Application Note: 382

# Stable Isotope Labeled Fatty Acid Analysis in Plasma Using LC-FAIMS-SRM

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**Key Words**

- TSQ Quantum Ultra™
- Surveyor™ HPLC
- Improved selectivity
- Isobaric interferences

## Introduction

The analysis of fatty acids is generally performed using GC-MS methods. While these methods provide the necessary sensitivity, sample preparation involves derivatization, which is time consuming and labor intensive. In comparison to GC methods, fatty acid analysis by LC-MS is simplified because derivatization is not required. The resulting LC-MS chromatogram, however, often exhibits a high chemical background that ultimately limits detection.

In this work, the gas-phase separation of high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) is used in combination with LC and zero neutral loss tandem MS to increase the selectivity of the method. The chemical background in the resulting LC-FAIMS-SRM chromatograms is significantly reduced with respect to that collected using the LC-SRM method. The result is improved detection limits for fatty acid analyses.

## Goal

To reduce the chemical background in the analysis of stable isotope labeled stearic and oleic acids by FAIMS with LC and tandem MS.

## Experimental Conditions

### Sample Preparation

Rat plasma samples containing labeled stearic and oleic acids were prepared at a concentration level of 1000 nM.

### HPLC

10 µL samples were separated on a 2.1×50 mm C8 column. A binary gradient was formed using the Surveyor MS Pump (Thermo Fisher Scientific, San Jose, CA) delivering mobile phases A (0.1% ammonium hydroxide in water) and B (0.1% ammonium hydroxide in methanol) at a flow rate of 250 µL/min as described in Table 1.

| Time (min.) | %B |
|---|---|
| 0 | 60 |
| 4 | 74 |
| 4.1 | 95 |
| 7 | 95 |
| 8 | 60 |
| 14 | 60 |

Table 1. Gradient profile

### MS

MS analysis was carried out on a TSQ Quantum Ultra triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe (Thermo Fisher Scientific, San Jose, CA). See Figures 1 and 2.

## The MS and FAIMS Conditions were as Follows

### MS Conditions

Ion source polarity: Negative ion mode
Spray voltage: 4000 V
Vaporizer temperature: 250 °C
Sheath gas ($N_2$): 40
Auxiliary gas ($N_2$): 40
Ion transfer tube temperature: 300 °C
Q1 peak width: 0.7 u FWHM
Collision energy: 10 eV
Scan time: 100 ms
Scan type: SRM

The SRM transitions were survivor ion scans for the analytes of interest. Survivor ion scanning, also known as zero neutral loss tandem MS, involves Q1 and Q3 of a triple quadrupole MS. Both mass resolving quadrupoles are set to the same *m/z*. The labeled oleic acid transition that was monitored was *m/z* 288.2 → *m/z* 288.2. The labeled stearic acid transition monitored was *m/z* 290.2 → *m/z* 290.2.

### FAIMS Conditions

Dispersion voltage: +4500 V
Outer bias voltage: –35 V (identical to ion transfer tube voltage offset)
Inner electrode temperature: 50 °C
Outer electrode temperature: 80 °C
FAIMS gas: 50% He in $N_2$ at 4.5 L/min

Implementing FAIMS requires the establishment of conditions for the transmission of the desired analyte(s) through the interface. Stable conditions for ion transmission is expressed as the compensation voltage (CV). The maximum response for the infusion of labeled oleic and stearic acid reference standards occurred at –14 V, and this value indicated the appropriate CV for LC-FAIMS-SRM analysis. See Figure 3.

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## Results and Discussion

Representative LC-SRM chromatograms for the analysis of labeled oleic acid ($t_R$ 5.6 min) and labeled stearic acid ($t_R$ 5.8 min) in rat plasma extracts are presented in Figure 4. Both chromatograms show that between 0.5 min. and 4.0 min. multiple species elute from the column and are transferred to the mass spectrometer. As a result, the baselines of the chromatograms are very high.

An increase in selectivity is achieved by using the FAIMS device to improve ion separation. Representative LC-FAIMS-SRM chromatograms from the injection of a rat plasma extract of labeled oleic acid ($t_R$ 5.6 min) and stearic acid ($t_R$ 5.8 min) are shown in Figure 5. The use of FAIMS provides enrichment of the analytes by removing some of the endogenous isobaric interferences and by reducing the chromatographic baseline.

Figure 6 shows the effect of FAIMS on an absolute scale. A representative LC-SRM chromatogram for labeled oleic acid is compared with an LC-FAIMS-SRM chromatogram from the injection of identical rat plasma extract. The signal heights are approximately identical, but the baseline for the LC-FAIMS-SRM chromatogram is greatly reduced.

