A NEW ION MOBILITY BASED METHOD UTILIZING TIME VARYING COLLISION ENERGY TO IMPROVE THE FRAGMENTATION EFFICIENCY OF MULTIPLE PRECURSOR IONS

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OVERVIEW

PURPOSE

- Investigate the potential improvement in fragmentation efficiency of a wide range of precursor ion masses using a novel IMS "Lift" approach.

METHODS

- Waters Synapt HDMS and Acquity Nanoscale LC IMS-MS and LC IMS-MSMS and pseudo parallel MS^3.

RESULTS

- Significant increase in fragmentation efficiency in both shotgun and pseudo parallel MS^3 was observed

INTRODUCTION

Ion mobility spectrometry (IMS) may be used to determine the interaction cross-sections between an ion and a neutral gas, thereby providing ionic structural information for comparison with, or validation of, calculated values. The separation afforded by ion mobility (IM) broadly correlates with both mass and charge, a characteristic which has previously been exploited to enhance the transmission of a Quadrupole - IMS - oaToF and to reduce undesirable chemical noise. Here we report a new method exploiting these correlations where the potential difference between the IMS cell and a downstream fragmentation cell is varied over the IMS time so that the collision energy (CE) is optimised for ions exiting the IMS at a given time.

METHODS

A Synapt HDMS (Waters Corporation) was used in these studies, figure 1. In operation alternate scans of Low CE (non-fragmenting) and elevated CE (fragmenting) were acquired. When enabled, IMS was performed in three T-wave devices; Trap, IMS and Transfer. The system pressures during IMS operation were ~ 10^{-2} mbar of Ar in the Trap and Transfer regions and 0.5 mbar of N_2 in the IMS T-Wave. The pressure during ToF only operation was ~ $8x10^{-3}$ mbar of Ar in the Trap and Transfer T-Wave regions. In this mode of operation the Trap CE potential was ramped between 12 and 35 eV during the Elevated CE scan.

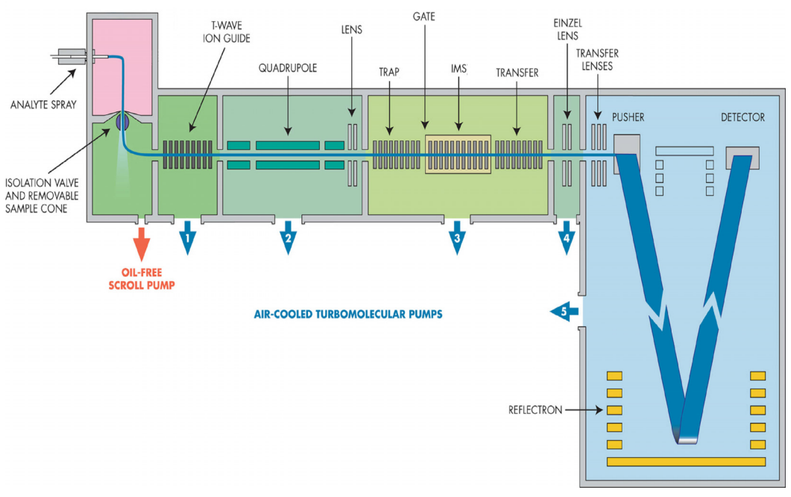

Figure 1 Diagram of the Synapt HDMS System instrument.

The Synapt utilizes a novel IM linked CE ("Lift") mode of operation[1,2]. When using this mode, ions are enter the IMS T-Wave as in normal operation and a pre-programmed DC potential profile is applied to the IMS T-Wave only. The start of the profile is synchronized with the start of the IMS experiment. This IMS "Lift" potential is defined as a look-up table with a Transfer CE defined for every IMS channel/ spectrum to allow maximum flexibility. To maintain system dynamic range the Transfer T-Wave pulse voltage may be reduced to produce a pseudo-continuous beam. Alternating Low and IMS-Profiled CE scans may be acquired.

Nanoscale LC Waters NanoAcquity UPLC

Trap Column	180µm ID x 20mm long, Symmetry C18
Analytical Col.	75µm ID x 200mm long, BEH 1.7µm
Solvent A	Aqueous 0.1% formic acid
Solvent B	Acetonitrile + 0.1% formic acid
Injection	Partial Loop mode
Trapping	100% solvent A at 15µL/min for 1 min
Gradient	1—40% B in 30 minutes at 300nL/min

For Nanoscale LC experiments 0.5 µl (total of 12.5 fM) of an equimolar mixture of four protein tryptic digests; Bovine Serum Albumin, Yeast Enolase, Yeast Alcohol Dehydrogenase and Rabbit Phos. B was injected.

