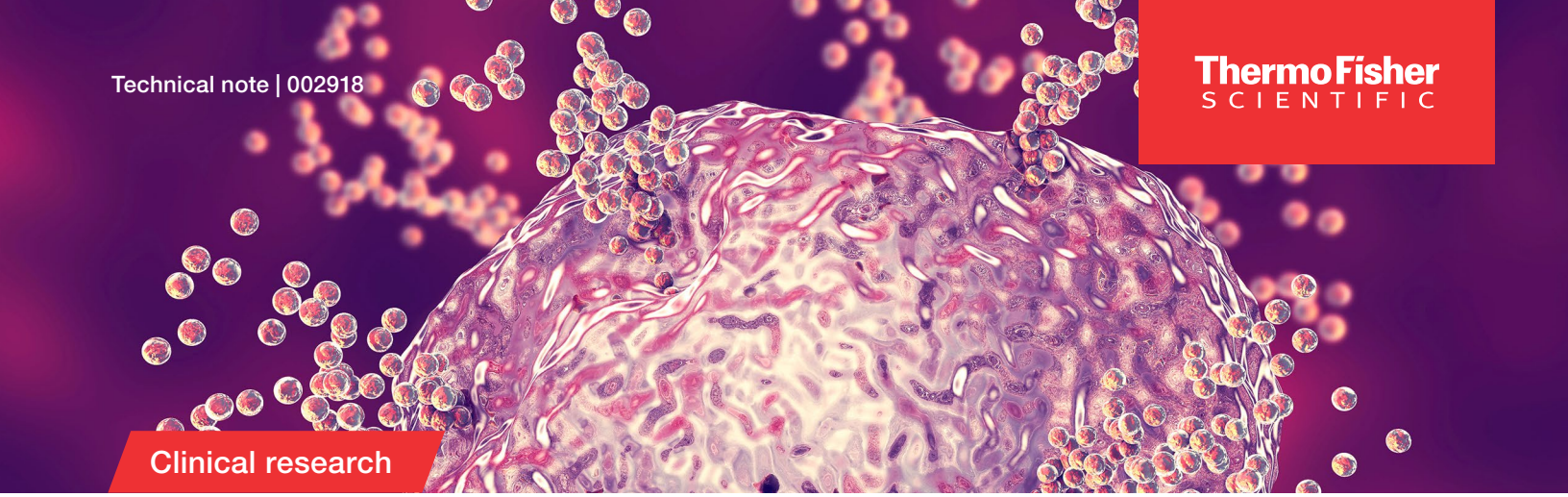

Technical note | 002918

Clinical research

Improved specificity for targeted LC-MS/MS measurements of 2,3-dinor-11β-prostaglandin F2α in urine using FAIMS technology

Authors

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Keywords

FAIMS Pro Duo interface, TSQ Altis Plus MS, 2,3-dinor-11β-prostaglandin F2α in urine, improved signal-to-noise ratio

Introduction

Mast cells play vital roles in immune responses, wound healing, and allergic reactions. Mast cell activation disorders arise when excessive mast cell activation releases inflammatory mediators, leading to conditions like mast cell activation syndrome, with symptoms ranging from subtle recurrent allergic reactions to life-threatening hypotension or anaphylaxis.[1,2] The diagnosis of mast cell disorders is challenging due to varied symptoms and the need for prompt blood sampling. An alternative and noninvasive approach is urine testing of excreted mediator metabolites. One example is the prostaglandin metabolite 2,3-dinor-11β-prostaglandin F2α (BPG) by liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, this method is sometimes severely impaired by the high prevalence of chromatographic interferences that result in reduced detection accuracy.

