TECHNICAL NOTE 73300

Improving limit of quantification in clinical research by combining PaperSpray with FAIMS technology

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Application benefits

- Fast analysis with no sample preparation
- Reduced background interference
- Improved LOD and LOQ in matrix samples with no sample preparation

Goal

In PaperSpray analysis, signal-to-noise is often the limiting factor in achieving lower LODs and LOQs. The goal of this technical note is to show the benefit of coupling PaperSpray with FAIMS technology to reduce background interferences and increase signal-to-noise, thereby lowering the limit of detection.

Introduction

PaperSpray mass spectrometry (PS-MS) is a rapid technique for analysis of compounds directly from unprocessed dried sample spots. Because minimal to no sample preparation is required, the technique is particularly beneficial for biological sample matrices, which normally require time-consuming and labor-intensive sample cleanup when analyzed by LC-MS. However, because of the lack of sample cleanup, PaperSpray can produce a high background ion signal. High background noise can limit the signal-to-noise ratio (S/N) and compromise the LOQ and LOD of the method. 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 differential mobility. It operates by applying an asymmetric waveform between a set of electrodes. Alternating between high and low field strengths impacts the mobility of ions through a carrier gas. By applying an additional, optimized offset voltage (compensation voltage, or CV), ions of particular mobility pass through the electrodes, while background ions get neutralized on the electrode walls.

By combining PaperSpray and FAIMS technology, background noise can be reduced, and signal-to-noise ratio enhanced. Here, we demonstrate this principle by analyzing the antidepressant drug amitriptyline using the new Thermo Scientific™ VeriSpray™ PaperSpray ion source, both with, and without the Thermo Scientific™ FAIMS Pro™ interface.

Experimental

Amitriptyline was spiked into donor human blood at concentrations ranging from 0.1 to 500 ng/mL to construct calibration curves. Amitriptyline-d_3 was used as internal standard and spiked into the blood at 50 ng/mL. QC samples were prepared at three additional concentrations (20, 100, and 400 ng/mL). Ten microliters of each sample were spotted onto VeriSpray sample plates and oven-dried for 30 min at 45 °C. Each concentration was spotted onto three different paper strips. A mixture of 95% methanol, 5% water, and 0.01% acetic acid was used as both rewet and spray solvent. The wetting steps are shown in Table 1.

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

Rewetting dispense delay		Solvent dispense delay	
Dispense	Delay (s)	Dispense	Delay (s)
1	1	1	1
		2	1
		3	1
		4	1
		5	2
		6	5
		7	5
		8	5
		9	5
		10	0

Data was acquired on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to the VeriSpray ion source and the FAIMS Pro interface. Three transitions were monitored per compound (Table 2), at a collision gas pressure of 2 mTorr. The ion transfer tube temperature was set to 350 °C. Data were acquired for 2 min per sample. The spray voltage was turned on at 0.1 min and turned off at 1.9 min to create a chronogram, which can easily be integrated (Figure 1). The spray voltage was set to 4 kV for runs without the FAIMS Pro interface, and 3.3 kV for runs with the FAIMS Pro interface. The distance of the paper tip with respect to the ion transfer tube, or FAIMS Pro entrance plate, was as follows: 4.5 mm without the FAIMS Pro interface, 2.4 mm with the FAIMS Pro interface (Figure 2). When the FAIMS Pro interface was in use, the CV was optimized by infusion with the HESI source. The optimum value for both amitriptyline and amitriptyline-d_3 was -29.5 V. The CV optimization scan for amitriptyline is shown in Figure 3. The FAIMS Pro interface was operated in normal resolution mode, and no additional user gas flow was set.

