Owlstone Nanotech Inc.

An Introduction to Ion Mobility Spectrometry with UltraFAIMS

ultrafaims@owlstone.co.uk
www.ultrafaims.com

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The UltraFAIMS microchip is a miniaturized ion mobility device that interfaces with a mass spectrometer to provide additional in-source separation of ions.

Mass spectrometry is an incredibly powerful analytical technique, but as with all analytical techniques, it has certain inherent limitations. Chief amongst these is the inability to differentiate between compounds that have the same mass-to-charge ratio. It can also be very difficult to pick out the signal from a particular compound in a complex background.

These weaknesses can often be overcome by adding an additional filtering stage to the mass spectrometer; one that separates compounds based on a property other than mass-to-charge ratio. UltraFAIMS provides just such a filter by separating ions according to their mobility through a buffer gas when exposed to an electric field.

What is ion mobility spectrometry?

When an ion in a vacuum is exposed to an electric field, it experiences a constant force, F=qE, and its motion will depend only upon its mass and charge. However, if it is surrounded by a buffer gas, when the electric field is applied, collisions with the atoms of the gas will rapidly cause it to stop accelerating. Its terminal velocity, v, is related to the electric field strength, E, by

$$v = KE$$

where K, the ion's mobility, depends on its collision cross-section. An ion with a larger cross-section will collide more often, and hence will have a lower terminal velocity, and lower K. For a given ion, K is not a constant, but is instead a function of the electric field strength i.e. K=K(E). Ion mobility spectrometry (IMS) uses measurements of ions' mobility to identify them. The simplest form of IMS uses a drift tube in a way almost exactly analogous to time-of-flight mass spectrometers. By measuring the amount of time it takes an ion to travel the length of a gas-filled drift tube when propelled by a constant electric field, its velocity, and hence its mobility, can be directly calculated.

Field Asymmetric Ion Mobility Spectrometry (FAIMS)

UltraFAIMS uses a form of IMS more analogous to quadrupole mass spectrometry, known as field asymmetric-waveform ion mobility spectrometry (FAIMS). By applying an alternating electric field across the path of the ions, a filter can be set up that allows only certain ions through. The FAIMS system comprises two parallel plates, forming a channel through which ions can pass. An alternating voltage is applied across the plates, creating an alternating electric field in the space between them. The form of the voltage, shown in Figure 1, is a short-duration, high-amplitude positive section followed by a long duration, low-amplitude negative section.
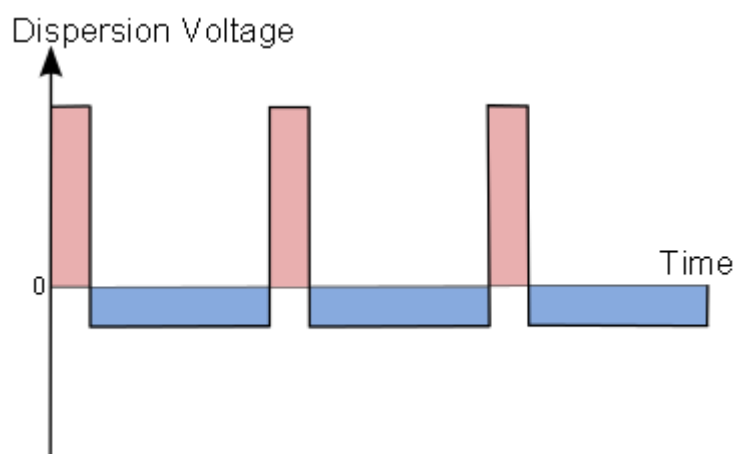
Figure 1 – Alternating Square Wave

