

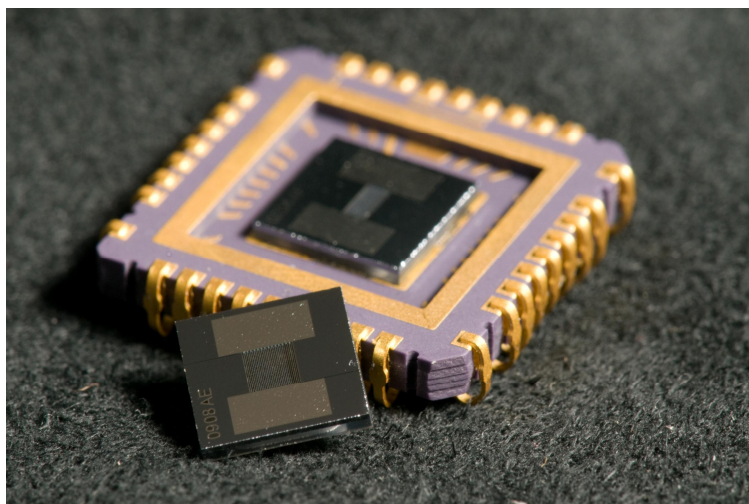
OWLSTONE LTD

Owlstone ultraFAIMS for LC-MS

White Paper

Owlstone Ltd

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CONTENTS

Introduction	3
FAIMS – the basics.....	4
What is different about Owlstone ultraFAIMS?	6
Why use ultraFAIMS with LC-MS?	9
References	18
Acknowledgements.....	18

INTRODUCTION



In the never-ending quest to analyze complex mixtures – pharmaceutical formulations, biological samples, food ingredients, environmental samples, and so on – the most powerful weapon available is the mass spectrometer. Since JJ Thomson first used his “mass spectrograph” in 1912 to distinguish the two isotopes of neon, scientists and inventors have striven to build more and more powerful instruments capable of penetrating the composition and structure of smaller amounts of more complicated analytes.

Key to being able to analyze a particular compound or molecule is whether or not it can be separated from other species that are also present in the sample. There are two ways of achieving this. Either you improve the resolution of the mass spectrometer, so that even smaller differences in the mass-to-charge ratio of the ions can be resolved, or you apply another separation technique upstream of the mass spectrometer to spread out the ions so they aren’t all arriving at the same time in the mass spectrometer, and then analyze them sequentially.

Both approaches have been important – mass spectrometers with the ability to resolve peaks separated by tiny fractions of a mass unit are now available, and upstream separation using gas- and liquid-chromatography is routine – and the combination of these approaches is extremely powerful. But despite this, the interest in alternative separation techniques continues, as chemists look for ways of tackling separation problems that existing techniques cannot solve. One of the alternative approaches that has elicited much interest in recent years is field-asymmetric waveform ion mobility spectrometry, or FAIMS.

FAIMS – THE BASICS

Ion mobility spectrometry (IMS) is a technique for distinguishing ions according to differences in the speed that they migrate through a buffer gas under the influence of an electric field.

If an electric field is applied to an ion surrounded by a buffer gas, the ion will drift in the direction of the field. Different ions reach different drift velocities and the ratio between the velocity, v , and the electric field, E , is known as the mobility, K .

$$K = \frac{v}{E}$$

Low-fields

At low electric fields, the mobility is independent of electric field. Linear drift tube IMS (DTIMS) exploits this to separate ions on the basis of the time taken to travel a fixed distance in a known field – ions with a higher mobility will travel faster than lower mobility ions and reach the detector sooner.

Travelling wave IMS (TWIMS) also operates in the low electric field regime – when the ratio of electric field strength to buffer gas density is small – but rather than applying a constant drift field, it works by applying travelling voltage waves to a stacked-ring ion guide. Ions with a lower mobility slip behind the waves and reach the detector later than ions with a higher mobility. The travelling wave technique has a high transmission efficiency compared with conventional drift tube IMS.

The downside of low-field mobility techniques is that in the low-field, the mobility of an ion is closely correlated to its mass, which means that ions that are not well-separated by m/z are also less likely to be separated by DTIMS or TWIMS.

High-fields

At high electric fields, the mobility of an ion is no longer constant but becomes a function of the electric field strength. Applying an alternating asymmetric high/low electric field to an ion creates a net drift in the direction of the field but, unlike in low-field IMS, the net drift velocity is now a function of the difference in high- and low-field mobility of the ion. This effect is the basis of field-asymmetric ion mobility spectrometry (FAIMS).

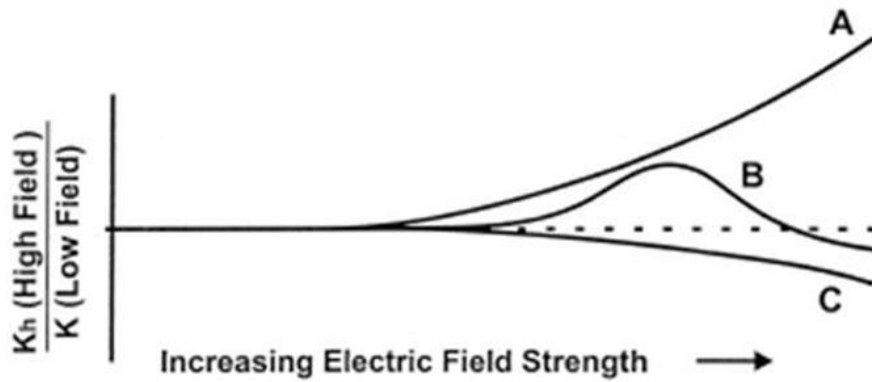


Figure 1: Variation of ion mobility K in increasing electric fields – at low fields, the mobility is essentially constant, but as the field continues to increase, the mobility starts to vary. A number of different types of behavior (A, B and C) have been observed (Purves 1999)

In FAIMS spectrometers, ions are carried by a buffer gas flow through a gap between two closely spaced electrodes. The separation field (also known as the dispersion field, or DF) is applied across the gap in a direction perpendicular to the gas flow. The field causes the ions to drift sideways, towards one or other electrode. To counteract this sideways drift, and allow the ions to pass through the electrode region, a DC field is applied in the opposite direction. This is called the compensation field (CF). Any given magnitude of CF will compensate only a specific drift velocity, and hence only ions that have this net drift velocity as a result of the applied DF will be transmitted. If the CF magnitude is varied, different sets of ions will be transmitted. In FAIMS devices, the CF is typically swept through a range of values to produce a spectrum of transmitted ions. This can be repeated for different DF magnitudes to produce a two-dimensional spectrum.