Field asymmetric ion mobility spectrometry (FAIMS) technology is a type of differential mobility spectrometry (DMS), an orthogonal ion separation mechanism that applies an asymmetric waveform between inner and outer electrodes with alternating high and low electric fields. Implementing an optimized compensation voltage tuned to the target compound can ensure stable transmission through the FAIMS interface prior to entering the mass spectrometer (MS), while destabilizing the transmission of unwanted ions to enhance the overall method selectivity. The cylindrical inner electrode benefits the targeted quantification assay over planar electrode DMS interfaces by refocusing the analyte ions before entering the ion transfer tube to improve the ion transmission efficiency.[3] Furthermore, FAIMS technology interfaces the electrospray and the entrance of the MS while blocking the neutral molecules from entering the MS, resulting in the improved robustness of the assay (Figure 1).[4]

In this technical note, we report the method and findings from an article published by Dr. Anthony Maus' team at Mayo Clinic in *Clinical Biochemistry* (DOI: 10.1016/j.clinbiochem.2024.110745) to demonstrate the improved signal-to-noise ratio of the quantification of BPG from donor urine samples utilizing the Thermo Scientific™ FAIMS Pro Duo interface on a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer. The enhanced specificity of BPG suggests that adding FAIMS selectivity can potentially reduce interferences without negatively affecting other aspects of analytical or clinical performance.

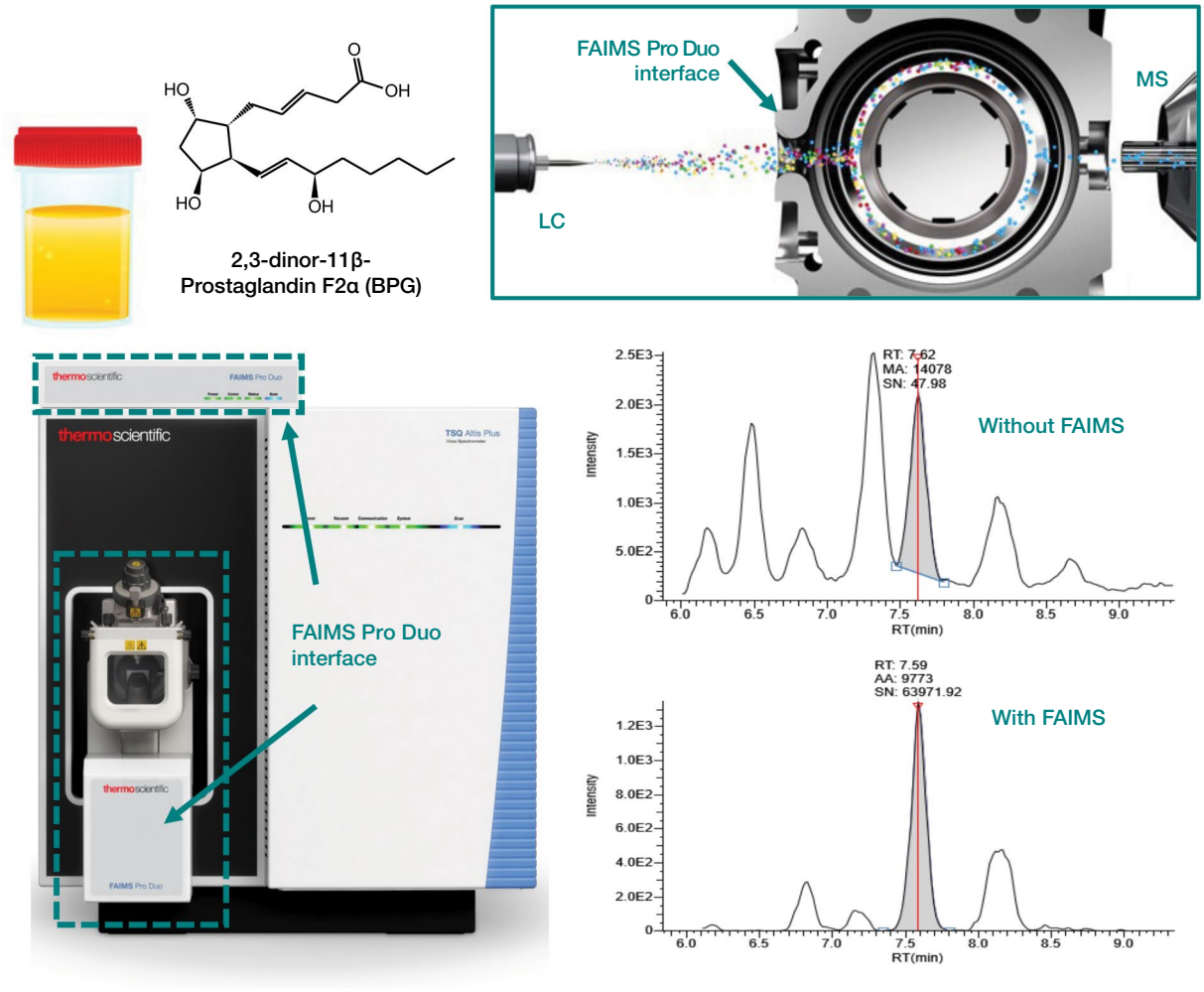

Figure 1. FAIMS selectivity reduces the chromatographic background in BPG quantification extracted from urine samples.