Figure 7 shows the effect of FAIMS on a relative intensity scale for labeled stearic acid. A representative LC-SRM chromatogram is overlaid with an LC-FAIMS-SRM chromatogram from the injection of identical rat plasma extract. The baseline for the LC-SRM trace shows a constantly varying baseline, but the LC-FAIMS-SRM trace shows resolved individual chromatographic peaks.

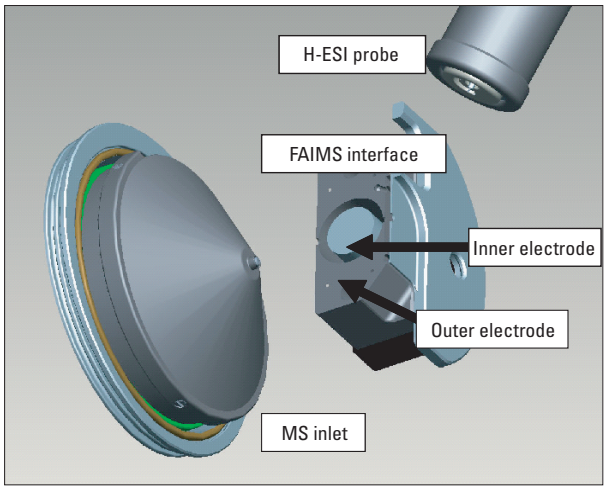


Figure 1: Drawing of the ion source (H-ESI probe), a cross-section of the FAIMS interface, and the MS inlet. FAIMS separation involves selective transfer of only a subset of the ions produced by the H-ESI probe.

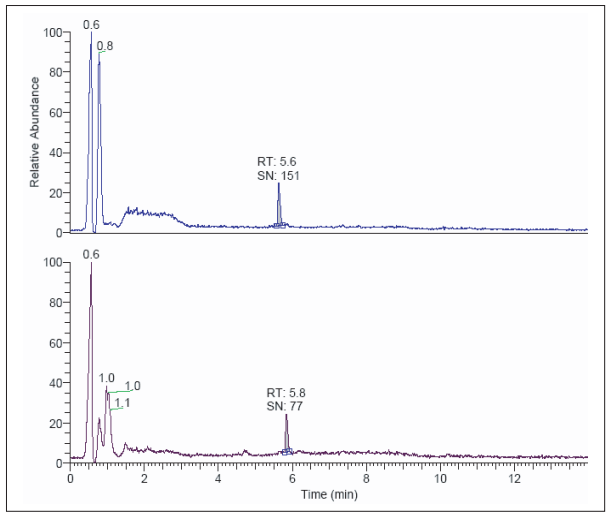


Figure 3: Representative LC-SRM chromatogram for labeled oleic acid (above) and stearic acid (below) in rat plasma extracts.

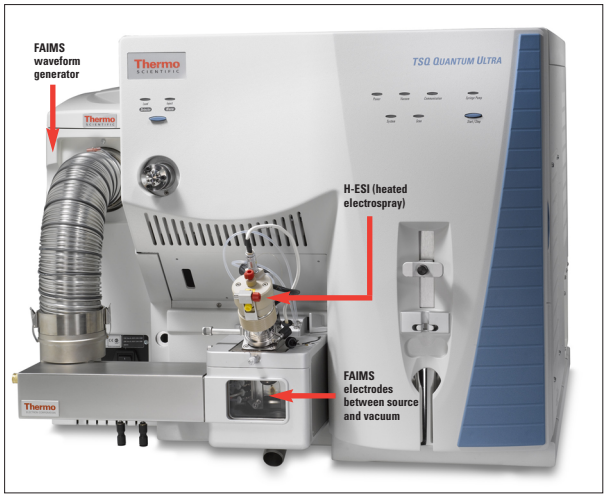


Figure 2: Photograph of FAIMS-enabled Quantum triple quadrupole MS showing the asymmetric waveform generator and the heated electrospray (H-ESI) source. FAIMS separates ions based on differences in mobility at very high vs. low electric fields.

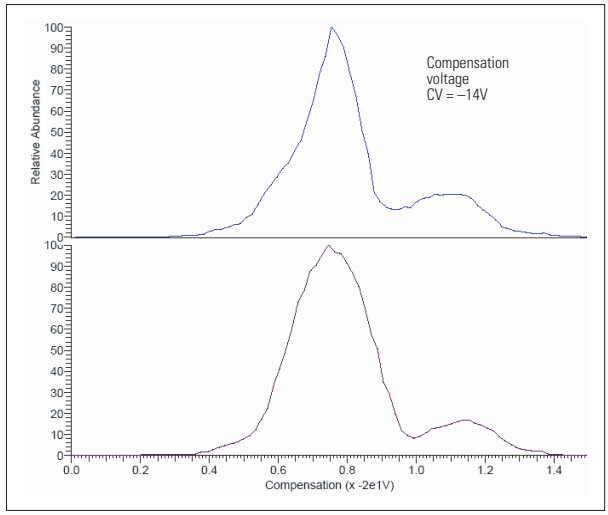


Figure 4: CV scan from the infusion of labeled stearic and oleic acids. The optimum CV at which the acids emerge from FAIMS is –14V.

