Application note | 000513

Mass Spectrometry

Quantification of sulfonamides in meat muscle matrix using FAIMS separation technology

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Keywords

FAIMS Pro Duo, Orbitrap Exploris 240, sulfonamide analysis, food safety

Goal

Improving the limit of quantification (LOQ) of sulfonamides in complex meat muscle extract by incorporating FAIMS technology into the LC/MS workflow

Introduction

Field asymmetric ion mobility spectrometry (FAIMS) is a differential ion mobility technique that spatially separates ions entering the gap between a set of two electrodes based on their mobilities in alternating high and low fields. This process can selectively transmit analyte ions of interest while attenuating the signal for matrix interferences. When coupled to Thermo Scientific™ Orbitrap™ mass analyzers, removing matrix compounds from entering the mass spectrometer can have the effect of increased accumulation of target ions in the IRM (Ion Routing Multipole). The result is often improved limit of quantification (LOQ) for target analytes, particularly when dealing with complex matrices such as meat muscle extract.

Sulfonamides are antibiotic agents used to treat bacterial infections in animals. Inappropriate use of sulfonamides in animals can result in adverse reactions in humans, such as antibiotic resistance, when consuming animal products. LC-MS/MS assays can detect and quantify sulfonamides in meat extracts; however, many assays are limited in reaching the desired detection levels by high matrix interference. Here, the Thermo Scientific™ FAIMS Pro Duo interface was coupled to the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer (Figure 1) for the analysis of low-level sulfonamides spiked into bovine meat muscle matrix with the goal to improve LOQ of sulfonamides using FAIMS technology.

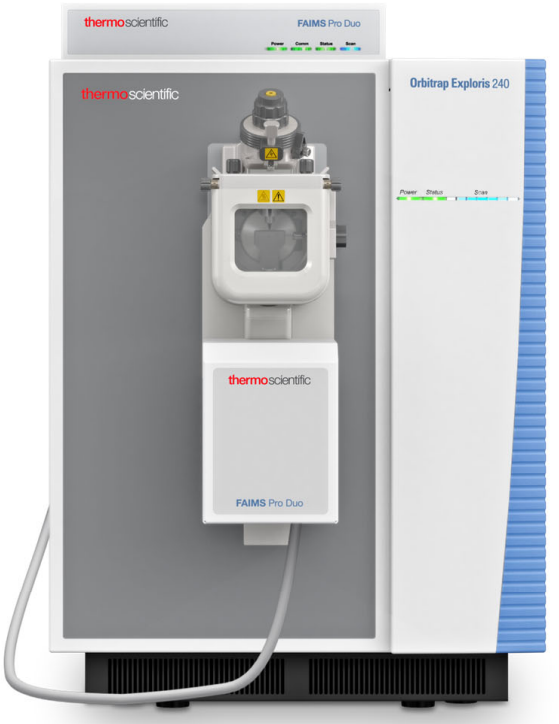

Figure 1. Orbitrap Exploris 240 mass spectrometer with the FAIMS Pro Duo Interface

Experimental

Sample preparation

Each of 17 sulfonamides was weighed and diluted in acetonitrile or acetone to generate 1 mg/mL solutions: sulfamethazine, sulfamethoxazole, sulfadoxine, sulfisoxazole, sulfadimethoxine, sulfaquinoxaline, sulfaguanidine, trimethoprim, sulfadiazine, sulfapyridine, sulfathiazole, sulfamerazine, sulfamoxole, sulfamethoxypyridazine, sulfamethizole, sulfachlorpyridazine, and sulfamonomethoxine. A master mix of the sulfonamide solutions was prepared in 90:10:0.01% acetonitrile:water:formic acid. Bovine meat muscle extract was prepared following a modified QuEChERs extraction. Five grams of tissue were added to a 50 mL Falcon tube. Next, 0.5 mL of 0.2 M ammonium oxalate/EDTA solution was added followed by acetonitrile to a total volume of 15 mL. The tubes were shaken at 2,500 rpm on a Fisherbrand™ Digital MultiTube Vortexer for 10 minutes. Then, 500 mg CEC18 was added to the supernatant. The tubes were vortexed for 30 seconds and then centrifuged at 3,000 rpm for 10 minutes. One milliliter 0.1% formic acid in water was added to 3 mL extract, which was then filtered and transferred to a 2 mL autosampler vial. The sulfonamide mix was spiked into this meat muscle extract at concentrations ranging from 0.1 to 1000 ng/mL.

