

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

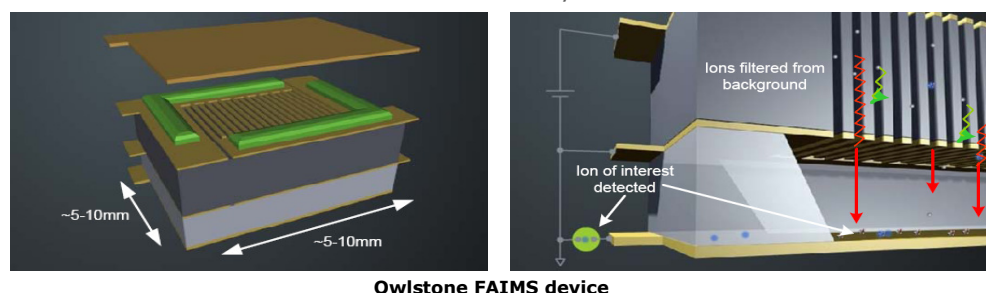
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Owlstone FAIMS Technology

Field Asymmetric Ion Mobility Spectrometry (FAIMS) represents an exciting and opportune technology in the world of scientific analysis, offering the ability to modify and control the behaviour of ions under investigation by varying the field applied to them.

FAIMS has been adapted to complex separation techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS), proving that such an add-on can enhance detection limits and resolution of analytes of interest.



The heart of the Owlstone detection technology is a microchip-sized spectrometer that allows the selective transmission of ions with a particular differential mobility. In FAIMS-MS, the FAIMS system is used as a tuneable filter, providing certain ions with a clear path to the mass spectrometer while blocking others. This means that in complex mixtures, ions of interest can be separated from the chemical background thus maximising the ability to detect a particular analyte.

The microscale size of the 'chip' allows higher separation fields to be reached with much simplified electronics, increasing analytical information and opening up new areas for research. The reduced ion residence time also allows faster analysis, making this system simple to incorporate with an LC-MS and fast enough to perform multiple sweeps on the LC timescale.

Advantages over Existing Systems

The small gap size within the device places it in a region of the air-breakdown voltage (Paschen) curve that allows much higher field strengths to be achieved in any other FAIMS device (Fig. 1). This means the Owlstone FAIMS device is capable of producing ~ 100 KV/cm dispersion field (DF), compared to ~ 30 KV/cm maximum for other systems (an energy equivalent of approximately 40 KJ/mol). It is this parameter that influences resolution, while a DC compensation voltage (CV) provides the ability to control which ion species pass through the filter.

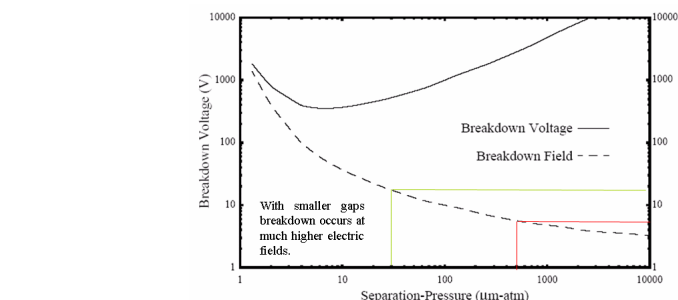


Fig. 1. Paschen Curve demonstrating the theoretical relationship for the DC breakdown voltage of two parallel-plane electrodes immersed in a gas, as a function of electrode separation.

Miniaturization also drives the ion residence times down which reduces the time for analysis – the shallow Owlstone silicon chips allow CV sweeps to be completed in under one second giving 5 – 10 CV sweeps for every LC peak. (Note that larger gap sizes require larger CVs to achieve separation, whereas a device with small gaps can provide the same separation with lower CV values).

The resolution determining dispersion field (DF) is also easier to generate, due to the dramatically lower voltages needed to achieve a given field strength. This reduces the size and complexity of the drive electronics, and this together with the small device size, leads to a system that is compact and easy to integrate with the mass spectrometer.

FMS-EV1: MS Integration

The proof-of-principle system (FMS-EV1) was developed with a custom made housing for the chip mounted as an extra layer within a standard 'IonMax' ion source available for all new Thermo mass spectrometers.

Ions were produced using a dynamic nanospray probe, filtered through an Owlstone chip device and directed into a Thermo LXQ mass spectrometer.

Nanospray was chosen for the lower liquid flow rates, lower voltages and lack of additional gas requirements.

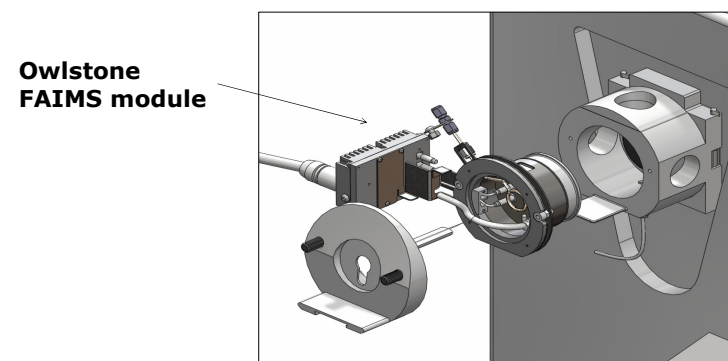


Fig. 2. Exploded CAD drawing of the FMS-EV1 system.

- A design that is mounted inside the source housing keeps the distance from probe tip to chip inlet equivalent to that of the MS interface without the FAIMS unit.
- An air tight seal can be created against the MS interface to allow for gas to be drawn through the FAIMS unit under a negative pressure.
- Further gas control floods the nanospray section with inert gas (Nitrogen) to aid the transport of ions through the device.
- A shoebox sized control box contains the gas management, signal generation and data collection systems all connected to a laptop PC so a FAIMS spectrum can be viewed in real-time alongside the mass spectral data.



