Quantitative analysis of sulfonamides in meat muscle matrix with a field asymmetric ion mobility interface on an Orbitrap Mass spectrometer

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Abstract

Often, mass spectrometric analysis for small molecules involves detection of low analyte amounts in high loads of matrix compounds. Matrices such as muscle meat matrix, milk, or blood may reduce the robustness and uptime of a mass spectrometer despite sample preparation. Still, good sensitivity is required. The field asymmetric ion mobility separation (FAIMS) interface coupled to LC-MS/MS allows orthogonal separation of matrix and analyte ions by their high-field vs. low-field mobility, with high transmission. This may reduce the matrix compounds entering the mass spectrometer and thus increase the robustness.

Introduction

- Sulfonamides are a class of antibiotics which are frequently used in animal husbandry. For food safety reasons, reliable analysis of these compounds is necessary.
- Mass spectrometry combined with HPLC is commonly the method of choice for quantification and confirmation of antibiotics in food matrices.
- The field asymmetric ion mobility interface coupled to LC-MS/MS allows orthogonal separation of matrix and analyte ions by their high-field vs. low-field mobility, with high transmission.
- Here a comparison of LOQs with and without FAIMS are shown
- Also, the data was evaluated for matrix compounds entering the mass spectrometer

Materials and Methods

Sample prep

- Calibration curves in meat muscle matrix extract – acetonitrile – from 0.1–1000 ng/mL. Meat muscle matrix was provided by Iowa state University. A modified QuEChERs extraction was used described below:
- Five grams of tissue was 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 2500 rpm on a Fisherbrand™ Digital MultiTube Vortexer for 10 minutes. 500 mg CEC18 was added to the supernatant and vortexed for 30 seconds, and then centrifuged at 3000 rpm for 10 minutes. 1 mL 0.1% formic acid in water was then added to 3 mL extract, filtered and transferred to a 2 mL autosampler vial.

LC-MS Method

- Prior to the sensitivity evaluation, for all compounds optimal compensation voltages (CV) are determined by running multiple experiments with different CV steps within one HPLC run in 3 iterations. The final method consists of 5 scan events with different CVs which are run in a loop.
- All samples were run on an Thermo Scientific™ Orbitrap Exploris™ 240 MS connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Separation was conducted with a Thermo Scientific™ Acclaim™ VANQUISH™ Polar Advantage II UHPLC Column and a gradient with 0.1 % formic acid in both methanol (solvent B) and water (solvent A). Injection volume was 2 µL. For comparison of LOQs all calibration standards were run twice, once with Thermo Scientific™ FAIMS Pro Duo and once without – each concentration in triplicate.

Table 1. HPLC gradient.

Time	Flow [mL/min]	%A	%B
0.0	0.400	100	0.0
2.2	0.400	100	0.0
11.0	0.400	5	95
13.0	0.500	5	95
14.4	0.500	5	95
14.5	0.450	100	0
16.6	0.400	100	0
17.0	0.400	100	0

Materials and Methods

Data Analysis methods

- Sensitivity is evaluated through processing the data in Thermo Scientific™ TraceFinder™ software. The criteria for the limits of quantification (LOQs) are < 20 % RSD of 3 injections and < 20 % difference from the theoretical amount
- The number of matrix compounds entering the mass spectrometer is evaluated through Thermo Scientific™ Compound Discoverer™ software, using an advanced data dependent acquisition mode for identification.
- Peak areas, determined in TraceFinder, were used to compare the CV optima of matrix versus analyte within single CV runs