The electric field is applied in a direction perpendicular to the ions' initial direction of travel through the channel, and as we have already seen, this will give an additional velocity v=KE to the ions in the direction of the field. As a result, the ions will travel along a saw-tooth trajectory in the channel, as shown in Figure 2.
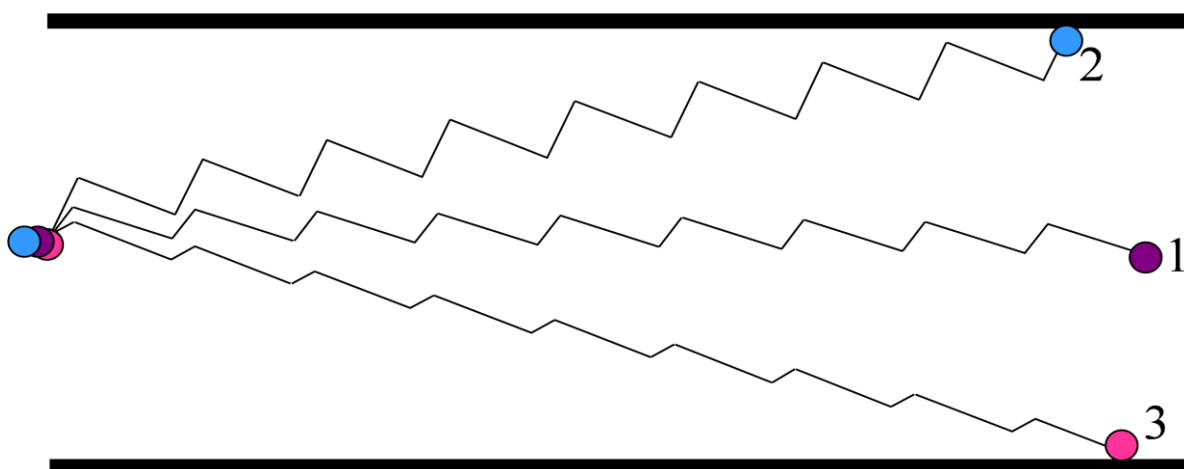

Figure 2 – Ion trajectories in FAIMS

The alternating voltage is designed so that the product of the voltage and the pulse duration is the same for the positive (pink in Figure 1) and negative (blue in Figure 1) sections. This means that if the ion's mobility, K, is the same under high-and low-field conditions, then the ion will move upwards during the positive section the same amount as it moves downwards during the negative section, giving a net vertical movement of zero, and allowing the ion to pass through the channel (along the path marked 1 in Figure 2). However, as mentioned earlier, the mobility will in general not be constant, but will instead vary with field

strength. If the mobility increases at high field strength, the ion will follow a path like that marked 2, and strike the top of the channel, while a decrease in mobility with increased field strength will result in an ion following a trajectory like that marked 3. An ion's path, then, is determined by its differential mobility (K_{high} –K_{low}); the greater the difference between the ion's high- and low-field mobilities, the more quickly it will hit the sides of the channel and be lost. Some examples of the ways in which differential mobility varies with field strength are shown in Figure 3.
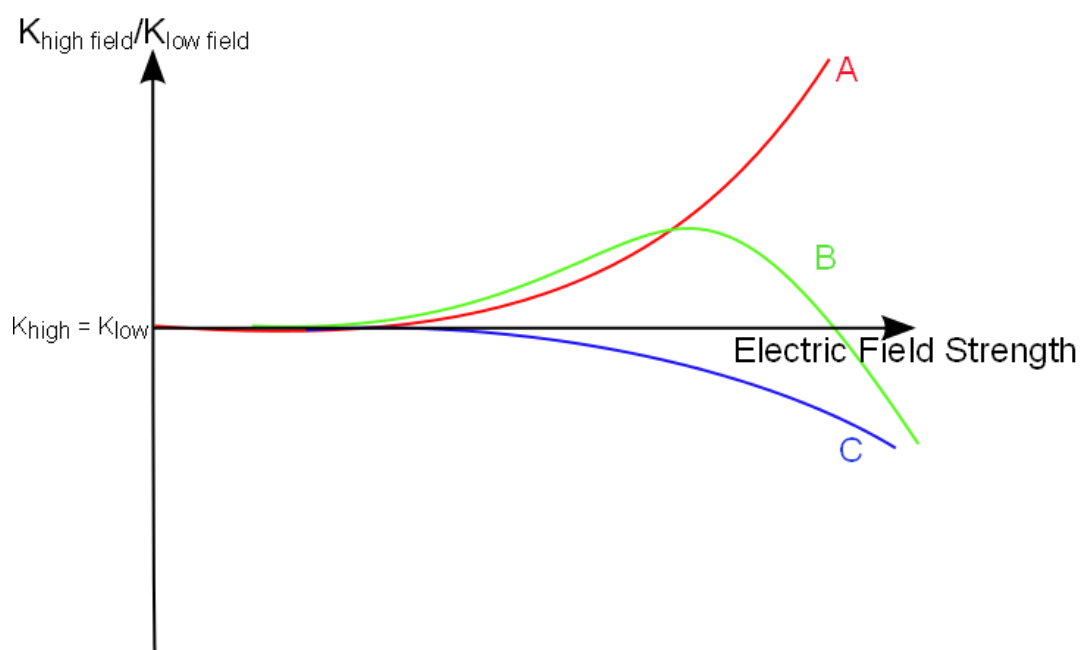