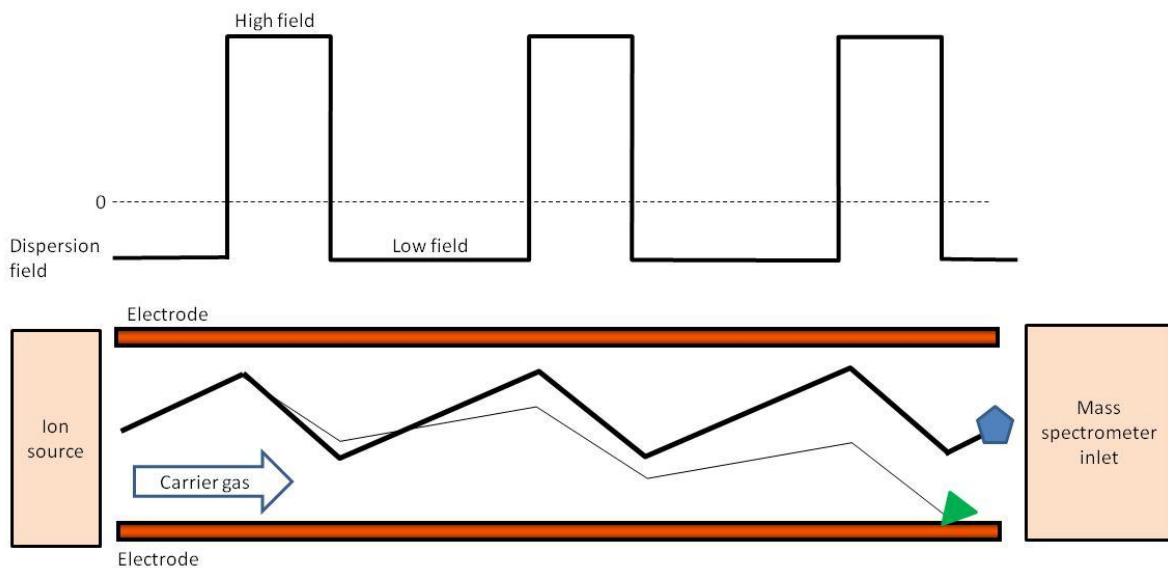


Figure 2: Schematic representation of how a FAIMS device works. Ions are carried through the device by the gas flow. Applying the high-frequency alternating dispersion field causes a net lateral drift of ions towards one or other electrode, depending on their mobility-field dependence. Most ions would thus be annihilated at the electrodes (e.g. the green ion). By applying a specific compensation field in the direction opposing the lateral drift, some ions (e.g. the blue ion) are enabled to pass through the FAIMS device. By sweeping the compensation field through its full range, all ions species are eventually transmitted.

Stand-alone versus hybrid FAIMS

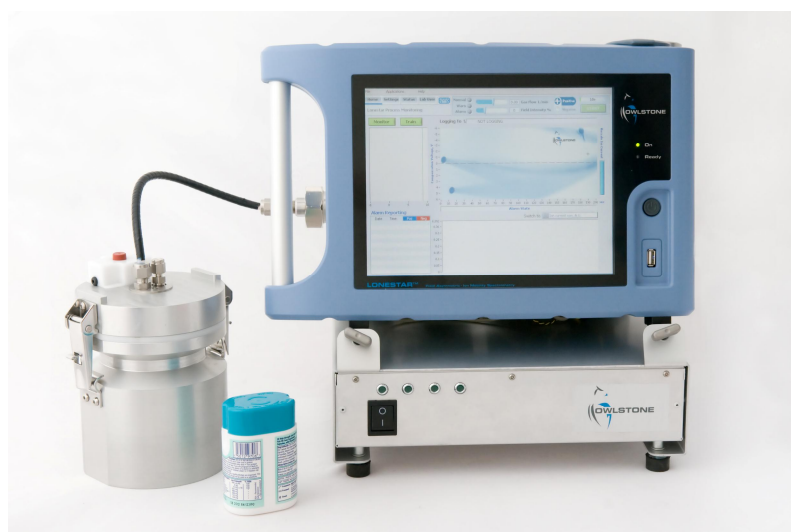


Figure 3: Owlstone Lonestar™ stand-alone gas analyzer

In stand-alone FAIMS devices, such as Owlstone's Lonestar™ product, ions passing through the electrodes reach a detector that measures the amount of charge accumulated over the time period corresponding to each CF step, producing a FAIMS spectrum that can be analyzed to detect and quantify specific analytes.

In hybrid FAIMS devices - for example FAIMS-Mass Spectrometer (MS) systems, the ions transmitted by the FAIMS device enter the downstream instrument for subsequent analysis. Used in this way, the FAIMS device pre-separates or filters the ions based on their differential mobility to enhance the subsequent analysis. Since the differential mobility of an ion tends not to be closely correlated to its mass (unlike the low-field mobility), a combined FAIMS-MS system has significantly increased ability to separate different ion species compared with the equivalent MS alone.

WHAT IS DIFFERENT ABOUT OWLSTONE ULTRAFAIMS?

Owlstone's ultraFAIMS spectrometer is a miniaturized FAIMS device. Using innovative manufacturing methods, the gap between the electrodes has been reduced to between 35 and 100µm – the smallest gap dimensions of any FAIMS spectrometer yet developed.

