

A New Ion Mobility Based Method Utilising 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 MS³.

RESULTS

- Significant increase in fragmentation efficiency in both shotgun and pseudo parallel MS³ was observed

INTRODUCTION

Ion mobility spectrometry 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 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 N₂ 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.

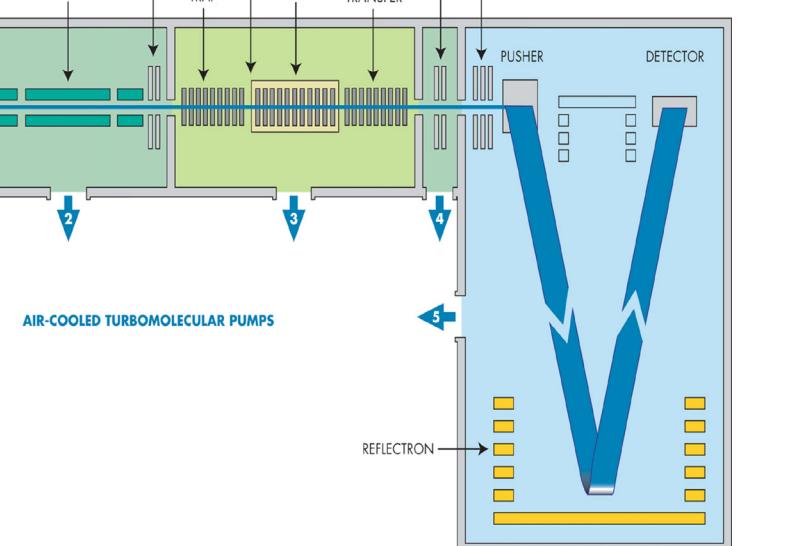


Figure 1 Diagram of the Synapt HDMS System instrument.

The Synapt HDMS mass spectrometer utilizes a novel Ion Mobility linked Collision Energy ("Lift") mode of operation^[1,2]. When operating in this mode, ions are infused at a rate of 1 μ l/min through the reference sprayer. This was chosen specifically to be different from the sample used in the fragmentation efficiency experiment to investigate the general applicability of this method. 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.

The instrument was calibrated using NaICsI.

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

For Nanoscale LC experiments 0.5 μ l (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,

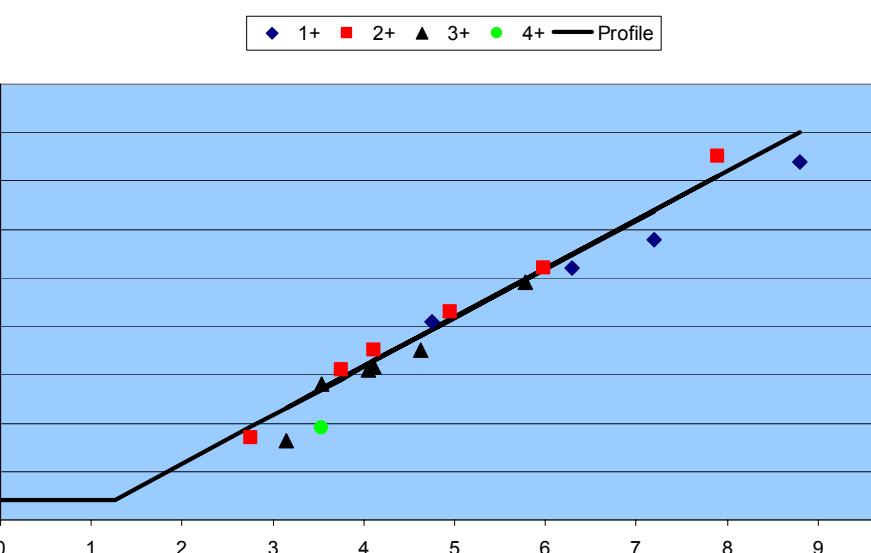


Figure 2 Graph showing optimum collision energy for various charge states as a function of arrival time and the profile applied.

intensity of the precursor ion prior to fragmentation. In all cases the ratio of the IMS profiled CE to the standard CE ramp fragmentation efficiency was greater than unity and averaged at 1.8 as shown in table 1.

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(CAN)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	EALDFRAR	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 the Synapt HDMS in a pseudo parallel MS³ mode. Here a precursor ion is selected using the quadrupole and fragmented in the Trap T-Wave. The fragments are then mobility 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 a pseudo parallel MS³ experiment using 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.