Figure 3 – Variation of mobility with increasing field strength

Selecting Ions

In order to control which ions pass through the system, an additional DC voltage can be applied between the plates. Often referred to as the compensation voltage (CV), its value can be tuned so as to exactly compensate for the net vertical drift of a particular ion. By scanning through values of the CV, and recording the ion current emerging from the channel at each, we can detect each of the ion types present.
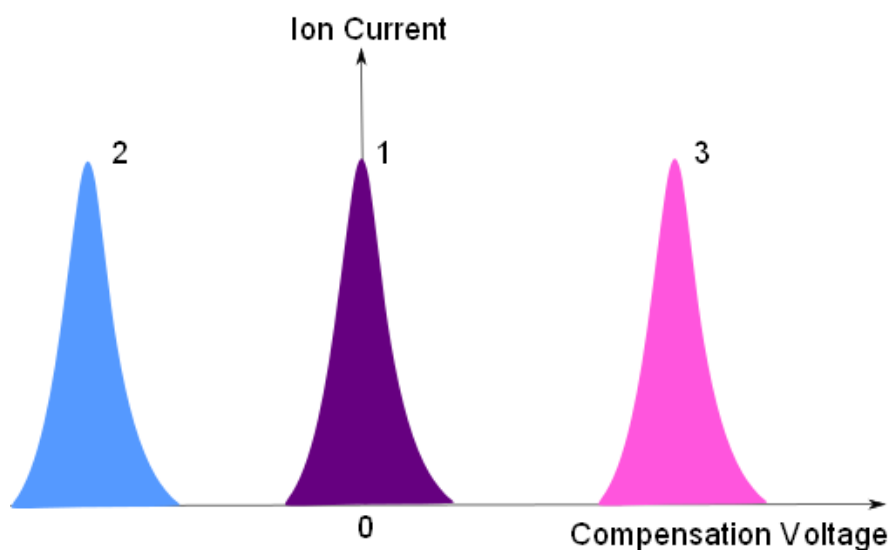
Figure 4 – Ion current peaks associated with ions 1, 2 and 3