"…. Incorporating DMS devices greatly improved the specificity of BPG measurements by LC-MS/MS, as evidenced by the comparison of chromatograms and fragment ion results. Validation studies showed exceptional performance for established analytical metrics, indicating that this technology can be used to minimize the impact of interferences without adversely impacting other aspects of analytical or clinical performance….. "

Experimental

Study approval

Institutional Review Board (IRB)/Ethics Committee from Mayo Clinic ruled that approval was not required for this study. Donor samples were deidentified prior to use in this study.

Sample preparation

BPG synthetic standard and its internal standard (IS) were purchased from Cayman Chemical (Ann Arbor, MI). For the calibrators, BPG was diluted and spiked into 1% BSA to the final concentration of 312.5, 625, 1,250, 2,500, 5,000, and 10,000 pg/mL. IS solution was prepared in methanol at a concentration of 20 ng/mL. The calibrators or the urine samples of 250 µL were mixed with 50 µL of IS solution, 50 µL of 1N sodium hydroxide, and 1 mL of water in a 2 mL 96-well deep well plate. BPG was extracted using a solid phase extraction (SPE) anion exchange plate and eluted with 200 µL of 1% acetic acid in methanol with 1 mg/mL estradiol. (For the full SPE method, see the original publication. DOI: 10.1016/j.clinbiochem.2024.110745) Twenty microliters of the eluant were then injected for the LC-MS/MS analysis. Calibration curves were built using a weighting factor of 1/x.

Liquid chromatography-mass spectrometry analysis of BPG with and without FAIMS

The LC separation was performed on a Thermo Scientific™ Vanquish™ Horizon UHPLC system with a CORTECS T3 column (2.1 x 50 mm, 1.6 µm). The mobile phase A was 0.02% acetic acid in water, and the mobile phase B was 0.02% acetic acid in methanol. The gradient is described in Table 1. The MS data was acquired from 6.0 to 9.5 min.

Table 1. The gradient for the quantification of BPG using the Vanquish Horizon UHPLC system

Start	Time (min)	Flow rate (mL/min)	Gradient	%A	%B
0.00	0.50	0.30	Step	80.0	20.0
0.50	0.17	0.30	Step	65.0	35.0
0.67	1.67	0.30	Ramp	63.0	37.0
2.33	6.83	0.30	Ramp	62.0	38.0
9.17	0.67	0.30	Ramp	50.0	50.0
9.83	1.67	0.30	Step	2.0	98.0
11.50	2.00	0.30	Step	80.0	20.0

Analyte detection was performed using a TSQ Altis Plus mass spectrometer equipped with a heated electrospray ionization probe (HESI) and operated in the Selected Reaction Monitoring (SRM) mode with and without the FAIMS Pro Duo interface. The MS global parameters and the SRM transitions are shown in Tables 2 and 3, respectively.

Table 2. MS global parameters for the quantification of BPG with and without FAIMS Pro Duo device

MS parameters	With FAIMS	Without FAIMS
Spray voltage (V)	3,500	3,000
Sheath gas (arb)	45	35
Aux gas (arb)	12	6
Sweep gas (arb)	NA	1
Ion transfer tube temperature (°C)	325	325
Vaporizer temperature (°C)	400	300
Collision gas pressure (mTorr)	1.5	1.5
Source fragmentation (V)	20	20
Total carrier gas flow (mL/min)	3.5	NA
FAIMS mode	Standard Resolution	NA
FAIMS compensation voltage (V)	10	NA

Table 3. The SRM transitions of BPG and BPG-IS

ID	Q1 mass	Q3 mass	Dwell time	CE (V)	RF Lens (V)
BPG, quantifier	325.3	145.1	375	18	50
BPG, qualifier	325.3	163.0	375	14	50
BPG-IS	334.3	145.1	375	18	50

The coarse value of compensation voltage (CV) for BPG was obtained via direct infusion of the analyte and 50% mobile phase A/B, and further optimized by analyte injection at varying compensation voltage from +5 to 15 with 1 V interval from the coarse value. The CV that yielded the maximum chromatographic peak intensity was used for validation. Post-acquisition data analysis was carried out using Thermo Scientific™ TraceFinder™ software (v. 5.1).

