In-source fragmentation of FAIMS-selected ions in combination with time-of-flight mass spectrometry

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Introduction

- In-source CID allows the acquisition of fragment data from intact electrospray ionisation (ESI) generated ions, but in the absence of precursor ion selection, complex mixtures yield overlapping product ion spectra.
- Field asymmetric waveform ion mobility spectrometry (FAIMS) separates gas phase ions based on differences in ion mobility under alternating high and low electric fields as they travel between two electrodes at atmospheric pressure.
- We present a tandem FAIMS-CID-MS approach that allows pre-selection of ions on the basis of differential mobility providing orthogonal separations prior to CID-MS.

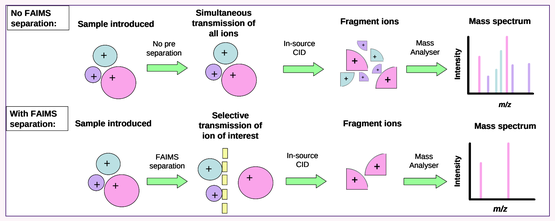

Figure 1. Schematic representation of CID-MS and FAIMS-CID MS data acquisition.

Experimental

A miniaturised FAIMS device (Owlstone Ltd. ultra-FAIMS) has been interfaced to an Agilent 6230 series ToF MS with a Jet Stream ESI source and an Agilent 1200 series LC (Figure 2). Previously the hyphenated system has been shown to enhance the mass spectral detection of peptides[1]

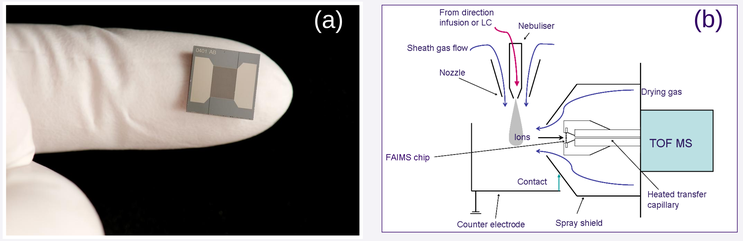

Figure 2. (a) The ultra-FAIMS chip **(b)** and interface with the Agilent 6230 series TOF.

- The ultra-FAIMS consists of multiple parallel planar electrode channels, 35 µm wide, allowing higher electric field intensities (60 kV cm^{-1}) to be applied and allows for a shorter ion residence time (~20 µs) suitable for fast scan rates compatible with LC[2].
- The interleaved electrode pairs enable multiple, simultaneous ion transmission through the electrodes, reducing the charge capacity constraints and improving transmission.
- Leucine enkephalin (Leu Enk), bradykinin, bombesin, luteinising hormone releasing hormone (LHRH) and MRFA were prepared at a concentration of 10 pmol $µL^{-1}$.
- 2-hydroxy-(4-octyloxy) benzophenone (HOBP) and PEG 400 were analysed at 5.1 pmol/µl and 104 pmol/µl concentrations, respectively.
- Mass spectrometer source conditions and ion optics were optimised prior to FAIMS analysis with the fragmentor voltage varied between 150 and 400 V for the transmission and fragmentation of ions, respectively.

Results: FAIMS-CID-MS analysis of a peptide mixture

- Tandem FAIMS-CID-TOFMS was applied to the analysis of a peptide mixture (Figure 3a).
- The $[M+2H]^{2+}$ bradykinin ion was isolated from the other singly and multiple charged peptide ions by FAIMS at a CV of + 3.95 - 4.05 V (Figure 3b),
- In-source CID-MS of the peptide mixture without FAIMS pre-selection gives a complex product ion spectrum (Figure 3c) with many fragment ions of interest not observed.
- Using FAIMS to selectively transmit the bradykinin $[M+2H]^{2+}$ ion prior to CID filtered out unrelated precursor ions producing a product ion mass spectrum containing the characteristic fragments of bradykinin (Figure 3d).

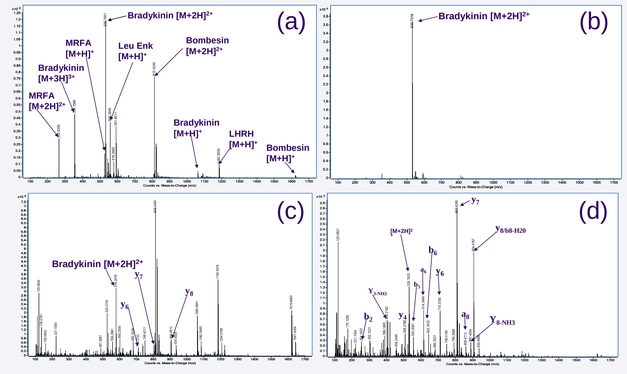

Figure 3. CID-MS and FAIMS-CID-MS spectra of peptide mixture.

