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LIQUID CHROMATOGRAPHY COUPLED WITH A MASS SPECTROMETER INCORPORATING A TRAVELLING WAVE ION MOBILITY SPECTROMETER FOR ANALYSING COMPLEX PEPTIDE MIXTURES

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OVERVIEW

In this poster we describe the use of an ion mobility spectrometer coupled with time-of-flight mass spectrometry for the separation and mass analysis of peptides.

IMS provides an additional stage of separation and can improve signal to noise by the removal of singly charged background ions from multiply charged peptides.

Post IMS fragmentation can be used to associate fragment ions with their precursor ions, providing extra specificity.

INTRODUCTION

The coupling of liquid chromatography with mass spectrometry is firmly established for the analysis of complex peptide mixtures. In this technique, species in a mixture are resolved by chromatographic retention time and mass to charge ratio and this can be considered as a two dimensional separation. The use of ion mobility spectrometry adds another dimension of separation, providing separation of species by their associated drift time, a factor which is dependant on ion charge state, mass and shape.
During an LC-MS experiment, there is an inherent background comprised predominantly of singly charged species. The acquisition of mobility separated MS data allows the data to be viewed in a three dimensional manner (m/z, drift time and HPLC retention time). Within this plot mobility separation of singly and multiply charged species can be clearly observed. This allows multiply charged species, which may be initially hidden by singly charged background ions, to be subsequently extracted thus improving the signal to noise within a mass spectrum.
This poster describes the preliminary results obtained by coupling a nanoscale liquid chromatograph to an experimental instrument incorporating IMS and Tof MS.

METHODS

IMS Enabled Mass Spectrometer The instrument used in these studies was an experimental hybrid Quadrupole/TWIMS/oa-Tof mass spectrometer, **Figure 1**. Briefly, ions produced by an ESI source are sampled by a z-spray source and pass through a quadrupole that may be set to select a particular m/z or pass a substantial mass range. They then enter a novel TWIMS device. This comprises three T-Wave [1] devices, the first device (accumulation T-Wave) accumulates ions and releases them in a short pulse (200µs) into the next device (IMS T-Wave) in which the mobility separation is performed. The final device (transport T-Wave) is used to transport the separated ions into the oa-ToF for subsequent analysis. Ions may be fragmented on entrance to the accumulation T-Wave and/or the transport T-Wave.

The pressure in the accumulation and transport T-Wave regions was ~ 10^{-2} mbar of Ar and the pressure in the IMS-T-Wave was 0.5 mbar of N_2.

The T-Wave pulse velocity and voltage were optimised to provide adequate ion mobility separation. Ion transit times through the system are recorded by the synchronisation of the oa-Tof mass spectral acquisition with the gated release of ions into the TWIMS. In a single IMS acquisition 200 pusher pulses are collected, each IMS experiment was 20ms in duration and the time between pushes was 60µs.

The instrument can be operated in High Duty Cycle mode. In this mode, the pusher is synchronised to the release of ions from the transport T-Wave device with a delay that is dependent upon the mobility separation such that the ion of interest is within the oa-extraction region when the oa-field is applied. This consequently leads to a signal increase over the entire mass range for a selected charge state.

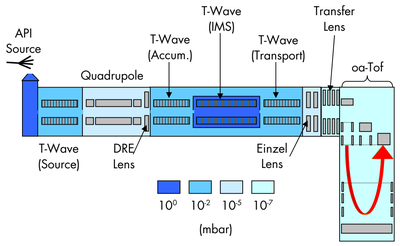


Figure 1. Schematic diagram of experimental instrumentation incorporating IMS and Tof MS.

Nanoscale Liquid Chromatography

Waters NanoAcquity UPLC operated in trapping mode.

Trap column	180µm ID x 20mm long, Symmetry C18
Analytical col.	75µm ID x 100mm long, Atlantis dC18
Solvent A	Aqueous 0.1% formic acid
Solvent B	Acetonitrile + 0.1% formic acid
Injection mode	2µL Full loop with 2X loop overfill
Trapping	100% solvent A at 5µL/min for 5 mins.
Gradient	1—40% B in 30 minutes at 300nL/min.

