

Enhanced Analyte Detection Using In-Source Fragmentation of Field Asymmetric Waveform Ion Mobility Spectrometry-Selected Ions in Combination with Time-of-Flight Mass Spectrometry

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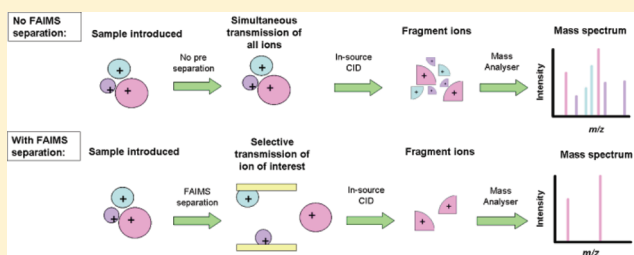
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ABSTRACT: Miniaturized ultra high field asymmetric waveform ion mobility spectrometry (FAIMS) is used for the selective transmission of differential mobility-selected ions prior to in-source collision-induced dissociation (CID) and time-of-flight mass spectrometry (TOFMS) analysis. The FAIMS-in-source collision induced dissociation-TOFMS (FISCID-MS) method requires only minor modification of the ion source region of the mass spectrometer and is shown to significantly enhance analyte detection in complex mixtures.

Improved mass measurement accuracy and simplified product ion mass spectra were observed following FAIMS preselection and subsequent in-source CID of ions derived from pharmaceutical excipients, sufficiently close in m/z (17.7 ppm mass difference) that they could not be resolved by TOFMS alone. The FISCID-MS approach is also demonstrated for the qualitative and quantitative analysis of mixtures of peptides with FAIMS used to filter out unrelated precursor ions thereby simplifying the resulting product ion mass spectra. Liquid chromatography combined with FISCID-MS was applied to the analysis of coeluting model peptides and tryptic peptides derived from human plasma proteins, allowing precursor ion selection and CID to yield product ion data suitable for peptide identification via database searching. The potential of FISCID-MS for the quantitative determination of a model peptide spiked into human plasma in the range of 0.45–9.0 $\mu\text{g/mL}$ is demonstrated, showing good reproducibility (%RSD < 14.6%) and linearity ($R^2 > 0.99$).



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 collision-induced dissociation (CID) or cone voltage fragmentation, may be used to induce fragmentation of ions passing through the interface.¹ The use of elevated interface voltages in the mass spectrometer interface allows product ion spectra to be generated for structural analysis of intact protonated molecules generated by electrospray ionization.² However, in the absence of precursor ion preselection, this can result in a complex mass spectrum containing overlapping precursor and product ions, which presents a challenge for spectral interpretation. Information regarding the relationship between precursor and product ions may also be lost because different precursor ions are fragmented simultaneously. Tandem mass spectrometry (MS/MS), in which a precursor ion is first selected on the basis of mass-to-charge (m/z) ratio and then subjected to CID with the resulting fragment ions identified by a second mass analyzer, is therefore the method of

choice for obtaining fragmentation data for elucidation of structure and quantitative analysis. An alternative approach is to combine mass spectrometry analysis with the separation of gas-phase ions by ion mobility spectrometry or field asymmetric waveform ion mobility spectrometry (FAIMS).^{3–7}

Field asymmetric waveform ion mobility spectrometry (FAIMS) is a gas-phase atmospheric pressure separation technique that exploits the difference in the mobility of an ion in alternating low and high electric fields.^{8,9} The alternating electric fields are generated in the gap between two closely spaced electrodes by the application of an asymmetric RF waveform, causing ions to oscillate between the electrodes. Ions with different mobilities under low and high field conditions accumulate a net drift toward an electrode eventually resulting

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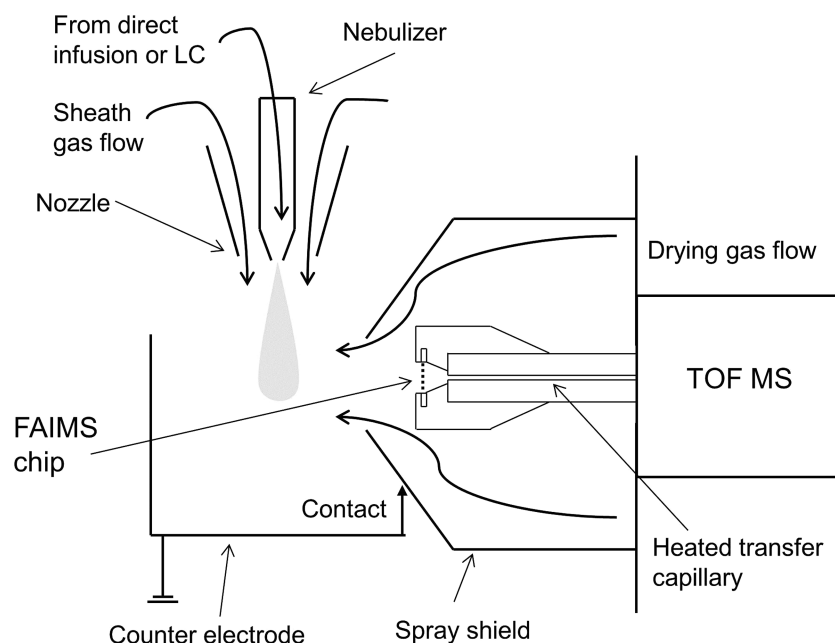


Figure 1. Schematic diagram of the FAIMS-MS interface.

in collision and neutralization. A small DC voltage is applied to one of the electrodes to reverse the drift of the ion so that collision with the electrode is avoided.⁵ This is termed the compensation voltage (CV)⁸ and allows ions to pass between the electrodes and be detected. The CV for ion transmission is compound specific, resulting from the difference in mobility in high and low fields.