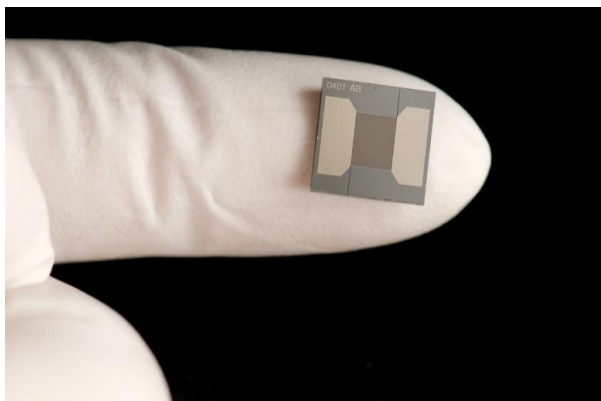


Figure 4: Owlstone ultraFAIMS device

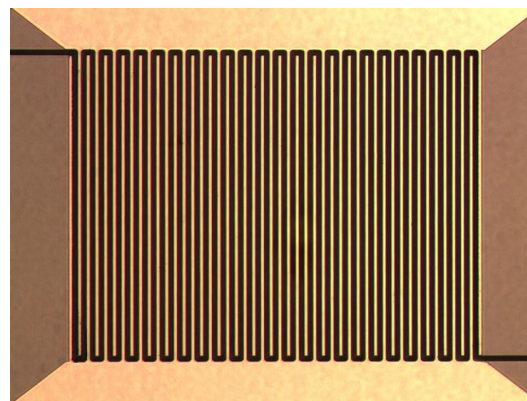


Figure 5: Microscope image of Owlstone ultraFAIMS device, showing the inter-digitated electrode structure

So, why is a small gap good?

Fundamentally, a small gap enables the device to separate a wider range of analytes more quickly.

Reducing the width of the gap means that higher dispersion fields can be used in the device. Ultimately, the maximum dispersion field that can be applied across an air gap is limited by the point at which the applied voltage causes the air to break down, leading to arcing between the electrodes. The physics of the breakdown process means that as the gap width narrows, the breakdown field limit increases – so for example, whereas with a 1mm gap the limit is $\sim 30\text{kV/cm}^1$, with a $100\mu\text{m}$ gap, the limit rises to $\sim 100\text{kV/cm}$. It is also much more practical to generate these higher fields in the ultraFAIMS device, since the small size means that the voltages needed to produce the higher fields are actually much lower than in macro-scale FAIMS instruments.

Reaching these extreme dispersion fields increases the range of analytes that can be separated. This is because the access to higher fields leads to greater scope for changes in mobility between the high- and low-field portions of the cycle (e.g. due to changes in shape, clustering, dipole formation etc), thus a greater possibility of moving the ion away from the zero compensation field point on the spectrum. This increases the likelihood of the ion of interest being separable from other background ions.

Another important benefit of reaching these extreme dispersion fields is that it dramatically speeds up the separation stage (Shvartsburg 2009a) since the residence time that is required to achieve a given resolution is proportional to the fourth to sixth power of the dispersion field – so, for example, a doubling of the dispersion field increases the speed of separation by a factor of $\sim 15-60$. This makes the ultraFAIMS device fast enough that the FAIMS stage is no longer the limiting factor in an LC-FAIMS-MS analysis and you can add combine it with LC separation in real-time without the need for multiple infusion experiments to establish the CF settings needed.

To compensate for the narrowness of the channel (which would otherwise limit the number of ions that could be transmitted), the device actually uses an inter-digitated electrode structure to create

¹ All figures are for DC fields (Paschen curve). Literature suggests that the limits are lower for high-frequency AC fields.

multiple parallel gaps with identical fields applied. Figure 5 shows the design of the inter-digitated electrodes. The use of multiple channels means space-charge effects are also reduced – it is more effective to transmit ions through several channels than through a single channel of equivalent cross-section – and ensures that the device does not reduce the conductance of the mass spectrometer inlet.

WHY USE ULTRAFAIMS WITH LC-MS?

Combining separation techniques is most beneficial when the separations from each stage are based on unrelated properties of the analytes, for example the chemical affinity to a stationary phase (in LC) and mass-to-charge ratio (in MS) – the separations are described as being orthogonal. The major benefit of combining FAIMS separation with LC-MS is that the differential mobility property is not highly correlated to m/z and therefore it has a good potential to separate peaks that would otherwise not be resolved. This contrasts with low-field IMS techniques, which are not very orthogonal to mass spectrometry.

But to be able to benefit from this orthogonality, you need to be able to do the FAIMS separation quickly enough so that it fits into the LC-MS timescale. This is why ultraFAIMS is so powerful in this context – the ion separation time is so short (fractions of milliseconds) that the full compensation field can be swept within the duration of a single LC peak, so you get the benefit of the full peak capacity of the FAIMS device rather than being limited to selecting only a few compensation field settings. The ultraFAIMS system can cycle through up to 500 independent CF values per second (for MRM analysis), or in continuous scanning mode, can produce up to 4 full spectra per second.

