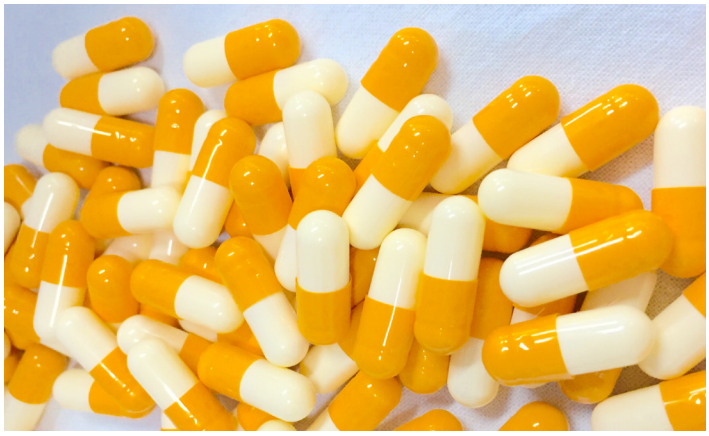
TECHNICAL NOTE 73307

Combined PaperSpray and FAIMS technology for rapid quantification of immunosuppressants in whole blood for clinical research

Authors: Katherine Walker, Cornelia Boeser, Rae Ana Snyder, Neloni Wijeratne, Debadeep Bhattacharyya
Thermo Fisher Scientific, San Jose, CA

Keywords: VeriSpray, PaperSpray, FAIMS Pro, improved S/N, TSQ Altis, immunosuppressant



Goal

The goal of this technical note is to show the synergy between PaperSpray and FAIMS technology in quantifying the concentration of immunosuppressants in whole blood for clinical research.

Introduction

The rapid quantification of immunosuppressant drugs from blood is a key interest in the clinical research community. Common methods use immunoassays or LC/MS. Immunoassays are expensive, may suffer from antibody cross-reactivity, and have limited dynamic range. While LC/MS run times can be very short, protein crash and other sample preparation and clean-up steps lengthen the overall duration of the analytical method and solvent waste is generated.

PaperSpray-MS is a technique for rapidly quantifying analytes in dried matrix spots such as urine or whole blood. Little or no sample preparation is required, and sample analysis times are 2 minutes or less. The new Thermo Scientific™ VeriSpray™ PaperSpray ion source system utilizes 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 Thermo Scientific™ VeriSpray™ plate loader (Figure 1A). The VeriSpray plate loader holds up to 10 VeriSpray sample plates (Figure 1B). Each VeriSpray sample plate contains

24 single-use paper strips (12 on each side, A and B, Figure 1C). The plate loader allows the full 10-plate magazine to be run without user intervention.

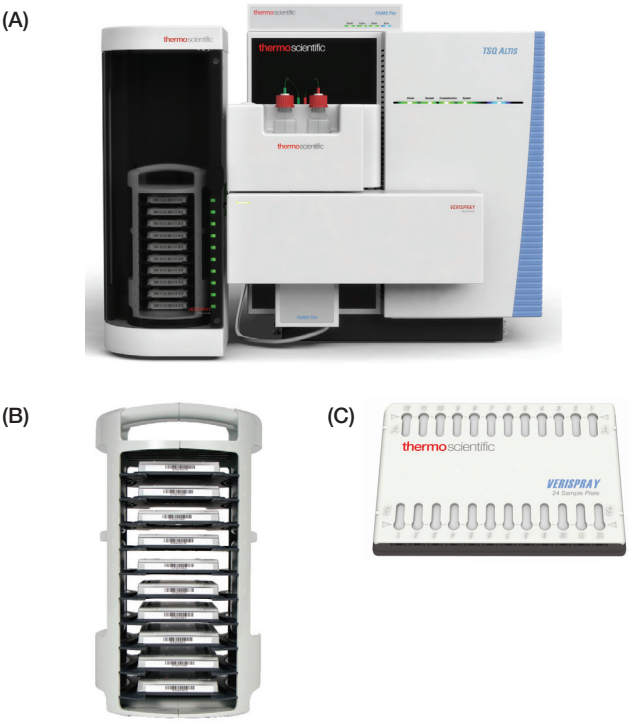


Figure 1. (A) VeriSpray ion source and plate loader, FAIMS Pro interface mounted onto TSQ Altis MS, (B) plate loader magazine, and (C) VeriSpray sample plate