FAIMS has been employed as an ion filter to select precursor ions prior to mass spectrometry analysis, because differential mobility is largely independent of m/z allowing orthogonal separations.⁹ The ability to preselect using FAIMS has been shown to simplify mass spectra by reducing background and isobaric ions.^{10–13} The combination of FAIMS with tandem mass spectrometry (FAIMS-MS/MS) has also been reported for the structural and quantitative analysis of small molecules and peptides, with CID of mass-selected ions occurring in the collision cell located between the mass analysers of a Q-time of flight (Q-TOF) or triple quadrupole spectrometer or after mass isolation in an ion trap.^{13–15}

The in-source fragmentation of ions in FAIMS-MS was reported by Guevremont et al.,¹⁶ who observed a change in cluster distributions of leucine enkephalin ions under low and high energy in an electrospray ionization (ESI) ion source. It was concluded that fragmentation comparable to MS/MS analysis was induced under high energy conditions. Subsequent fragmentation studies were pursued using MS/MS of mass-selected ions in a triple quadrupole spectrometer rather than through in-source CID. Eiceman et al. also described fragmentation of chlorocarbon ions in the interface of an atmospheric pressure ionization mass spectrometer, which complicated the identification of FAIMS-selected ions.¹⁷ Measurements were carried out under conditions designed to minimize rather than enhance ion decomposition in the mass spectrometer interface. The use of FAIMS with in-source CID and selected reaction monitoring MS/MS was used by Xia and Jemal to determine the location of source fragmentation in the analysis of ifetroban and its acylglucuronide metabolite.¹⁸ FAIMS was used to transmit either the parent drug or its acylglucuronide, from which it was concluded that fragmenta-

tion inducing the conversion of the acylglucuronide metabolite to the parent drug form occurred almost entirely after the inlet orifice of the mass spectrometer. The objective of this work was to minimize the effect of in-source CID on the LC-MS/MS with selected reaction monitoring (SRM) for the quantitative analysis of prodrugs and metabolites. The targeted use of in-source CID-MS using FAIMS to isolate ions from a mixture prior to fragmentation was reported by Coy and co-workers.¹⁹ The separation of a simple mixture containing five isobaric model compounds (m/z 316) was achieved by direct infusion into the ESI source and differential mobility spectrometry. Ions were transmitted at their optimum CV and fragmentation induced by increasing the inlet cone voltage in the interface of a quadrupole MS.

Here, we describe the development of generic FAIMS-in source CID-MS procedures (which we term FISCID-MS) that exploit the ability to control the fragmentation of FAIMS-selected ions in the interface of the mass spectrometer, by combining the orthogonal separation characteristics of a miniaturized high field FAIMS device and in-source CID with time-of-flight mass spectrometry. The technique requires only minor modification of the ion source region of the mass spectrometer to incorporate the FAIMS device but is shown to provide major enhancements for the analysis of complex mixtures. FISCID-MS is demonstrated for the separation of pharmaceutical excipients²⁰ and for the qualitative and quantitative analysis of model and tryptic peptides derived from human plasma.

■ EXPERIMENTAL SECTION

Chemicals and Reagents. HPLC grade methanol (MeOH), acetonitrile (ACN), water, and formic acid (FA) were purchased from Fisher Scientific (Loughborough, UK). Leucine enkephalin (YGGFL), bradykinin (RPPGFSPFR), bombesin (EQRLGNQWAVGHLM), luteinizing hormone releasing hormone (EHWSYGLRPG), and the tetrapeptide MRFA were obtained from Sigma Aldrich (Gillingham, UK). All peptide standards were prepared at a concentration of 10

pmol/ μ L in 50/50 (v/v) methanol/water containing 0.1% FA. 2-Hydroxy-(4-octyloxy) benzophenone and polyethylene glycol (PEG) 400, obtained from Sigma Aldrich (Gillingham, UK), were prepared in 50/50 (v/v) methanol/water with 0.1% FA at respective concentrations of 5.1 and 104 pmol/ μ L (1:20 molar ratio).

Sample Preparation. Human blood samples were collected from volunteers in lithium heparin tubes (BD, Oxford, UK) and centrifuged at 1500g for 15 min at 4 °C. ACN (400 μ L) was added to an aliquot of the collected plasma (200 μ L) before being vortex-mixed for 30 s and sonicated for 10 min to precipitate the proteins. The supernatant was removed, and proteins were reconstituted in 2 mL of water. Trypsin Gold Mass Spectrometry grade (100 μ g; Promega, Southampton, UK) was dissolved in 100 mM ammonium bicarbonate, and 6 μ L was added to the plasma extract. The sample was digested overnight at 37 °C. The reaction was quenched with 200 μ L of 1% trifluoroacetic acid (TFA) solution (Sigma Aldrich, Gillingham, UK) in water, and the digested plasma was prepared for LC analysis via solid phase extraction (SPE) using 3 mL volume Oasis C₁₈ solid phase microextraction cartridges (Waters, Manchester, UK). The cartridge was conditioned with 3 mL of 50% ACN in water and equilibrated with 3 mL of 0.1% TFA in water. Peptides generated by protein digestion and retained on the SPE cartridge were washed with 2 mL of 0.1% TFA in water. The retained peptides were then eluted in 200 μ L of 20:80 water/ACN (v/v) with 0.1% FA. The eluate was dried down under argon and reconstituted in 90:10 water/ACN (v/v) with 0.1% FA. Standard solutions of Gramicidin S (cyclo(VOLDFP)₂) were prepared for quantitative analysis in 10:90 water/ACN (v/v) with 0.1% FA at concentrations in the range of 5–500 ng/ μ L. Aliquots (20 μ L) of these standards were spiked into 200 μ L of the plasma tryptic digest to give concentrations of 0.45–45 μ g/mL.

