Isolation of peptide ions for sequence analysis using microchip FAIMS-in source CID-MS (FISCID-MS)

Peptide identification and structural elucidation without MS/MS

Introduction

Collision-induced dissociation in the intermediate pressure region between an atmospheric pressure ion source and the vacuum of a mass analyzer, commonly referred to as ***in-source CID*** or cone voltage fragmentation, is a well-known method of inducing fragmentation of ions passing through this region.[1] This can be achieved through the use of elevated interface voltages in the mass spectrometer interface, and the product ion spectra generated can be used for structural analysis of intact protonated molecules generated by electrospray ionization.[2]

In the absence of precursor ion selection, in-source CID can result in a complex mass spectrum containing overlapping precursor and product ions, which makes spectral interpretation a challenge. Information regarding the relationship between precursor and product ions may also be lost because different precursor ions are fragmented simultaneously. Hence, tandem MS, which allows selection of a single precursor *m/z*, is usually the method of choice for this type of analysis. However when a tandem mass spectrometer is not available, the use of a FAIMS pre-filter prior to in-source CID provides a lower-cost alternative for peptide identification and structural analysis.

This application note demonstrates the use of FAIMS-in source CID-MS (FISCID-MS) for qualitative analysis of model and tryptic peptides.

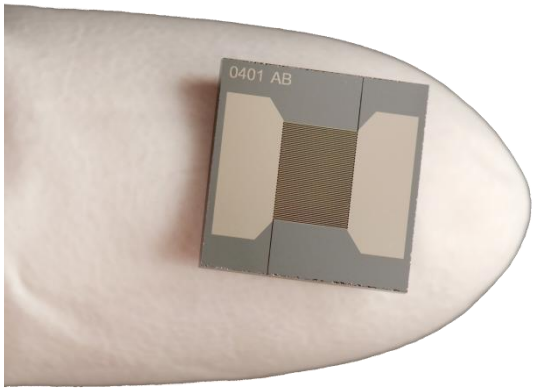

Figure 1: The miniature UltraFAIMS microchip (35µm gap design)

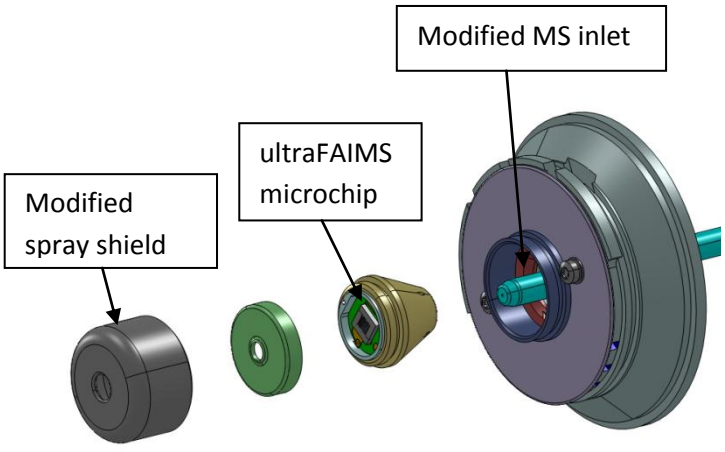

Figure 3: Exploded view of UltraFAIMS interface to Agilent 6230 TOF

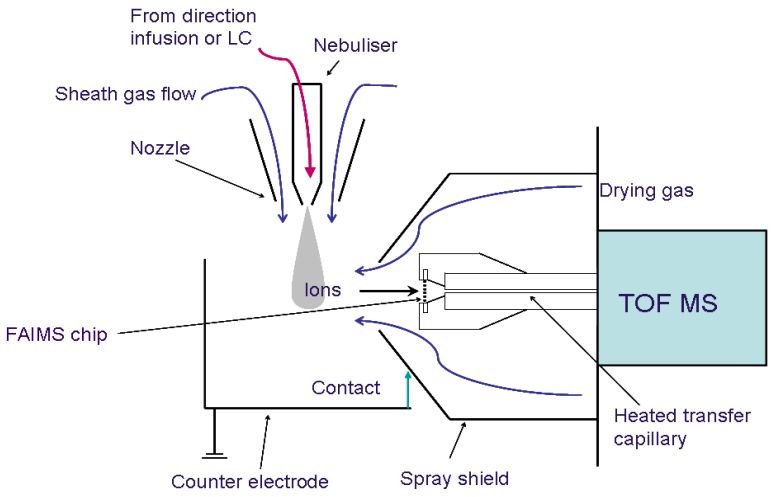

Figure 2: Schematic of Agilent JetStream ionization source with UltraFAIMS interface

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Sample preparation

The model peptides used were Leucine enkephalin (YGGFL), bradykinin (RPPGFSPFR), bombesin (EQRLGNQWAVGHLM), lutenizing hormone releasing hormone (EHWSYGLRPG) and MRFA. These were obtained from Sigma Aldrich (Gillingham, UK) and prepared at a concentration of 10pmol/L in 50/50 (v/v) methanol/water containing 0.1% formic acid (FA).