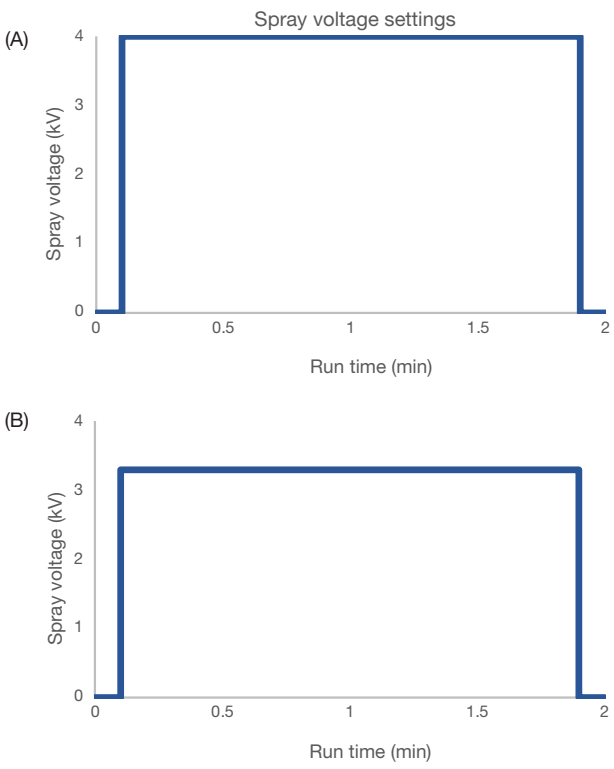


Figure 1. Time-dependent spray voltage settings for the analyses (A) without the FAIMS Pro interface, (B) with the FAIMS Pro interface

Table 2. Optimized mass spectrometer transitions for amitriptyline and the deuterated internal standard amitriptyline-d_3

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Amitriptyline	278.2	117.1	24	65
	278.2	191.1	26	65
	278.2	233.1	18	65
Amitriptyline-d_3	281.2	117.1	24	62
	281.2	191.1	26	62
	281.2	233.1	18	62

(A)

(B)

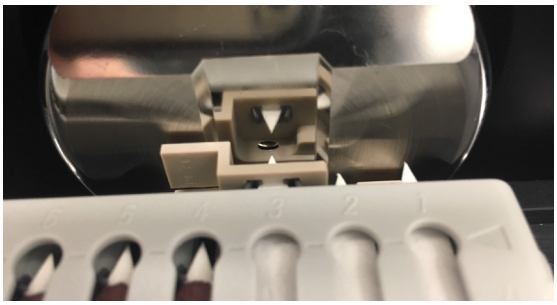


Figure 2. (A) VeriSpray PaperSpray system and FAIMS Pro interface mounted to TSQ Altis triple quadrupole mass spectrometer, (B) VeriSpray tip positioned in front of FAIMS Pro entrance plate

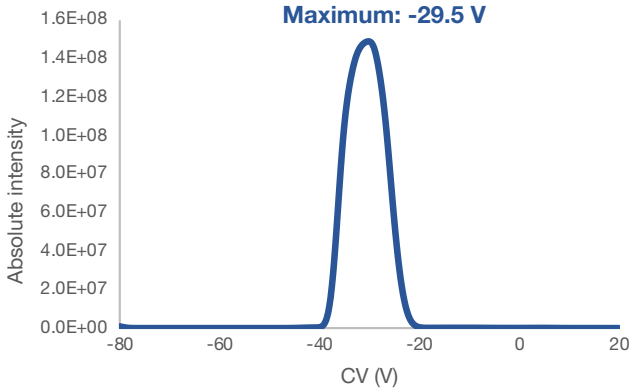
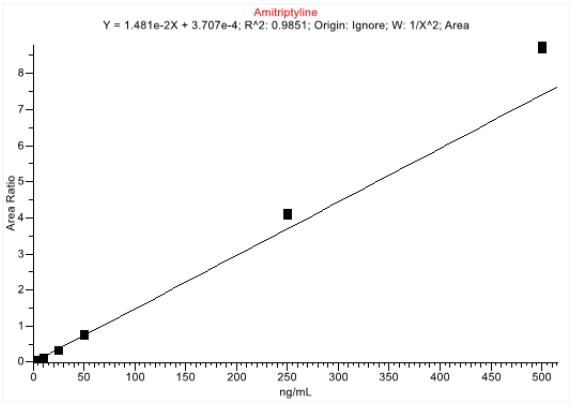


