Enhancing Mass Spectrometric Performance with Field Asymmetric Waveform Ion Mobility Spectrometry

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There is growing interest among mass spectrometrists in field-asymmetric waveform ion mobility spectrometry (FAIMS) as a technique that can potentially provide fast orthogonal separation in line with a mass spectrometer. To explore the capabilities of this approach, a miniaturised FAIMS device combined with mass spectrometry (MS) has been used for the separation and determination of a wide range of analytes, including active pharmaceutical ingredients and excipients, drug metabolites, potentially genotoxic impurities, peptides and proteins. The system proved to be compatible with sample introduction via direct infusion, HPLC and thermal desorption. On a single mass analyser, FAIMS-selection of precursor ions before in-source dissociation improved product ion spectra, aiding identification of a drug metabolite in urine and of plasma proteins.

This article reviews the advantages of incorporating miniaturised, chip-based field asymmetric waveform ion mobility spectrometry with mass spectrometry (FAIMS-MS). FAIMS, also known as differential mobility spectrometry, exploits the structural and ion mobility differences between ions under high and low electric fields, allowing a rapid separation that is highly orthogonal to mass spectrometry and liquid chromatography. FAIMS acts as an on-line filter for ions entering the MS and is used to select ions and remove interferences prior to mass analysis.

Ions are carried between a pair of electrode plates positioned between the ion source and mass spectrometer inlet by a flow of neutral gas (typically nitrogen at atmospheric pressure). Alternating high and low electric fields cause ions to oscillate between the electrodes. The magnitude of the asymmetric waveform (dispersion field or DF) can be optimised for separation, as a result of differences in ion mobility at high and low field strengths. Differential mobility causes a drift towards one of the electrodes, resulting in a neutralising collision. This sideways drift can be cancelled out, guiding ions through the device by using an opposing compensation field (CF) which can be held static or scanned to produce a spectrum. Peak location in the CF spectrum is characteristic of ion structure and can be used as a dimension of separation or to preferentially transmit analyte ions whilst other interfering ions are neutralised and filtered out.

The ability to separate isobaric ions is a feature of FAIMS that makes hyphenation with mass spectrometry ideal [1-4]. FAIMS can also be used to rapidly remove interferences from sample matrices that would otherwise require extra steps of sample preparation and separation which increase the analysis time. A further advantage of using FAIMS to remove interferences is that lower limits of quantitation can be achieved [1-3]. FAIMS selection of ions prior to in-source collision-induced dissociation (FISCID-MS) enables tandem experiments to be performed on a single mass analyser platform, significant enhancements in fragmentation spectra were achieved by the addition of a FAIMS separation. The ability to select precursor ions based on differential mobility reduces the complexity of product ion spectra by removing interferences from multiple precursors, similar to mass-selection for selected reaction monitoring performed with a tandem mass spectrometer [2,3]. The enhancement in selectivity and improved quantitative performance offered by combining these techniques is demonstrated for a variety of analytes and matrices.

Experimental

Materials and Methods

The chip-based FAIMS spectrometer (Owlstone Ltd., UK) features multiple planar electrode channels (100 µm gap) to enable high dispersion fields (<300 Td) with a 700 µm path length to give short ion residence times (50 – 250 µs) for rapid compensation field (CF) scanning *(Figure 1)*. FAIMS was combined with an Agilent 6230 TOFMS (Agilent technologies, UK). Samples have been introduced by direct infusion, thermal desorption (Unity1, Markes International, UK) and high performance liquid chromatography (HPLC 1200 series, Agilent Technologies, UK). The materials and methods vary for each application, the details of which can be found in corresponding references.