Instrument conditions

Samples were analyzed both with and without the FAIMS Pro Duo interface installed. The injection volume was 2 µL. A Thermo Scientific™ Vanquish™ Flex binary UHPLC system was used with a Thermo Scientific™ Acclaim™ Vanquish™ Polar Advantage II UHPLC Column (P/N 071401-V) installed. The chromatographic conditions are shown in Table 1. The Thermo Scientific™ OptaMax™ ion source HESI sprayer was positioned at L-M (vertical alignment) and 1 (front/back alignment). The Orbitrap Exploris 240 mass spectrometer was used in data-dependent mode with a targeted inclusion list. Mass spectrometer settings are shown in Tables 2 and 3. The FAIMS Pro Duo interface was operated in standard resolution mode with an inner electrode temperature of 100 °C and an outer electrode temperature of 100 °C. The total carrier gas was set to 1.7 L/min. The sweep cone was installed for experiments without the FAIMS Pro Duo interface.

Table 1. Chromatographic conditions

Parameter	Value		
Column	Thermo Scientific™ Acclaim™ Vanquish™ Polar Advantage II, 2.2 µm, 120 Å, (2.1 × 150) mm (P/N 071401-V)		
Mobile phase	A: Water with 0.1% formic acid B: Methanol with 0.1% formic acid		
Gradient conditions	Time (min)	Flow (mL/min)	%B
	0	0.4	0
	2.2	0.4	0
	11	0.4	95
	13	0.5	95
	14.4	0.5	95
	14.5	0.45	0
	16.6	0.4	0
	17	0.4	0
Autosampler temperature	10 °C		
Column temperature	40 °C		
Injection volume	2 µL		

Table 2. Ion source properties

Ion source properties	
Ion source type	H-ESI
Positive ion spray (V)	3,500
Sheath gas (Arb)	50
Aux gas (Arb)	13
Sweep gas (Arb, if FAIMS is not installed)	1
Ion transfer tube temperature (°C)	280
Vaporizer temperature (°C)	400
FAIMS mode	Standard resolution
Total carrier gas flow (L/min)	1.7

Table 3. Scan settings: (A) Full scan settings (*CV value was defined individually for each experiment), (B) Data-dependent MS² settings, and (C) Targeted inclusion list

A

Full scan properties	
Orbitrap resolution	30,000
Scan range (*m/z*)	120–1,000
FAIMS voltages	On
FAIMS CV (V)	*
RF lens (%)	70
AGC target	Standard (1e6)
Max injection time mode	Auto
Polarity	Positive

B

ddMS² properties	
Isolation window (*m/z*)	2
Collision energy type	Absolute
HCD collision energies (V)	21, 38, 56
Orbitrap resolution	15,000
Scan range mode	Auto
AGC target	Standard (1e5)