Figure 3. CV Scan for amitriptyline (*m/z* 278.2, acquired in SIM mode), resulting in an optimized CV value of -29.5 V

Results and discussion

Calibration curves were generated for amitriptyline in human whole blood, acquired both without and with FAIMS technology (Figure 4). Both curves were linear over the measured concentration range. LOQs were based on the following criteria: precision and accuracy at the LOQ must be ≤15% and within ±20%, respectively. Further, the S/N at the LOQ must be ≥4. Without the FAIMS Pro interface, an LOQ of 2.5 ng/mL was obtained. With the FAIMS Pro interface, the LOQ was 1.0 ng/mL (Table 3).

(A) Amitriptyline without FAIMS



(B) Amitriptyline with FAIMS

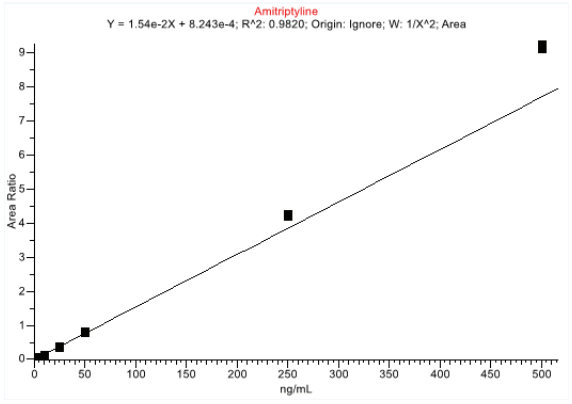


Figure 4. Calibration curves for amitriptyline, obtained from human whole blood (A) without the FAIMS Pro interface, and (B) with the FAIMS Pro interface

Table 3. LOQ obtained for amitriptyline, without and with the FAIMS Pro interface