Since PaperSpray is a direct analysis technique with no chromatographic separation and minimal sample cleanup, MS signals can have high background, which may limit the LOQ due to the signal-to-noise (S/N). Field asymmetric ion mobility spectrometry (FAIMS) is a technique that enhances selectivity of an analytical method by adding an additional dimension of separation based on ion mobility. It operates by applying an asymmetric waveform between a set of electrodes. Alternating between high and low field strengths impacts mobility of ions through a carrier gas. By applying an optimized compensation voltage (CV), target ions pass through the electrodes, while ions not of analytical interest are neutralized on the electrode walls.

By combining PaperSpray and FAIMS technology, the background noise can be reduced and the signal-to-noise ratio enhanced, thereby achieving lower limits of detection. Herein we analyze cyclosporin A, tacrolimus, and everolimus in whole blood using the new VeriSpray PaperSpray ion source, both with and without the Thermo Scientific™ FAIMS Pro™ interface.

Experimental

Three immunosuppressants—cyclosporin A, tacrolimus, and everolimus—were spiked into whole human donor blood at calibration levels ranging from 10 to 1600 ng/mL for cyclosporin A, 0.5 to 80 ng/mL for tacrolimus, and 2.5 to 80 ng/mL for everolimus. Their corresponding internal standards—cyclosporin A-D_4, tacrolimus-$^{13}C,D_2$, and everolimus-D_4—were also spiked into the blood samples at 640 ng/mL, 32 ng/mL, and 32 ng/mL, respectively. Ten microliters of each blood sample were spotted onto VeriSpray sample plates and oven-dried for 30 min at 45 °C. Five replicates of each calibration level were analyzed, both with and without the FAIMS Pro interface installed.

Dried plates were placed in the VeriSpray ion source plate loader. Before analysis, the source applies a rewet solvent 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. A mixture of 60% methanol, 40% chloroform, and 0.1% sodium acetate was used as both the rewet and spray solvent. The wetting protocol and delays between solvent dispenses are shown in Table 1.

Data were acquired on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to the VeriSpray ion source with and without the FAIMS Pro interface using Thermo Scientific™ Xcalibur™ software. Two optimized transitions were monitored per analyte (Table 2)

Table 1. VeriSpray solvent application parameters. Each rewet and spray solvent dispense is 10 µL.

Rewet solvent dispense		Spray solvent dispense	
Dispense	Delay (s)	Dispense	Delay (s)
1	5	1	1
		2	1
		3	1
		4	1
		5	5
		6	5
		7	5
		8	5
		9	5
		10	5
		11	5
		12	10
		13	10
		14	10
		15	10

Table 2. Optimized SRM transitions and CV parameters for cyclosporin A, tacrolimus, everolimus, and their corresponding internal standards: cyclosporin A-D_4, tacrolimus-$^{13}C,D_2$, and everolimus-D_4

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	CV (V)
Tacrolimus	826.471	616.387	34.91	106	-22
Tacrolimus	826.471	443.304	46.58	106	-22
Tacrolimus-$^{13}C,D_2$	829.487	619.417	35.45	113	-22
Everolimus	980.57	389.292	55	163	-26
Everolimus	980.57	409.292	53.99	163	-26
Everolimus-D_4	984.599	393.321	54.45	161	-26
Cyclosporin A	1224.831	1112.917	55	223	-15
Cyclosporin A	1224.831	1207.042	55	223	-15
Cyclosporin A-D_4	1228.859	1112.774	55	153	-16