The tryptic peptides were derived from human blood samples. Acetonitrile (ACN, 400ul) was added to plasma (200µL) to precipitate proteins prior to digestion with trypsin (Promega, UK) in 100mM ammonium bicarbonate 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.

Instrumentation and Methods

The Owlstone ultraFAIMS system was installed on an Agilent 6230 TOF MS with JetStream ESI source. The FAIMS microchip forms an array of parallel channels (35µm x 300µm) across which an asymmetric dispersion field of 47kV/cm was applied. Selected ions were transmitted through the chip by application of an appropriate compensation voltage (CV). "Non-FAIMS" data was also collected for comparison purposes by setting the dispersion field to 0kV/cm and the CV to 0V. ESI was carried out in positive mode. LC and MS conditions are shown in Table 1.

FISCID-MS analysis of model peptides

First, the effect of adding FAIMS to in source-CID-MS was tested with the directly infused model peptide mixture. The precursor ion spectrum (no in-source CID) is shown in Figure 4a.

- Without FAIMS pre-selection, in-source CID-MS of the peptide mixture gives a complex product ion spectrum (Figure 4b) with many fragment ions of interest not observed
- Using FAIMS, the $[M+2H]^{2+}$ bradykinin ion could be isolated from the other singly and multiply charged peptide ions at a CV of +3.95-4.05V (Figure 4c)
- FAIMS-selected transmission of the bradykinin $[M+2H]^{2+}$ ion prior to CID filtered out unrelated precursor ions and produced a product ion mass spectrum containing the characteristic fragments of bradykinin (Figure 4d)

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

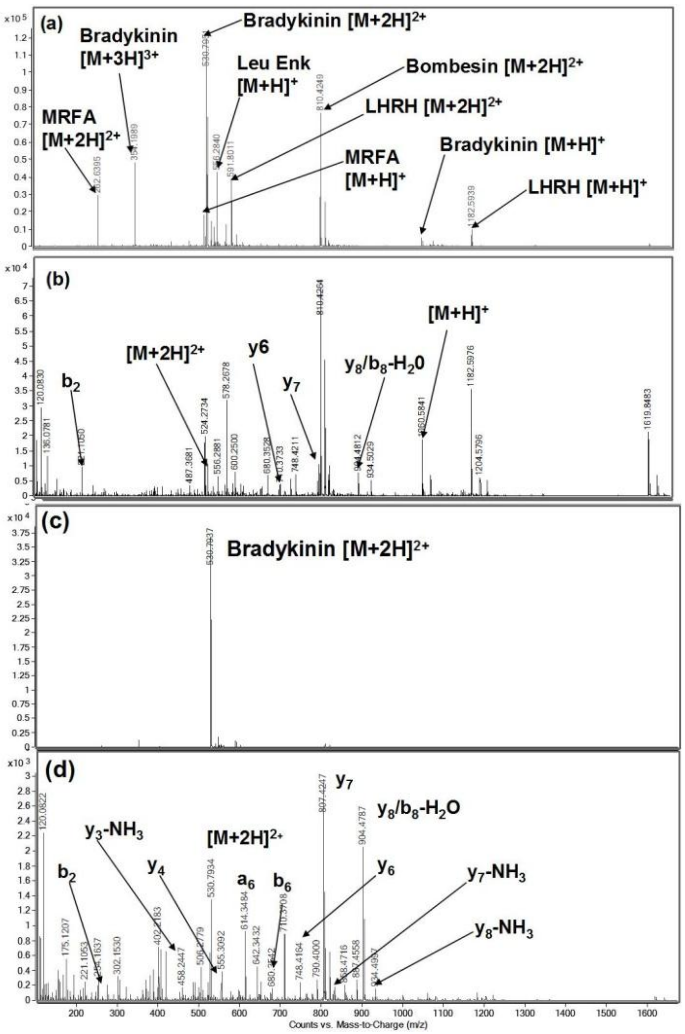

Figure 4: MS, FAIMS-MS and FISCID-MS analysis of a peptide mixture, (a) mass spectrum without FAIMS selection or in-source CID (b) in-source CID-MS (350V) without FAIMS selection; (c) FAIMS-selection of the $[M+2H]^{2+}$ bradykinin ion (CV = 2.6 – 2.7V) and (d) FISCID-MS product ion spectrum of the $[M+2H]^{2+}$ bradykinin ion (CV = 2.6 – 2.7V, fragmentor voltage 350 V).

