A rapid, high-field Field-Asymmetric Ion Mobility pre-filter for improved LC-MS analysis of complex samples

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Introduction

Field-Asymmetric Ion Mobility Spectrometry (FAIMS) offers the potential to enhance LC-MS analysis of complex samples. A FAIMS filter positioned between the LC and MS systems can suppress chemical noise, filter ions with different numbers of charges and separate ions that cannot be differentiated on the basis of retention time or mass/charge. This additional separation becomes significantly more useful if performed fast enough to be carried out simultaneously with the LC-MS assay.

FAIMS devices achieve separation by applying an orthogonal high-frequency asymmetric electric field (dispersion field, or DF) that deflects ions travelling through the device, with the degree of deflection depending on the differential mobility of the ion. By adding a DC compensation voltage (CV), ions with a given differential mobility can be brought back on course. These ions emerge from the device while all others are lost. The CV can be gradually swept through a range of values, allowing ions of different differential mobility to emerge at different times, hence separating ions that would otherwise enter the mass spectrometer simultaneously.

Faster sweep speed can only be achieved by reducing the time the ion spends travelling through the device. The Owlstone FAIMS "chip" has typical ion residence times of 30us, which is 30-3500 times shorter than in other commercial FAIMS devices. This is achieved by fabricating the device by etching the gap into a silicon wafer, reducing both path length and gap width by many orders of magnitude.

An important consequence of the smaller gap size is that the device can support higher field strengths without air breakdown – in the FAIMS device presented here (**Figure 1a/e**), maximum field strength is approximately 4 times that in other existing devices. This gives the potential to resolve species (typically heavier ions) that cannot be separated at lower fields. The small form factor also allows easier integration of the device into the mass spectrometer ion source.

Objective

Our goal was to integrate the miniaturized FAIMS filter onto a mass spectrometer and use it to demonstrate rapid sweeping within LC peak timescales, suppression of chemical noise and elimination of singly charged protein ions.

Instrument Setup

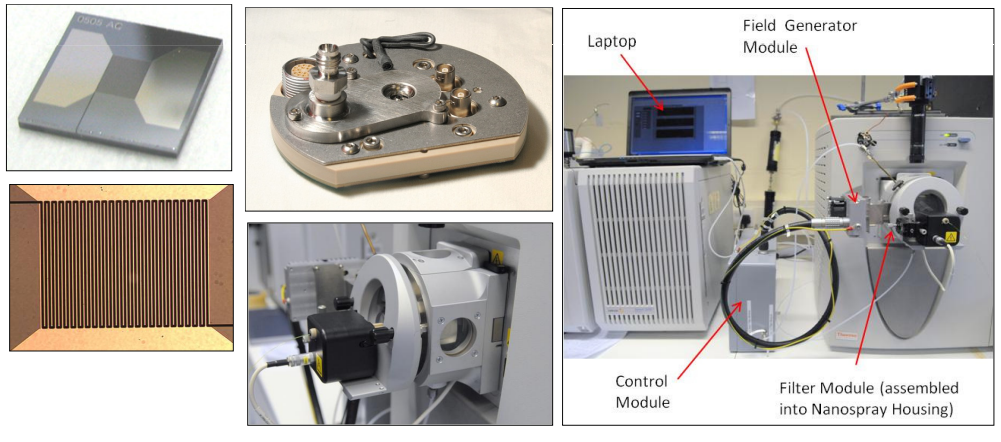

Figure 1: (Clockwise from top left) a: Owlstone FAIMS chip; b: FAIMS cartridge holding chip; c: complete FAIMS-MS System installed on Thermo LXQ; d: filter module integrated into Ion Max source; e: microscope image of top surface of chip showing serpentine analytical gap

The mass spectrometer used was a Thermo LXQ ion trap with an Ion Max Nanospray source. The FAIMS chip was mounted into a "cartridge" (**Figure 1b**) that provided electrical connections to drive the electric fields. On the front of the cartridge was mounted an attachment designed to channel a flow of auxiliary gas to form a curtain flow opposing the ion flow. The cartridge was mounted into a holder (the Filter Module, **Figure 1d**) that could be inserted into the Ion Max housing. This insert formed a seal against the mass spectrometer inlet, allowing carrier gas to be drawn through the chip. The flow rate through the chip could be increased above the baseline flow produced by the mass spectrometer using an additional pump connected to the source housing downstream of chip.