C

Compound	Formula	Adduct	*m/z*	z	t start (min)	t stop (min)
Sulfaguanidine	$C_7H_{10}N_4O_2S$	+H	215.0597	1	1.53	3.53
Sulfachlorpyridazine	$C_{10}H_9ClN_4O_2S$	+H	285.0208	1	6.45	8.45
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	+H	251.0597	1	4.9	6.9
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	+H	311.0809	1	7.3	9.3
Sulfadoxine	$C_{12}H_{14}N_4O_4S$	+H	311.0809	1	6.45	8.45
Sulfamerazine	$C_{11}H_{12}N_4O_2S$	+H	265.0754	1	5.38	7.38
Sulfamethazine	$C_{12}H_{14}N_4O_2S$	+H	279.091	1	5.74	7.74
Sulfamethizole	$C_9H_{10}N_4O_2S_2$	+H	271.0318	1	5.98	7.98
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	+H	254.0594	1	6.49	8.49
Sulfamethoxypyridazine	$C_{11}H_{12}N_4O_3S$	+H	281.0703	1	5.88	7.88
Sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$	+H	281.0703	1	6.38	8.38
Sulfamoxole	$C_{11}H_{13}N_3O_3S$	+H	268.075	1	5.58	7.58
Sulfapyridine	$C_{11}H_{11}N_3O_2S$	+H	250.0645	1	5.15	7.15
Sulfaquinoxaline	$C_{14}H_{12}N_4O_2S$	+H	301.0754	1	7.54	9.54
Sulfathiazole	$C_9H_9N_3O_2S_2$	+H	256.0209	1	5.18	7.18
Sulfisoxazole	$C_{11}H_{13}N_3O_3S$	+H	268.075	1	6.74	8.74
Trimethoprim	$C_{14}H_{18}N_4O_3$	+H	291.1452	1	4.52	6.52

CV determination

FAIMS compensation voltage (CV) optimization was performed by creating methods with multiple parallel full scan experiments that only differ in the CV value. At high chromatographic flow rates, it is important to optimize CV values on-line by injection because CV position can be impacted by sprayer position and/or solvent composition at time of compound elution. In this experiment, two CV optimization injections were performed, each covering different CV ranges and/or step sizes. First, a coarse optimization was performed covering a CV range of -45 to 0 V in steps of 5 V. Then, a fine optimization injection was performed covering a CV range of -29 to -5 V in steps of 2 V (Figure 2).

Using Thermo Scientific™ FreeStyle™ software (version 1.8 SP1), CV plots were generated for each sulfonamide by using the 'CV merge' function under 'Auto Filter', specifying the accurate mass of each compound under 'Chromatogram Ranges' and generating a CV plot (see Figure 3 for an example of CV plots for five sulfonamides). Due to overlap in CV space between the sulfonamides, five CV values were selected that were appropriate for all sulfonamides (Table 4). The method for all subsequent FAIMS experiments contained 5 full scan-ddMS^2 experiments (Figure 4), each applying a different CV value (-7, -17, -19, -21 and -25 V).

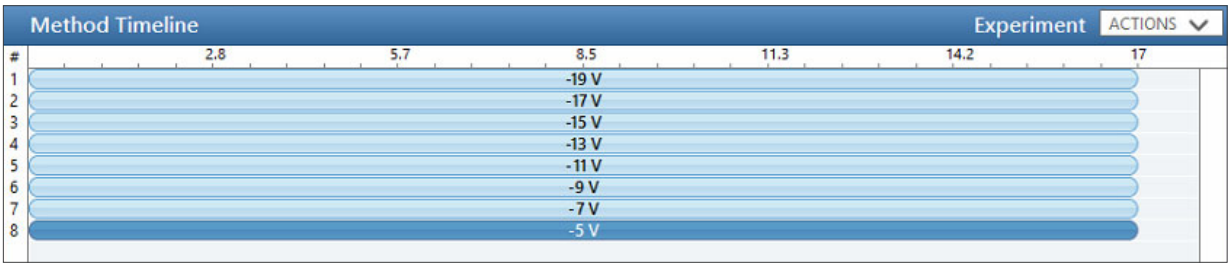

Figure 2. CV optimization method with multiple parallel full scan experiments that only differ in CV value, in this example covering a CV range of -19 to -5 V in steps of 2 V