Samples

1 Tryptic digest of an *E. Coli* cytosolic fraction (Waters Corporation)
2 Digest of a mixture of four standard proteins; Alcohol Dehydrogenase, Phosphorylase B, Yeast Enolase and Bovine Serum Albumin (Waters Corporation).

Nano ESI Source

The analytical column output was coupled to a NanoLC sprayer. Sprayer position and source parameters were optimized by infusing a solution of 100fmol/µL of Glu-Fibrinopeptide B. A capillary voltage of 3.95kV was applied and a nebulising gas flow of approximately 5psi assisted nebulisation. The source temperature was set to 70C.

Mass Spectrometer Acquisition Parameters

The MS was set to acquire from 200-1000amu in 1 second with a 1 second interscan delay. Each 1 second integration represents the accumulation of fifty 20ms mobility events.

High Duty Cycle Mode Calibration

Calibration of the relationship between the pusher and transport T-Wave was achieved by infusion of a tryptic digest of Yeast Enolase (Waters Corporation), and the centre of the drift time window for doubly charged species over the mass range recorded.

Viewing Of Raw Data

Raw data is loaded into mobility viewer software which allows the visualisation of m/z vs. drift time plots. The separation of singly and multiply charged species in these plots can be clearly observed. Regions of interest can be highlighted and subsequently extracted as a new two dimensional raw file. This output file can be viewed as a typical chromatogram of intensity versus retention time (RT).

RESULTS

A Total Ion Chromatogram (TIC) from an LC-IMS MS experiment from the injection of 0.1µg of *E. Coli* tryptic digest is shown in **Figure 2** and loading these data into the viewer generates the m/z vs. drift time plot shown in **Figure 3**.

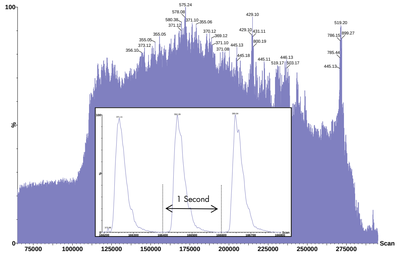


Figure 2. Background: Mobility enabled raw chromatogram obtained from the injection of 0.1 µg of E. Coli tryptic digest. In the foreground is the exploded view of the region 166200 to 166800 scans. Each 1 second section corresponds to the accumulation of fifty 20ms mobility experiments.

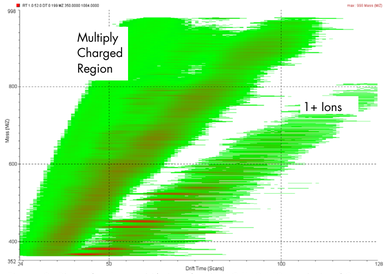
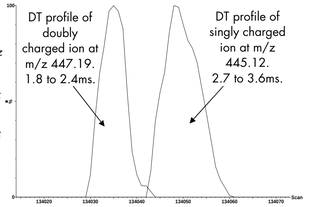


Figure 3. Plot of m/z vs. drift time for the LC-IMS MS analysis of 0.1µg of an E. Coli tryptic digest. The singly charged band and multiply charged regions are clearly separated and indicated on the plot.

Selecting only the portion of the data where multiply charged ions are present allows the removal of singly charged species and the detection of ions that may have been initially obscured by background in the TIC. This can dramatically enhance the signal to noise; **Figures 4** and **5**.

Figure 4. Drift time profiles, showing separation of a background singly charged ion at m/z 445.1 from a doubly charged peptide ion at m/z 447.19, which is obscured in the LC-MS data. A total of 5fmol of each protein in sample 2 was injected on column.



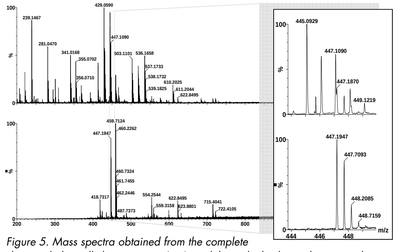


Figure 5. Mass spectra obtained from the complete data, including all charge states, (top), and the multiply charged region only (bottom). Removal of the singly charged species clearly enhances the multiply charged spectrum. The expanded section around m/z 445 on the right shows the emergence of the doubly charged species at m/z 447.19, which in the upper spectrum is masked by the isotope of the singly charged m/z 445.12 ion. The same intensity vs. RT chromatogram scan numbers are combined to produce each spectrum and a total of 5fmol of each protein in sample 2 was injected on column.