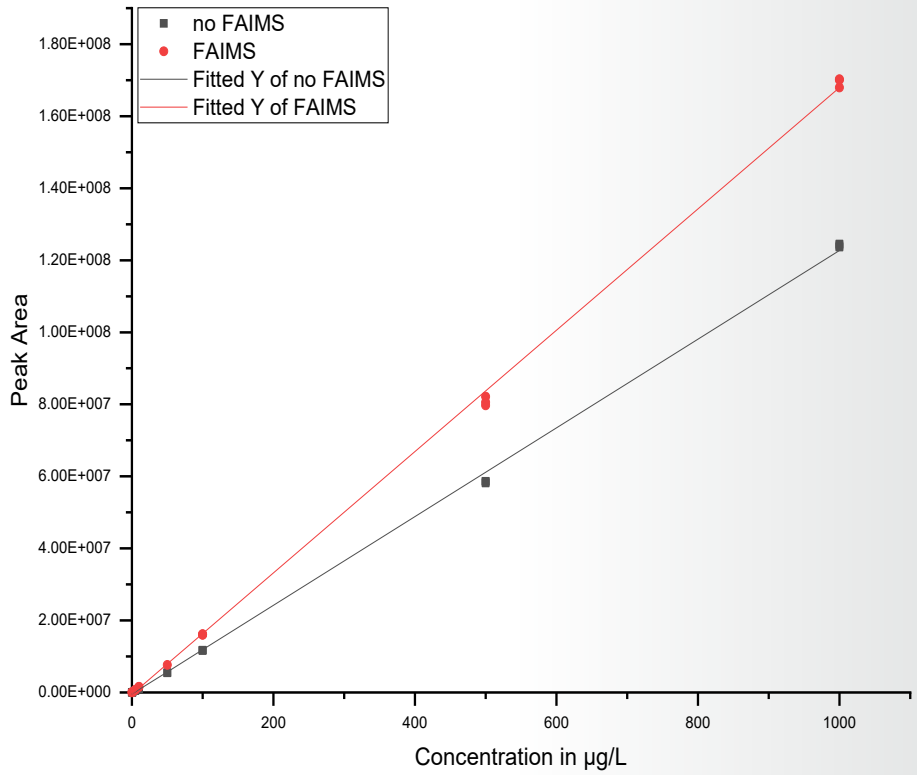
Results

Table 2 shows the limits of Quantification for the sulfonamides with and without FAIMS. LOQs were at least the same with FAIMS applied. Some LOQs were improved due to mobility separation between the matrix and target compounds within the FAIMS device prior to entering the mass spectrometer.

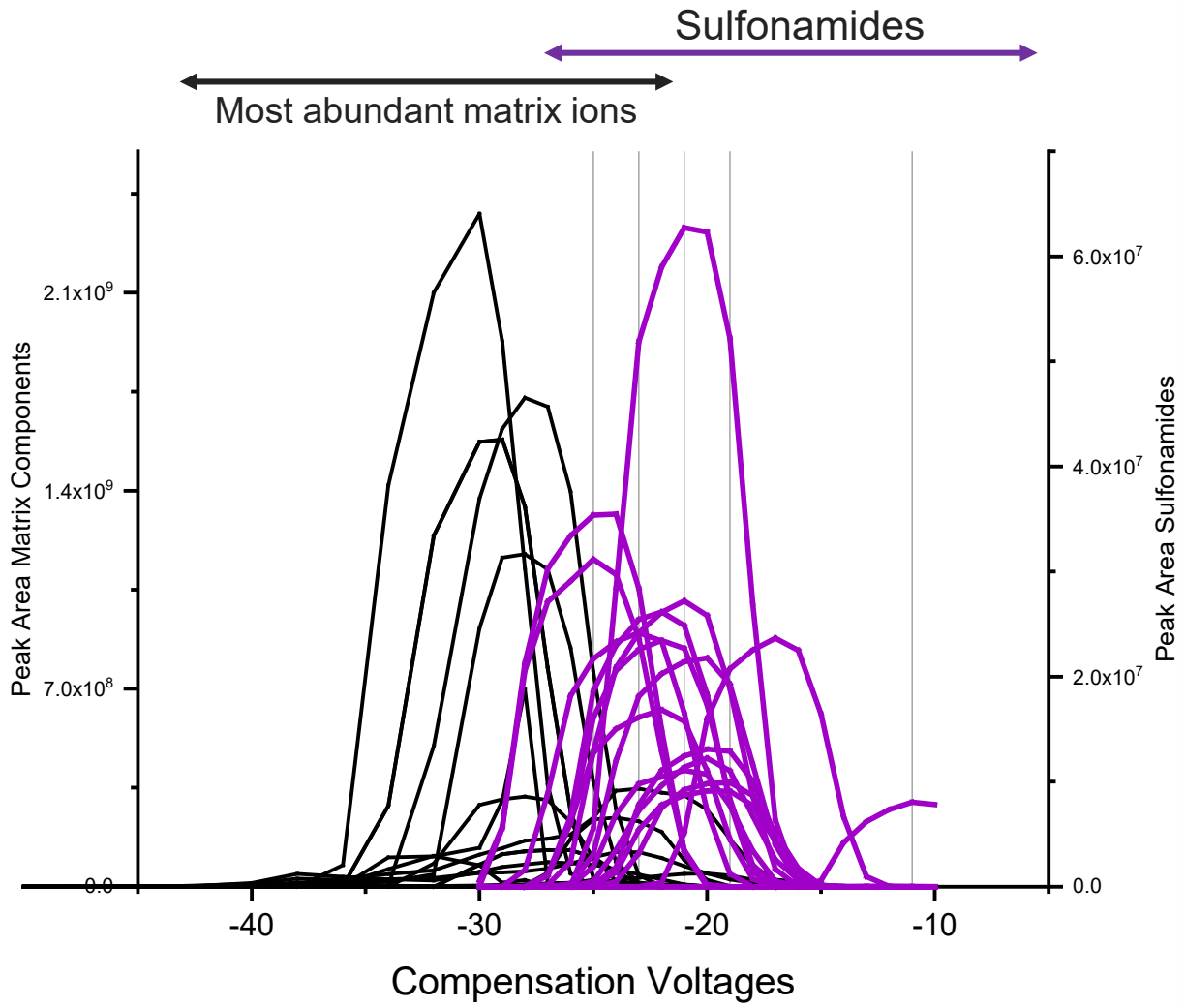
Table 2. Limits of Quantification with and without FAIMS