The magnitude of the AC signal may also be altered, meaning the strength of the electric field (also known as the dispersion field, DF) in the high- and low-field sections can change (although the ratio remains constant). Because the mobility is a complicated function of field strength, this will result in a change in the differential mobility for each ion. This is important because a) as can be seen from Figure 3, at low field values, the differential mobility for all ions is the same (≈0) and b) even at high field values, two particular ions may happen to have the same differential mobility at a particular DF value (as can be seen from the fact that the lines marked A and B cross in Figure 3). By varying the DF, we can find a DF value at which any two compounds can be separated, as shown in Figure 5.
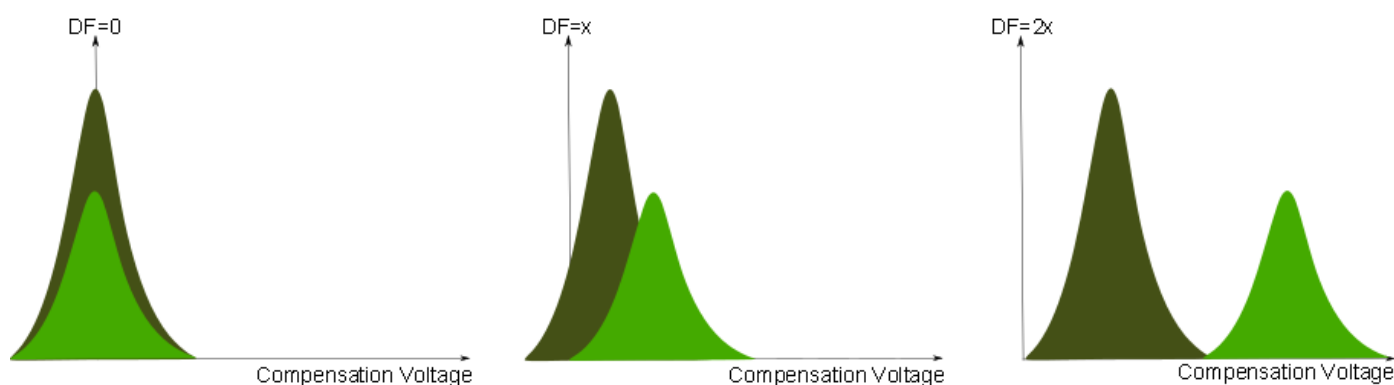

Figure 5 – Varying the dispersion field alters the compensation voltage necessary for different compounds to pass through the system

Why Does Mobility Change with Field Strength?

An ion's mobility is determined by how likely it is to collide with atoms/molecules of the buffer gas. A number of physical effects that affect this likelihood occur as field strength increases.

1. Ion Declustering

At low field strengths, each ion may be surrounded by atoms/molecules of the drift gas, increasing its effective cross-section and lowering its mobility by making collisions more likely. As field strength increases, partial or total declustering occurs, lowering the collision cross-section and increasing mobility.
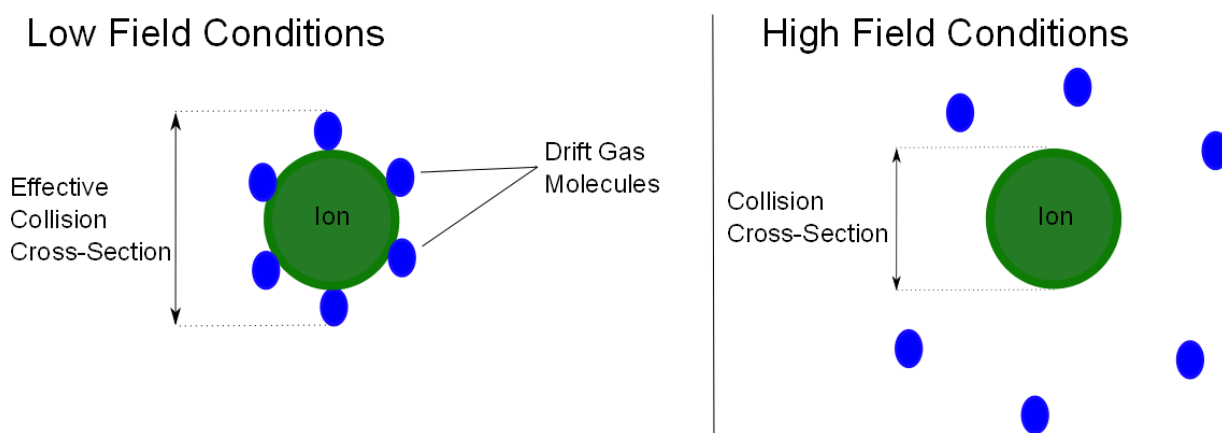

