Enhanced selectivity in the qualitative and quantitative analysis of peptides using in-source fragmentation of FAIMS-selected ions combined with LC-MS

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Introduction and overview

- In-source CID allows the acquisition of fragment ion 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 pass between two electrodes at atmospheric pressure.[1,2]
- We present a tandem FAIMS-in source CID-MS approach, termed FISCID-MS (Figure 1), that exploits the pre-selection of ions on the basis of differential mobility prior to in source CID-MS for the qualitative and quantitative analysis of peptides in human plasma.[3]

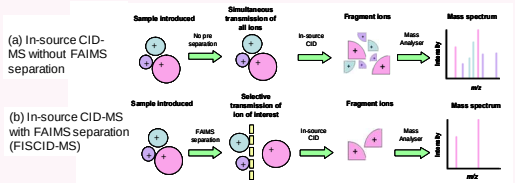

Figure 1. Schematic representation of in-source CID-MS and FISCID-MS data acquisition.

Methods

A prototype miniaturised FAIMS device (Owlstone Ltd.) was interfaced to an Agilent 6230 series TOF MS with a Jet Stream ESI source and an Agilent 1200 series LC (Figure 2). The FAIMS device consists of an array of parallel electrodes (35 µm x 300 µm) to which an asymmetric dispersion field (DF = 47 kV/cm) was applied. Ions of a selected differential ion mobility are transmitted through the electrodes by application of a compensation voltage (CV).

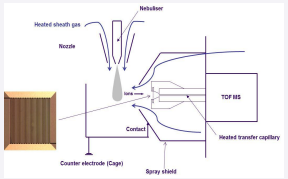

Figure 2. Schematic diagram of the miniaturised FAIMS chip interfaced with a TOF mas spectrometer.

Bradykinin and LHRH (EHWSYGLRPG) peptides (Sigma Aldrich, UK) were prepared at 10 pmol μL^{-1} in 50/50 (v/v) methanol/water + 0.1 % formic acid (FA). Acetonitrile (ACN, 400 μL) was added to plasma (200 μL) to precipitate proteins prior to digestion with trypsin (Promega, UK) in 100 mM NH_4HCO_3 at 37 °C and cleaned-up using a C18 SPE cartridge. The eluate was evaporated to dryness and reconstituted in 90:10 water:ACN with 0.1% FA.

References

1. Brown, L.J.; Toutoungi, D.E.; Devenport, N.A.; Reynolds, J.C.; Kaur-Atwal, G.; Boyle, P.; Creaser, C.S.; *Anal. Chem.*, **2010**, 82, 9827. **2.** Shvartsburg, A.A.; Smith, R.D.; Wilks, A.; Koehl, A.; Ruiz-Alonso, D.; Boyle, B.; *Anal. Chem.*, **2009**, 81, 6289. **3.** Brown, L. J. et al., *Anal. Chem.*, **2012**, 84, 4095. **4.** MASCOT search engine, http://www.matrixscience.com/, accessed 27/10/11

Acknowledgements

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LC-FISCID-MS analysis of a peptide mixture

The LC-FISCID-MS method is demonstrated in Figure 3. Co-eluting bradykinin and LHRH (Figure 3a) produced a mass spectrum containing ions from both peptides (Figure 3b). Applying a CV of 1.7-1.8 (FAIMS spectra shown in Figure 3a (insert)), the $[M+2H]^{2+}$ ion of LHRH is preferentially transmitted by the FAIMS chip (Figure 3c). The LC-in source CID-MS spectrum of the co-eluting peptides (Figure 3d) is simplified by LC-FISCID-MS with FAIMS selection of LHRH (Figure 3e).

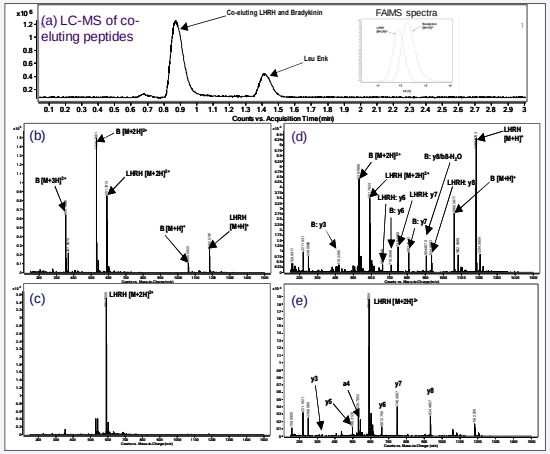

Figure 3. LC-MS and LC-FISCID-MS analysis of co-eluting peptides

Results: Peptide quantification by LC-FISCID-MS

The quantitative potential of LC-FISCID-MS was evaluated for the determination of an exogenous peptide, gramicidin S, spiked into human plasma. Calibration graphs for the LC-FAIMS-MS analysis of the gramicidin $[M+H]^+$ ion (*m/z* 571) and the LC-FISCID-MS analysis of the four FAIMS-selected in-source CID product ions *m/z* 311, 424, 685 and 798) show a linear response (R^2 > 0.99) in the range 0.45-9.0 µg/mL (Figure 6). The selected ion response (*m/z* 311) fragment of the $[M+2H]^{2+}$ ion of gramicidin obtained from the LC-FISCID-MS analysis of the plasma is shown in Figure 4.