Instrumentation. FISCID-MS analysis was carried out in positive ion electrospray ionization mode using a prototype miniaturized FAIMS device (Owlstone, Cambridge, UK), interfaced to an Agilent 6230 orthogonal acceleration time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The electrospray source of the TOF mass spectrometer was modified to accommodate the presence of the FAIMS chip within the spray shield (Figure 1). The FAIMS chip has been described in detail elsewhere^{21,22} and consists of 47 electrode pairs with a channel width of 35 μ m and a channel length of 300 μ m. The FAIMS chip was connected to a field generator module, containing the electrical circuitry required to generate field asymmetric waveforms. The frequency of the applied asymmetric waveform was 25 MHz in a roughly 2:1 ratio of low field to high field. Comparative non-FAIMS data were acquired with analytes transmitted through the FAIMS chip with the dispersion field (DF) and CV set to 0 kV/cm and 0 V, respectively. FAIMS separation was carried out at a 47 kV/cm DF, over a CV range of –1 V to +4 at 0.5 V/s CV sweep rate. For LC-FISCID-MS analysis, FAIMS-selected analytes were transmitted into the mass spectrometer interface by scanning a 0.1 V window around the optimum CV for transmission. The exact CV values and scan windows were experiment dependent and as such are indicated in the text where appropriate. Data were processed using Mass Hunter version B.01.03 (Agilent Technologies, Santa Clara, CA, USA) and Excel 2007 (Microsoft, Seattle, USA).

TOF spectral acquisitions were performed at a rate of 10 spectra/s for the analysis of peptide standards and 2 spectra/s for the analysis of plasma samples. Analytes were infused into the JetStream-ESI ion source at 50 μ L/min and ionized using a voltage of 1.5 kV. Source conditions for all experiments were nozzle voltage, spray shield, and counter electrode, 400 V; skimmer voltage, 65 V; drying gas temperature, 150 °C; sheath gas temperature, 250 °C; nebulizer gas pressure, 25 psig. HOBP/PEG analysis was carried out with the inlet capillary voltage set to –29 V, the drying gas flow set to 4.6 L/min, and the sheath gas flow set to 7 L/min. Peptide analysis was carried out with the inlet capillary voltage set to –15 V, the drying gas flow set to 10 L/min, and the sheath gas flow set to 12 L/min. The fragmentor voltage was set to 150 V for transmission of intact analytes without fragmentation and increased to the region of 350–400 V in order to induce in-source fragmentation by CID. The fragmentor voltage was compound dependent and is indicated in the text where appropriate.

LC separation of peptide standards was carried out using an XBridge C₁₈ 5 μ m column, with dimensions 2.1 $\times$ 50 mm, 5 μ m (Waters, Manchester, UK), operated at a flow rate of 0.2 mL/min using an isocratic mobile phase of 30:70 water/ACN (v/v) with 0.1% FA, with a run time of 3 min and a 2 μ L injection volume. Analysis of the spiked plasma tryptic digest was carried out using a Poroshell 300SB-C₁₈ column, with dimensions 2.1 $\times$ 7.5 mm, 5 μ m (Agilent, Santa Clara, CA, USA), at a flow rate of 0.4 mL/min with a 20 μ L injection volume. The gradient elution program consisted of a linear increase from 95:5 water/ACN (v/v) to 60:40 water/ACN (v/v) in 10 min and then to 10:90 water/ACN (v/v) in 2 min. The gradient was then returned to the initial conditions giving a total run time of 15 min.

RESULTS AND DISCUSSION

The control of fragmentation of FAIMS-selected ions in the interface of a TOF mass spectrometer yields product ion information on the selected precursor ions. The potential of this FAIMS-in-source CID-MS (FISCID-MS) approach to enhance the analysis of complex mixtures using a single mass analyzer has been evaluated for the separation of pharmaceutical excipients and for peptide sequencing and quantification.

Separation of Pharmaceutical Excipients. Ions derived from the pharmaceutical excipients 2-hydroxy-4-octyloxybenzophenone (HOBP, m/z 327.1955) and PEG 400 were chosen as test analytes because the protonated HOBP and PEG $n = 7$ oligomer (m/z 327.2013) are sufficiently close in mass (17.7 ppm mass difference) that these ions could not be resolved by the reflectron TOF mass analyzer (resolving power required $\sim$ 130K). Robust accurate mass measurement of these ions is therefore not possible without separation prior to mass analysis. The two components were analyzed as a mixture containing a 20-fold molar excess of the PEG (Figure 2). CV sweeps (–1 to +4 V) with the DF set to 48 kV/cm were used to determine the optimum CV required for selected transmission and subsequent in-source fragmentation. The selected ion response for m/z 327.2 (Figure 2) shows that the protonated PEG $n = 7$ and HOBP ions are resolved by FAIMS.

The mass spectrum of the mixture without FAIMS separation (Figure 3a) shows the typical polymer distribution of PEG ions, with the HOBP unresolved from the protonated PEG ($n = 7$) ion. The measured mass of the overlapping peaks is m/z 327.1994, a mass difference of 11.9 and –5.8 ppm from HOBP and PEG, respectively. In-source CID-MS of this

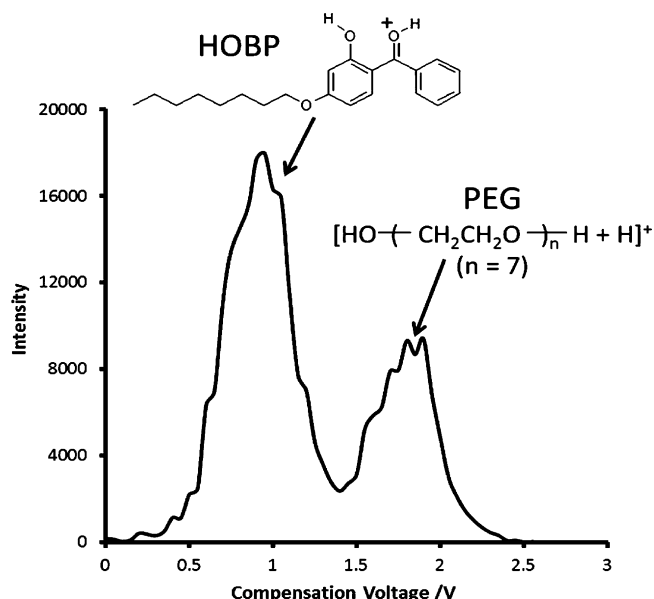


Figure 2. FAIMS-MS CV spectrum (m/z 327.2) of HOBP and PEG 400.