Figure 6 – Ion declustering

2. Field-Induced Structural Changes

The applied electric field may cause a change to the charge distribution of the ion, which may in turn cause a change to the 3D structure of the ion. This could either increase or decrease its collision cross-section, lowering or raising its mobility respectively.
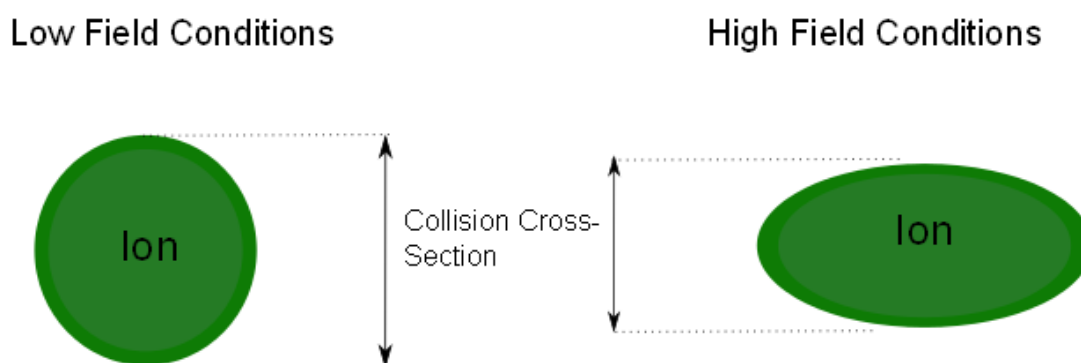

Figure 7 – Structural change caused by applied field

3. Collision-Induced Structural Change

At high field strengths, collisions between ions and drift gas molecules may be sufficiently energetic to cause structural changes to the ions. In extreme cases, fragmentation of the ions may be possible.

4. Orientation of Ions with Field

In low electric field conditions, the orientation of ions will be random, so their effective collision cross-section will be the average of their cross-sections in all possible orientations. In high fields, on the other hand, any ions with a permanent dipole will preferentially align along the direction of the field, and the cross-section in that orientation will determine their mobility.
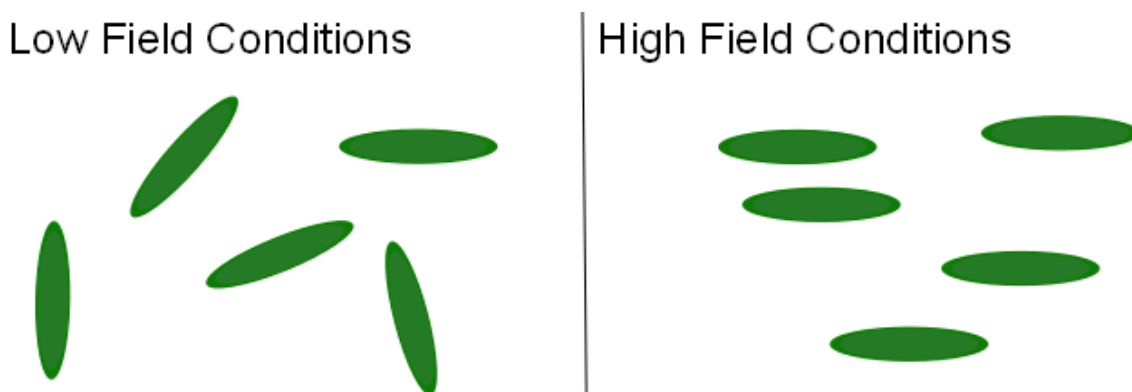

Figure 8 – Ions align with the external field

5. Thermal Heating of the Drift Gas

At high field strengths, the ions are accelerated more strongly by the field between collisions with drift gas molecules. This means that the resulting collisions are more energetic, and more energy is transferred to the drift gas molecules. This in turn increases the drift gas temperature, meaning the drift gas molecules are moving faster, and are therefore more likely to collide with ions, lowering the ions' mobility.

Using UltraFAIMS with a Mass Spectrometer

So how can FAIMS be used to improve results from a mass spectrometer? There are three general ways to do so: by using it to separate a small number of ions of interest; by using it to reduce background noise; or by using it to simplify complex spectra.