Compound Name	FAIMS LOQ µg/L	LOQ µg/L
Sulfamethazine_M+H	~5	5
Sulfamethoxazole_M+H	0.5	1
Sulfisoxazole_M+H	1	1
Sulfadimethoxine_M+H	0.5	0.5
Sulfaquinoxaline_M+H	5	5
Sulfaguanidine_M+H	5	10
Trimethoprim_M+H	1	1
Sulfadiazine_M+H	5	5
Sulfapyridine_M+H	0.5	0.5
Sulfathiazole_M+H	1	5
Sulfamerazine_M+H	0.5	5
Sulfamoxole_M+H	0.5	0.5
Sulfamethoxypyridazine_M+H	1	5
Sulfamethizole_M+H	5	5
Sulfachlorpyridazine_M+H	1	5
Sulfamonomethoxine_M+H	0.5	1

The graph shows improved response overall with better linearity at the low end of the curve.



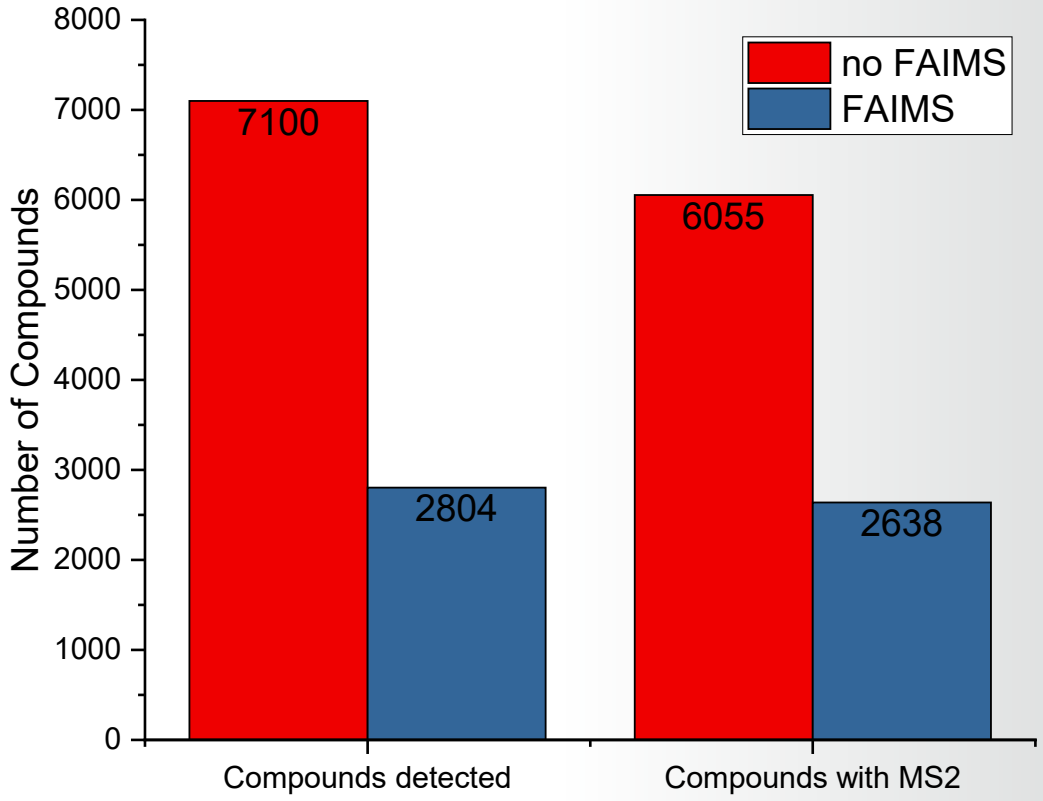
Results



- Here the CV optima of the sulfonamides and the 21 most abundant compounds can be seen in comparison
- The vertical lines indicate which CVs were applied during the quantitative experiments
- The 21 most abundant matrix compounds were derived from the Compound Discoverer results
- The CV optima for the most abundant matrix ions are on average different than the optima for the target sulfonamindes, allowing potentially better detection/matrix elimination prior to MS