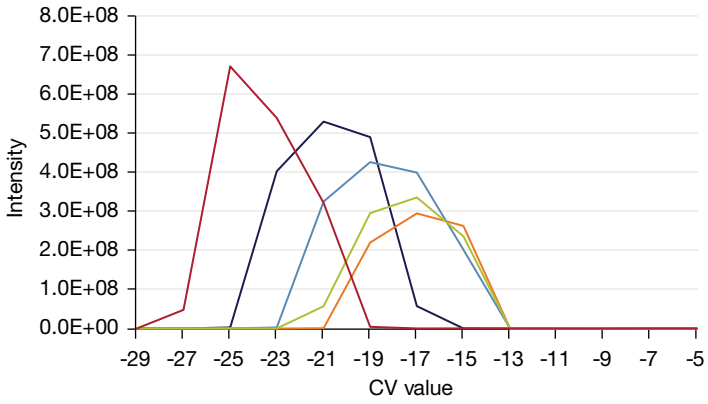

Figure 3. CV plots for sulfamethazine (red), sulfamerazine (dark blue), sulfamethoxazole (light blue), sulfathiazole (green), and sulfachloropyridazine (orange) generated by running a CV optimization injection cycling through CV values in the range of -29 to -5 V in steps of 2 V

Table 4. FAIMS CV values determined for all sulfonamides.
The following CV values were selected for subsequent FAIMS analysis: -7, -17, -19, -21 and -25 V.

Compound	CV value (V)
Sulfamethazine	-25
Sulfamethoxazole	-19
Sulfadoxine	-21
Sulfisoxazole	-7
Sulfadimethoxine	-25
Sulfaquinoxaline	-19
Sulfaguanidine	-7
Trimethoprim	-17
Sulfadiazine	-19
Sulfapyridine	-17
Sulfathiazole	-17
Sulfamerazine	-21
Sulfamoxole	-21
Sulfamethoxypyridazine	-19
Sulfamethizole	-17
Sulfachlorpyridazine	-17
Sulfamonomethoxine	-19

Figure 4. Method used for sulfonamide analysis that includes five parallel experiments differing only in the CV value

Data acquisition

Triplicate injections of the nine calibrators and a matrix blank were made both with and without the FAIMS Pro Duo interface installed. Then, a 50 ng/mL QC was injected 240 times over 3 consecutive days with the FAIMS Pro Duo interface installed. Thermo Scientific™ TraceFinder software was used for quantitative data analysis. For FAIMS data, a FAIMS raw file was associated with the Data Analysis Method to select scan filters with CV information.

Results and discussion

Untargeted screening and quantification methods of multi-class veterinary drugs using HRAM MS evolve continuously to extend LOQs in the presence of complex matrices. This requires simple methods to suppress matrix ions while enhancing the detection efficiency of veterinary drugs. Introduction of the FAIMS Pro Duo interface provided the orthogonal selectivity needed to improve LOQ while maintaining instrument performance and robustness over the entire study.

The simplicity of method development and optimization is also crucial when modifying existing methods. Optimization routines for the FAIMS Pro Duo interface enable online CV determination for all of the sulfonamides by stepping through CV settings per full scan HRAM MS acquisition. In general, the maximum number of CV steps that can be performed in a single optimization injection depends on the peak width. In order to obtain an accurate determination of CV optimum for a compound, at least 5-6 full scan spectra should be acquired at each CV value over the course of the analyte peak, using a full scan method without MS2 fragmentation to maximize time available for CV switching. For the data presented, chromatographic peak widths were about 6 seconds at the base enabling 12 or more CV settings per optimization injection. Two sample injections enable the coarse and then fine CV determination for the final method. In addition, simplified data processing routines were demonstrated using FreeStyle software. Processed results identified CV optima for each compound. Five CV values were selected that best transmit the sulfonamides and added to the data-dependent MS^2 method as parallel experiments (Figure 4). It should be noted that for quantitative analytical methods, as opposed methods merely used for CV optimization, the number of CV values that can be fitted into one injection is more limited because of the additional data dependent MS^2 scans that reduce time available for CV switching, as well as the need for additional scans across the peak.

