Application Note: 431

Enhanced LC-MS/MS Selectivity for the Analysis of Human Urinary 8-isoprostane, using FAIMS

Kazuyo Nakagawa, Thermo Fisher Scientific, Yokohama, Japan

Key Words

- Biomarkers
- FAIMS
- Increased Selectivity
- Ion Mobility
- Targeted Metabolomics

Introduction

A series of isoprostanes is generated non-enzymatically in humans as peroxidation reaction products of arachidonic acid. The 8-isoprostane (8-epi PG $F_{2\alpha}$) is a prostaglandin formed by free radical oxidation and has been proposed as a marker of antioxidant deficiency and oxidative stress. Elevated levels of this compound have been found in heavy tobacco smokers and it can also appear in plasma and urine under normal conditions. Evaluating oxidative stress (lipid oxidation) *in vivo* is possible by measuring the concentration of 8-isoprostane in urine. Isoprostanes are present in very low concentrations with high background; therefore, a highly sensitive and selective assay is needed to quantify these compounds accurately.

High-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS, Figure 1) is a differential ion mobility technique. The compensation voltage (CV) parameter is determined in part by the ion mobility of each compound. The selectivity of LC/MS can be improved with FAIMS. If the chemical background has a different CV from the target compound, then the signal for the target compound can be detected without interference from the background. The analysis is performed at the optimized CV for the target compound.

A newly developed LC-FAIMS-MS/MS method for 8-isoprostane in human urine extract described here provides high selectivity with short chromatographic separation time. The chromatogram of 8-isoprostane using FAIMS shows significantly improved selectivity and higher sensitivity than the conventional technique. LC-FAIMS-MS/MS data showed better signal-to-noise ratios than LC-MS/MS data mainly due to the reduction of background noise.

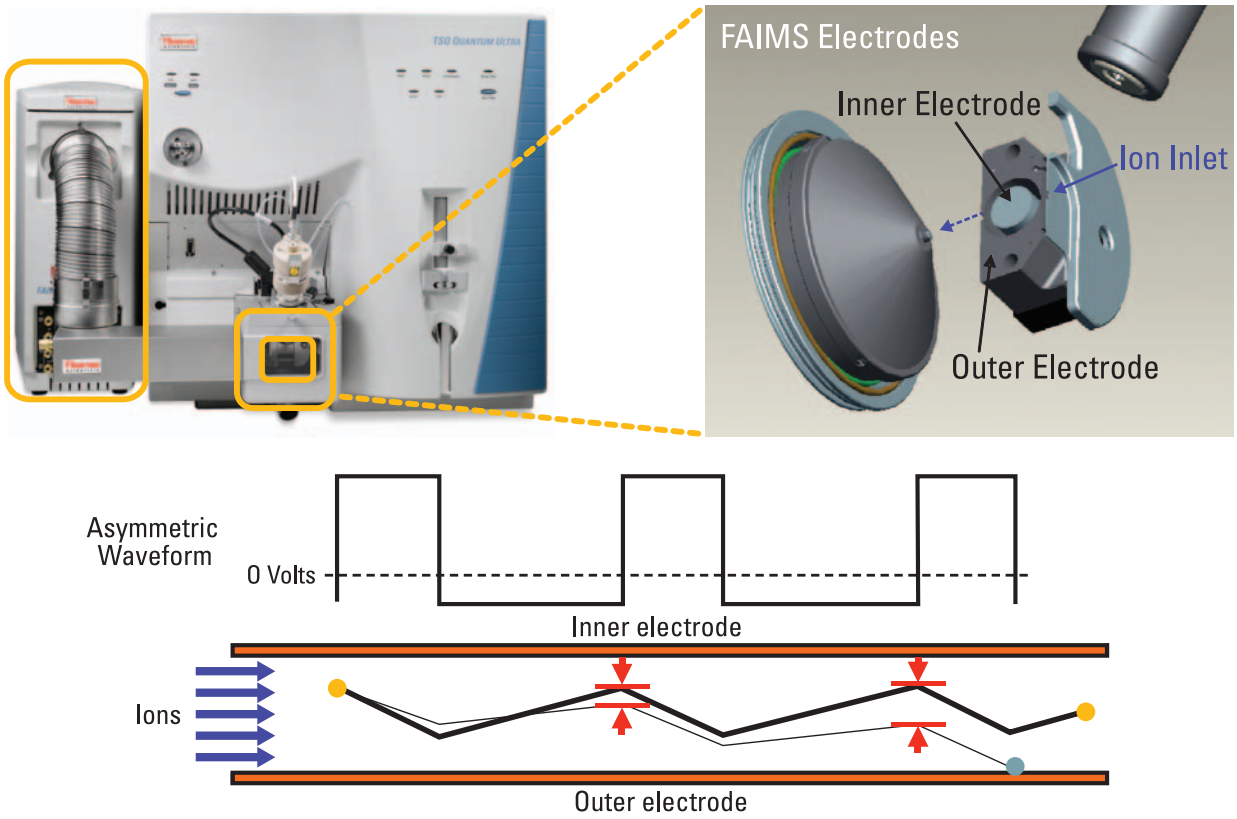

Figure 1: Illustrations of the FAIMS-enabled TSQ Quantum Ultra and the FAIMS mechanism. FAIMS separates ions based on differences in ion mobility at high versus low electric fields, and operates at atmospheric pressure. The temperature is fully controllable. The optimum compensation voltage transmits an ion through the electrodes and into the mass spectrometer. Other ions cannot pass through because they collide with one of the electrode walls.