mixture gives a complex product ion spectrum dominated by PEG fragment ions (Figure 3b). Quasi-static FAIMS filtering (CV range 0.6–0.7 V) was used to transmit the HOBP ion selectively, which appears as the base peak in the resulting mass spectrum (Figure 3c), with an observed mass of 327.1966 (3.3

ppm). The FISCID-MS product ion spectrum of the FAIMS-selected HOBP ion (Figure 3d) shows the HOBP fragment ions free of interference from PEG fragment ion peaks, reducing the complexity of the mass spectrum and allowing unambiguous identification of the HOBP fragments. These observations demonstrate the mass spectral enhancement possible using FISCID-MS, through the FAIMS preselection of a precursor ion in a complex mixture, on the basis of differential mobility, prior to in-source CID. FISCID-MS allows the unresolved ions to be separated and a clean product ion spectrum to be obtained for HOBP using a single mass analyzer with only minor modification of the ion source to incorporate the miniaturized FAIMS device.

Isolation of Peptide Ions for Sequence Analysis. The FISCID-MS method was applied to the analysis of a mixture of peptide standards, MRFA, bradykinin, luteinizing hormone releasing hormone peptide (LHRH), leucine enkephalin, and bombesin (Figure 4a). The mixture was first infused directly into the ESI source of the mass spectrometer without FAIMS separation, producing a mass spectrum containing the $[M + H]^+$ ions of the four peptides, the $[M + 2H]^{2+}$ ions of bradykinin, LHRH, and bombesin, and the $[M + 3H]^{3+}$ ion of bradykinin, resulting in a complex mass spectrum (Figure 4a). FISCID-MS of the peptide mixture using a fragmentor voltage of 350 V without FAIMS preselection results in a complex product ion spectrum (Figure 4b) with a predominance of singly charged ions which do not readily fragment. FAIMS selection of singly charged peptide ions to generate a pseudo-peptide mass fingerprint for protein identification has been