RESULTS

In order to set up the Transfer CE look-up table a standard peptide mix solution (MassPrep -Waters Corp) containing equimolar amounts of nine peptides (RASG-1, Angiotensin frag. 1-7, Bradykinin, Angiotensin I & II, Renin substrate, Enolase T35 & T37 and Melittin) was infused at a rate of 1µl/min through the reference sprayer. This was chosen specifically to be different from the sample used in the fragmentation efficiency (FE) experiment to investigate the general applicability of this method. The optimum CE required to fragment a range of m/z and charge states was noted along with their peak arrival time. The optimum CE being defined as the energy required to fragment 90 to 95 % of the selected ion as shown in figure 2 along with the ramp chosen.

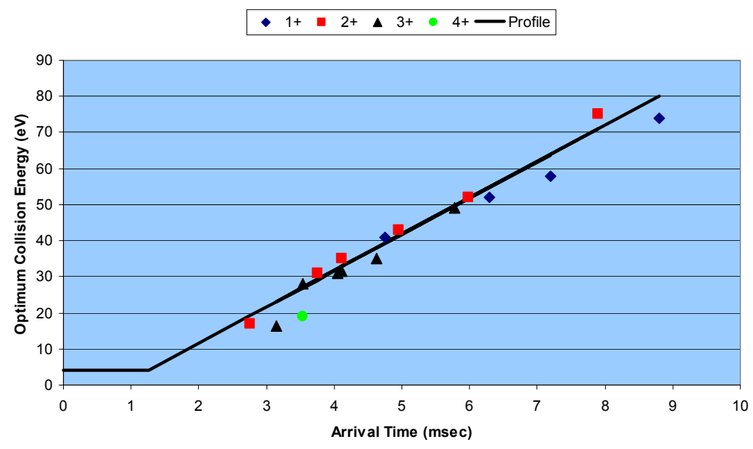

Figure 2 Graph showing optimum collision energy for various charge states as a function of arrival time and the profile applied.

The CE profile remains at 4 eV, which is the value used in the non-fragmenting mode, until 1.3 msec to allow all of the ions to be transferred into the IMS T-Wave before the "Lift" profile starts. A typical Low CE BPI chromatogram is shown in figure 3 highlighting the ion species that were used in the FE calculations. In this study the FE was defined as the intensity sum of the fragment ions belonging to the precursor chosen divided by the intensity of the precursor ion prior to fragmentation. In all cases the ratio of the IMS profiled CE to the standard CE ramp FE was greater than unity and averaged at 1.8 as shown in table 1.

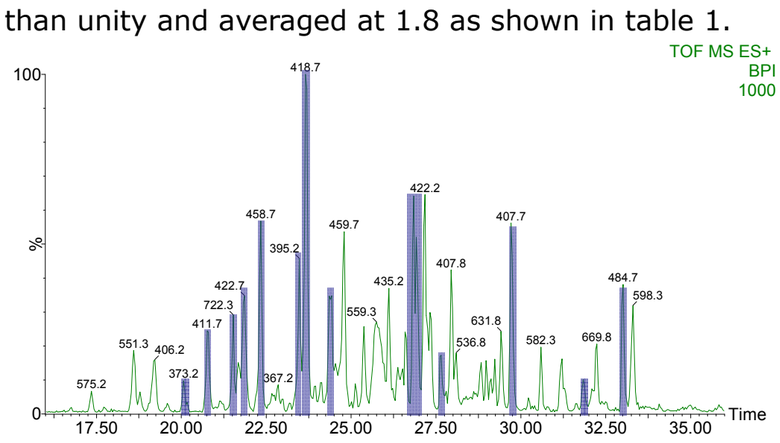

Figure 3 Shows a BPI chromatogram highlighting the ion species used in the efficiency calculations.