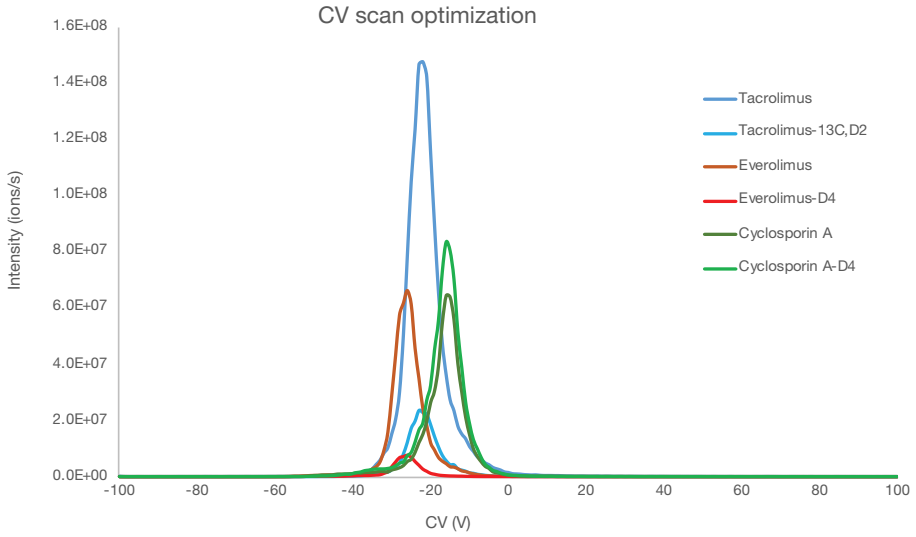


Figure 2. Scan of CVs from -100 to 100 V to determine optimized CV values for immunosuppressants and their internal standards

at a collision gas pressure of 1.5 mTorr. The ion transfer tube temperature was set to 350 °C. The distance of the paper tip with respect to the ion transfer tube, or FAIMS Pro entrance plate, was as follows: 5 mm without the FAIMS Pro interface, 2.5 mm with the FAIMS Pro interface. Data was acquired for 1 min per sample. The spray voltage, which was set at 3400 V for both experiments with and without the FAIMS Pro interface, was turned on at 0.1 min and turned off at 0.9 min to produce a chronogram. Chronograms were integrated using Thermo Scientific™ TraceFinder™ software to determine the area-under-the-curve (AUC). A summary of the TSQ Altis system settings are in Table 3.

The CV (compensation voltage) for each compound was optimized by infusion with the HESI source (Table 2 and Figure 2). The FAIMS Pro interface was operated in normal resolution mode at 100 °C and no additional user gas flow was set.

Table 3. (A) TSQ Altis MS parameters for the analysis of immunosuppressants and (B) time-dependent spray voltage settings

(A)

TSQ Altis MS Parameter	Value
Spray voltage	Time-dependent
Positive ion	3400 V
Sweep gas	0 Arb
Ion transfer tube temperature	350 °C
Q1 resolution	0.7
Q3 resolution	1.2
CID gas	1.5 mTorr

(B)

Time (min)	Voltage (V)
0	0
0.1	3400
0.9	0

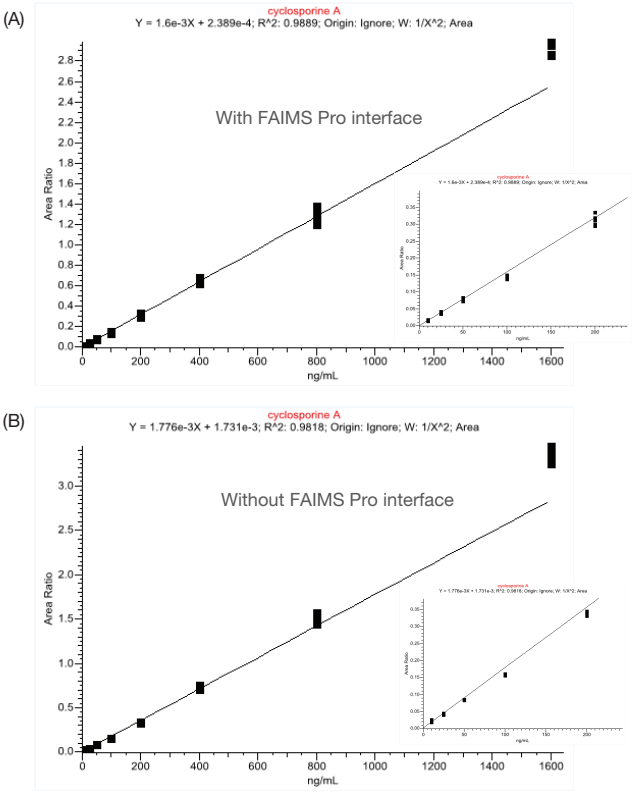


Figure 3. (A) Cyclosporin A calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Inset: calibration levels from 10 to 200 ng/mL