Introducing the FAIMS Pro Duo interface into the UHPLC-HRAM MS method increased the quantitative performance for most of the sulfonamides in meat matrix. Exemplary calibration curves for sulfadimethoxine and sulfamoxole are shown in Figure 5 for both experiments, with and without the FAIMS Pro Duo interface. For both compounds, LOQ was improved from 0.5 ng/mL to 0.1 ng/mL using the FAIMS interface. Acceptance criteria for LOQ were based on accuracy (± 20%), linearity of the calibration curve ($R^2 > 0.98$), and relative standard deviation (≤ 15%). LOQs for all sulfonamides measured with and without FAIMS technology are summarized in Table 5. For most compounds, an improvement in LOQ was observed using the FAIMS Pro Duo interface. The improvement in LOQ observed with the FAIMS Pro Duo interface is attributed to attenuation of signal coming from matrix components while selectively increasing target ion accumulation. Figure 6 visualizes this behavior for the detection of sulfachloropyridazine. Continuous formation of solvent ions (for example, *m/z* 150 and *m/z* 164) and co-elution of matrix compounds (for example, *m/z* 271) limit intra-scan dynamic range and prevent detection of low abundance compounds of interest. When using FAIMS technology, the abundance of solvent and co-eluting matrix ions is significantly attenuated, enabling detection of sulfachloropyridazine at lower levels compared to unfiltered detection without the FAIMS interface.