Compound	Precursor m/z		Efficiency Improvement
Phos. B	411.73	TIAQYAR	1.75
	422.25	VLVDLER	1.42
	458.75	NLAENISR	2.34
	527.75	TNFDAFPDK	1.88
	721.85	VLYPNDNFFEGK	2.81
BSA	395.24	LVTDLTK	1.89
	461.75	AEFVEVTK	2.18
	722.33	YIC(CAM)DNQDTISSK	2.30
Enolase	373.23	IATAIEK	1.01
	644.86	VNQIGTLSESIK	1.65
ADH	407.76	DIVGAVLK	1.25
	418.73	IGDYAGIK	1.65
	484.75	EALDFFAR	1.53
Average Improvement			1.81

Table 1 Shows the fragmentation efficiency improvement factor for the various species selected in this study.

A more challenging situation for fragmentation occurs when operating in a pseudo parallel MS^3 mode. A precursor ion is selected using the quadrupole and fragmented in the Trap T-Wave. The fragments are then IM separated in the IMS T-Wave and are further fragmented on entry to the Transfer T-Wave. By alternating between a Low and an Elevated Transfer CE, 2nd generation product ions may be assigned to 1st generation product ions based upon their arrival times. Figure 4 shows results obtained from Renin substrate ($(M+3H)^{3+}$ = 586.9).

In the Low Transfer CE data (4eV), two clear bands containing $(M+2H)^{2+}$ and $(M+H)^{1+}$ 1st generation fragment ions can be observed. As the CE increases, fragments at higher m/z from the doubly charged ions may be observed time aligned with their precursor ion. However higher m/z singly charged ions are not fragmented until a higher CE ~ 70eV is reached at which point a significant proportion of the shorter arrival time ions have been over fragmented.

Figure 5 shows results obtained using the same Transfer CE profile as used previously. The data clearly shows that even higher m/z singly charged

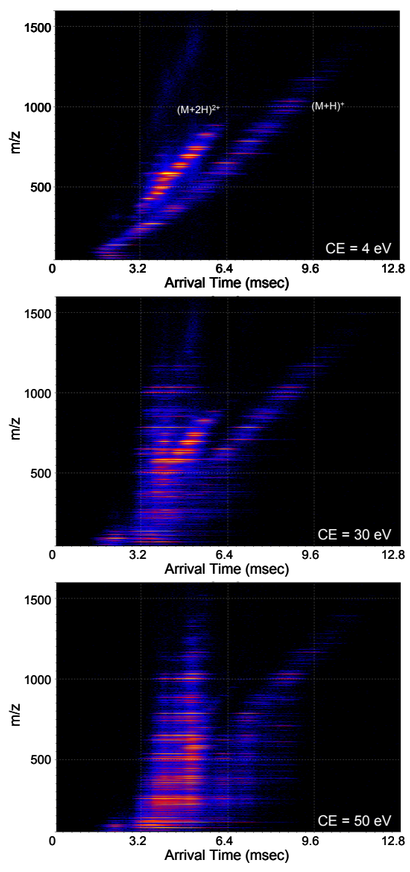

Figure 4 Shows m/z v's arrival time obtained using various static Transfer CE values.

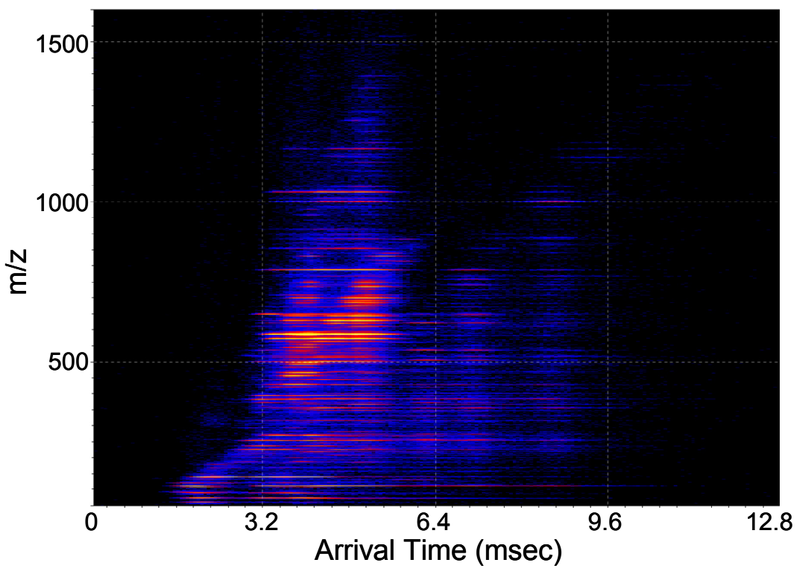

Figure 5 Shows m/z v's arrival time obtained using an IMS linked Transfer CE.

ions may be fragmented without over fragmenting the ions at shorter arrival times. This is further illustrated in figure 6 where mass spectra are shown taken from arrival times corresponding to (i) b_5^+,(ii) b_8^+,(iii) b_9^{+2} and (iv) b_{12}^{+2} ions for different Transfer CE conditions.