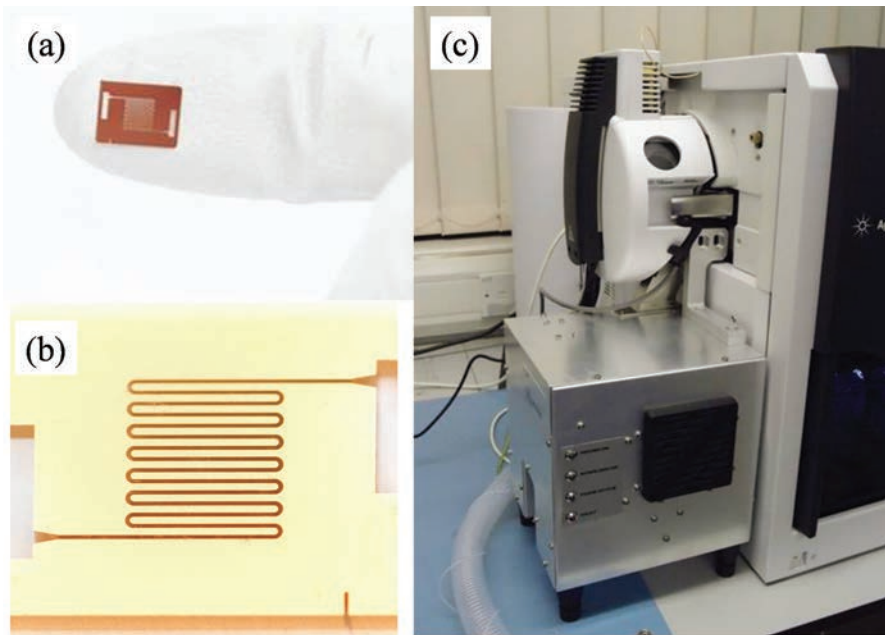

Figure 1. Photographs showing chip-based FAIMS (a) on a finger, showing the scale of the miniaturised device; (b) close-up of FAIMS chip showing the multiple parallel gaps between the electrode plates (ions travel through the device into the plane of the page); (c) incorporated into ESI source of TOF MS, with FAIMS control system positioned below the source.

Results and Discussion

Separation of Isobaric Compounds

2,4,6-trimethylaniline (2,4,6-TMA) and N,N-dimethyl-m-toluidine (N,N-DMT) are potentially genotoxic impurities (PGIs), which are also isobaric. The level of PGIs needs to be monitored in active pharmaceutical ingredients (APIs) because they possess structural characteristics that may exhibit carcinogenicity. Conventional chromatographic techniques (GC-MS and LC-MS) are widely used for monitoring levels of PGI compounds in APIs; however lengthy sample preparation and chromatographic separation reduce sample throughput. There is a need for new analytical strategies to meet the needs of the fast-paced pharmaceutical research and discovery environment [5,6]. Thermal desorption-mass spectrometry (TD-MS) offers an alternative to conventional methods but lacks the selectivity of separation prior to detection. A TOF MS provides qualitative information with moderate quantitative performance, although isobaric analytes cannot be distinguished. The addition of FAIMS separation gives an extra stage of selectivity without increasing analysis time.

A FAIMS scan of 2,4,6-TMA and N,N-DMT at DF 230 Td *(Figure 2 insert)* shows that it is possible to select each PGI at a static CF while filtering out interference from the other. The appropriate FAIMS conditions can be combined with thermal desorption for the rapid detection of the PGIs – demonstrated here in a surrogate API (starch). The thermal desorption peak for the FAIMS-selected PGI can be integrated and quantified *(Figure 2)*. Selective detection of N,N-DMT and 2,4,6-TMA provides limits of quantification at 0.13 and 0.19 ppm (w/w) respectively, approximately an order of magnitude lower than the required 1.5 ppm, the threshold of toxicological concern assuming a 1 g per day dose (European Medicines Agency). Precision was evaluated at 1 ppm, giving RSDs of 7.5 and 8.4% for N,N-DMT and 2,4,6-TMA. The requirements for screening PGIs are easily met by combining FAIMS with TD-MS with significantly less sample preparation and analysis times.