Method validation

Intra-assay and inter-assay precision were tested using 20 replicate measurements of four urine pools with concentrations from the LLOQ and spanning the range of concentrations observed in most clinical patient samples. The acceptance criterion for imprecision was less than or equal to 20% CV for all levels. Accuracy was confirmed by comparing results with a reference LC-MS/MS method currently performed clinically on another vender's triple quadrupole MS without ion mobility device. Results were compared using Passing-Bablok regression analysis. The acceptance criteria were a slope of 1 ± 0.20 and an $r \geq 0.95$.

Results and discussion

The quantification of BPG in urine samples is analytically challenging as the analyte detection can be severely affected by the co-eluting background interferences, even after extensive sample preparation. The addition of FAIMS selectivity significantly reduced matrix interferences observed in the chromatograms of many of the 114 donor samples. Representative extracted ion chromatograms (EIC) of BPG quantification from two donor urine samples with and without FAIMS selectivity are shown in Figure 2, where peak fronting and high background were notably reduced. Similarly, FAIMS selectivity improved the signal-to-noise ratio for the lowest calibration samples, and strong linearity was obtained with R2 values > 0.99 (Figure 3).

When performing clinical testing using LC-MS/MS, a commonly accepted quality control practice involves verifying that the ion ratios or quantification results from at least two fragment ions for each analyte are consistent within 20% of one another. As demonstrated in Figure 4, when analyzed without the FAIMS Pro Duo interface, 46 out of the 114 donor urine samples had the BPG quantifier/qualifier ion pair results that differed greater than 20%. In contrast, employing FAIMS selectivity resulted in BPG ion pair measurements within 20% for all 114 donor samples. This improvement has tremendous clinical implications because these results can be confidently reported without the need for repeat measurements, reducing the turnaround time and labor required for the testing.

The inter- and intraday imprecision results for 20 replicates from four levels of pooled urine samples are below 6.5% (Figure 5). Accuracy was compared to the reference method that was performed on a validated existing method using another vendor's triple quadrupole MS without an ion mobility device. The resulting Passing-Bablok regression analyses can be seen in Figure 6. The results generated from the described method with the FAIMS Pro Duo interface showed good agreement with those from the reference method.