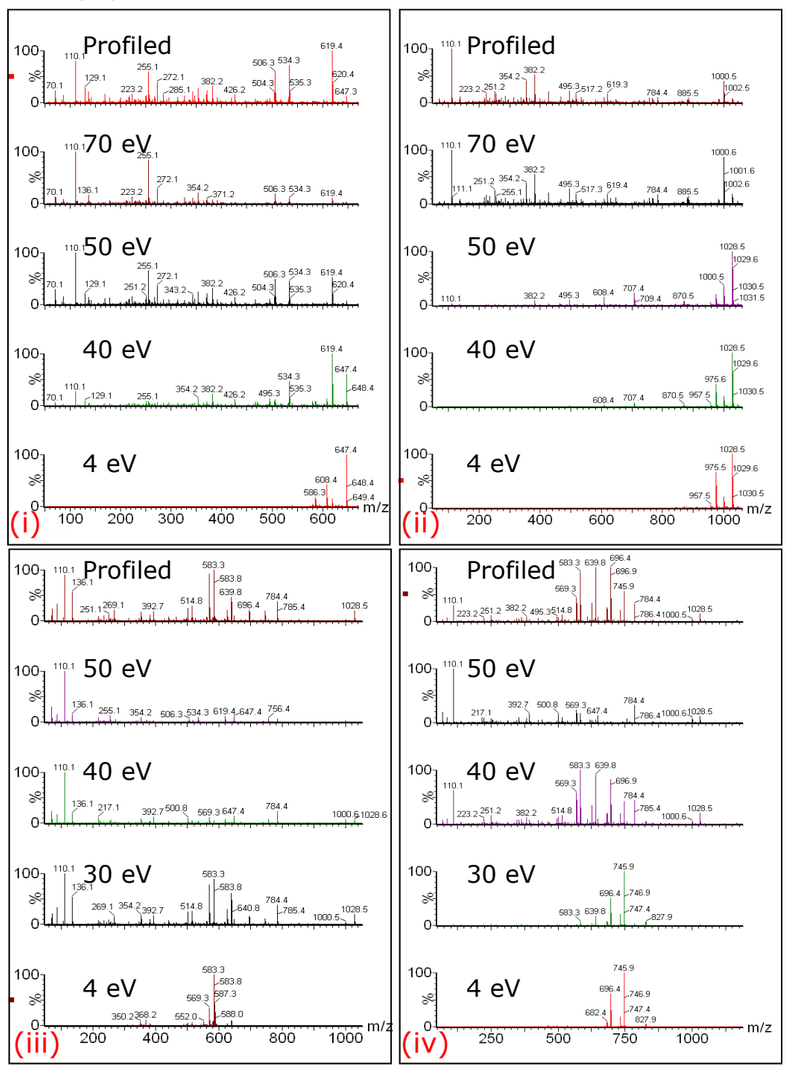

Figure 6 Shows example mass spectra taken from arrival times corresponding to (i) b_5^+,(ii) b_8^+,(iii) b_9^{+2} and (iv) b_{12}^{+2} ions for different Transfer collision conditions.

For example the optimum CE for the b_5^+ ion is ~ 50 eV, the b_8^+ ~ 70 eV, the b_9^{+2} ~ 30 eV and the b_{12}^{+2} ~ 40 eV, the profiled CE gave results similar to the static values stated above.

CONCLUSION

- Application of an Ion Mobility Linked Transfer T-Wave CE improved the Fragmentation Efficiency of a wide range of precursor ion masses in a nano-scale LC shotgun type experiment by an average of 1.8 with no detrimental side effects.
- The overall fragmentation quality in pseudo parallel MS3 type experiments was improved.

REFERENCES

[1] Wildgoose, Pringle, Giles, and Bateman, Patent Application WO 2006 / 0302505 A2 published 23th March 2006.

[2] Bateman, Giles, Pringle, and Wildgoose, Patent Application GB 2 439 814 published 9th January 2008.

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