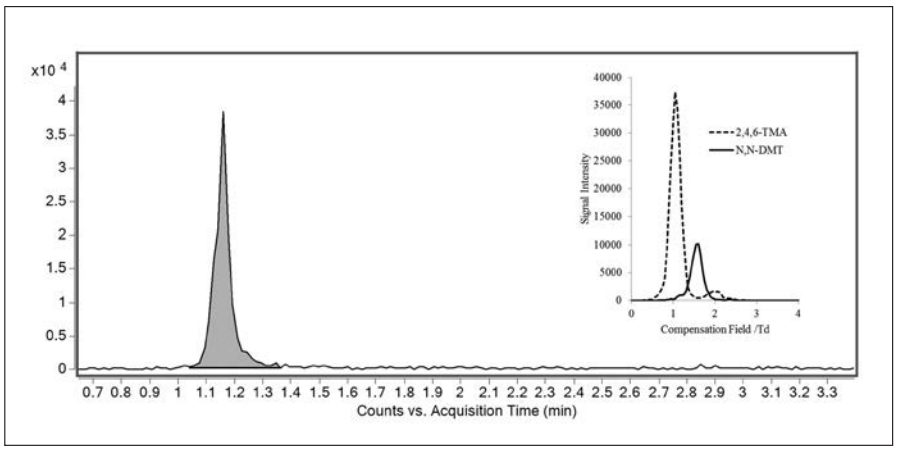

Figure 2. Thermal desorption profile for FAIMS-selected N,N-DMT and (insert) FAIMS spectra for 2,4,6-TMA and N,N-DMT at DF 230 Td. (Reprinted with permission from reference [4]. Copyright 2013, Royal Society of Chemistry).

Reducing Sample Complexity and Chemical Noise

Polyethylene glycol is a component of many pharmaceutical formulations, added to act as solubilizers, dispersants and lubricants, a polyethylene glycol (PEG) matrix gives a complex mass spectrum, masking analytes of interest *(Figure 3a)*. A pharmaceutical intermediate, 2-hydroxy-(4-octyloxy) benzophenone (HOBP) is close enough in mass (17.7 ppm mass difference) to a PEG400 (n=7) peak, which cannot be resolved by a TOF mass analyser (requiring a resolving power of ~130K). A FAIMS scan of HOBP and PEG (n=7) at DF of 48 kV/cm shows 2 peaks for the selected ion response m/z 327.2 *(Figure 3.b insert)*; HOBP is sufficiently separated from the PEG matrix to improve the mass accuracy for HOBP from 11.9 ppm for the overlapping HOBP/PEG peak to 3.3 ppm for the FAIMS-selected HOPB peak *(Figure 2b)*. The removal of the PEG matrix also gives a simpler mass spectrum of HOBP [2].