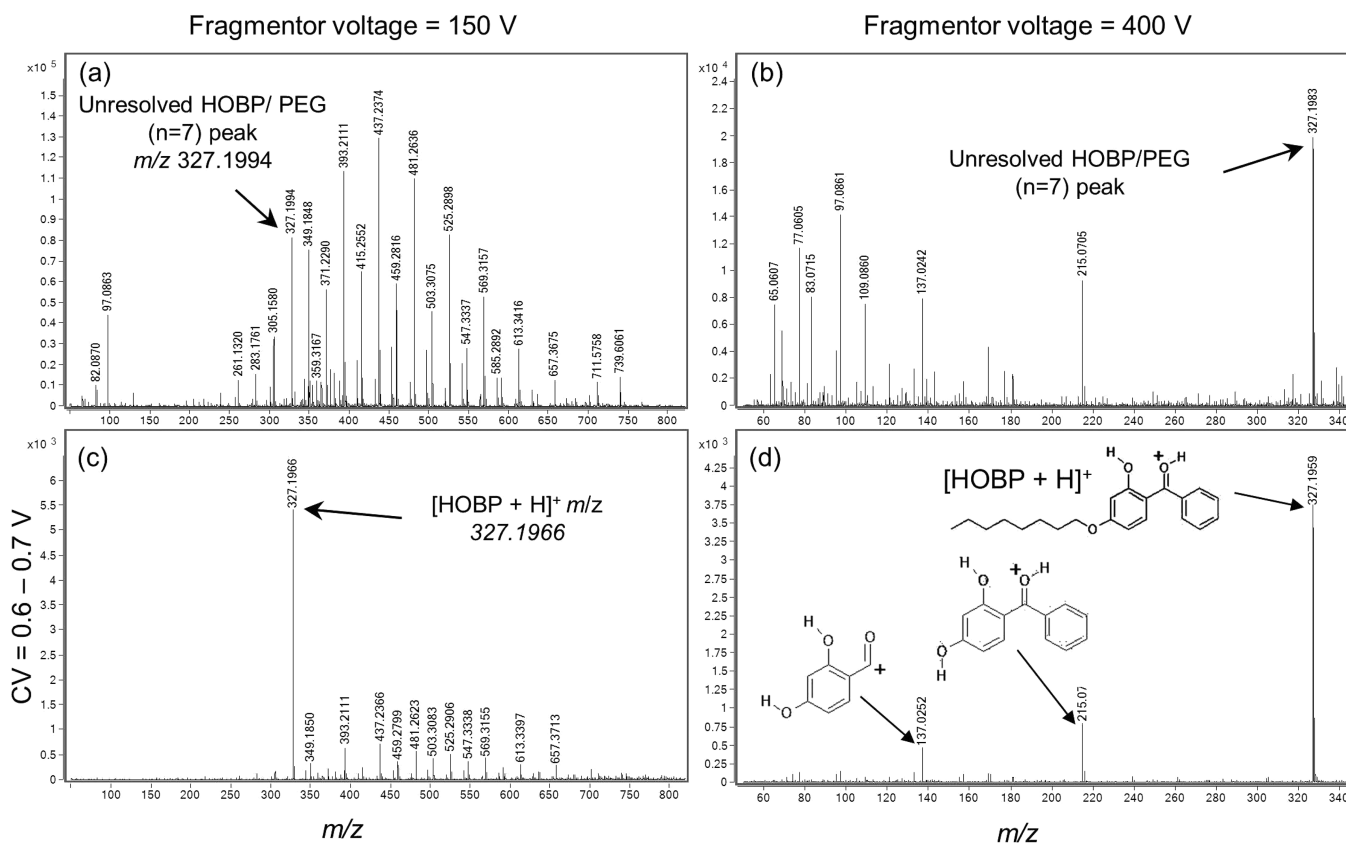


Figure 3. MS, FAIMS-MS, and FISCID-MS mass spectra of a mixture of HOBP and PEG 400 (1:20 molar ratio): (a) without FAIMS separation or in-source CID, (b) in-source CID-MS without FAIMS separation, and (c) FAIMS-selected HOBP ion (CV = 0.6–0.7 V) without in-source CID; (d) FISCID-MS product ion spectrum of the FAIMS-selected HOBP ion with in-source CID.

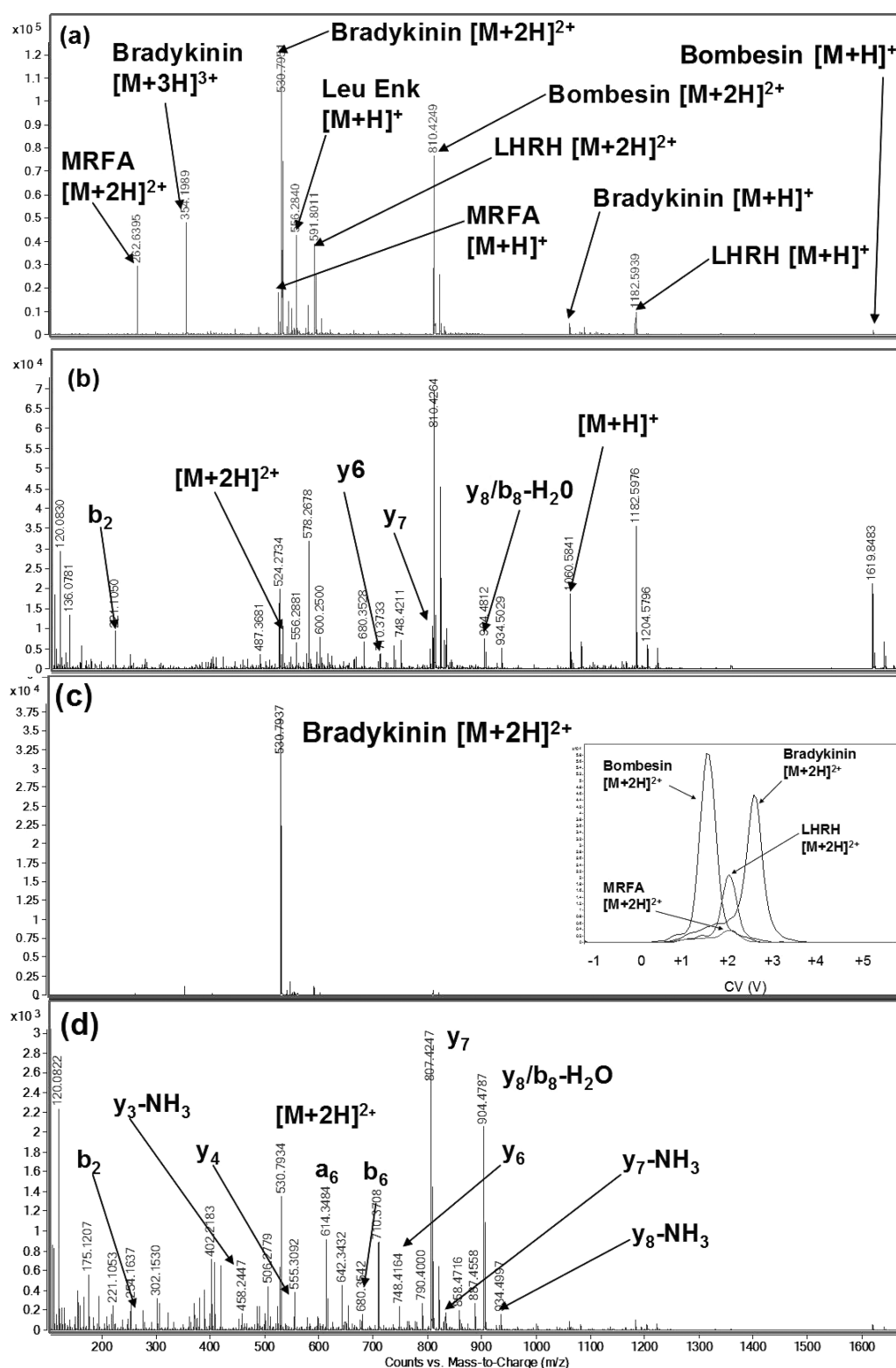


Figure 4. MS, FAIMS-MS, and FISCID-MS analysis of a peptide mixture: mass spectrum (a) without FAIMS selection or in-source CID, (b) in-source CID-MS (350 V) without FAIMS selection, and (c) FAIMS-selection of the $[M + 2H]^{2+}$ bradykinin ion (CV = 2.6–2.7 V) with (inset) mass-selected CV spectra;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

reported previously,¹³ but higher charge state ions are preferred for sequence analysis by CID. A FAIMS CV spectrum was obtained by scanning the CV voltage in the range of 0 to +4 V with a DF of 48 kV/cm, and the selected ion responses for the $[M + 2H]^{2+}$ ions were extracted from the total ion response.