The LC-SRM ion chromatograms for both of the labeled fatty acids demonstrate that a high chemical background is present, limiting the level of quantitation. Signal-to-noise values of 155 and 77 were obtained for labeled oleic and labeled stearic acid, respectively. See Table 1. The ion filtering action of FAIMS provided a reduction in chemical background of approximately 10-fold, while the ion signal was maintained for labeled oleic acid and reduced by 50% for labeled stearic acid. The signal-to-noise values with FAIMS were 688 and 173 for labeled oleic and labeled stearic acids, respectively. Therefore, FAIMS provided a 4-fold improvement in the signal-to-noise ratio for labeled oleic acid and a 2-fold improvement in the signal-to-noise ratio for labeled stearic acid.

## Conclusion

The use of FAIMS significantly reduced the chemical background and enriched the labeled fatty acid chromatograms by partially removing endogenous isobaric interferences. This reduction in chemical background helped to define the chromatographic peaks, which resulted in a more reliable integration of the ion signal. The overall result was an improved assay for the detection of tracer fatty acids in fat metabolism studies.

### References


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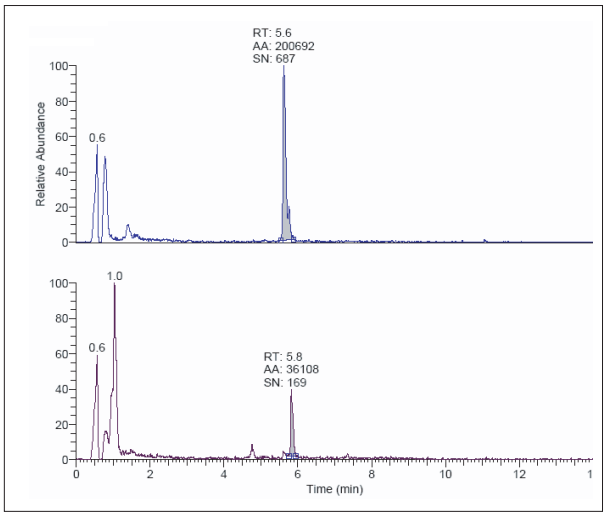


Figure 5: Representative LC-FAIMS-SRM chromatogram for oleic and stearic acid in rat plasma extract.

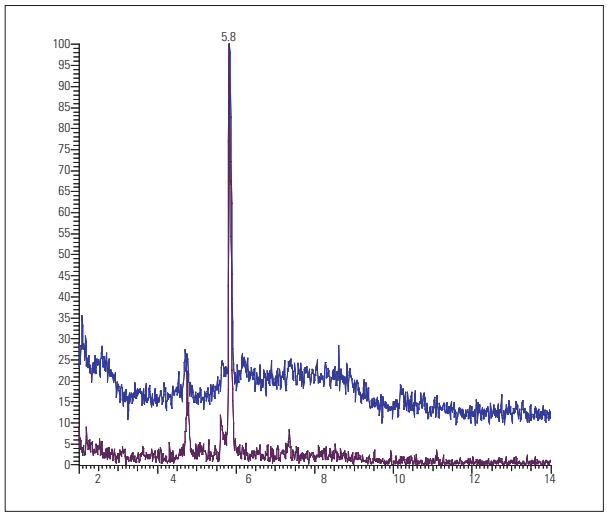


Figure 7: Overlayed chromatograms of labeled stearic acid with and without FAIMS.

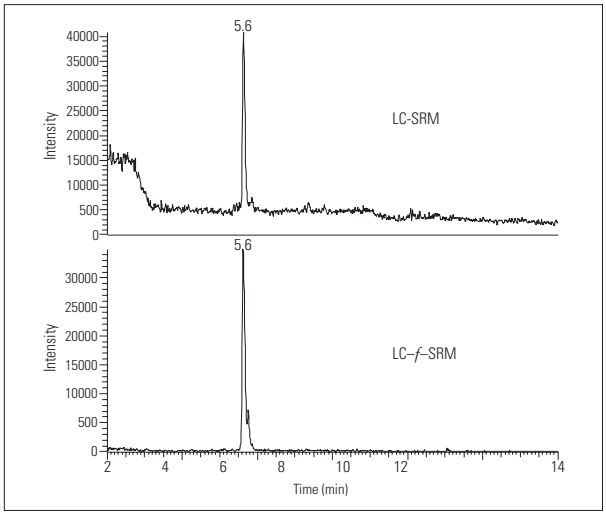


Figure 6: Labeled oleic acid chromatograms from LC-SRM (above) and LC-FAIMS-SRM (below) analysis. The absolute height of these peaks is approximately equal, but the background is reduced with FAIMS.

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