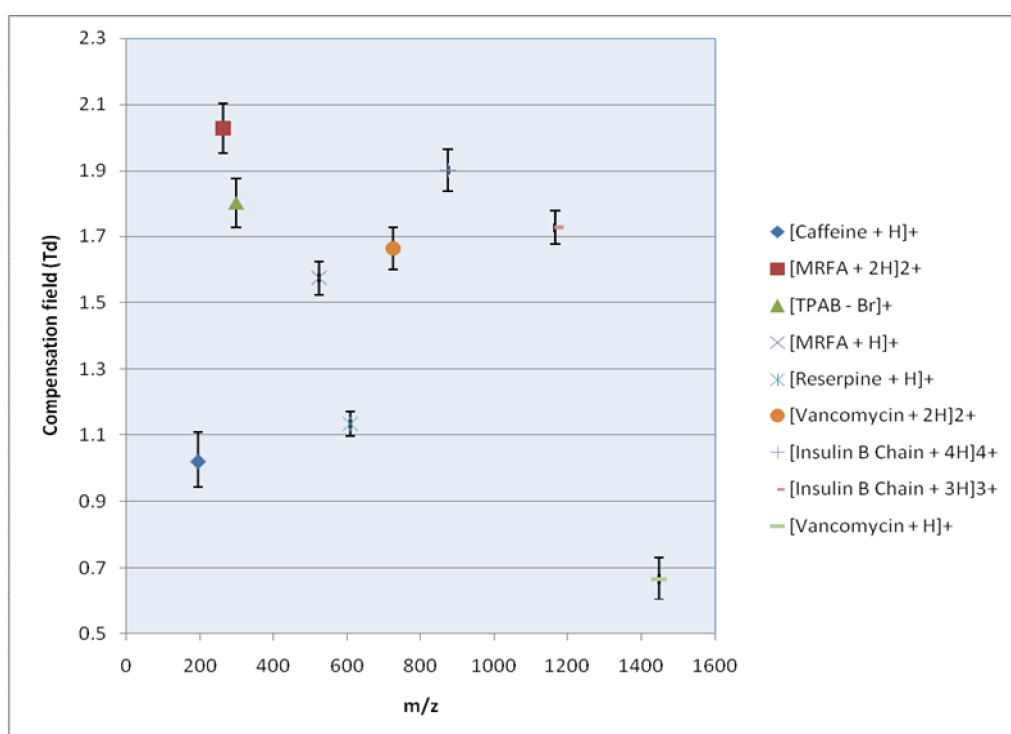


Figure 6: Experimentally determined ultraFAIMS CF values for a range of ions, showing high orthogonality (low correlation) between CF and m/z (Data provided by Colin Creaser and Rob Smith, University of Loughborough)

So what can ultraFAIMS-MS do?

UltraFAIMS separation is a novel technique, and applications are being explored. The following sections illustrate some of these applications.

1. Improve signal-to-noise of low abundance analytes

UltraFAIMS can be used in a targeted mode to filter out chemical noise in order to improve detection of low abundance ions. In this mode, a compensation field value (or series of values) is applied that transmits the ion(s) of interest. Ions that require different compensation field values cannot pass through the device, and so the mass spectra acquired are much cleaner. The following two examples demonstrate this.

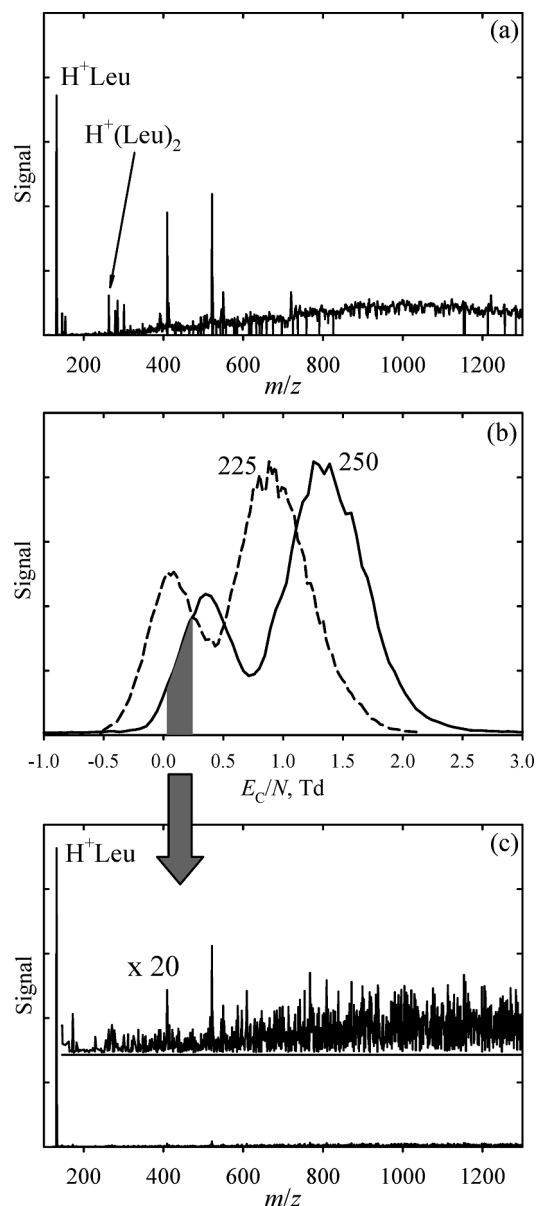


Figure 7: Data for leucine solutions in a complex matrix: (a) total MS spectrum; (b) FAIMS spectra for two dispersion field values as labeled (Q_{out} 1.1 L/min); (c) MS spectrum integrated over the compensation field (CF) range of 0.03-0.24 Td marked in (b) with an inset showing the baseline magnified by x20 – this figure shows that by selecting a small part of the CF range, much of the background is filtered out, enhancing the signal to noise ratio of the Leucine H^+ ion dramatically (Shvartsburg 2009b)

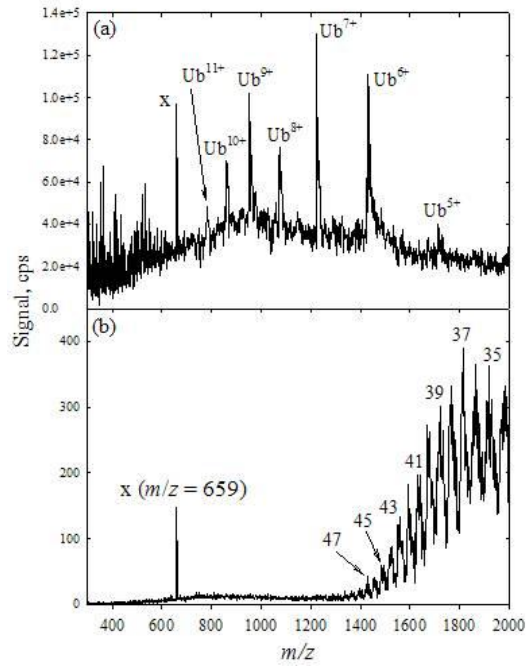


Figure 8: Mass spectra for solution of ubiquitin plus trace concentration of BSA in acidified dirty water (a) without FAIMS and (b) with FAIMS using a dispersion field of 250Td and compensation field of 0Td. In (b) the low abundance BSA ions (and an unknown ions at $m/z=659$) which are otherwise hidden by the complex background are now visible.

