MS/MS		PaperSpray-FAIMS-MS/MS	
	LOD (ng/mL)	**LOQ (ng/mL)**	**LOD (ng/mL)**	**LOQ (ng/mL)**
Everolimus	11.7	20	4.6	5
Sirolimus	16.6	40	6.1	10
Tacrolimus	4.3	20	0.8	1.25
Cyclosporin A	25.4	25	1.7	12.5

CONCLUSIONS

- The FAIMS Pro interface reduced background interferences from the dried blood spot matrix for all four immunosuppressant drugs analyzed.
- The FAIMS device improved sensitivity of the PaperSpray method for Everolimus, Sirolimus and Tacrolimus and linearity for Everolimus and Sirolimus.
- The use of FAIMS improved accuracy in the analysis of Everolimus, Sirolimus and Tacrolimus with limited negative impact on precision.
- The FAIMS Pro interface improved limits of detection and quantitation for all immunosuppressant drugs analyzed.

REFERENCES

1. Manicke, N. E., Belford, M. "Separation of opiate isomers using electrospray ionization and paper spray coupled to high-field asymmetric waveform ion mobility spectrometry." Journal of the American Society for Mass Spectrometry 26.5 (2015): 701-705.
2. Wang, H., et al. "Paper spray for direct analysis of complex mixtures using mass spectrometry." Angewandte Chemie International Edition 49.5 (2010): 877-880.
3. Pierson-Perry, J. F., et. al. Evaluation of detection capability for clinical laboratory measurement procedures; approved guideline. CLSI, 2012.

For Research Use Only. Not for use in diagnostic procedures.

TRADEMARKS/LICENSING

PO65513-EN0519S

ThermoFisher SCIENTIFIC