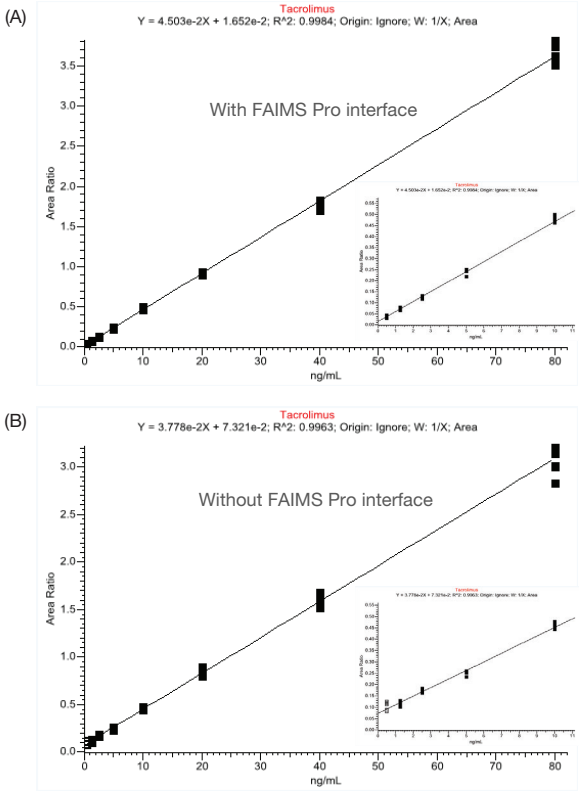


Figure 4. (A) Tacrolimus calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Open circles are excluded calibration levels that had accuracy ≥20%. Inset: calibration levels from 0.5 to 10 ng/mL

Results and discussion

The VeriSpray ion source was used to extract analytes from whole human blood and introduce ions into the mass spectrometer with very little sample preparation. Calibration curves were constructed for cyclosporin A, tacrolimus, and everolimus in human whole blood, acquired with the VeriSpray ion source both without and with the FAIMS Pro interface (Figures 3–5). With the FAIMS Pro interface installed, good precision, accuracy, and linearity were achieved for each immunosuppressant across the measured range. Without the FAIMS Pro interface, the lowest calibration level for tacrolimus and everolimus was excluded based on poor accuracy; this is due to this calibration level being close to the background signal.

With the FAIMS Pro interface, the LOQ (limit of quantitation) was equal to or lower than the LOQ without the FAIMS Pro interface. LOQs were determined based on the following criteria: S/N at the LOQ must be ≥4, precision and accuracy at the LOQ must be <15% and ≤20%, respectively, and the ion ratio of the target ion AUC to confirming ion AUC must be consistent. The LOQs for tacrolimus and everolimus were lowered to 0.5 ng/mL from 10 ng/mL and 5 ng/mL from 20 ng/mL, respectively, when the FAIMS Pro interface was used. The LOQ for cyclosporin