[Data provided by Alex Shvartsburg, PNNL]

2. Assist metabolite characterization

In this example, the ultraFAIMS device was used to reduce the intensity of the excipient ions present in the sample in order to improve the signal-to-noise ratio of the parent drug ion and metabolites

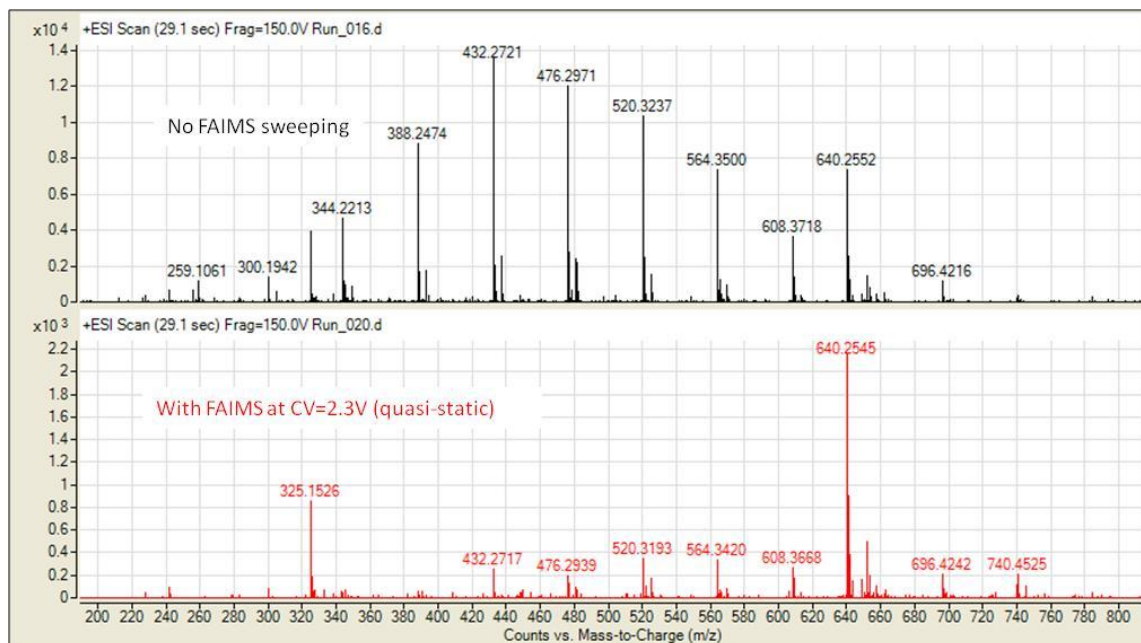


Figure 9: 10 μ l injection of sample containing parent drug (m/z 640) and metabolite ions (m/z 242 and 325) plus PEG 400 (a) with no FAIMS sweeping, and (b) with FAIMS compensation field of 2.9Td and a dispersion field of 260Td. In (b) the intensity of the PEG ions is significantly reduced, improving detection of the parent and metabolite ions.

[Data supplied by Dan Weston, Astra Zeneca, UK]

3. Improve mass measurement

Since there is low correlation between the compensation field that allows an ion to pass through the FAIMS device and its mass-to-charge ratio, it is often possible to use FAIMS to separate isobaric ions, enabling the accuracy of mass measurement to be improved.

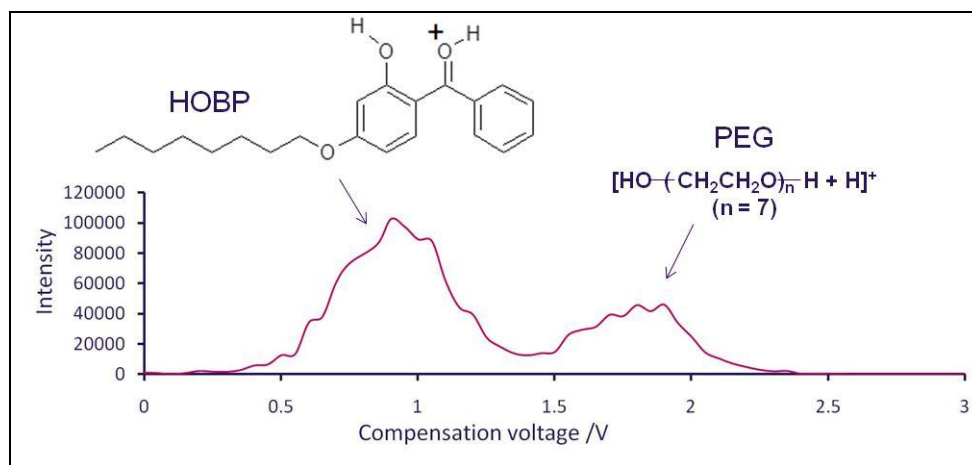


Figure 10: CV scan of m/z 327.2 with resolved protonated PEG 400 ($n=7$, m/z 327.2013) and protonated HOBP (m/z 327.1955) ions

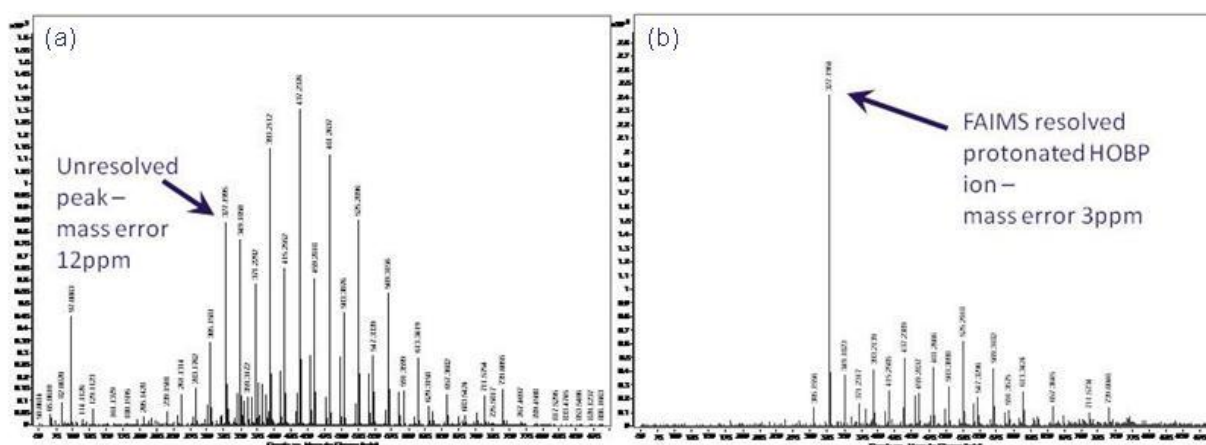


Figure 11: Mass spectra of the mixture of PEG400 and HOBP (a) without FAIMS sweeping, and (b) with FAIMS compensation field of 0.75-0.88Td and dispersion field of 260Td, showing improved mass accuracy for the HOBP ion.