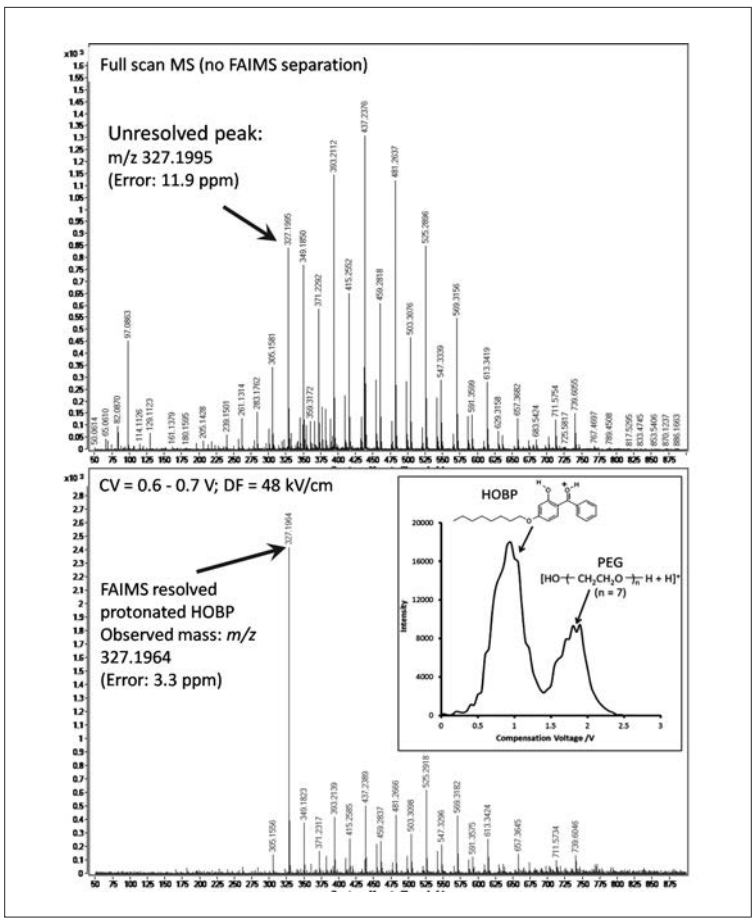

Figure 3. Mass spectra of a mixture of HOBP and PEG 400 (1:20 molar ratio): (a) without FAIMS separation; (b) FAIMS-selected HOBP ion (CV = 0.6-0.7V); and (b insert) CV spectrum (m/z 327.2). (Reprinted (adapted) with permission from reference [2]. Copyright 2012, American Chemical Society).

The complexity of biological matrices makes the detection of compounds at low levels challenging even for UHPLC separation. Ibuprofen 1-β-o-acyl glucuronide (IAG), a metabolite of ibuprofen, was spiked into a pooled urine sample (healthy male and female urine) and filtered before injection onto a C-18 extended Zorbax column (4.6 mm x 50 mm, 1.8 µm). The UHPLC-MS analysis of IAG in urine results in chemical interference from the urine matrix co-eluting with the IAG peak in the selected ion chromatogram (m/z 381), making peak integration challenging and increasing the limit of quantification (Figure 4a). The introduction of more sample preparation steps to remove the matrix interference, changing the chromatography to increase the IAG elution time or using a narrower mass window could resolve IAG from the chemical interference. However, the former two options are often undesirable because they increase analysis time, and the latter option failed to remove chemical interference without also reducing the IAG response. The application of static FAIMS separation upon peak elution reduces the interference observed in the selected ion chromatogram *(Figure 4b)* without prolonging the analysis time.

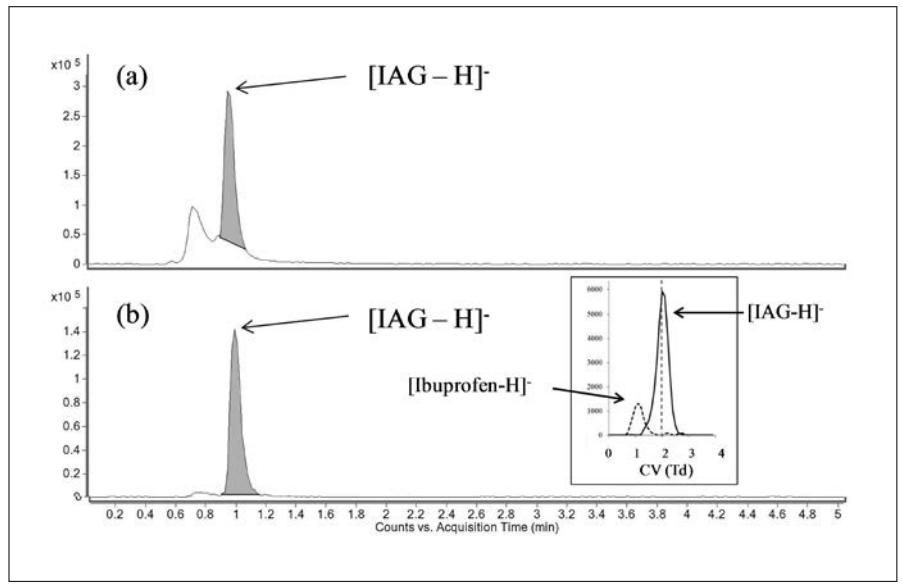

Figure 4. Selected ion chromatograms (m/z 381 ±0.02) for IAG (highlighted) spiked into urine (0.55 µg/ml) analysed by (a) UHPLC-MS (FAIMS off); (b) UHPLC-FAIMS-MS (FAIMS on) with selective transmission of IAG (DF 260 Td, CF 2.2 Td); and (b insert) CF spectrum of IAG (m/z 381, solid line) and ibuprofen (m/z 205, dashed line). (Reprinted with permission from reference [3]. Copyright 2013, Elsevier).