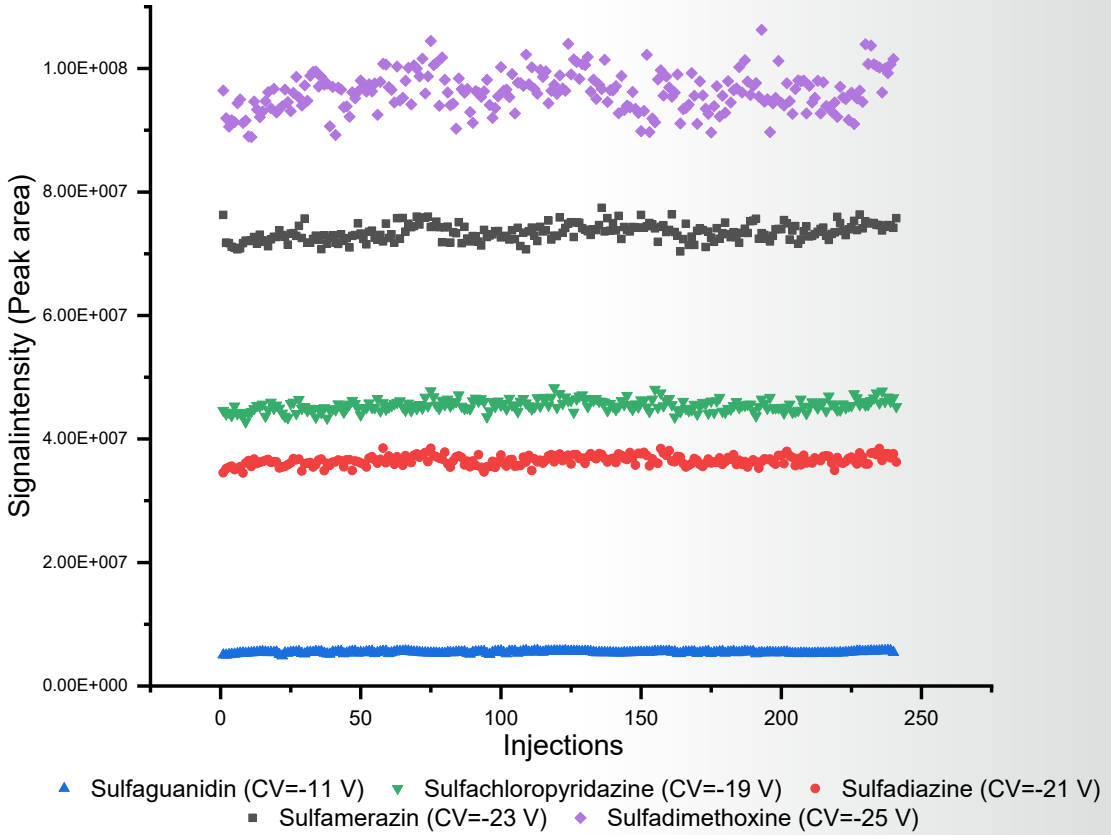
Results

The graph below represents the total number of compounds identified in the extract. For FAIMS it is the number detected with all 5 CVs combined. FAIMS can reduce matrix co-extractives which can allow for improved detection in targeted quantitation.



The graph below shows the robustness and reproducibility of the instrument (FAIMS + Orbitrap Exploris 240) for all 5 CVs in use.

The data shown was acquired over 84 hours of continuous measurement. The signal did not diminish over 4 days, even though a complex matrix was used.



Conclusions

- In LC-MS analysis matrix compounds may lead to frequent cleaning of the instrument
- We show that we can reduce the number of matrix compounds entering the mass spectrometer while maintaining and/or improving the sensitivity for sulphonamides by using the FAIMS Pro Duo interface

References and Acknowledgements

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