[Data supplied by Colin Creaser & Lauren Brown, University of Loughborough]

4. Enhance peptide identification when using in-source CID

FAIMS followed by in-source CID may be used as a means of isolating and fragmenting ions in the absence of MS/MS capability. The sequence of spectra below shows the use of ultraFAIMS to isolate a single charge state of a peptide, followed by in-source fragmentation using the TOF fragmentor voltage, as a means of producing a peptide mass fingerprint. Without the FAIMS separation, the fragment ions are masked by the presence of other ions, but by applying the correct CV to isolate the target parent ion, many more fragments can be identified. The following example demonstrates this for a single peptide in a mixture, but the fast stepping capability of the ultraFAIMS device means that the same process could be applied to each of the peptides in sequence.

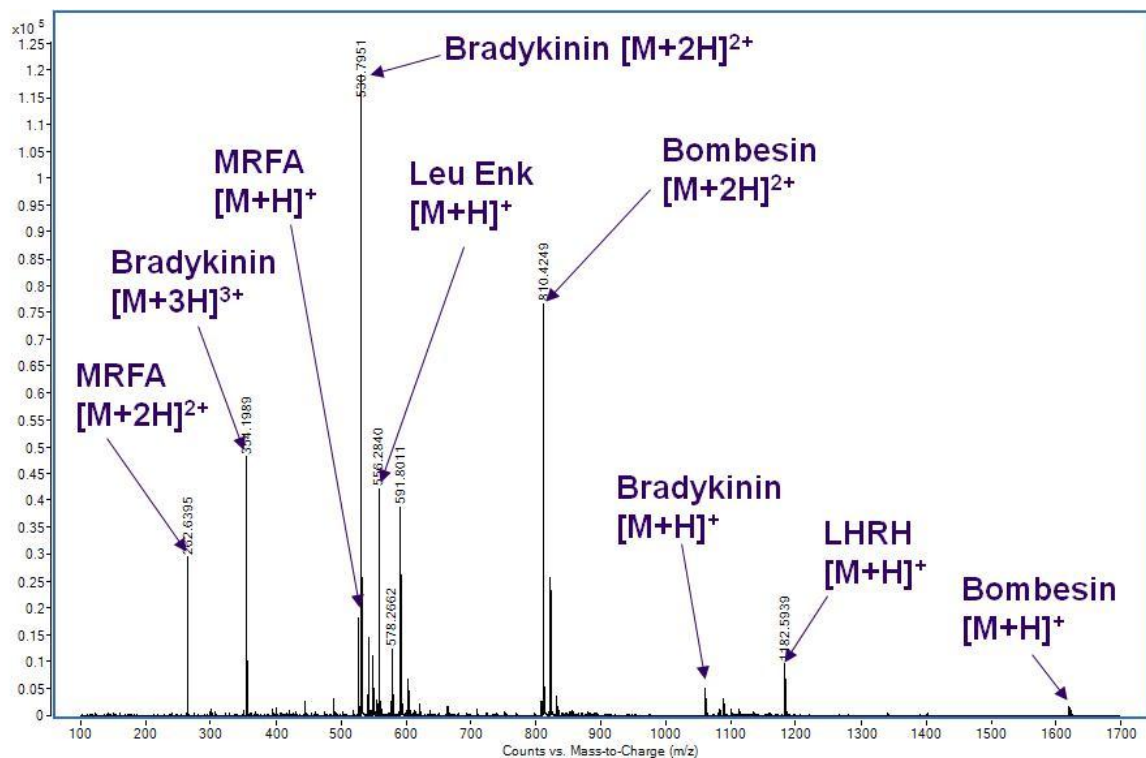


Figure 12: TOF mass spectrum of directly infused peptide mixture without FAIMS separation, showing the presence of multiple peptide ions including different charge states.