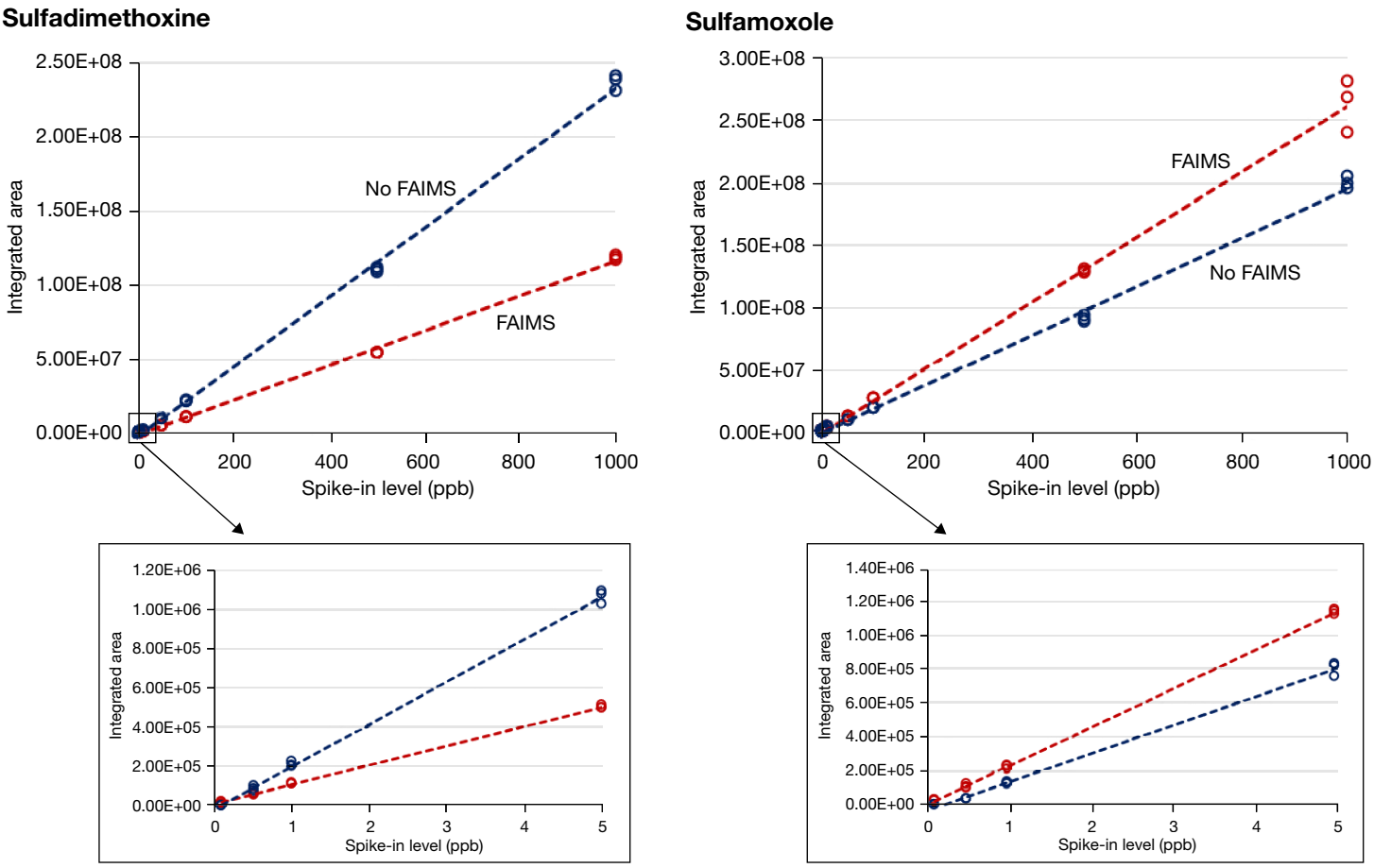

Figure 5. Calibration curves for sulfadimethoxine (left) and sulfamoxole (right), measured in triplicate both with (red), and without (blue) the FAIMS Pro Duo interface. For sulfadimethoxine, the absolute area count when using FAIMS technology is lower compared to without; whereas for sulfamoxole, the absolute area count is higher when using FAIMS technology. For both compounds, using FAIMS technology improved the LOQ from 0.5 ng/mL to 0.1 ng/mL.

Table 5. LOQ of all sulfonamides measured both with and without the FAIMS Pro Duo interface

Compound	LOQ (ng/mL)	
	Without FAIMS technology	With FAIMS technology
Sulfamethazine	1	0.1
Sulfamethoxazole	0.5	0.5
Sulfadoxine	0.5	0.1
Sulfisoxazole	0.5	0.1
Sulfadimethoxine	0.5	0.1
Sulfaquinoxaline	0.5	0.1
Sulfaguanidine	5	1
Trimethoprim	0.5	0.5
Sulfadiazine	0.5	0.5

Compound	LOQ (ng/mL)	
	Without FAIMS technology	With FAIMS technology
Sulfapyridine	0.5	0.1
Sulfathiazole	0.5	0.1
Sulfamerazine	0.5	0.1
Sulfamoxole	0.5	0.1
Sulfamethoxypyridazine	0.5	0.1
Sulfamethizole	1	0.5
Sulfachlorpyridazine	1	0.1
Sulfamonomethoxine	0.5	0.1