Setup

The ultraFAIMS device is an add-on component that can be attached to suitable mass spectrometers when extra separation is needed. It consists of the ultraFAIMS chip (the FAIMS device itself) mounted in a housing that is designed to fit the mass spectrometer inlet (atmospheric pressure interface), and a connected ultraFAIMS controller module where the FAIMS waveforms are generated. The ultraFAIMS chip housing is installed between the ionisation source and the MS inlet, as illustrated in Figure 9. The ionisation source

operates as normal, and the ions are then pulled through the ultraFAIMS chip by the gas flow into the mass spectrometer.
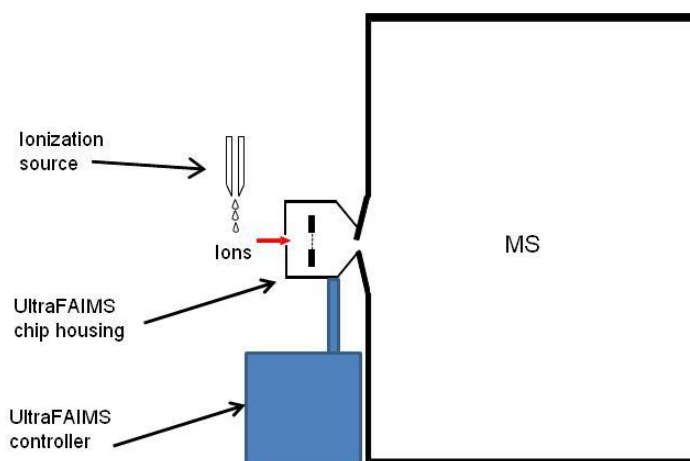

Figure 9 – Schematic of Mass Spectrometer with ultraFAIMS installed (components not to scale)

Software is provided to control the system. There are two basic modes of operation:

- **Sweep mode:** here the compensation field (CF) is ramped continuously between two points with the dispersion field (DF) held constant during the ramp. Subsequent ramps may be at the same or different DFs
- **Static/hop mode:** here the CF and DF are held constant for a defined period of time, and then stepped to a different pair of values for the next time period

Sweep mode is often used as the first step in method development, when trying to determine what FAIMS conditions give the best performance. It can also be used for untargeted analysis, where you want to add an extra dimension of nested separation to the MS or LC/MS data.

Static mode is most applicable to targeted analysis where you are mainly interested in transmitting a relatively small number of specific analytes. These two cases are explained in more detail below.

Separating specific ions of interest

If several different compounds with the same mass-to-charge ratio are present in an experimental sample, single stage mass spectrometry will be unable to distinguish between them. However, if these isobaric compounds have non-identical differential mobilities, the FAIMS device can be used to separate them before they enter the MS.

To do this, you would initially use sweep mode to scan through CV values, recording MS data for the ions of interest (or all ions). Plotting the MS data against time (which in this mode is equivalent to CV) produces a FAIMS spectrum. For each compound, there will be a peak in the FAIMS spectrum centred on the particular

CV that allows that particular ion to pass through the FAIMS electrodes. Figure 10 shows a representation of a FAIMS spectrum you might see for a pair of isobars transmitted at two different CVs. Repeating this with a variety of different conditions (e.g. different dispersion fields, temperatures, etc) would allow you to find the combination that offers best separation of the various ions of interest.
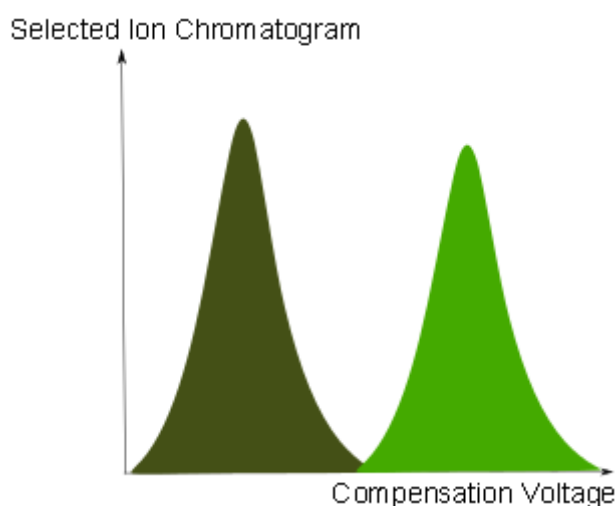