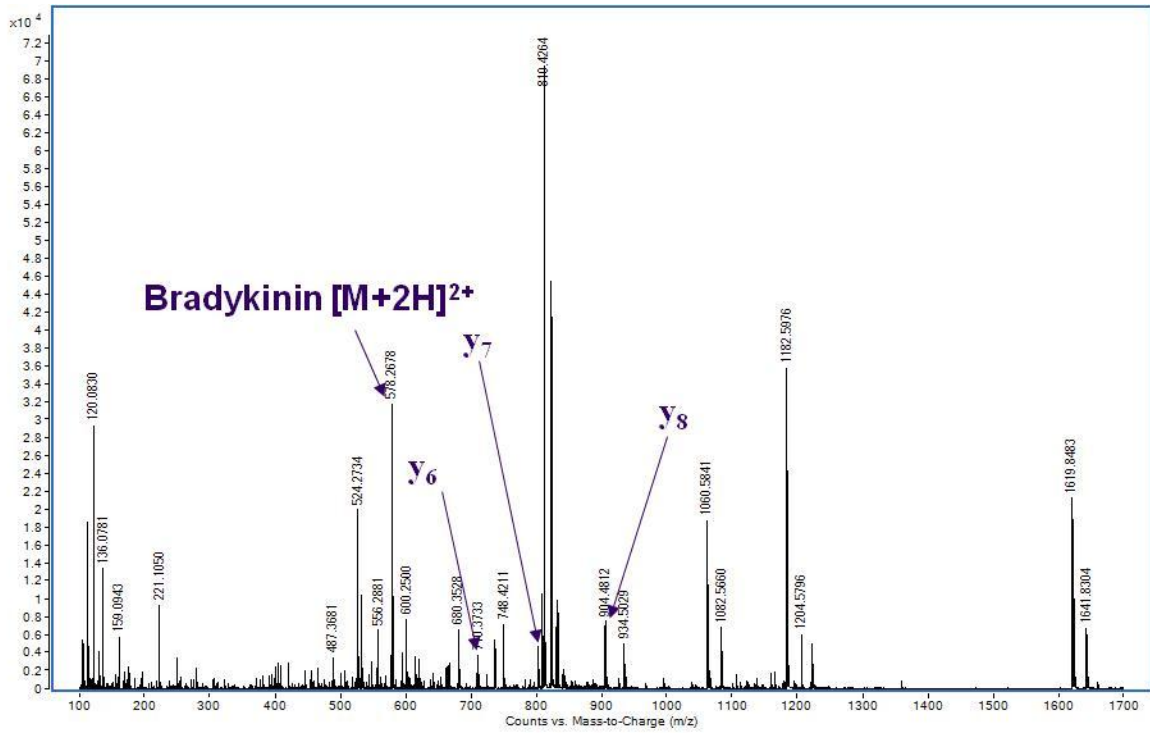


Figure 13: TOF mass spectrum without FAIMS after in-source CID (using fragmentor voltage of 350V) – some of the Bradykinin y-fragment ions are visible, but other fragment ions are masked

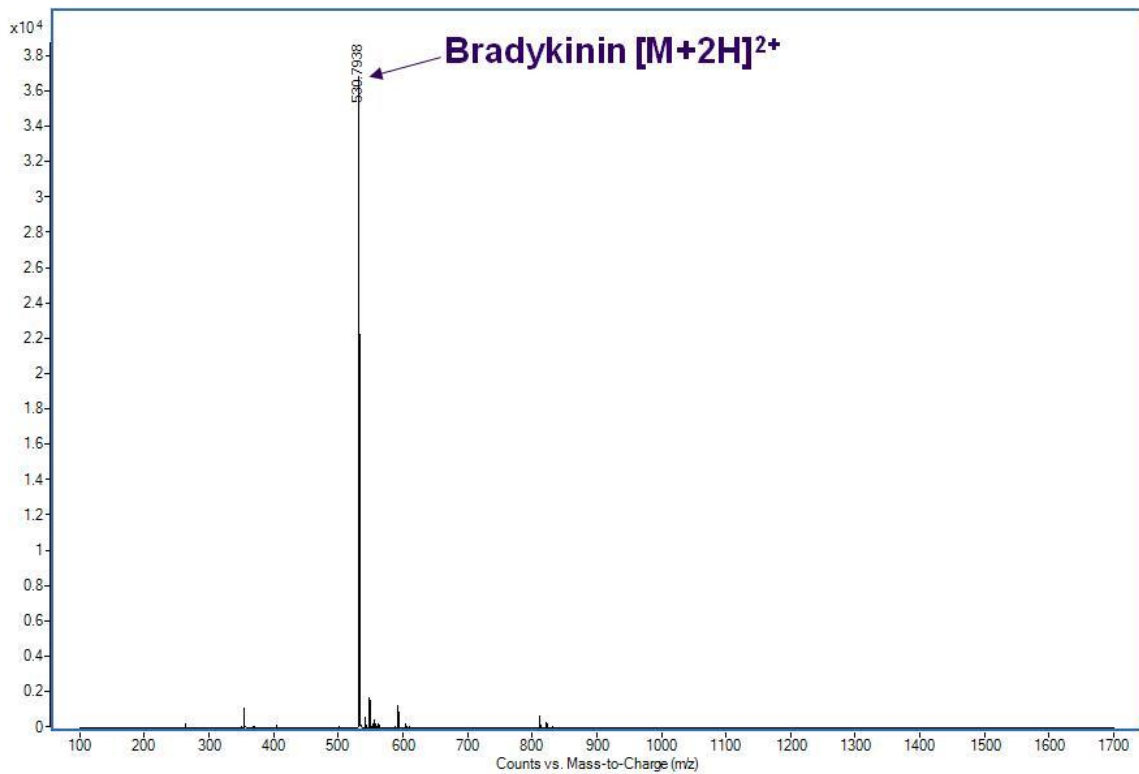


Figure 14: FAIMS CV of 2.6-2.7V is used to isolate the Bradykinin [M+2H]²⁺ ion (without CID), showing the isolation of a single peptide ion