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The FISCID method was then combined with LC (Figure 5a). Co-eluting peptide peaks produce a mass spectrum containing singly and multiply charged ions of LHRH and bradykinin (B) peptides (Figure 5b). By applying a CV of 1.7-1.8V, $[M+2H]^{2+}$ LHRH was isolated from the other peptide ions (Figure 5c). The complex LC-CID-MS spectrum of the peptide mixture (Figure 5d) is simplified by FAIMS pre-selection enhancing the detection of the characteristic product ions of LHRH (Figure 5e).

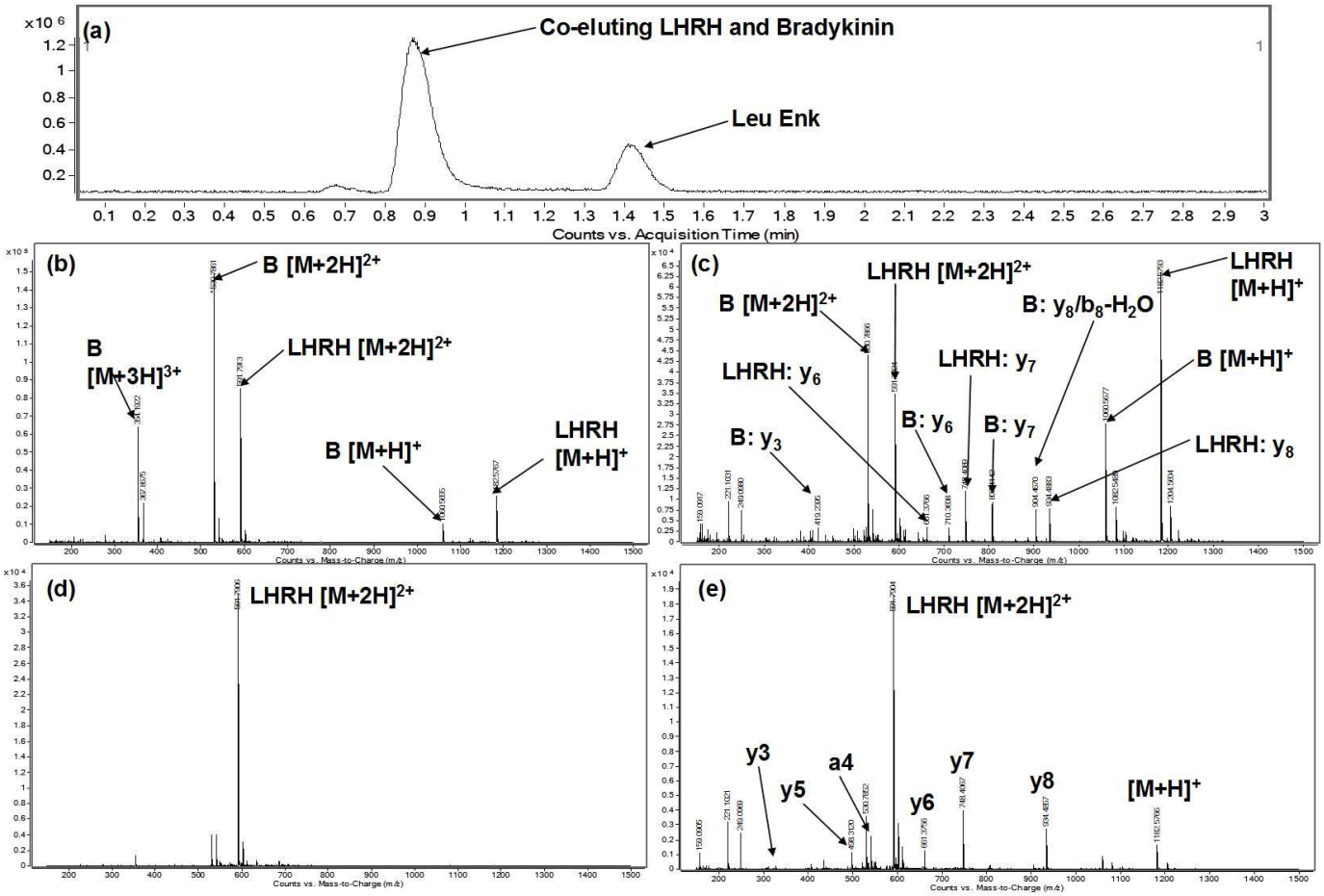

Figure 5: LC-MS and LC-FISCID-MS analysis of a peptide mixture, (a) TIC of peptide mixture, (b) mass spectrum of LC peak at ~0.9min without FAIMS selection or in-source CID, (c) FAIMS pre-selection of LHRH $[M+2H]^{2+}$ ion from co-eluting peptides (CV = 1.8 – 1. 9V), (d) in-source CID-MS (fragmentor voltage 350 V) product ion spectrum without FAIMS pre-selection; (e) FISCID-MS product ion spectrum (CV = 1.8 – 1.9V, fragmentor voltage 350 V) of FAIMS-selected LHRH $[M+2H]^{2+}$ ion from co-eluting peptides.