The FAIMS device was then programmed to the appropriate CV for transmission of each of the $[M + 2H]^{2+}$ species. The isolation of the $[M + 2H]^{2+}$ bradykinin ion from the other singly and multiple charged peptide ions by FAIMS at a CV of +2.6–2.7 V is shown in Figure 4c. Using FAIMS to transmit the

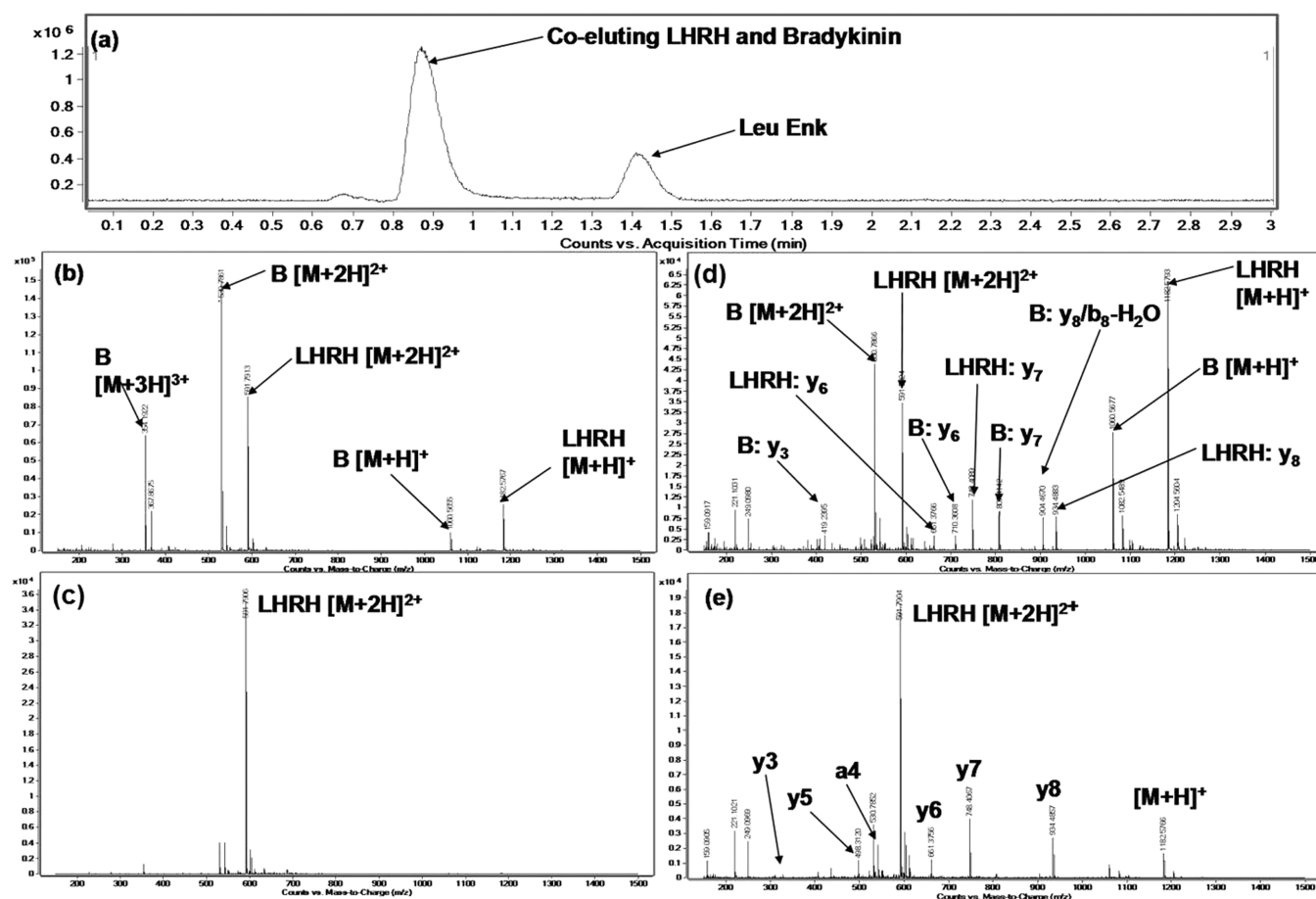


Figure 5. LC-MS and LC-FISCID-MS analysis of a peptide mixture: (a) TIC of peptide mixture; mass spectrum of (b) LC peak at ~0.9 min without FAIMS selection or in-source CID and (c) FAIMS preselection of LHRH $[M + 2H]^{2+}$ ion from coeluting peptides (CV = 1.8–1.9 V); (d) in-source CID-MS (fragmentor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

bradykinin $[M + 2H]^{2+}$ ion selectively in a FISCID-MS analysis filtered out unrelated precursor ions producing a product ion mass spectrum containing the characteristic fragments of bradykinin (Figure 4d). A comparison of peak lists generated by the TOFMS software identified 21 characteristic bradykinin fragment ions using the FISCID-MS method, compared to just 6 without FAIMS separation, enhancing confidence in the identification of bradykinin, based on sequence coverage.

FISCID-MS was then combined with an LC preselection to evaluate the potential of FAIMS to aid the identification of coeluting peptides. The coeluting bradykinin and LHRH peptide (RT 0.88 min; Figure 5a) produced a mass spectrum containing singly and multiply charged peptide ions from both peptides (Figure 5b). The $[M + 2H]^{2+}$ ion of LHRH was isolated from the other peptide ions by applying a CV of 1.7–1.8 V (Figure 5c). The complex, overlapping LC-in-source CID-MS spectrum of the coeluting peptides without FAIMS separation (Figure 5d) is simplified by FAIMS preselection of the LHRH $[M + 2H]^{2+}$ ion enhancing the detection of the characteristic product ions of LHRH (Figure 5e).