Fig. 3. (right) Photo of the FAIMS device mounted in the nanospray housing.
Fig. 4. (left) photo of the entire add-on system integrated on to the Thermo LXQ

Experimental Conditions

Solutions and Solvents	Nanospray conditions
<ul style="list-style-type: none"> 50:50 ACN:H₂O with 0.1% formic acid as the solvent Mixture of PEG 400 at 28.2 mM and Loperamide.HCl at 1.95 mM 	<ul style="list-style-type: none"> Positive ion mode + 1.6 kV spray potential Liquid flow: 50 nLmin⁻¹ Liquid delivered from syringe pump
FAIMS conditions	MS conditions
<ul style="list-style-type: none"> CV sweep start: -4 V CV sweep range: 8 V CV sweep rate: 2 V s⁻¹ DF value: 75.6 KVcm⁻¹ Auxiliary gas: Nitrogen (99.9995%) Aux. gas flow rate: 2.5 Lmin⁻¹ 	<ul style="list-style-type: none"> Inlet capillary voltage: -15 V Inlet capillary temp: 250°C Tube lens voltage: 90 V Scan range: 300 – 1000 amu Scan speed: Turbo

Results and Conclusions

The following case is an example of how the FAIMS pre-filter can improve analysis. Detection of Loperamide within a matrix of the excipient PEG 400 can be difficult due to the overlap of the Loperamide.H⁺ ion (477 amu) with the PEG 400 ions (440 – 520 amu). The FMS-EV1 has been employed to improve the resolution of these ions.

The following results are a combination of MS Total Ion Chromatogram (TIC) plots and mass spectral data all obtained from the FMS-EV1 and LXQ for the solution mentioned previously.

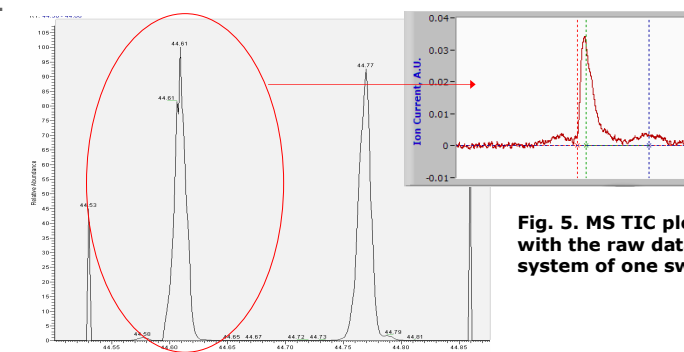


Fig. 5. MS TIC plot of two CV sweeps with the raw data from the FAIMS system of one sweep (insert).

Below, the same CV sweeps zoomed in to see the fine structure:

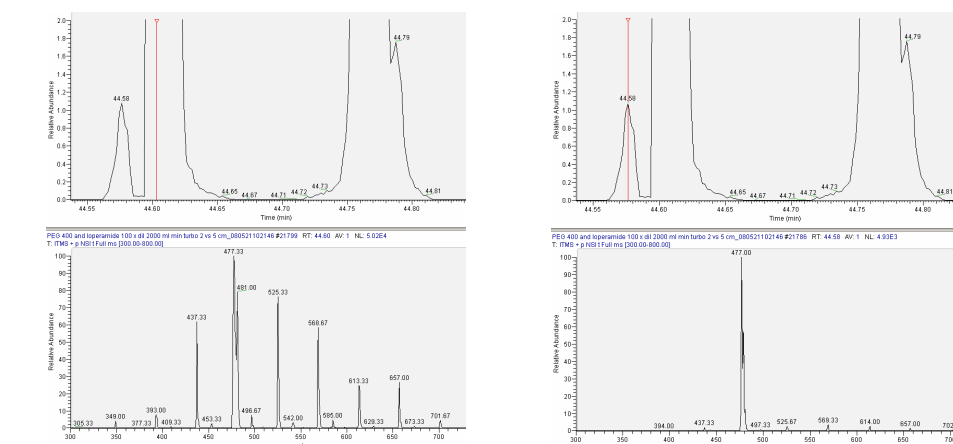


Fig. 6. Mass spectra from different points during the CV sweep. The red cursor shows the points where PEG 400 (left) and Loperamide (right) reach the mass spectrometer.

The main observation from the results is the improvement in detection capability for the protonated Loperamide ion at a CV position of -1.2V through the suppression of the complex PEG background (Fig 6, bottom right).

This compensation voltage sweep was performed in 12 seconds at higher resolution than necessary, with respect to the ion current generated. This timescale can be further reduced by altering parameters on the FAIMS and MS systems.

However, it was not possible to isolate PEG alone suggesting that some Loperamide remained unresolved from the PEG, within the broad peak at 0V CV. This would impact on the quantification of components in the mixture. A possible reason for this effect may be insufficient desolvation within the ionisation region leading to charged droplets entering the analysis region of the FAIMS device. Large, charged droplets would show little or no movement under the influence of the electric fields within the FAIMS device and would remain a mixture of components until the MS analyser.

Further Work in Progress

- The introduction of a heating stage or curtain plate before the FAIMS device to increase desolvation efficiency
- Hyphenation with an LC system to ensure that the timescales for each instrument are compatible
- Design of assemblies for alternative mass spectrometer manufacturers with academic and commercial partners
- Implementation new electronic hardware to give more flexibility in operation and selection of FAIMS parameters