The quantitative performance observed for UHPLC-MS and UHPLC-FAIMS-MS is compared in *Table 1*. Despite a loss in signal, the limit of quantification for FAIMS-selected IAG is reduced 2-fold, increasing the linear dynamic range to 3 orders of magnitude; intra-day precision is also improved. The addition of a FAIMS separation was found to increase the selectivity and sensitivity of a UHPLC-MS system by significantly reducing chemical noise from the urine matrix.

Table 1. Quantitative UHPLC-MS and UHPLC-FAIMS-MS performance for IAG. (Reprinted with permission from reference [3]. Copyright 2013, Elsevier).

LC-MS	LC-FAIMS-MS	
LOQ (µg/ml)	0.018	0.010
LDR (µg/ml)	0.018-11	0.010-11
R2	0.9991	0.9987
Intra-day (% RSD)	5.0	2.7

Combining FAIMS with In-source CID

FAIMS-selected in-source collision-induced dissociation-mass spectrometry (FISCID-MS) is a process of fragmenting FAIMS-selected ions via in-source CID followed by the mass analysis of the fragments. In-source CID is not a commonly used method for product ion analysis because the absence of precursor ion selection results in complex product ion spectra making interpretation difficult. Product ion analysis is therefore usually performed on instruments with precursor ion mass-selection capabilities (e.g. a triple quadrupole or quadrupole-TOF). However, the addition of a FAIMS device to a single mass analyser has the added benefit of increasing selectivity and making product ion analysis possible by combining FAIMS precursor ion selection and in-source CID.

The FAIMS-selected transmission of HOBP from a PEG matrix was presented earlier *(Figure 3)*. Increasing the voltage of a skimmer (fragmentor) lens causes fragmentation of FAIMS-selected HOBP to give easily-identifiable, characteristic fragments which would have otherwise been hidden by interfering fragments from multiple precursors *(Figure 5)*. FISCID-MS was also used to determine fragments of IAG, where the relative intensity of characteristic fragments increased 2-fold (data not shown). FAIMS removes multiple precursors to simplify fragment spectra and aid structural identification of the FAIMS-selected analyte.