The LC-FISCID-MS method was applied to the analysis of a complex mixture of tryptic peptides derived from human plasma proteins. The plasma tryptic digest was initially analyzed by LC-MS without FAIMS separation or in-source fragmentation (Figure 6a). The complex nature of the sample resulted in the coelution of several tryptic peptides, even after LC

separation. One such example was m/z 480.7889, observed at a retention time of 3.5 min. The selected ion chromatograms obtained in the retention time range of 3.4–3.6 min without FAIMS-selection shows overlap with other coeluting peptide ions (Figure 6b). This can be seen on the corresponding mass spectrum (Figure 6c) averaged across the m/z 480 LC peak at half height. The mass spectrum, acquired with the FAIMS programmed to the optimum CV (2.5–2.6 V) for transmission of the m/z 480 peptide ions, is shown in Figure 6d. By applying a FAIMS preselection, the coeluting m/z 564, m/z 707, and other ions are filtered out, with the m/z 480 ion preferentially transmitted, resulting in a much simpler mass spectrum (Figure 6d). In order to evaluate whether spectral quality could be enhanced by FAIMS-selected ion transmission prior to in-source CID, the peptides were subjected to a fragmentor voltage of 340 V to induce fragmentation of the coeluting peptides from the LC-MS analysis with and without FAIMS separation. A complex product ion spectrum is obtained without FAIMS selection (Figure 6e), compared to the product ion mass spectrum observed using LC-FISCID-MS with FAIMS selection at a CV of 2.5–2.6 V (Figure 6f).

The prominent peaks in the LC-FISCID-MS product ion spectrum (Figure 6f) suggested the data acquired would be suitable for database searching to identify the unknown plasma peptides from the observed b and y fragments. Peptide identification was carried out via the MASCOT search engine,²³

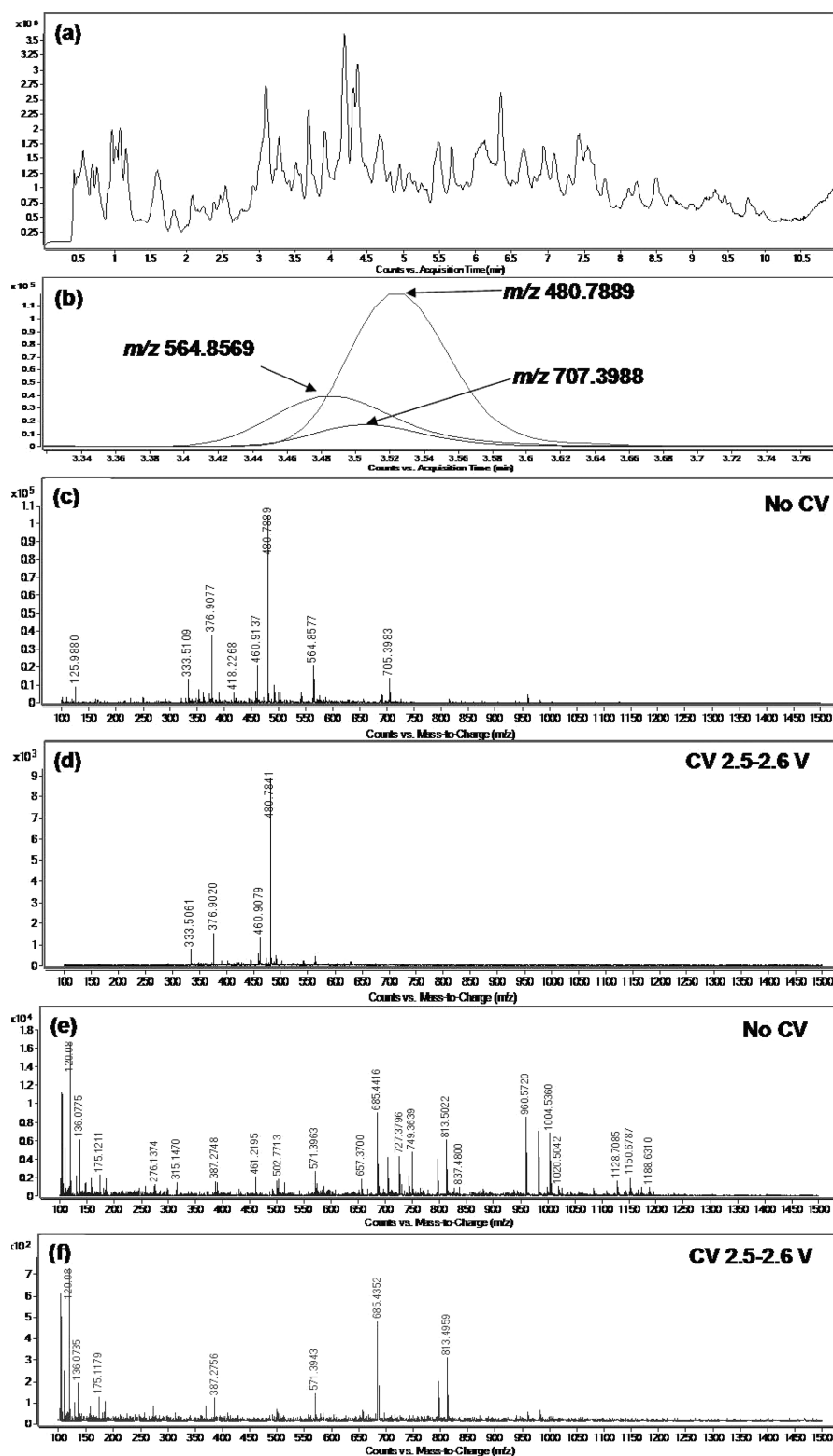


Figure 6. LC-MS and LC-FISCID-MS analysis of human plasma tryptic digest: (a) TIC, (b) selected ion chromatograms at 3.4–3.6 min, (c) LC-MS spectrum of peaks at 3.52 min without FAIMS separation, (d) LC-FAIMS-MS spectrum with FAIMS selection of the m/z 480 ion (CV of 2.5–2.6 V), (e) LC-in-source CID-MS 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, fragmentor voltage 340 V).