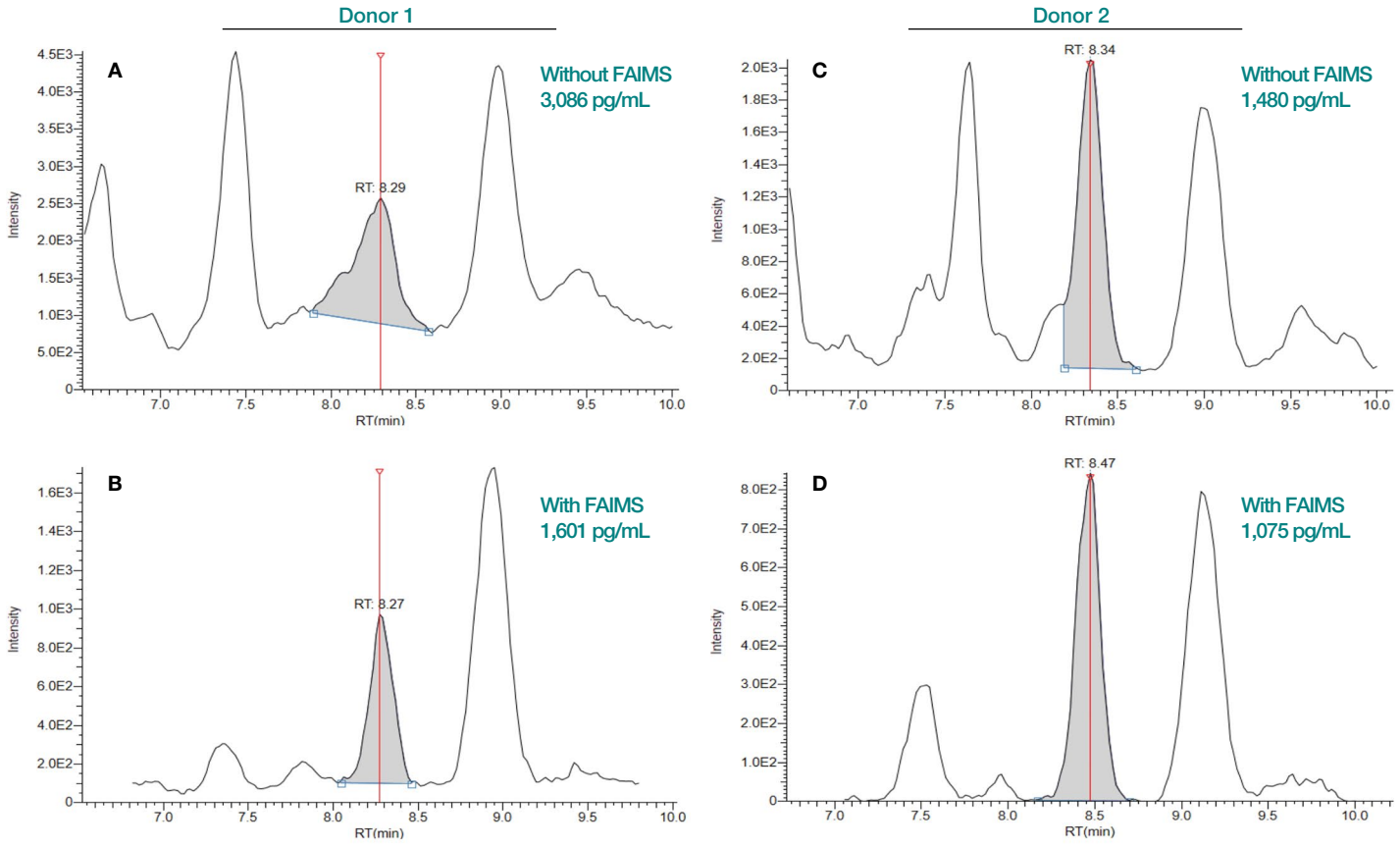

Figure 2. Example EIC of BPG from two donor urine samples without (A, C) and with (B, D) implementing FAIMS selectivity