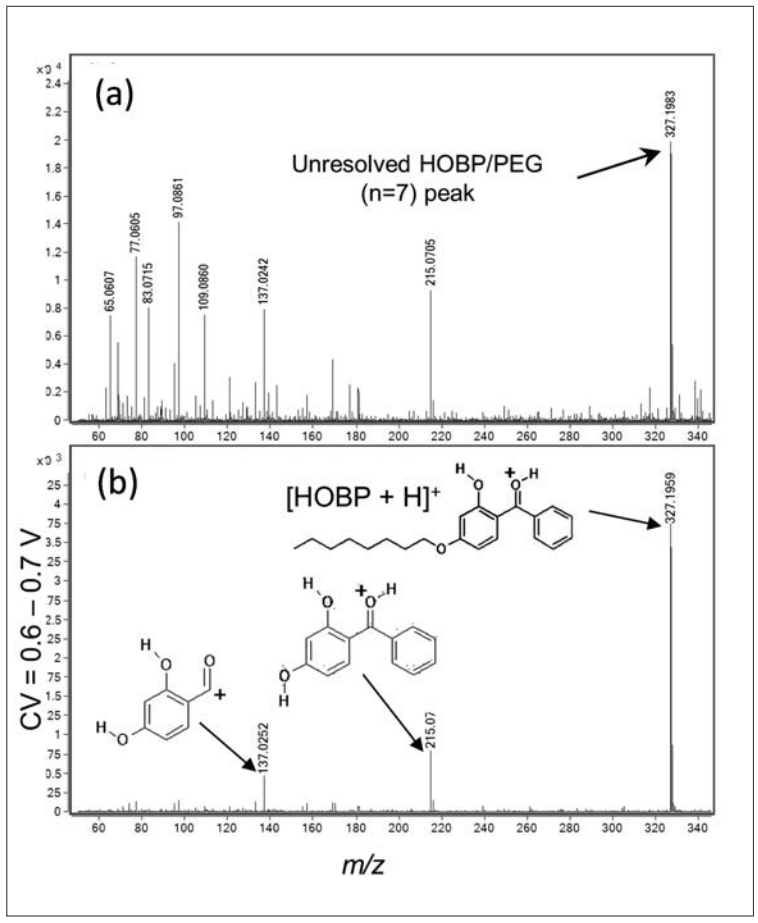

Figure 5. Product ion mass spectra of a mixture of HOBP and PEG 400 (1:20 molar ratio): (a) in-source CID without FAIMS separation; (b) FISCID-MS of selected HOBP ion (CV = 0.6-0.7V). (Reprinted (adapted) with permission from reference [2]. Copyright 2012, American Chemical Society).

The potential of using FISCID-MS for peptide identification was tested using peptide standards. A peptide mixture containing bradykinin, tetrapeptide MRFA, leucine enkephalin (LeuEnk), bombesin, and leutenising hormone releasing hormone (LHRH) produced complex mass spectra with multiple charge states for each peptide. Isolation of [Bradykinin+2H]2+ from the other singly and multiply charged peptides by FAIMS selection prior to in-source CID resulted in the identification of 21 characteristic bradykinin fragments due to significantly simplified fragment data, compared to just 6 without FAIMS pre-selection (data not shown).

The quantitative performance of LC-FISCID-MS was evaluated by spiking gramicidin S, not present in human plasma, into a human plasma tryptic digest. The relative standard deviation (RSD) for the precursor ion [gramicidin S+2H]2+ (9 ng on-column, m/z 571, n=6) was 5.1%, and less than 15% for the fragment ions (m/z 311, 424, 685 and 798) generated by FISCID-MS. The gramicidin precursor and product ions gave a linear response in the range 0.45-9 µg/ml for gramicidin S.

The LC-FISCID-MS method was also applied to the analysis of a complex mixture of tryptic peptides derived from depleted human plasma proteins to test the ability for peptide identification in a real sample. LC-MS of the plasma sample shows the presence of many components *(Figure 6.a)* resulting from the co-elution of tryptic peptides. The ion, m/z 480.8, at retention time of 3.5 min co-elutes with other compounds *(Figure 6.b)*, which are observed in the mass spectrum taken across the SIC m/z 480.8 peak at half height *(Figure 6.c)*. FAIMS selection (CV 2.5-2.6 V) removes the interfering ions (e.g. m/z 564 and 707) from the mass spectrum *(Figure6.d)*. Product ion peaks from LC-CID-MS *(Figure 6.e)* and LC-FISCID-MS *(Figure 6.f)* were used for peptide identification. LC-FISCID-MS provided a simpler product ion spectrum with fewer but more prominent peaks. Peptide identification was carried out via the MASCOT search engine [7], using the SwissProt protein database. No significant hits were yielded from the analysis without a FAIMS separation, however LC-FISCID-MS gave human serum albumin (HSA), present at <0.4% in the depleted serum, as the top hit (confidence score 34, 27 or above deemed significant). The enhancement in selectivity, reproducibility and linear response shows that LC-FISCID-MS has the potential to offer significant benefits over a single mass analyser.