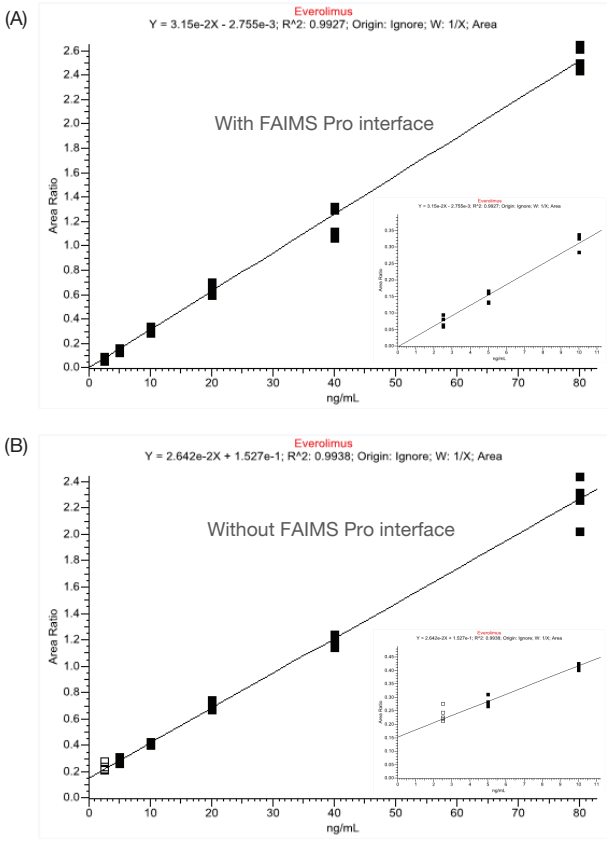


Figure 5. (A) Everolimus calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Open circles are excluded calibration levels that had accuracy ≥20%. Inset: calibration levels from 2.5 to 10 ng/mL

Table 4. Clinically relevant target level for tacrolimus, everolimus, and cyclosporin A and the LOQ (ng/mL) obtained using the VeriSpray ion source with and without FAIMS Pro interface

Compound	Target range (ng/mL)	LOQ with FAIMS Pro interface (ng/mL)	LOQ (ng/mL)
Tacrolimus	5-20	0.5	10
Everolimus	3-8	5	20
Cyclosporin A	100-400	25	25

Table 5. Comparison of average blank AUC, average cal level AUC, and S/N for immunosuppressants (A) with FAIMS Pro interface and (B) without the FAIMS Pro interface. Cal level is 800 ng/mL for cyclosporin A and 40 ng/mL for tacrolimus and everolimus.

(A)

With FAIMS Pro interface	Blank AUC	Cal AUC	S/N
Cyclosporin A	8.1	332528	41053
Tacrolimus	585	136378	233
Everolimus	27	27549	1024

(B)

Without FAIMS Pro interface	Blank AUC	Cal AUC	S/N
Cyclosporin A	1494	932426	624
Tacrolimus	11434	217741	19
Everolimus	8579	55477	6.5

A remained at 25 ng/mL because the LOQ was limited by the ion ratio not the S/N (Table 4). With the FAIMS Pro interface, the ranges of quantitation cover the clinically relevant ranges of immunosuppressants in human whole blood.

The FAIMS Pro interface filters interfering compounds based on differences in ion mobility. This feature is particularly useful for PaperSpray samples, which have no chromatographic separation. With the FAIMS Pro interface, the LOQs improved due to significant reduction in background signal of the matrix blank. Table 5 shows the average blank AUC, an average high cal level (800 ng/mL for cyclosporin A, and 40 ng/mL for tacrolimus and everolimus) AUC, and the S/N for samples with and without the FAIMS Pro interface. The high cal level AUCs show some reduction in signal, which is expected. However, the many-fold decrease in the blank signal leads to an improvement in the signal-to-noise. For cyclosporin A and everolimus, the FAIMS Pro interface almost entirely eliminates the transmission of interfering ions in the matrix blank. In Figures 6-8, example chronograms of the matrix blank and the LOQ calibrator for each immunosuppressant with and without the FAIMS Pro interface are shown. All chronograms have the typical square shape that results

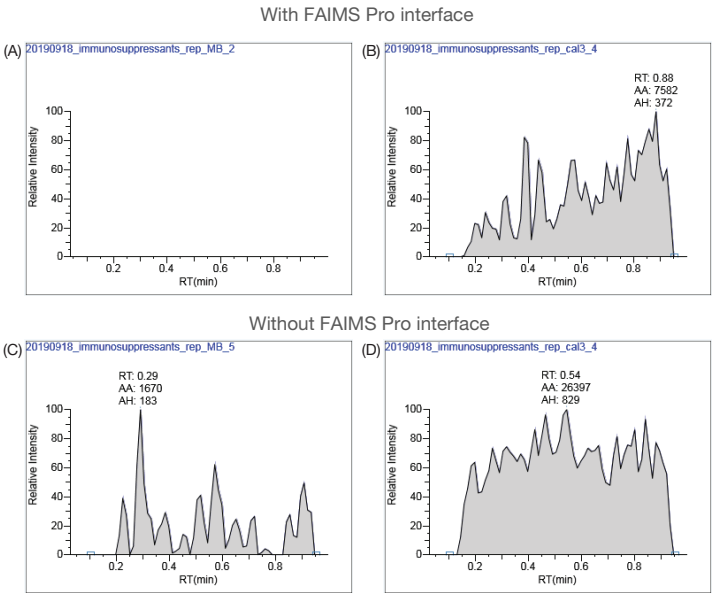


Figure 6. Cyclosporin A chronograms: A) matrix blank with FAIMS Pro interface, B) 25 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 25 ng/mL calibrator