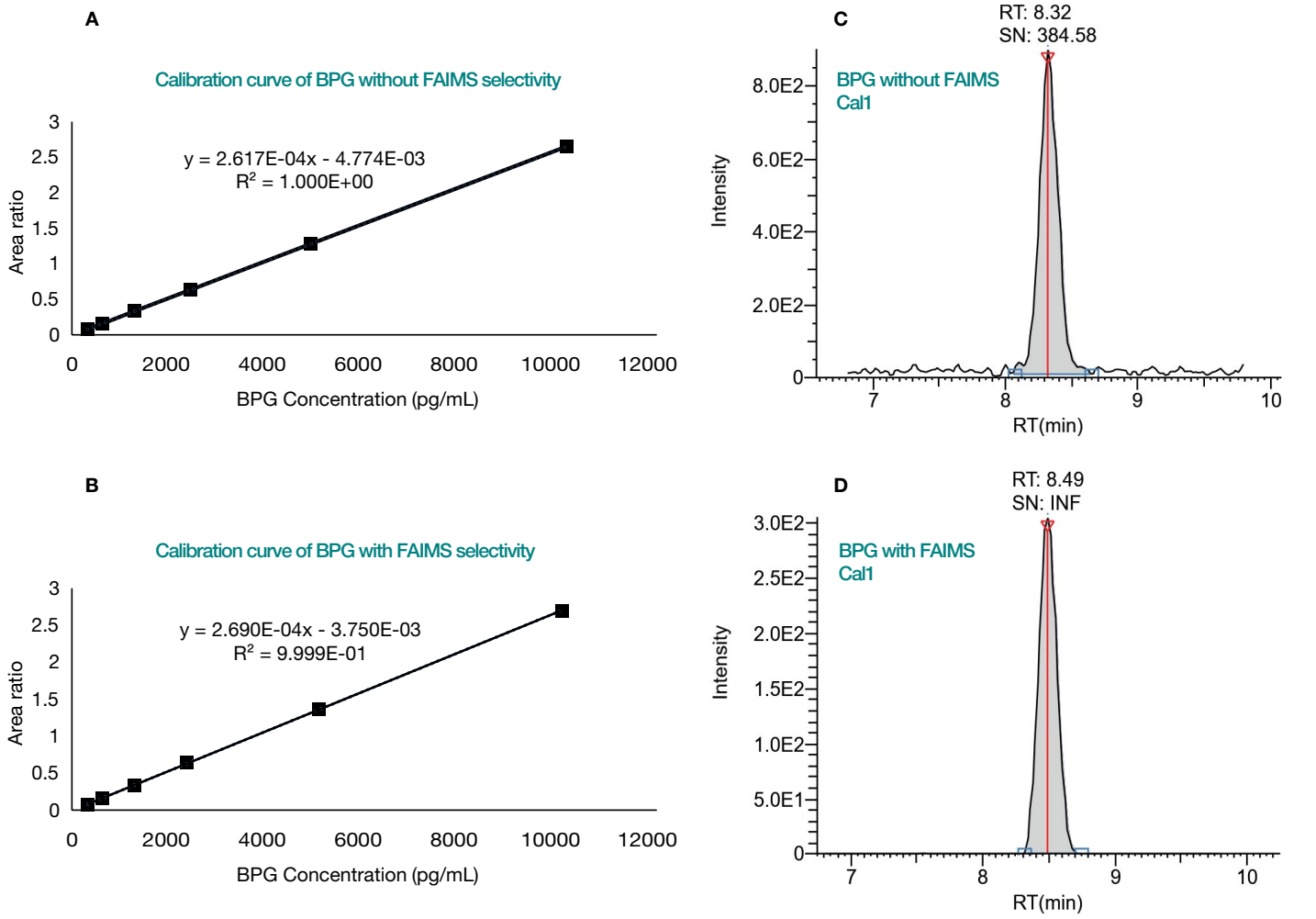

Figure 3. Calibration curve of BPG in urine sample without (A) and with (B) FAIMS selectivity. EIC of BPG at the lowest calibration level from the two curves are shown to the right, where FAIMS selectivity improved signal-to-noise ratio (D, compared to C).

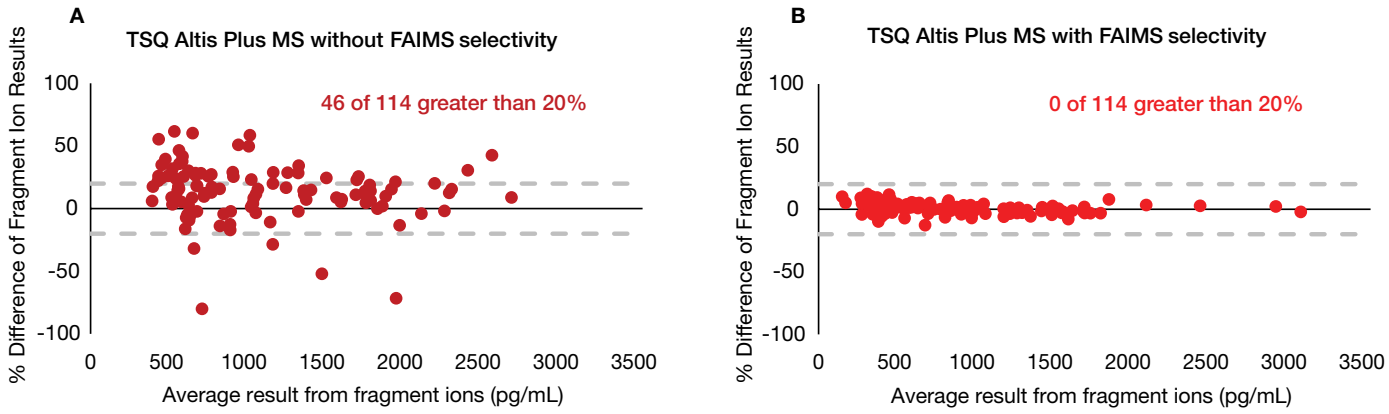

Figure 4. Percent difference plots comparing the calculated concentrations from the two BPG fragments analyzed without (A) and with (B) FAIMS selectivity from the 114 donor urine samples