and all ions with intensities greater than 10% of the base peak were included in the peak list generated by the TOF-MS software for the data obtained both with and without FAIMS filtering. The peak list was searched against the SwissProt protein database. With no FAIMS separation, the LC-CID-MS

method yielded no significant hits on the database, and therefore, no protein was identified. However, with the CV set to 2.5–2.6 V, 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

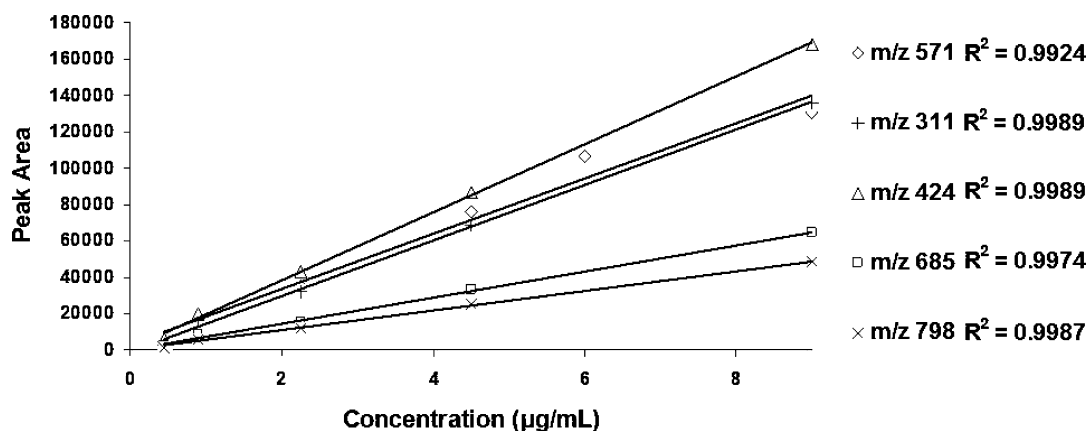


Figure 7. Calibration graphs for the quantitative LC-FISCID-MS analysis of the FAIMS-selected (CV 1.75–1.85 V) $[M + 2H]^{2+}$ precursor ion (m/z 571) and four product ions (m/z 311, 424, 685, and 798) of gramicidin S spiked in human plasma tryptic digest.

a 95% confidence interval), based on the fragmentation of the doubly charged FQNALLVR tryptic fragment (m/z 480.7854).

Peptide Quantification. The quantitative characteristics of LC-FISCID-MS were evaluated for the determination of a peptide spiked into human plasma. The peptide gramicidin S was chosen as a test compound, as it is not present in plasma and is therefore unaffected by natural fluctuations in abundance between plasma samples. A reproducibility study was performed using aliquots of human plasma tryptic digest spiked with 9 ng on column mass (0.45 µg/mL) of gramicidin S to determine the stability of LC-FISCID-MS for replicate injections of plasma. The % relative standard deviation (%RSD) for the peak area of the FAIMS-selected (CV +1.75–1.85 V) $[M + 2H]^{2+}$ precursor ion (m/z 571) without in-source fragmentation was 5.1% ($n = 6$). LC-FISCID-MS peak area precision for the fragment ions at m/z 311, m/z 424, m/z 685, and m/z 798, generated by in-source fragmentation of the FAIMS-selected m/z 571 precursor ion gave %RSDs ($n = 6$) of 13.3%, 14.6%, 13.1%, and 8.2% respectively, demonstrating good reproducibility of LC-FISCID-MS for quantitative measurement at this concentration.

Calibration graphs for the LC-FAIMS-MS analysis of the gramicidin precursor ion (m/z 571) and the LC-FISCID-MS analysis of the four product ions (m/z 311, 424, 685, and 798) were prepared by spiking the plasma tryptic digest with different concentrations of gramicidin S. A linear response ($R^2 > 0.99$) was observed in the range of 0.45–9.0 µg/mL for the precursor ion and all four product ions (Figure 7). The enhanced selectivity, reproducibility, and linear response to the spiked peptide in the plasma extract suggest that LC-FISCID-MS has the potential to offer significant benefits compared to the use of a single mass analyzer. This approach may also be an alternative to LC-MS/MS with SRM for quantitative analysis, while still being able to perform qualitative structural characterization and elemental formula analyses in the same experiment. This potential paradigm shift mirrors current trends within the pharmaceutical industry, where time-efficient use of one high-resolution mass analyzer for quantitative bioanalysis and qualitative metabolite characterization is receiving much attention.^{24,25}

CONCLUSIONS

FAIMS preselection of precursor ions using a miniaturized high field FAIMS device followed by in-source CID-MS requires only minor modification of the ion source region of the mass

spectrometer but provides enhanced selectivity for the qualitative and quantitative analysis of analytes in complex mixtures. Thus, FISCID-MS successfully separated pharmaceutical excipient ions that are too close in m/z value to be resolved by TOF-MS alone, providing improved accurate mass measurement and a product ion spectrum free from interferences. Transmission of FAIMS-selected peptide ions from a mixture on the basis of differential mobility facilitated peptide identification by enhancing the characteristic fragment ions of the selected precursor ion in the FAIMS-selected product ion spectrum. The LC-FISCID-MS analysis of these model peptides and a human plasma tryptic digest reduced the mass spectral complexity of coeluting peptides. The 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. The quantitative potential of LC-FISCID-MS has also been demonstrated for the determination of a FAIMS-selected peptide in a spiked plasma tryptic digest sample. 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, and has potential as an alternative to LC-MS/MS for qualitative and quantitative analysis or for further enhancing selectivity using LC-FISCID-MS/MS routines.

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Notes

The authors declare no competing financial interest.

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