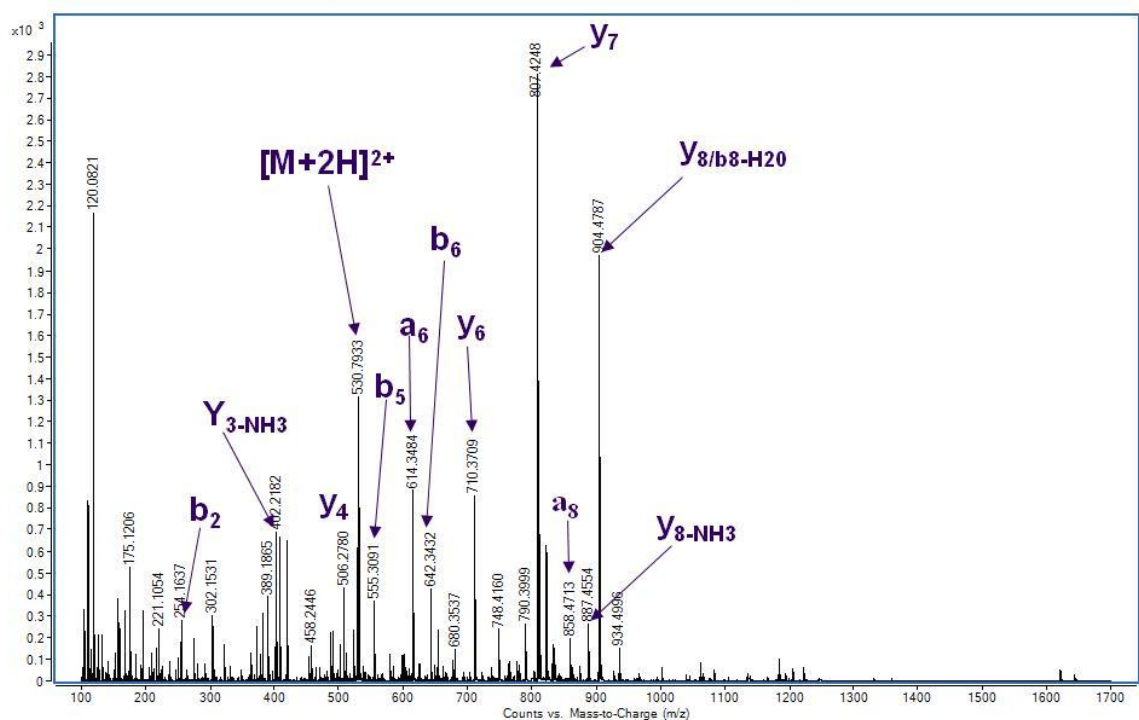


Figure 15: Applying the same CV as above, together with a fragmentor voltage of 350V, produces a spectrum with only the fragments from the Bradykinin $[M+2H]^{2+}$ parent ion

m/z	Assignment
530.7955	$[M+2H]^{2+}$
614.3497	a6
710.3733	y6
807.4273	y7
904.4812	y8
1060.584	$[M+H]^+$

m/z	Assignment
254.1637	b2
305.1642	y2-NH3
322.1906	y2
380.245	a4
402.2182	y3-NH3
408.2403	b4
419.2452	y3
489.2515	y4-NH3
506.278	y4
527.315	a5
530.7933	$[M+2H]^{2+}$
555.3091	b5
614.3484	a6
642.3432	b6
653.3496	y5
710.3709	y6
790.3999	y7-NH3
807.4248	y7
887.4554	y8-NH3
858.4712	a8
b8+H2O/y8	b8+H2O/y8

Figure 16: The peak lists generated from the spectrum produced without FAIMS show that 6 ions associated with Bradykinin could be identified

[All data supplied by Colin Creaser & Lauren Brown, University of Loughborough]

Figure 17: By using FAIMS, the software was able to identify 21 ions

5. Separate co-eluting compounds

Because the ultraFAIMS device can carry out complete CF sweeps very fast, it can be used to separate compounds that co-elute from the LC column, without the need for preliminary infusion experiments to establish the compensation fields required for each compound of interest. This makes it much quicker to establish whether the ultraFAIMS stage will enhance a particular LC-MS assay. The following example shows the separation of two of the charge states of [Val⁴]-Angiotensin from a PEG 415 background.

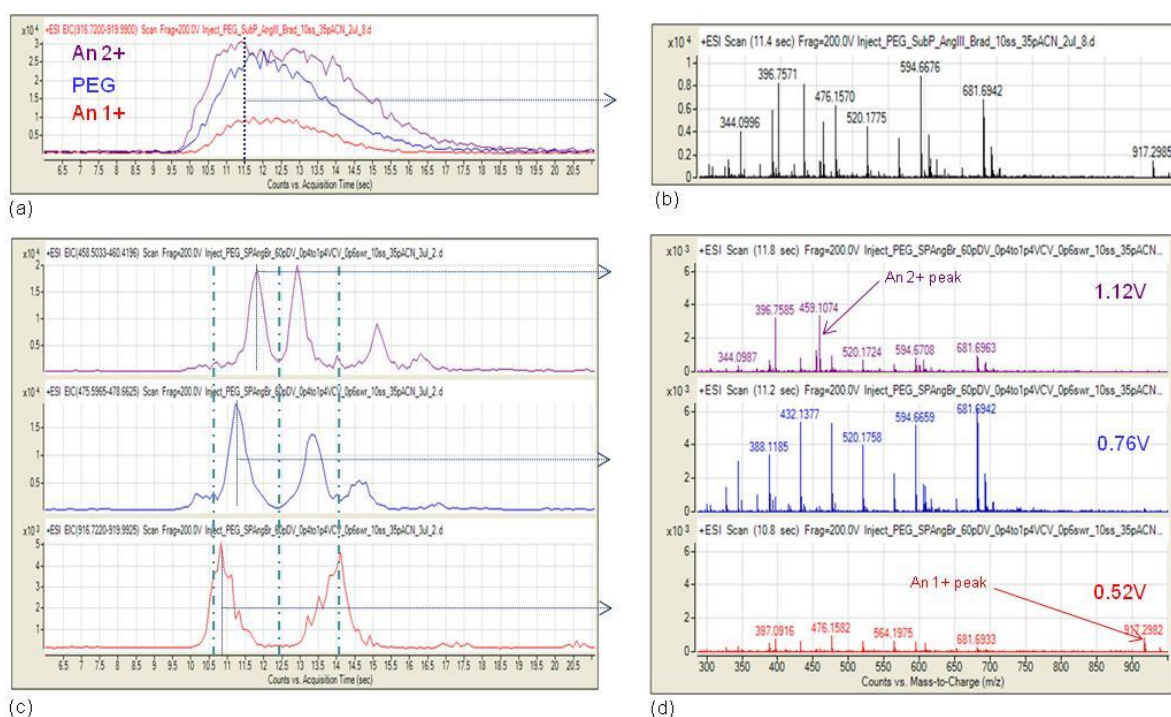


Figure 18: LC injection of [Val⁴]-Angiotensin and PEG 415 (Column: 75mm x 5mm Poroshell 300SB-C8; mobile phase: gradient water:acetonitrile mixture with 1% formic acid) (a) extracted chromatograms for the two angiotensin charge states and PEG 415 ion (as labelled) without FAIMS separation showing co-elution, (b) mass spectrum at the time marked in (a), (c) extracted ion chromatograms of the same 3 ions during an ultraFAIMS compensation field sweep with dispersion field set at 156Td, showing the different ions appearing at different compensation field values (time axis is equivalent to CF axis), (d) mass spectra at the times marked in (c), showing the angiotensin ions (top and bottom plots) separated from the PEG background (middle plot).

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