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 6).

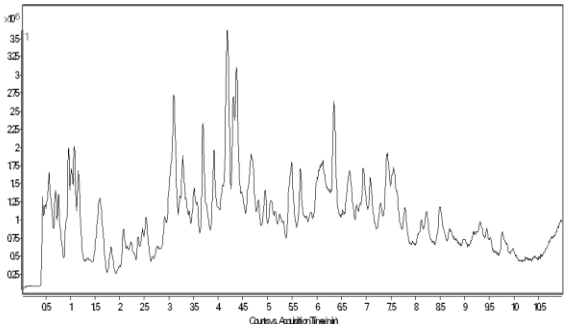

Figure 6: TIC of tryptic peptides from LC-MS analysis of human plasma

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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 7). This can be seen in the mass spectrum (Figure 8a) obtained by summing the spectra across the *m/z* 480 peak at half height. Data acquired with the FAIMS set to a CV of 2.5-2.6V 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 8b).

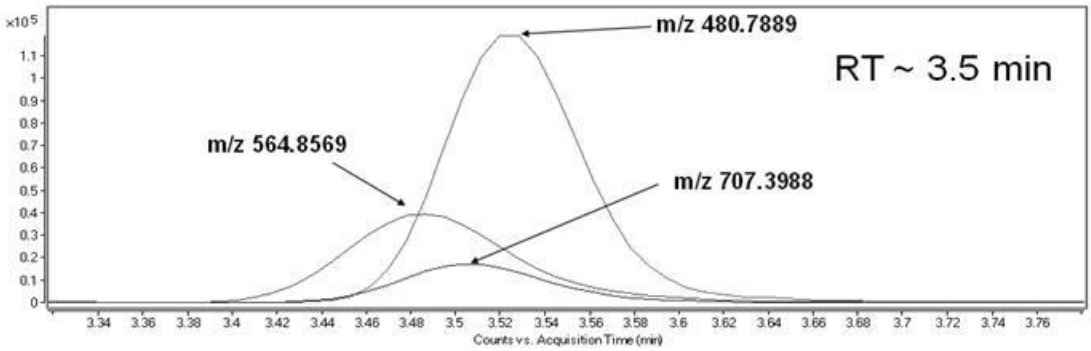

Figure 7: SIC of three tryptic peptides from human plasma co-eluting at a retention time of ~3.5 minutes

The peptide ions were subjected to in-source CID (fragmentor voltage of 340V) both with, and without, prior FAIMS separation. The LC-CID-MS mass spectrum (Figure 8c) shows spectral overlap of fragments from the co-eluting peptides, compared to the LC-FISCID-MS spectrum (CV 2.5-2.6V) (Figure 8d) containing far fewer fragments, due to the simplification of the mass spectra prior to fragmentation.