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Goal

To compare LC-MS/MS and LC-FAIMS-MS/MS for the detection of 8-isoprostane in human urine by using Selected Reaction Monitoring (SRM) and FAIMS.

Experimental Conditions/Methods

Sample Preparation

8-isoprostane (Figure 2) was purchased from Cayman (MI, USA). 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-hydroxy-TEMPO) was purchased from Calbiochem (Darmstadt, Germany) and ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA) was from Dojindo Laboratories (Kumamoto, Japan). The urine samples were collected from nine healthy human male volunteers into vials containing 4-hydroxy-TEMPO / EDTA and quickly stored at -30 °C until analysis.

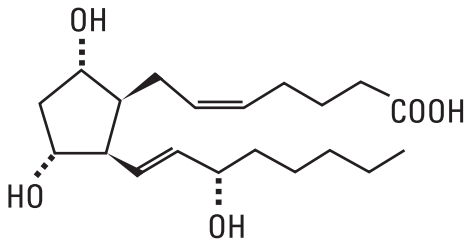

Figure 2: Chemical structure of 8-isoprostane

1 mL urine was extracted with 1 mL ethyl acetate and then the aqueous layer was washed with 1 mL hexane. The aqueous layer was acidified with 20 µL 1N hydrochloric acid and then extracted with 1 mL ethyl acetate. The resulting organic layer was evaporated during centrifugation and the residue was dissolved in 50 µL of starting mobile phase (55% A, 40% B, 5% C, see Gradient Method at right).

HPLC Conditions

A Thermo Scientific Accela™ HPLC System was used. Samples were injected onto a 2 x 150 mm C18 column (5 micron particles). The injection volume was 20 µL. A gradient elution was performed by using mobile phases A (Water), B (Methanol) and C (1% formic acid in water) at a flow rate of 200 µL/min.

Standard negative ion FAIMS conditions were used as described in the publication, "FAIMS on the TSQ Quantum: Tuning for Selective Quantitation Quick Reference Guide."

Gradient Method

Time	%A	%B	%C
0	55	40	5
3	33	62	5
12	23	72	5

FAIMS Conditions

Compensation Voltage (CV)	15V

Mass Spectrometer Conditions

Mass Spectrometer	Thermo Scientific TSQ Quantum Ultra™
Ion Source and Polarity	H-ESI, negative ion mode
Spray Voltage	4000 V
Sheath Gas	40 units, nitrogen
Aux Gas	35 units, nitrogen
Vaporizer Temperature	400 °C
Transfer Tube Temperature	270 °C
Collision Gas Pressure	1.5 mTorr, argon
Collision Energy	26eV
SRM Setting	*m/z* 353.2 → *m/z* 193.1

Results and Discussion

FAIMS provides selectivity without the need to change sample preparation and LC conditions. Implementing FAIMS requires transmission of the target analyte through the interface. Transmission is achieved by setting the CV to the optimum value for the analyte.

Figure 3 shows how the optimum CV was determined. The CV was automatically optimized during infusion of 8-isoprostane reference solution by ramping the CV. The signal maximum determines the optimum CV.

Representative mass chromatograms for the 8-isoprostane analysis in human urine are shown in Figure 4 for LC-MS/MS and LC-FAIMS-MS/MS analyses. Although LC-MS/MS is a selective technique, there are many isobaric (nominal mass) interferences and co-eluting peaks observed. These interference peaks make 8-isoprostane detection and quantification difficult.

For an LC-FAIMS-MS/MS analysis of 8-isoprostane in human urine, the CV was set to 15 V. A representative LC-FAIMS-MS/MS chromatogram for the 8-isoprostane analysis in human urine is shown in Figure 4 (lower chromatogram). The selectivity of FAIMS provides reduced background noise and removes interference peaks compared with the corresponding conventional LC-MS/MS chromatograms (upper chromatogram in Figure 4).