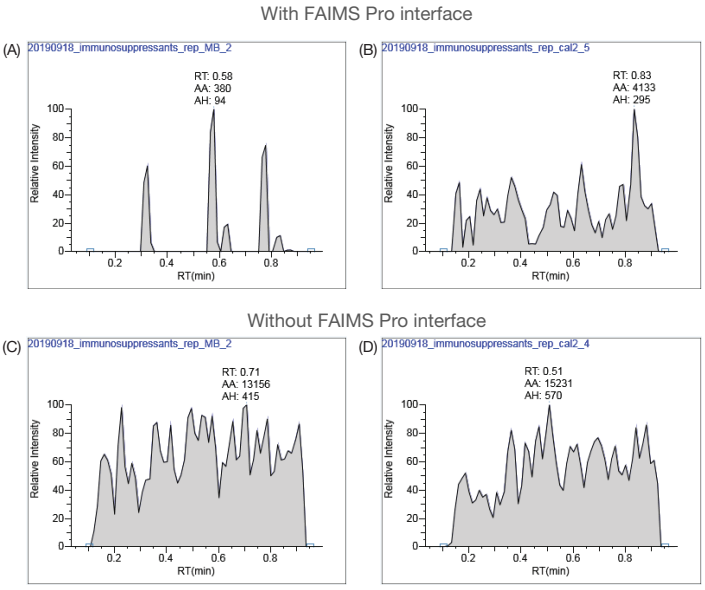


Figure 7. Tacrolimus chronograms: A) matrix blank with FAIMS Pro interface, B) 0.5 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 0.5 ng/mL calibrator

from ion generation only when voltage is applied except those for matrix blanks with the FAIMS Pro interface. The chronograms of matrix blanks with the FAIMS Pro interface have low intensity signal spikes or no signal at all because of the reduction in the transmission of interfering ions.

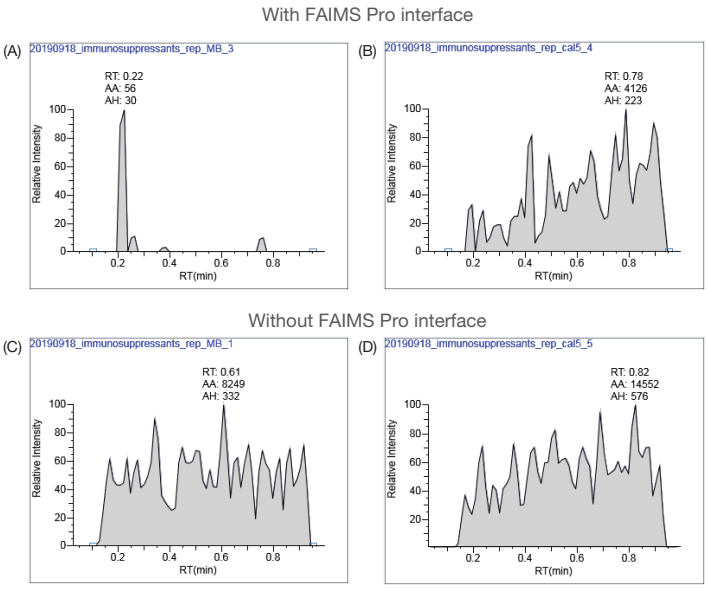


Figure 8. Everolimus chronograms: A) matrix blank with FAIMS Pro interface, B) 5 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 5 ng/mL calibrator

Conclusion

The VeriSpray ion source extracts immunosuppressants from whole human blood and introduces ions into the mass spectrometer with little or no sample preparation. When using the FAIMS Pro interface, the background signal is significantly reduced and the LOQ is lowered. The combination of FAIMS Pro and PaperSpray technologies yields an easy-to-use, sensitive, fast analytical method for clinical research.

Find out more at **thermofisher.com/FAIMSPro**
thermofisher.com/VeriSpray
thermofisher.com/Altis

ThermoFisher SCIENTIFIC