Figure 10 – Peak height versus compensation voltage

Depending on your experiment, you might then switch to "static/hop" mode, and set up a table of CF/DF values corresponding to best transmission of each ion of interest. The FAIMS device can hop between different set-points within milliseconds, so this table can be executed very rapidly. At each set-point, one of the ions of interest would be transmitted and the others excluded. This can be useful for example for quantitation.

Reducing Background Noise

Reducing background noise uses a similar approach to that described above. Again, the user would run sweeps to find the CVs at which ions of interest are transmitted. At any given CV, a subset of ions will be transmitted along with the ion of interest, but many others will not. This tends to lead to an improvement in signal-to-noise of the target compared to the matrix background. Different FAIMS conditions (DFs, temperatures etc) can be tested to find where the best improvement in signal-to-noise happens. The user can then use these conditions in static/hop mode to achieve lower limits of quantitation or detection.

This approach may be particularly useful as a way of reducing LC run times, or in cases where LC is not an option, for example with direct ionisation sources.
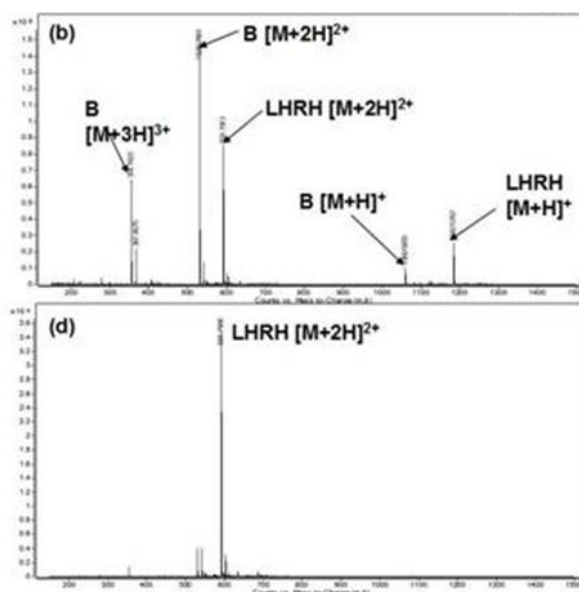
Figure 11 – Mass spectra (b) without and (d) with ultraFAIMS optimized for LHRH [M+2H]$^{2+}$

In proteomics, it also provides a means of selecting specific charge states of a protein or peptide for downstream analysis, since different charge states of an ion typically appear at different CVs.

Simplifying complex spectra

The final general scenario is when the aim of the analysis is not to detect or quantify specific target analytes, but to identify as many as possible of the ions in a particular sample. In this case, rather than looking for a needle in a haystack, the aim is more akin to examining every stalk of hay in the stack. FAIMS can provide benefit here too, by adding another dimension of separation.

In practical terms, the process is similar to the approach for the previous two scenarios. Initial sweeps can be used to determine the range of CVs across which ions are transmitted. The next step is slightly different. Rather than picking a small number of specific CVs to step between, you would either simply continue using sweep mode, possibly with the CV range narrowed to eliminate regions where nothing is transmitted, or divide the CV range into a number of blocks and use static mode to step through each block, holding CV fixed at the centre point of each block for a short period. Within each block, a subset of ions is transmitted – this helps break up a complex spectrum into several simpler spectra, and increases the likelihood of identifying low abundance ions that might otherwise be hidden in the noise.

This approach is likely to be applicable, for example, in a bottom-up proteomics workflow, where the aim is to maximize coverage of peptides in a sample.

If you have any further questions, even more information is available from www.ultrafaims.com or by contacting us at ultrafaims@owlstone.co.uk .