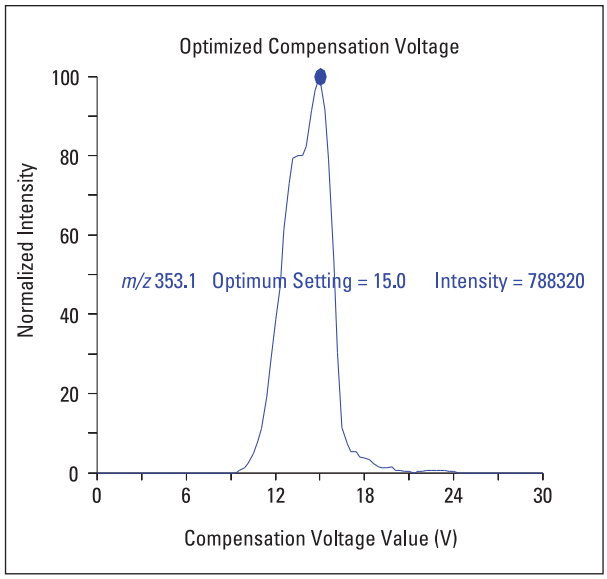

Figure 3: The compensation voltage (CV) scan experiment for 8-isoprostane, which was scanned from 0 to 30 V automatically

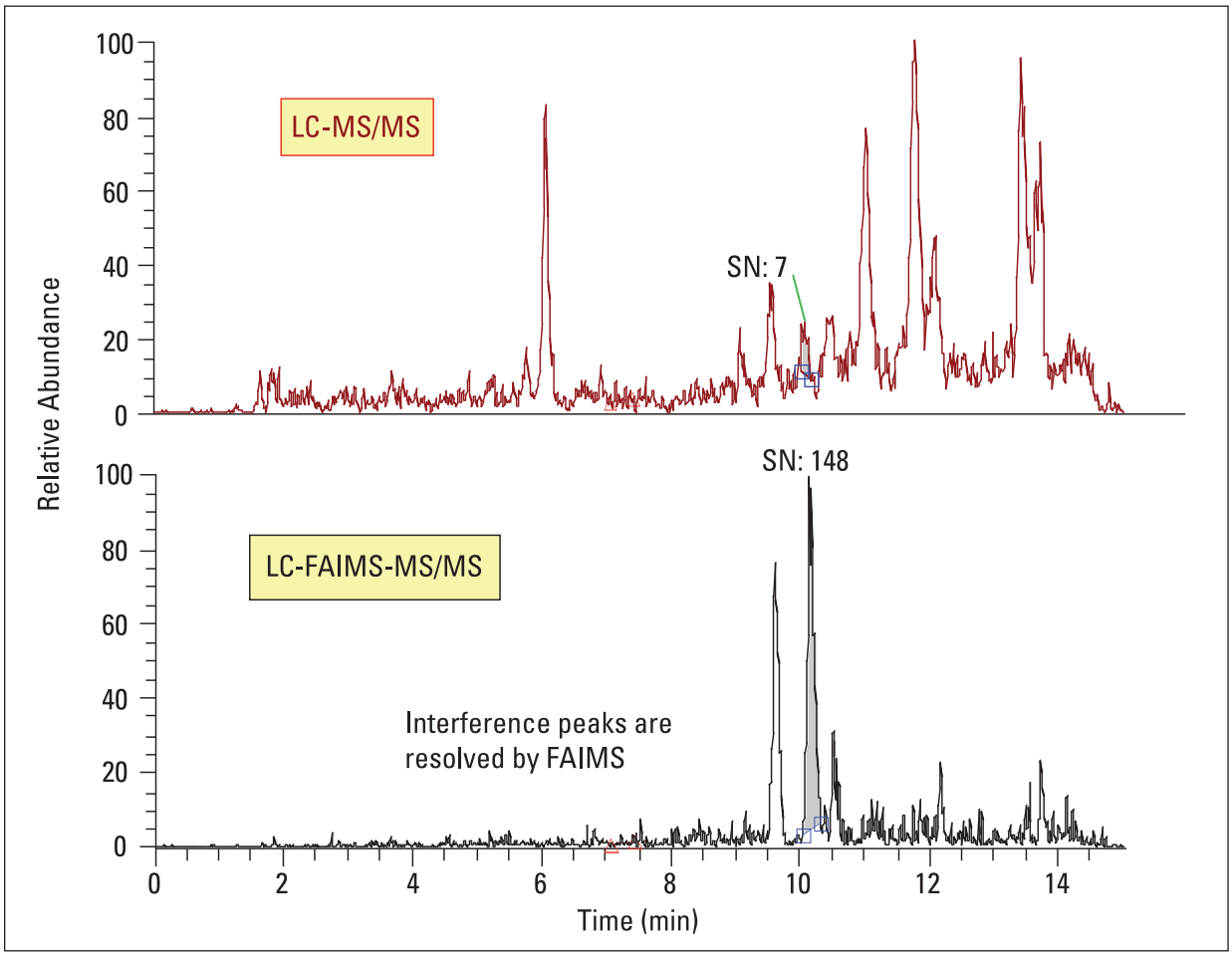

Figure 4: Representative mass chromatograms for 8-isoprostane in human urine samples (upper trace LC-MS/MS, lower trace LC-FAIMS-MS/MS)

Conclusion

LC-FAIMS-MS/MS provided greater selectivity than conventional LC-MS/MS. The selectivity as measured by signal-to-noise ratio was improved by 21-fold by using FAIMS. The improved selectivity was a direct result of removing interferences and chemical background from the analysis of 8-isoprostane.

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