A recent study published by Arthur et al. in Analytical Chemistry has found that peak capacity can be improved in non-targeted ‘omics analyses by incorporating Owlstone Medical’s ultraFAIMS ion mobility device as an additional separation stage between liquid chromatography (LC) and mass spectrometry (MS) steps. The addition of ultraFAIMS increased the number features detected threefold, compared to LC-MS alone (Figure 1).

By increasing the number of identifiable analytes in complex biological samples, ultraFAIMS addresses one of the major challenges facing researchers undertaking non-targeted mass spectrometric analyses focused on metabolites, proteins, lipids or other sample components.

Introduction

Approaches that use MS typically employ chromatographic techniques (e.g. LC) to improve separation between components of the sample. However, difficulty resolving analytes hidden by coeluting isobaric species is common in non-targeted ‘omics studies. Detection is also often hampered by the presence of significant chemical noise.

Attempts to improve “peak capacity”; - the number of discrete analyte peaks that “fit” in a chromatogram - have included difficult sample preparation steps and lengthy chromatographic gradients. However, these approaches are often unsuccessful and can be unsuitable for non-targeted studies that aim to profile, for instance, a whole metabolome or proteome.
Improving peak capacity in ‘omics applications with ultraFAIMS

ultraFAIMS acts like a tuneable filter, helping resolve more features by reducing chemical noise and separating coeluting isobaric species across the entire analytical space. This increases selectivity and lowers the limit of detection (LOD), making more peaks ‘visible’ to the mass spectrometer, thus increasing peak capacity. Figure 1 shows that incorporating ultraFAIMS into the LC-MS workflow allowed many more features to be resolved in human urine.

How does ultraFAIMS work?

Field Asymmetric Ion Mobility Spectrometry (FAIMS) distinguishes ions according to differences in the speed that they move through a buffer gas under the influence of an asymmetric oscillating electric field.

As the mobility of the ions during the two parts of the waveform is rarely equal, there is usually a net drift towards one of the electrodes. In FAIMS, this net drift is corrected for by applying an additional DC voltage, known as the compensation field (CF), focussing specific ions through the device to the detector.

Unlike drift tube ion mobility spectrometry, FAIMS is orthogonal to both LC and MS because it is able to separate compounds that coelute from an LC column based on their differential mobility, which is not strongly related to their mass-to-charge ratio (m/z).

Figure 3: Electrospray ionization (ESI)-ultraFAIMS-MS analysis of a urine extract. (a) Direct infusion ESI-ultraFAIMS-MS heat plot of DF vs CF (with percentage intensity on the color scale); (b) three-dimensional plot with two-dimensional projection of retention time vs CF for LC-ultraFAIMS-MS; and (c) mass spectra extracted from LC-ultraFAIMS-MS data set at the same retention time and four different CF values.
**ultraFAIMS enables ultra-fast separation compatible with standard UHPLC run times**

Previous non-targeted ‘omics studies using macro-scale FAIMS instruments\(^2,^3\) have been limited by the time it takes the FAIMS device to sweep through the complete range of possible CF voltages: macro-scale FAIMS systems are only capable of making a complete CF scan in around 3 seconds. These slow scan speeds are incompatible with modern ultra high performance liquid chromatography (UHPLC) which produces narrow peak widths of 5 - 10 seconds. Incorporating macro-scale FAIMS systems into these workflows requires the use of limited CF scan ranges or very long LC run times. This results either in missing data or extremely time consuming analyses.

In contrast, the miniaturized ultraFAIMS device has extremely short ion residence times, so it can perform a full CF scan in ~1s. This makes ultraFAIMS compatible with UHPLC peak widths, and allows full LC-FAIMS-MS datasets to be collected using standard LC run times (~10 minutes) (Figure 4).

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**Figure 4: ultraFAIMS is able to scan across the full CF range in ~1 second making it compatible with UHPLC peak widths**

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**Summary**

Adding ultraFAIMS to your ‘omics workflow introduces a separation stage orthogonal to both LC and MS, enabling you to:

- Detect more features in ‘omics applications without affecting the run time
- Reduce chemical noise from complex samples
- Improve signal-to-noise ratios
- Increase sensitivity and dynamic range
- Detect previously hidden features by separating isobaric interferences
Retrofit ion mobility to your mass spectrometer
Learn more about ultraFAIMS

owlstonemedical.com/ultraFAIMS

References