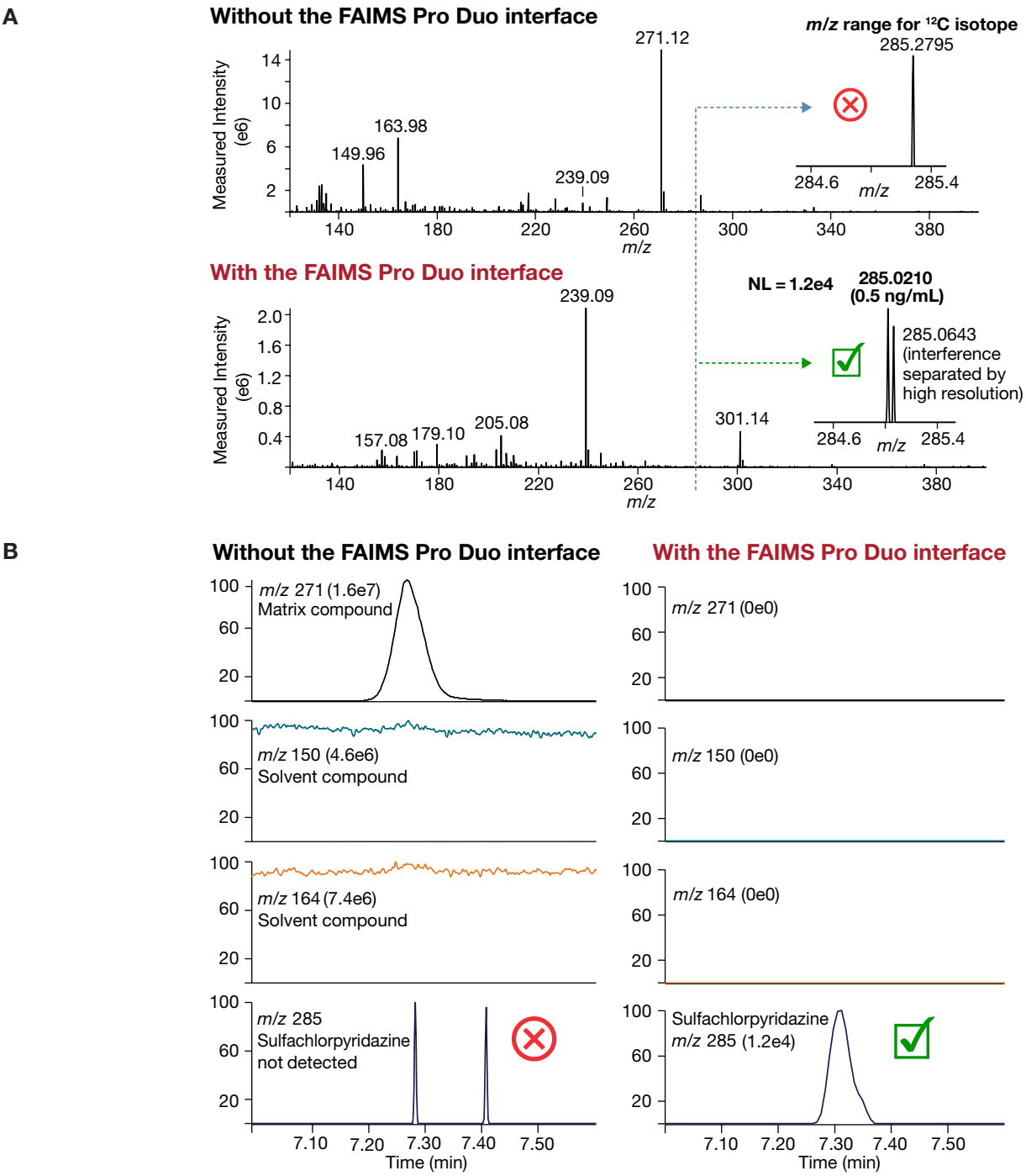

Figure 6. Analysis of sulfachlorpyridazine (0.5 ng/mL) spiked into acetonitrile extract of meat muscle matrix. (A) Full scan spectra without (top) and with (bottom) the FAIMS interface. (B) Extracted ion chromatograms for co-eluting matrix and solvent ions, as well as sulfachloropyridazine both without (left) and with (right) the FAIMS interface. Without the FAIMS interface, several solvent and co-eluting matrix ions are detected with high abundance, preventing the detection of sulfachloropyridazine. With the FAIMS interface, signal for the solvent and matrix ions is attenuated, enabling the detection of the target compound.

The LC-FAIMS-MS system remained robust over three days where 240 QC samples in meat matrix extract were injected. Neither the FAIMS electrodes nor the ion transfer tube were cleaned over this duration. As an example, the peak areas of sulfapyridine and sulfadoxine are shown in Figure 7. The % RSDs of the absolute peak areas for the QC injections were below 5% for all compounds and are summarized in Table 6. It should be noted that if the study was extended, simple maintenance could be performed on the FAIMS electrodes and ion transfer tube without breaking vacuum or requiring instrument calibration, maximizing productivity.