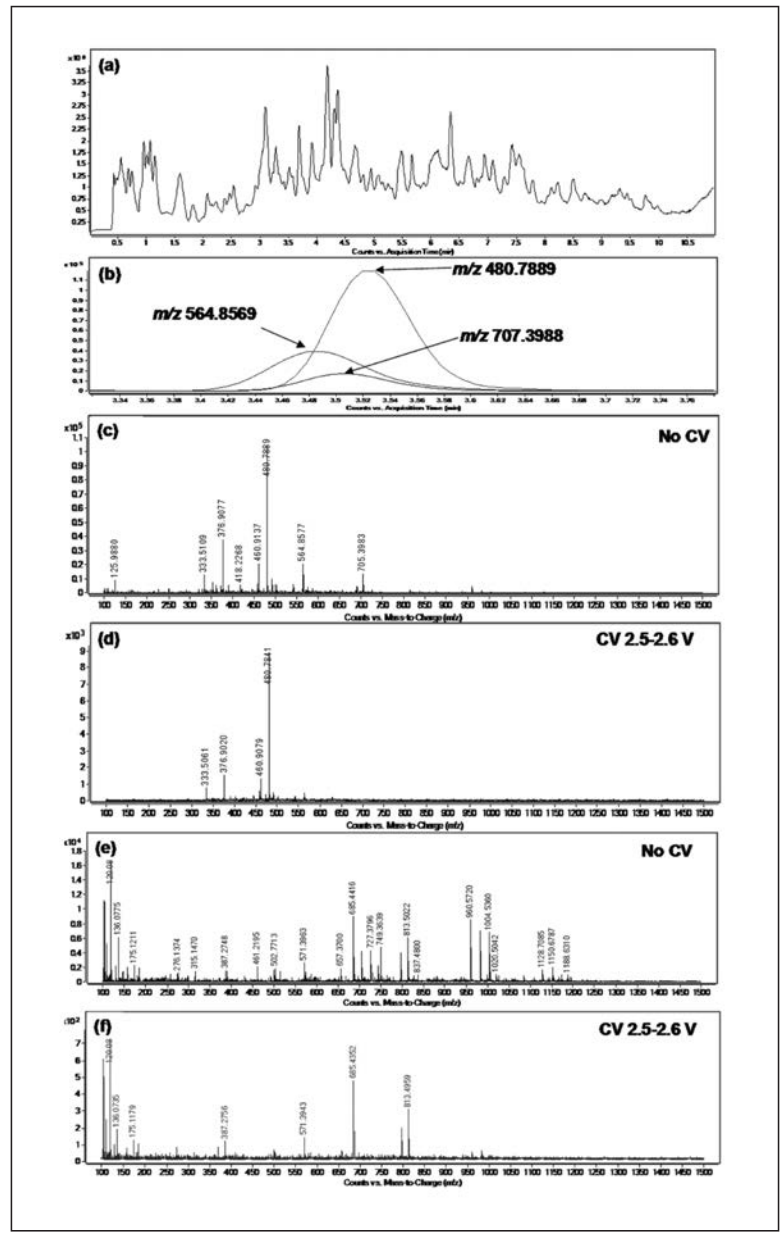

Figure 6. LC-MS and LC-FISCID-MS analysis of human plasma tryptic digest: (a) TIC, (b) selected ion chromatograms at 3.4-3.6 min, (c) LC-MS spectrum of peaks at 3.52 min without FAIMS separation, (d) LC-FAIMS-MS spectrum with FAIMS selection of the m/z 480 ion (CV 2.5-2.6 V), (e) LC-in-source CID-MS spectrum without FAIMS selection, and (f) LC-FISCID-MS spectrum with FAIMS selection of the m/z 480 ion and in-source CID (CV 2.5-2.6 V). (Reprinted (adapted) with permission from reference [2]. Copyright 2012, American Chemical Society).

Conclusion

The integration of a miniaturised chip-based FAIMS device with mass spectrometry has been shown to enhance analytical capabilities in a number of ways, including improving limits of quantitation by reducing chemical noise, increasing selectivity by removing sample complexity and by offering the ability to distinguish between isobaric ions. Rapid FAIMS separation can be combined with chromatographic techniques to enhance detection and in some cases can provide an alternative to LC. The additional separation stage can also provide pre-selection prior to fragmentation via in-source CID, enabling quasi-tandem experiments on a single-stage MS. These results suggest that FAIMS has the potential to become a versatile tool for enhancing mass spectrometry.

Acknowledgements

The authors thank Owlstone Ltd. and Loughborough University for financial support. We thank Owlstone Ltd and Agilent Technologies for the provision of instrumentation and technical support and AstraZeneca for provision of chemicals.

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