The drive circuitry for generating the alternating fields (the Field Generator Module) was mounted next to the Filter Module, to keep the path length for the RF signals as short as possible. A compact Control Module situated nearby housed the pump, the mass flow controllers, sensors monitoring temperature, pressure and humidity in the downstream gas flow, and PCBs handling control of peripherals and communication with the PC-based software. **Figure 1c** shows the complete system mounted onto the Thermo LXQ mass spectrometer.

Table 1 shows the MS and FAIMS device settings used in the experiments.

MS Settings	FAIMS settings
Ionization: Positive mode	CV sweep range: -4.2V to +4.2V
Spray voltage: 1.6kV	Sweep rate: 1 - 5V/s
Liquid flow rate: 0.5ul/min	Maximum dispersion field: 75 kV/cm
Inlet capillary temp: 250°C	Carrier gas: Nitrogen (99.9995%)
Scan range: 150 – 1500 amu	Carrier gas flow rate: 2.5L/min
	Curtain gas flow rate: 0.7L/min

Table 1: Instrument settings

Experimental results

A. Suppression of chemical noise and separation of singly- and multiply-charged ions

Solvent: 50:50 water:acetonitrile with 0.1% formic acid
Analyte: 74uM Substance P (molar mass 1347.63 g/mol) in a background matrix of PEG400

CV sweeps from -4.2Vto +4.2V were carried out at 1V/s, with dispersion field increased after each sweep from 0-75kV/cm in 25 steps.

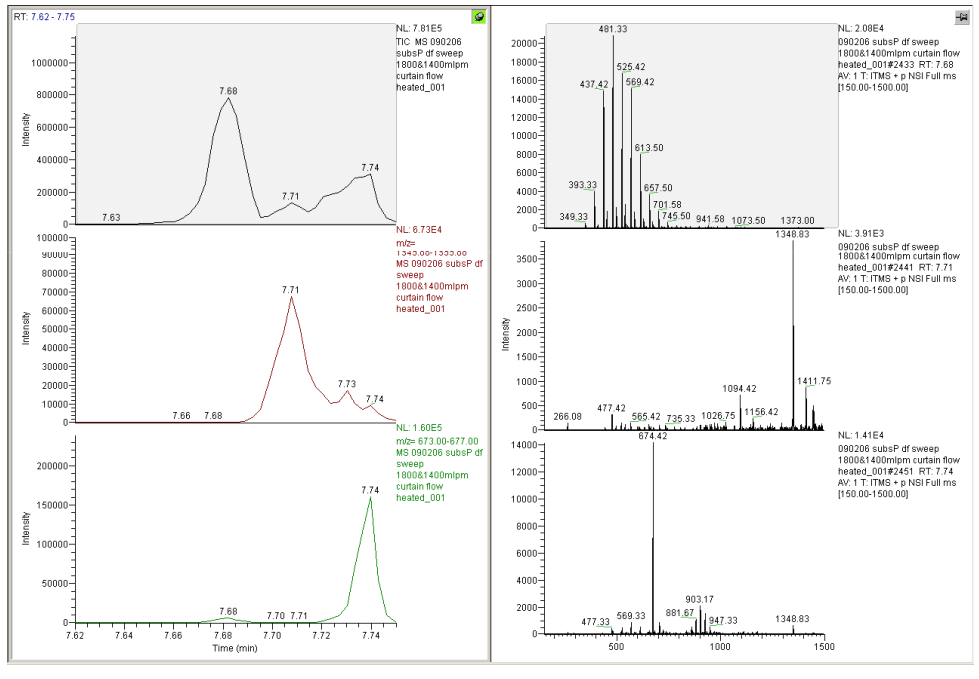

Figure 2: Separation of singly- and doubly-charged Substance P at 75kV/cm FAIMS DF. (Left, top to bottom) TIC; XIC for m/z=1345-1355; XIC for m/z=673-677; (Right, top to bottom) m/z spectra at times corresponding to main peaks in each of the 3 left-hand plots, showing that the single- and doubly-charged ions appear at different times during the CV sweep and that both are separated from the PEG400 background

As seen in **Figure 2**, when the analyte passes through the FAIMS filter with a dispersion field set to 75kV/cm, there is a clear separation in time between the appearance of the singly-charged (m/z=1348) and doubly-charged (m/z=674) forms of Substance P. The singly-charged ion appears predominantly at CV of 1.8V (t=7.71mins) while the doubly-charged ion appears only at a CV of 3.6V (t=7.74mins). The TIC shows that there is a residual peak at a CV of 0.12V (t=7.68mins), consisting mainly of the PEG400.

This demonstrates both how the system can be used to separate singly-charged from doubly-charged ions, and how it can help clear away a complex background.

