Demonstration of FAIMS-MS Separation on Chromatographic Timescales

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Overview

The use of Field-Asymmetric Ion Mobility Spectrometry (FAIMS) devices in line with LC-MS is of growing interest due to the orthogonal separation that FAIMS can provide. To be practically useful, the FAIMS separation should occur fast enough to be carried out in real-time during an LC-MS assay. The goal of this project was to demonstrate high-speed FAIMS sweeping during LC assays.

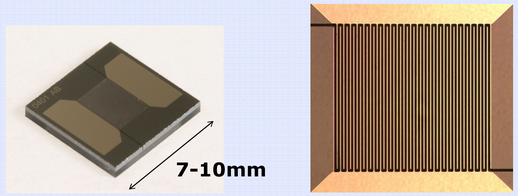


Figure 1: (left) example FAIMS chip, (right) microscope image of example FAIMS chip

Introduction

FAIMS devices have recently started to be used in line with LC-MS systems, acting as a band-pass filter that selectively allows only certain analytes to pass into the MS. Current systems have the following limitations:

- Full compensation voltage sweeps take up to 1min, significantly longer than the duration of most LC peaks, so real-time LC-FAIMS-MS scanning is not possible
- Preliminary experiments are needed to find the required compensation voltage (CV) setting for a given analyte
- Effective FAIMS peak capacity is severely restricted because only a few CV points can be explored during a single LC peak

The factor that fundamentally limits sweep speed is the ion residence time – the time taken for the ion to travel through the device. In the miniature FAIMS device presented here, the small size of the device means ion residence time is reduced to tens of microseconds, 100 times shorter than in other devices. This means CV sweeps can take less than 1 second – fast enough to be carried out in real-time during an LC peak.

As a result:

- Preliminary steps and repeated experiments can be eliminated
- Complete sweeps can be completed within the duration of individual LC peaks, so the full peak capacity of the device is used
- Rather than simply acting as a fixed filter, the FAIMS device now adds an extra dimension of separation to the LC-MS analysis (see Figure 2)

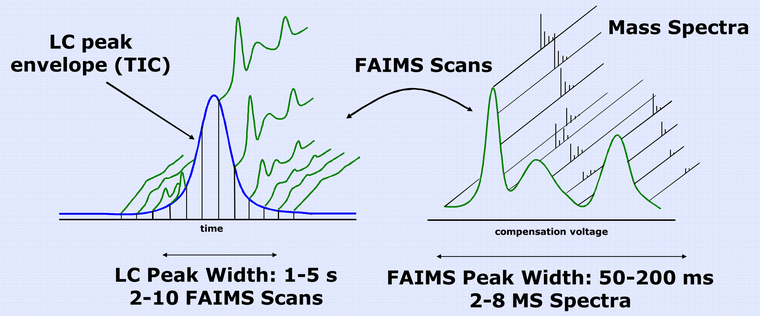


Figure 2: schematic illustration of the extra degree of separation provided by the FAIMS chip. (Left) multiple FAIMS scans are carried out within each LC peak; (right) within each FAIMS sweep, mass spectra are generated at a frequency of up to 20Hz.

Method

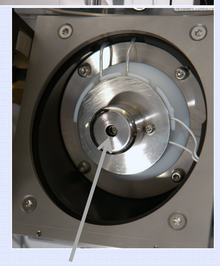
FAIMS chip built into a housing on the Agilent 6230 TOF inlet

ESI with Agilent Jet Stream used, with the standard voltage configuration reversed: 1.5kV applied to nebulizer, capillary inlet held at -100V

TOF MS acquisition rates up to 20Hz

FAIMS RF drive electronics

Gas flow rate through chip matched to the MS inlet flow rate (to minimise ion losses)



Reverse flow of heated drying gas at 150°C assists desolvation

FAIMS parameters:
- dispersion field frequency 25MHz
- dispersion field amplitude up to 300Td
- CV sweep range: -10 to +10V
- CV sweep speed: up to 10V/s

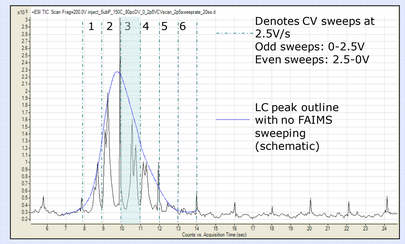
The following analytes were tested with the following conditions:

Analyte	FAIMS sweep conditions
(a) Peptide [Met-Ome11]-substance P	0-2.5V at 2.5V/s, 80% maximum dispersion field
(b) Mixture of peptides including [Val4]-Angiotensin III and PEG415	0.4-1.4V at 0.6V/s, 60% maximum dispersion field

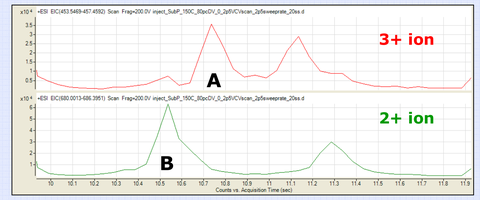
Analyte was injected by LC (Column: 75mm x 5mm Poroshell 300SB-C8; mobile phase: water/acetonitrile mixture with 0.1% formic acid) resulting in typical peak widths of a few seconds.

Results

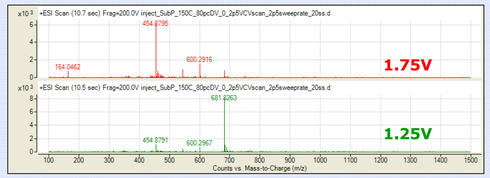
a. Separation of charge states of [Met-OMe[11]]-substance P



Total ion chromatogram (TIC) for the injection, with active FAIMS sweeping

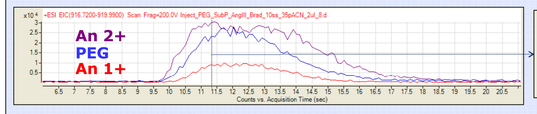


Above: Extracted ion chromatograms (EICs) for the 2+ and 3+ ions during FAIMS sweep #3

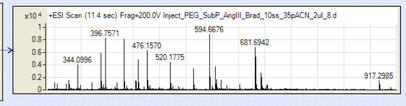


Above: m/z spectra at times A and B, equivalent to CVs 1.75V (top) and 1.25V (bottom)

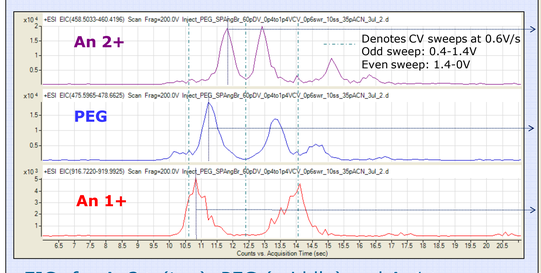
b. Separation of [Val[4]]-Angiotensin III and PEG415



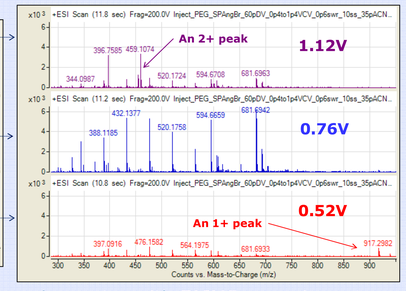
EICs with no FAIMS sweeping; PEG and Angiotensin elute at the same time and their spectra overlap (right)



m/z spectrum with no FAIMS sweeping



EICs for An2+ (top), PEG (middle) and An1+ (bottom) with FAIMS sweeping at 0.6V/s; the FAIMS field produces separation of the analytes.



m/z spectra with FAIMS sweeping, at times marked on EICs (left), equivalent to CVs 1.12V (top), 0.76V (middle) and 0.52V (bottom); Angiotensin peaks are clear of the PEG background

Conclusions

- A miniaturized FAIMS device integrated with a time-of-flight mass spectrometer has been used to demonstrate *real-time* in-line LC-FAIMS-MS separations
- The sweep speed of the FAIMS device is up to 100 times faster than existing commercial devices, allowing multiple scans within even short LC peaks
- The device has demonstrated the ability to separate different charge states of peptides, and to separate peptide ions from background matrices
- Future work will focus on enhancing transmission of lower mass analytes and increasing FAIMS peak capacity

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