	Without FAIMS	With FAIMS
LOQ (ng/mL)	2.5	1.0

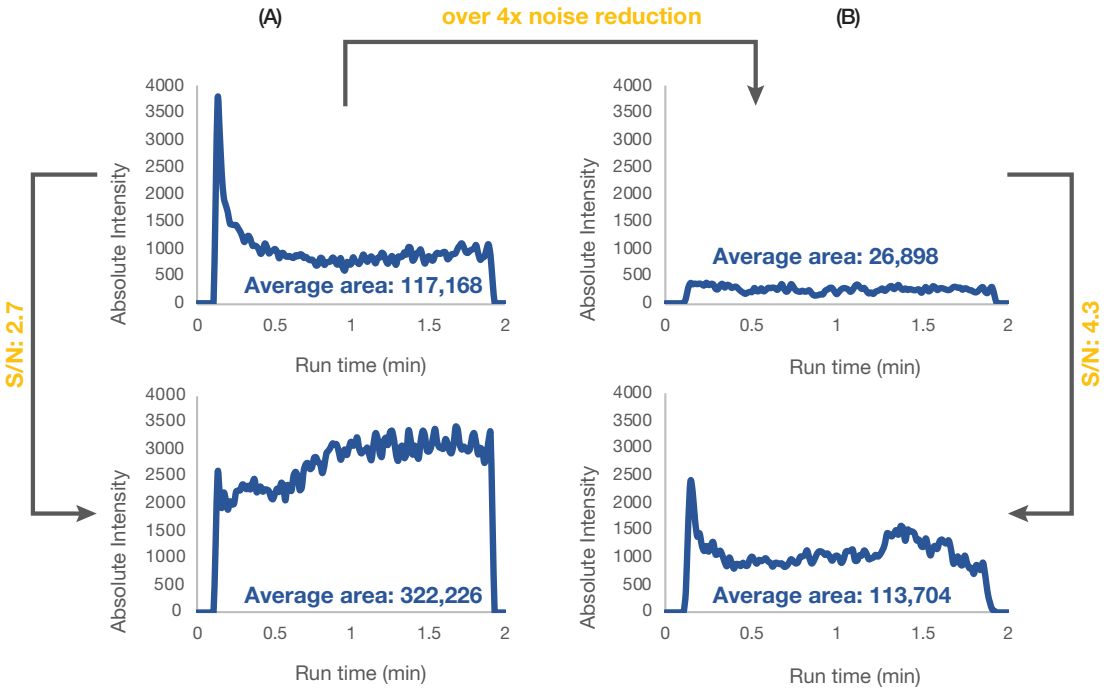


Figure 5. Chronograms of a matrix blank (top) and 1 ng/mL amitriptyline in whole blood (bottom), acquired (A) without the FAIMS Pro interface, and (B) with the FAIMS Pro interface. Because of significant reduction in background interference, the data obtained with the FAIMS Pro interface meets the LOQ requirement of S/N ≥ 4 at a concentration of 1 ng/mL.

Figure 5 shows a comparison of the chronograms of both a 1 ng/mL sample and a matrix blank sample obtained both without and with the FAIMS Pro interface. The background signal in the matrix blank (absolute area under the trace) obtained with the FAIMS Pro interface was over four times lower compared to the background obtained without the FAIMS Pro interface. Because ions of different structures have different ion mobilities through the FAIMS Pro device, it acts as a filter, which limits, or even eliminates, transmission of interfering ions. Although the signal for amitriptyline decreased slightly with the FAIMS Pro interface attached, the background decreased significantly more, thereby raising the S/N from 2.7 to 4.3 at a concentration of 1 ng/mL. This S/N improvement demonstrates the effectiveness of the FAIMS Pro interface in reducing background interferences, which cannot be separated by mass spectrometry alone.

Table 4 shows the precision of each calibrator sample, without and with the FAIMS Pro interface. For both experiments, %RSDs obtained are well below 10%. Table 5 shows the intra-day precision and accuracy of the QC samples. The precision was under 2% for three replicates, and accuracy was within ±20%. For the run without the FAIMS Pro interface, additional inter-day precision and accuracy were obtained using the mid-level QC sample (Table 6). On all three days, the precision was under 1% RSD and the accuracy was within ±12%.

Table 4. Precision of calibrator samples for amitriptyline, without and with the FAIMS Pro interface

Concentration (ng/mL)	%RSD	
	Without FAIMS	With FAIMS
1.0	N/A	7.1
2.5	3.6	9.9
5.0	0.5	0.7
10.0	1.4	0.4
25.0	0.7	0.4
50.0	1.2	0.9
250.0	0.8	1.0
500.0	0.4	0.6

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Table 5. Intra-day precision and accuracy of QC samples (three replicates per concentration) for amitriptyline, without and with the FAIMS Pro interface

Concentration (ng/mL)	%RSD		% Difference	
	Without FAIMS	With FAIMS	Without FAIMS	With FAIMS
20	0.5	1.7	- 15.7	- 10.2
			- 15.0	- 12.5
			- 15.5	- 13.0
100	0.2	0.2	- 10.0	- 9.3
			- 10.3	- 9.7
			- 10.2	- 9.5
400	0.2	1.0	1.7	1.1
			1.8	2.6
			2.2	3.0

Table 6. Inter-day precision and accuracy of the mid-level QC sample (100 ng/mL) on three consecutive days (three replicates per day) for amitriptyline, without the FAIMS Pro interface

Day	%RSD	% Difference
1	0.2	- 10.0
		- 10.3
		- 10.2
2	0.9	- 11.4
		- 10.0
		- 11.3
3	0.7	- 11.3
		- 10.2
		- 10.2

Conclusion

Combining FAIMS and PaperSpray technology leads to improved S/N and lowers the LOQ for amitriptyline, measured directly from human whole blood. Using the FAIMS Pro interface for this type of clinical research analytical method makes it possible to take advantage of short turnaround times and ease-of-use offered by the VeriSpray ion source, without limiting analytical method performance arising from interferences.

Acknowledgements

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