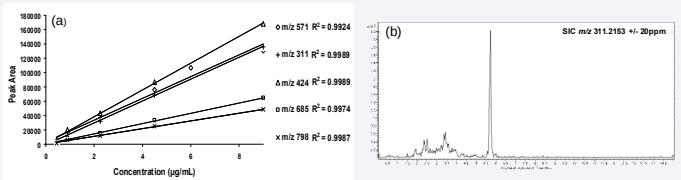

Figure 4. (a) Calibration graphs for the LC-FISCID-MS analysis of the FAIMS-selected (CV 1.75-1.85 V) $[M+2H]^{2+}$ precursor ion (*m/z* 571) and four product ions (*m/z* 311, 424, 685 and 798) of gramicidin S spiked in human plasma tryptic digest and (b) LC-FISCID-MS chromatogram for *m/z* 311 fragment ion.

The %RSD for the peak area of the FAIMS-selected $[M+2H]^{2+}$ precursor ion (*m/z* 571) without in-source fragmentation was 5.1% (n=6; 0.45 µg/mL). LC-FISCID-MS precision for the fragment ions at *m/z* 311, *m/z* 424, *m/z* 685 and *m/z* 798, was 13.3 %, 14.6 %, 13.1 % and 8.2 % respectively

Results: LC-FISCID-MS analysis of tryptic peptides in plasma

The LC-FISCID-MS method was applied to the analysis of a complex sample of tryptic peptides derived from depleted human plasma proteins. The plasma tryptic digest was initially analysed by LC-MS without FAIMS separation or fragmentation (Figure 5a).

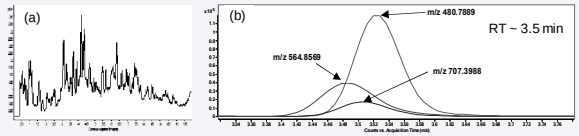

Figure 5. (a) TIC of tryptic peptides from LC-MS analysis of human plasma and (b) SIC of three tryptic peptides from human plasma co-eluting at a retention time of ~3.5 minutes.

The complex nature of the sample resulted in the co-elution of peptides. For example, the SIC for *m/z* 480, observed at RT 3.52 minutes, shows overlap with other co-eluting peptide ions (Figure 5b). This can be seen in the mass spectrum (Figure 6a) obtained by summing the spectra across the *m/z* 480 peak at half height. Data acquired with the FAIMS set to a CV of 2.5-2.6 V filtered out the co-eluting *m/z* 564 and 707 ions, with the *m/z* 480 species preferentially transmitted, resulting in a simplified mass spectrum (Figure 6b).

The peptide ions were subjected to in-source CID (fragmentor voltage of 340 V) both with, and without, prior FAIMS separation. The LC-CID-MS mass spectrum (Figure 6c) shows spectral overlap of fragments from the co-eluting peptides, compared to the LC-FISCID-MS spectrum (CV 2.5-2.6 V) (Figure 6d) containing far fewer fragments, due to the simplification of the mass spectra prior to fragmentation.

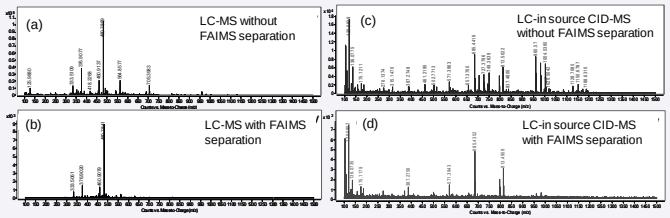

Figure 6 (a) LC-MS spectrum of peak at 3.52 min, (b) LC-FAIMS-MS spectrum of peak at 3.52 min with a CV of 2.5-2.6 V applied (c) LC-CID-MS spectrum of peak at 3.52 min, and (d) LC-FISCID-MS spectrum at a CV of 2.5-2.6 V, and fragmentor voltage of 340 V.

Peptide identification was carried out via the MASCOT search engine,[4] searched against the SwissProt protein database. All ions with greater than 10% of the base peak intensity were included in the search peak list. LC-in source CID-MS yielded no significant hits on the data base. However, with the CV of 2.5-2.6 V applied, human serum albumin (HSA) was identified as the top hit, the only significant match, with a confidence score of 34 (where 27 or above was deemed statistically significant at a 95% confidence interval). The ACN depletion procedure removes >99.6% of HSA from plasma, so the residual peptide concentration is <0.4%.

Conclusions

- **FAIMS has been combined with LC-MS for the selective transmission of differential mobility pre-selected ions prior to in-source CID (LC-FISCID-MS).**
- **Transmission of FAIMS-selected peptide ions enhances the detection of characteristic fragment ions of the selected peptides, generated by in-source CID, by eliminating fragments associated with co-eluting peptides**
- **The quantitative potential LC-FISCID-MS has been demonstrated for the determination of an exogenous peptide spiked into human plasma.**

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