High Duty Cycle mode (HDC)

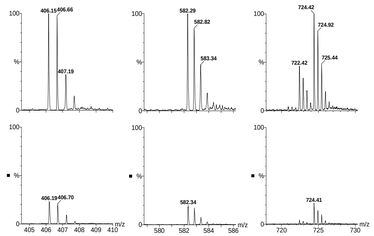


Figure 6. The effect of HDC mode (top) compared to the normal un-synchronised mode of operation. For a particular charge state, HDC mode gives a signal increase over the entire mass range. Data shown is from the injection of 5fmoles of sample 2 and three doubly charged ions, m/z 406, 582.3 and 724.4 are illustrated. The same number of scans are combined across the apex of each chromatographic peak and data is normalised to the HDC 'on' signal at each mass.

An important aspect of experiments [2] where there is no selection of precursor ions with the quadrupole and where all co-eluting precursors are fragmented simultaneously, is the correlation of fragments to their precursors.
By operating the experimental IMS enabled mass spectrometer such that fragmentation occurs after the IMS separation, the fragments will exhibit the same drift time characteristics as their precursor ions. This extra degree of separation, **Figure 7**, can help to determine the origin of the fragments and to produce different, specific, fragment ion spectra at the same retention time, **Figure 8**.

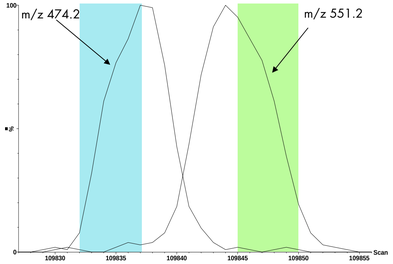


Figure 7. Post IMS fragmentation. A total of 20fmol of each protein in sample 2 was injected. At a retention time of 18.4minutes, the drift time profiles for two doubly charged co-eluting species of m/z 474.2 and 551.2 are shown. The shaded 300µs regions are the drift time windows selected to produce the elevated energy, fragment ion, spectra in figure 8.

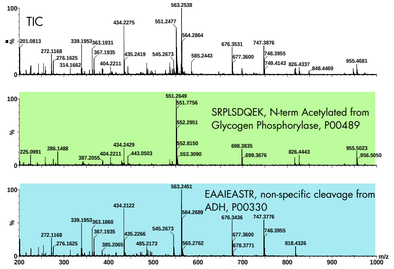


Figure 8. Post IMS fragmentation. Elevated energy spectra (+15eV) obtained by selecting the different drift time windows shown in figure 7. The TIC spectrum, which is produced by combining over all drift times, is equivalent to a spectrum acquired without IMS separation and is clearly a combination of the two lower spectra. The same intensity vs. RT chromatogram scan numbers are combined to produce each spectrum.

CONCLUSION

- **An experimental instrument incorporating IMS and TOF-MS, has been combined with nanoscale LC and used to analyse complex peptide mixtures.**
- **The mobility drift time of singly charged species is different from multiply charged ions of the same m/z. Through the post processing removal of singly charged ions from the data, dramatic signal to noise enhancements can be achieved.**
- **The High Duty Cycle mode of operation provides a signal increase for a chosen charge state.**
- **Inducing fragmentation after the IMS separation results in fragments and precursors which exhibit the same drift time, providing additional specificity.**
- **The extra dimension of separation provided by IMS could potentially have benefits in the processing of data generated in experiments where all ions are transmitted through the quadrupole and fragmented simultaneously.**

References

[1] "Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S.Pringle, K. Worthington and R. Bateman— Presented at the 51st ASMS Conference, Montreal, Canada 2003. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

[2] J. Silva *et al.* Anal. Chem., **2005**, 77, 2187.

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