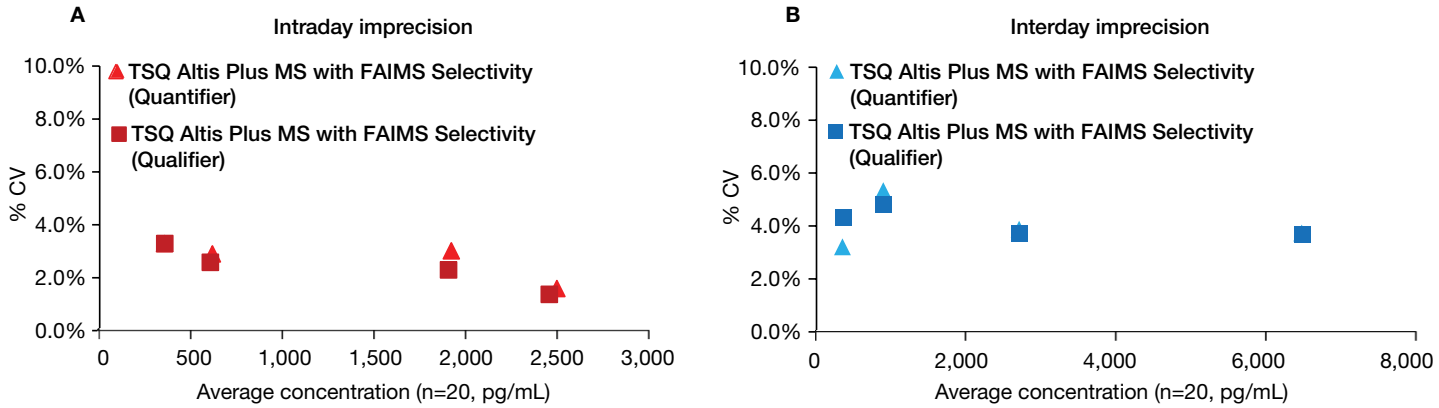

Figure 5. Intra- (A) and interday (B) imprecision of the BPG measurement from four pooled urine samples measured with FAIMS selectivity

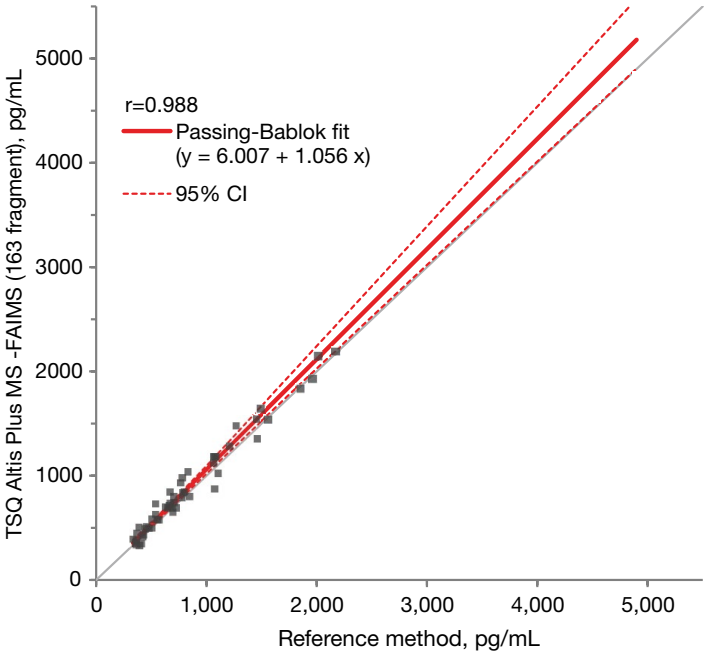

Figure 6. Passing-Bablok regression analysis between the LC-FAIMS-SRM method presented in this work and the reference method

Conclusions

Incorporating the FAIMS Pro Duo interface greatly improved the peak shape and reduced background of BPG when it was quantified in urine samples using LC-MS/MS. The enhanced signal-to-noise ratio provided better specificity, as evidenced by the fragment ion ratio results. Validation studies showed exceptional performance for established analytical metrics, indicating that this technology can be used to minimize the impact of interferences without adversely impacting other aspects of analytical or clinical performance.

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