B. Fast sweeping

Solvent: 50:50 water:acetonitrile with 0.1% formic acid
Analyte: 1mM Loperamide (molar mass 477.03 g/mol), as Loperamide HCl, in a background matrix of PEG400

CV sweeps from -4.2V to +4.2V were carried out first at 1V/s and then at 5V/s. Dispersion field was increased in steps after each sweep up to 75kV/cm.

Figure 3 shows the mass spectrometer TIC during the 75kV/cm DF sweep for the 1V/s and 5V/s scans (top and bottom left, respectively). As shown from the retention time axes, the complete sweeps took 9 seconds and 1.8 seconds, respectively. The peaks on the 5V/s sweep are less well-defined – this is due to the Ion Trap requiring around 150ms for each sample point at this signal intensity. The middle column shows the m/z spectra for the main peak of each TIC and the right-hand column shows the m/z spectra for the secondary peak in each TIC. As these show, the faster sweep is as effective at separating the Loperamide from the PEG400 contamination as the slower sweep.

Typical LC peak widths range from 2 seconds upwards, so at 1.8 seconds for a full FAIMS CV sweep, it should be possible to use the device to provide orthogonal separation in real-time during LC assays.

The FAIMS device itself is not limited to 5V/s – this system can scan at speeds up to at least 40V/s and the next-generation device currently being developed can reach rates at least an order of magnitude faster. This means that if transmission into the MS can be increased further, it will be possible to carry out multiple CV sweeps within a single LC peak.

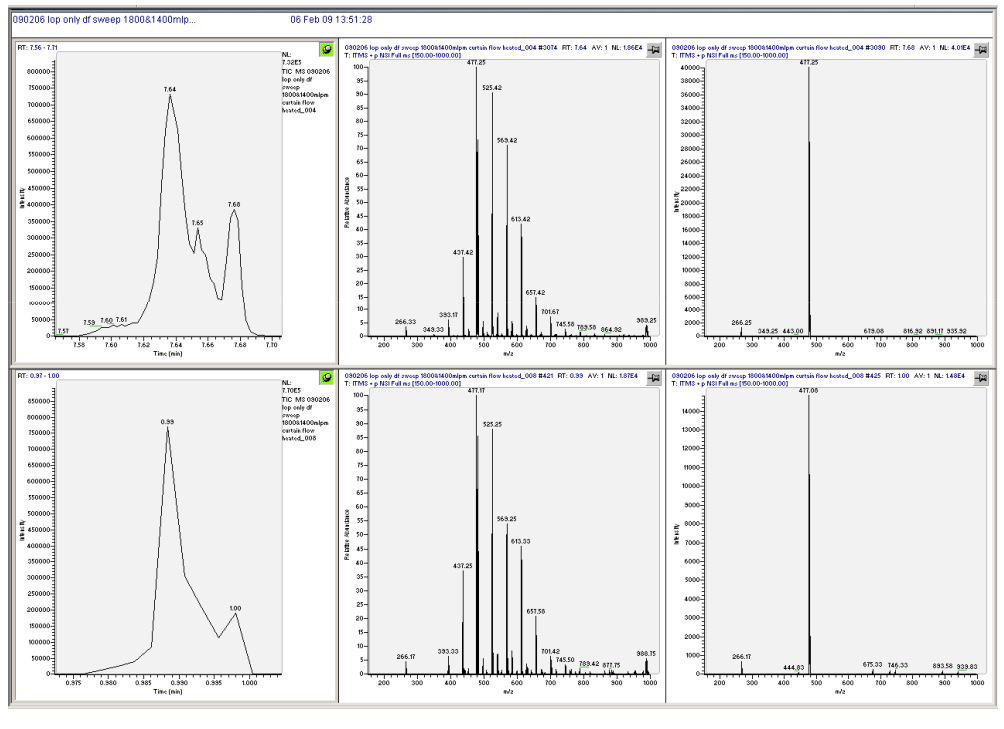

Figure 3: High speed separation of Loperamide from PEG400. Top: 1V/s sweep (9 seconds total time). Bottom: 5V/s sweep (1.8 seconds total time). Left-hand column shows TIC during the sweep. Middle column shows the m/z spectra for the TIC main peak (corresponding to CV of 0.12V). Right-hand column shows the m/z spectra for the left-hand peak (corresponding to CV of 2.4V)

Summary

- A miniaturized FAIMS device has been successfully integrated with an ion trap mass spectrometer
- The FAIMS device has demonstrated separation of singly-charged from multiply-charged ions and suppression of background matrices
- The device can carry out full sweeps in timescales of a few seconds, meaning for the first time, FAIMS separations can be carried out in real-time during LC-MS assays

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