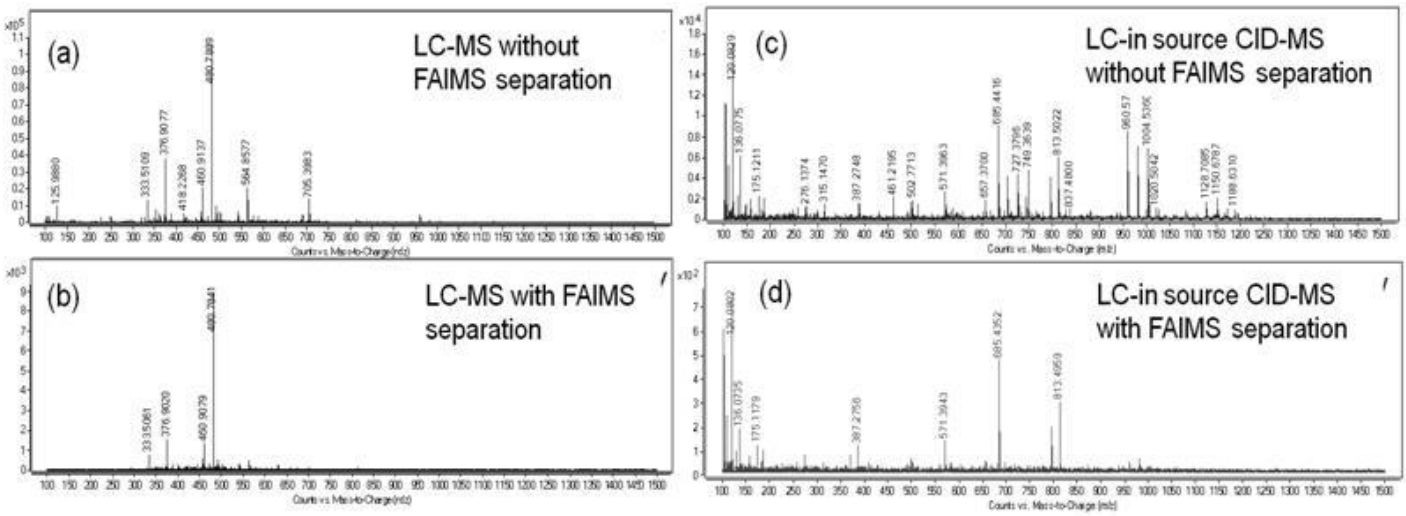

Figure 8: (a) LC-MS spectrum of peak at 3.52min, (b) LC-FAIMS-MS spectrum of peak at 3.52min with a CV of 2.5-2.6V applied (c) LC-CID-MS spectrum of peak at 3.52min, and (d) LC-FISCID-MS spectrum at a CV of 2.5-2.6V, and fragmentor voltage of 340V

Peptide identification was carried out via the MASCOT search engine3, 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 without FAIMS pre-filtering yielded no significant hits on the data base. However, with the CV of 2.5-2.6V 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%.

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Conclusions

- The ultraFAIMS microchip 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
- Product ion spectra obtained via LC-FISCID-MS enabled the identification of plasma proteins with high confidence that could not otherwise be identified without FAIMS selection of the precursor ion, because of the presence of unrelated fragment ions in the mass spectrum.

FISCID-MS therefore offers significant improvements in selectivity for analyses conducted using a mass spectrometer with a single mass analyzer, such as a TOF or quadrupole analyzer. In addition, due to the orthogonal nature of differential mobility separation, it has potential to provide additional selectivity when combined with tandem MS, and in some cases FAIMS-MS/MS or FISCID-MS/MS may provide a faster alternative to LC-MS/MS.

Table 1: MS and LC conditions

Parameter	Setting
TOF acquisition rate	10 scans/sec for model peptides; 2 scans/sec for analysis of plasma
Infusion rate	50uL/min
Nebulizer voltage	1.5kV
Spray shield voltage	400V
Skimmer voltage	65V
Drying gas temperature	150°C
Sheath gas temperature	250°C
Nebulizer pressure	25psig
Capillary voltage	-15V
Drying gas flow	10L/min
Sheath gas flow	12L/min
Fragmentor voltage	150V for intact ions; 340-400V for in-source CID
For analysis of peptide standards:	
LC column	XBridge C_{18} 5 µm column, 2.1x50mm
LC flow rate	0.2mL/min
Gradient	Isocratic 30:70 water:ACN (*v*/*v*) with 0.1% FA for 3 mins
Injection volume	2uL
For analysis of spiked plasma tryptic digest:	
LC column	Poroshell 300SB-C_{18}, 2.1 x 7.5 mm, 5μm
LC flow rate	0.4 mL/min
Gradient	Linear increase from 95:5 water:ACN (*v*/*v*) to 60:40 water:ACN (*v*/*v*) in 10 min, then to 10:90 water:ACN (*v*/*v*/) in 2 min, then initial conditions for 3 mins

References

1. McLuckey, S. A. J. Am. Soc. Mass Spectrom. 1992, 3, 599
2. Bure, C.; Lange, C. Current Organic Chemistry, 2003, 7, 1613.
3. Mascot search engine, http://www.matrixscience.com, date accessed: 27/10/2011.

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