- Comparison of peak lists generated by the Agilent Mass Hunter software identifies 21 characteristic bradykinin fragment ions using the FAIMS-CID-MS method, compared to just 6 without FAIMS separation.

The FAIMS-CID-MS method was also combined with LC. Co-eluting peptide peaks (Figure 4a) produce a mass spectrum containing singly and multiply charged ions of LHRH and bradykinin (B) peptides (Figure 4b). By applying a CV of 1.7-1.8 V $[M+2H]^{2+}$ LHRH was isolated from the other peptide ions (Figure 4c). The complex LC-CID-MS spectrum of the peptide mixture (Figure 4d) is simplified by FAIMS pre-selection enhancing the detection of the characteristic product ions of LHRH (Figure 4e).

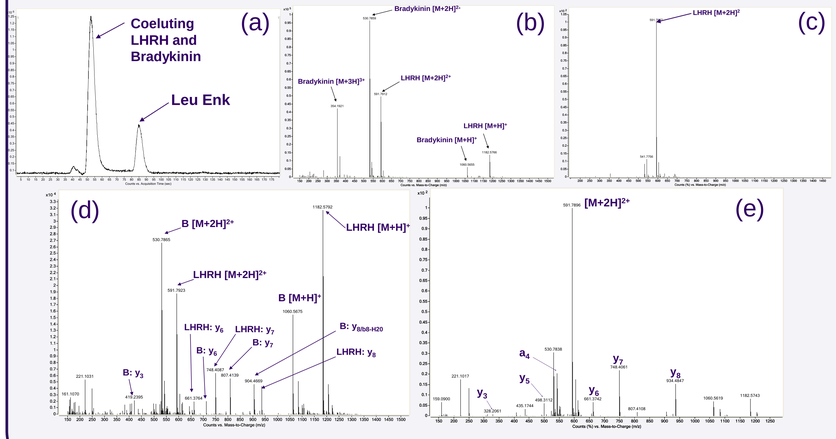

Figure 4. LC-CID-MS and LC-FAIMS-CID-MS spectra of co-eluting peptides.

Results: FAIMS-CID-MS analysis of isobaric species

- Isobaric ions derived from HOBP and PEG400 n=7 excipient (17.7 ppm mass difference) are sufficiently close in *m/z* value that they would not be resolved by a quadrupole mass analyser in MS/MS analysis (Figure 5a).
- The two compounds were separated by FAIMS on the basis of differential mobility. Figure 5b shows an extracted ion chromatogram for *m/z* 327.2 showing the resolution of PEG and HOBP ions by FAIMS-MS analysis. Quasi-static filtering (CV range 0.6– 0.7 V) selectively transmitted HOPB as the base peak.
- The mass measurement of the unresolved peak gave a 12 ppm error for HOBP. Selection on the basis of differential mobility identified HOBP with a mass accuracy of 3 ppm.

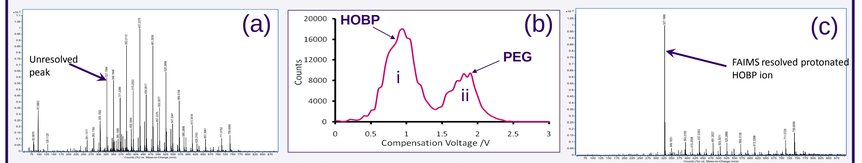

Figure 5. FAIMS-CID-MS analysis of HOBP and PEG mixture.

In-source CID-MS of the mixture gives a complex product ion spectrum (Figure 6a). A simpler CID product ion spectrum is observed when FAIMS is applied to transmit HOBP ions (Figure 6b, CV range 0.6 – 0.7V) by filtering out the PEG 400 ion peaks, reducing complexity of the mass spectrum, aiding the identification of HOBP fragments.

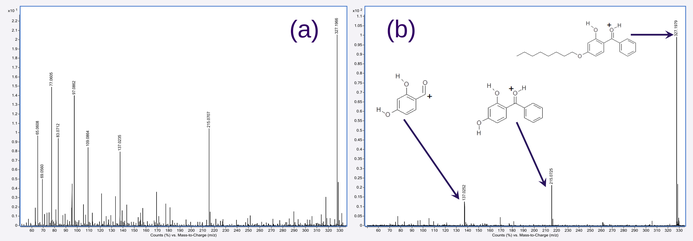

Figure 6. In-source CID-MS of PEG 400 and HOBP without (a) and with (b) FAIMS separation.

Conclusions

- FAIMS has been combined with MS and LC-MS for the selective transmission of pre-selected ions prior to CID within the MS source.
- Transmission of selected peptide ions from a mixture enhances the detection of characteristic fragment ions, facilitating peptide identification.
- The tandem FAIMS-CID-MS approach has been shown to resolve isobaric species sufficiently close in *m/z* value that they could not be resolved by low resolution mass-selection in a quadrupole mass analyzer in conventional MS/MS analysis giving increased confidence in the structural elucidation of molecules.

References

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