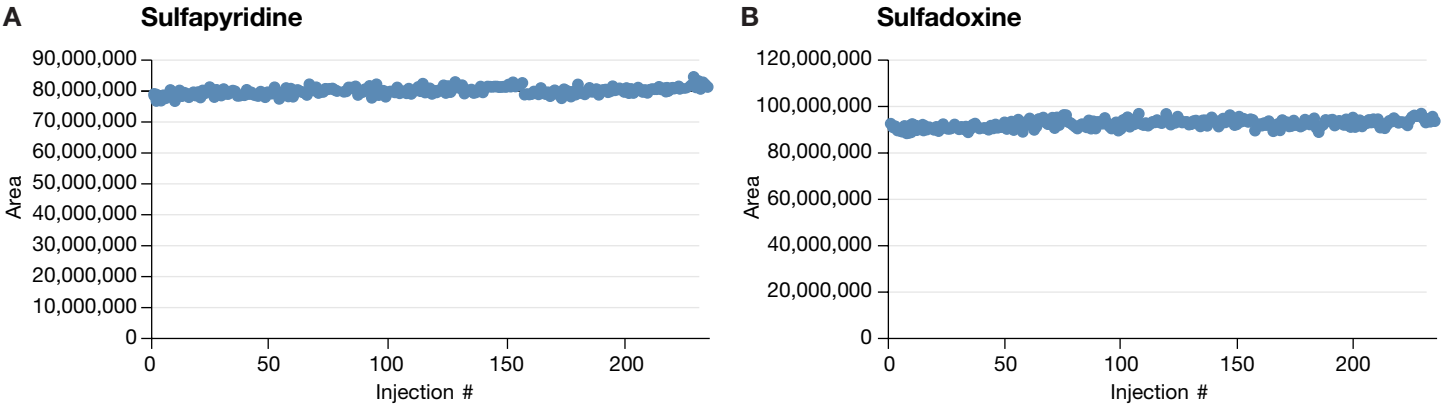

Figure 7. Absolute peak areas of sulfapyridine (A) and sulfadoxine (B) for 240 sample injections acquired over three days. The mass spectrometer was set to stand-by for at least two hours in between days.

Table 6. % RSD of the absolute peak areas for all sulfonamides by day over three days and 240 injections

Compound	% RSD		
	Day 1	Day 2	Day 3
Sulfamethazine	1.93	1.88	1.91
Sulfamethoxazole	2.24	2.17	1.87
Sulfadoxine	3.37	3.16	3.41
Sulfisoxazole	1.97	1.61	1.73
Sulfadimethoxine	3.04	2.39	2.44
Sulfaquinoxaline	1.91	1.70	1.64
Sulfaguanidine	2.86	2.18	2.96
Trimethoprim	4.39	3.25	3.56
Sulfadiazine	2.66	3.13	2.92
Sulfapyridine	1.78	1.53	1.72
Sulfathiazole	2.21	1.57	1.66
Sulfamerazine	1.94	1.67	1.75
Sulfamoxole	1.41	1.40	1.39
Sulfamethoxypyridazine	2.60	2.24	2.21
Sulfamethizole	1.73	1.48	1.70
Sulfachlorpyridazine	4.02	3.42	3.81
Sulfamonomethoxine	3.99	3.73	3.62

Conclusion

In LC/MS analysis, many matrix and solvent ions enter the system and are detected at high abundance, limiting intra-scan dynamic range and the ability to detect low abundant target compounds. Using the FAIMS Pro Duo interface coupled to the Orbitrap Exploris 240 mass spectrometer, matrix and solvent ion signals can be attenuated, leading to more efficient accumulation of compounds of interest and improved LOQ for many sulfonamides.

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