FAIMS Improves the Signal-to-Noise Ratio when Performing Targeted LC-MS/MS Measurements of Interference-Prone Analytes

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Introduction

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has established a crucial role in clinical laboratory testing primarily due to the exceptional specificity and sensitivity of the methodology. However, despite chromatographic separation and characteristic MS/MS fragmentation, the detection of some analytes is impaired by interferences and/or high background, which diminishes the specificity and signal-to-noise ratio of the measurement. However, ion mobility is capable of rapid separation based on charge and collisional cross-section, making it an ideal complement to liquid chromatographic separation. To demonstrate the potential of ion mobility paired with LC-MS/MS, we utilized the Thermo Scientific™ FAIMS Pro Duo interface for analysis of 2,3-dinor-11-β-prostaglandin F2α (2,3 BPG) in urine, a specificity-challenged and clinically-significant biomarker for diagnosis of systematic mastocytosis.

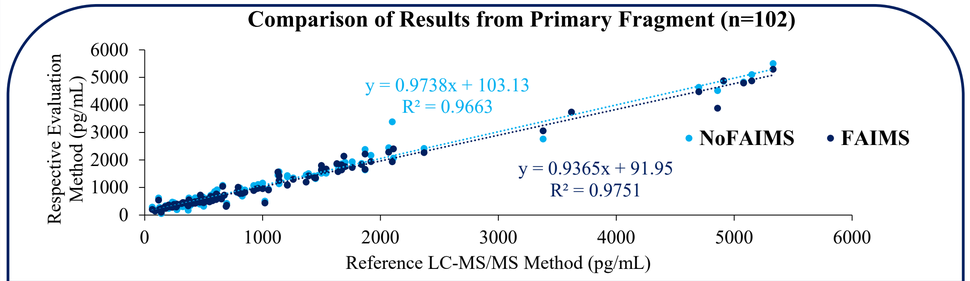
Experimental Methods

First, deuterium-labeled 2,3 BPG and 50 µL of 50% acetic acid is added to 1 mL of urine, followed by the addition of 1 mL of ethyl acetate for liquid-liquid extraction. Following vortexing and centrifuging, 500 µL of the organic layer was transferred, dried, and reconstituted in 135 µL of 60:40 water:methanol. Injection (10 µL) and separation were performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system. The analytical column was a 2.1x50 mm Waters T3 (1.6 µm). The LC method was 13.5 minutes long and utilized a shallow gradient from 40% B to 40.5% B to perform the desired separation. Mass spectrometry analysis was done using a Thermo Scientific™ TSQ Altis™ MS equipped with the FAIMS Pro Duo interface.

Results and Discussion

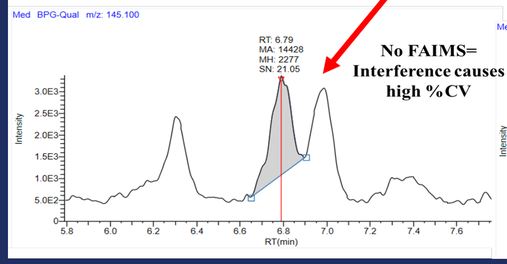
Despite the relatively long LC method and the high-efficiency LC column used, LC-MS/MS measurements of 2.3 BPG were still prone to interferences that reduced the signal-to-noise ratio and specificity of the measurements. This can be seen in the chromatograms shown on the right, which were in some cases difficult to integrate due to poor chromatographic performance. The ramification of these challenges are clearly shown in the comparison of the %CVs. The FAIMS Pro Duo greatly improved the precision of the replicate measurements, especially for the qualifier fragment of the medium pool, which had a visible interference. The benefits of FAIMS was also evident when looking at the patient sample comparison with a reference method. Use of the FAIMS Pro Duo produced much more favorable R^2 and slopes, especially for the chromatographically challenging qualifier fragment.

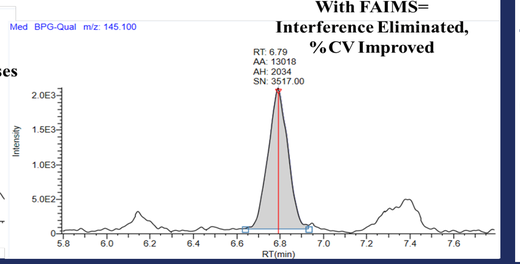
Comparison of 2,3-Dinor 11 β-Prostaglandin F2 Alpha Results



Comparison of Precision

%CV of Calculated Concentrations (n=4)				
Level	NoFaims-Quant	NoFaims-Qual	Faims-Quant	Faims-Qual
Low	18.6	16.0	6.0	4.7
Med	6.5	57.0	4.3	9.7
High	3.9	19.5	4.0	8.3





Chromatogram Comparison