This is further illustrated in figure 6 where mass spectra are shown taken from arrival times corresponding to (i) b₅⁺, (ii) b₈⁺, (iii) b₉⁺² and (iv) b₁₂⁺² ions for different Transfer CE conditions.

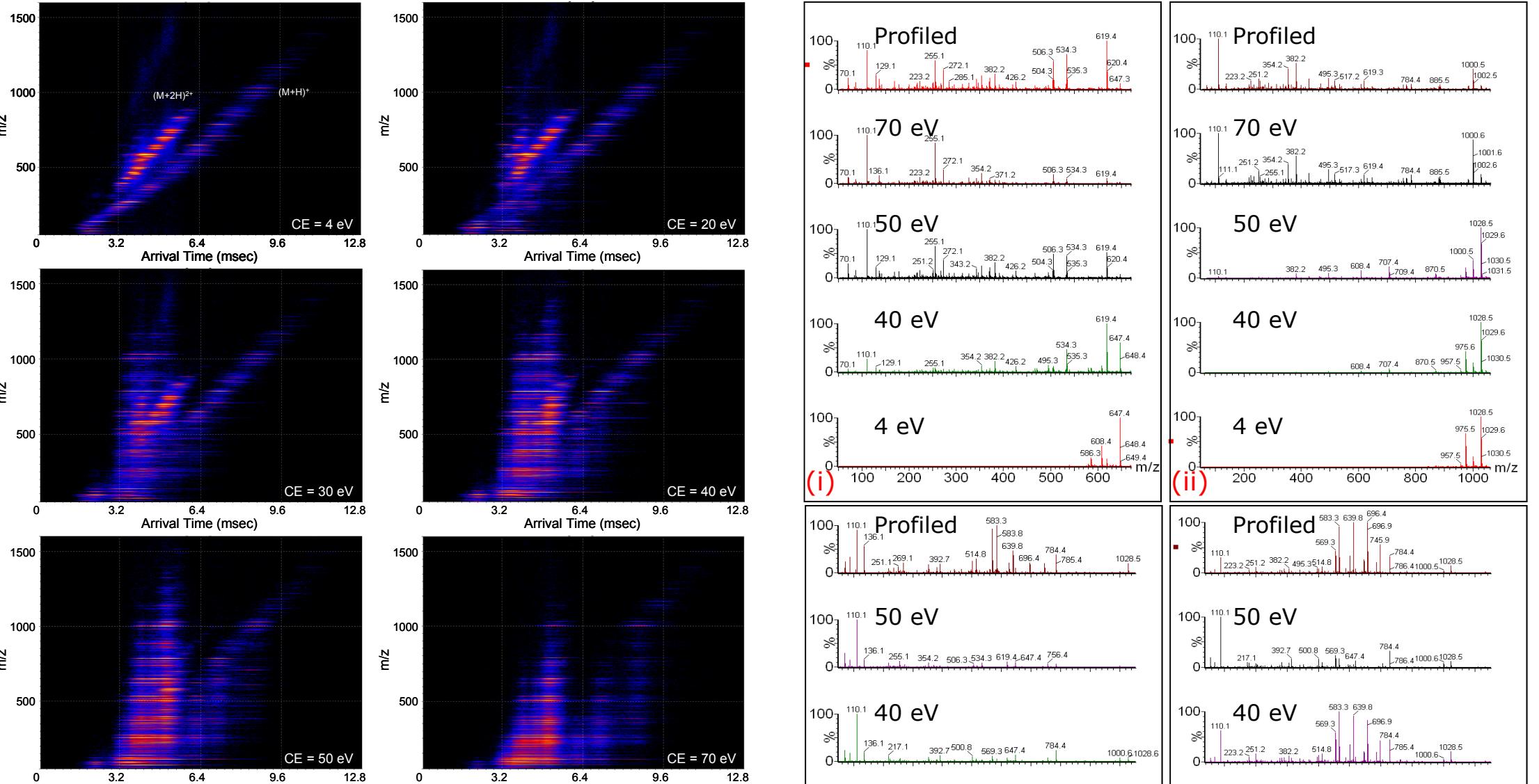


Figure 4 Shows m/z v's arrival time obtained using various static Transfer CE values.

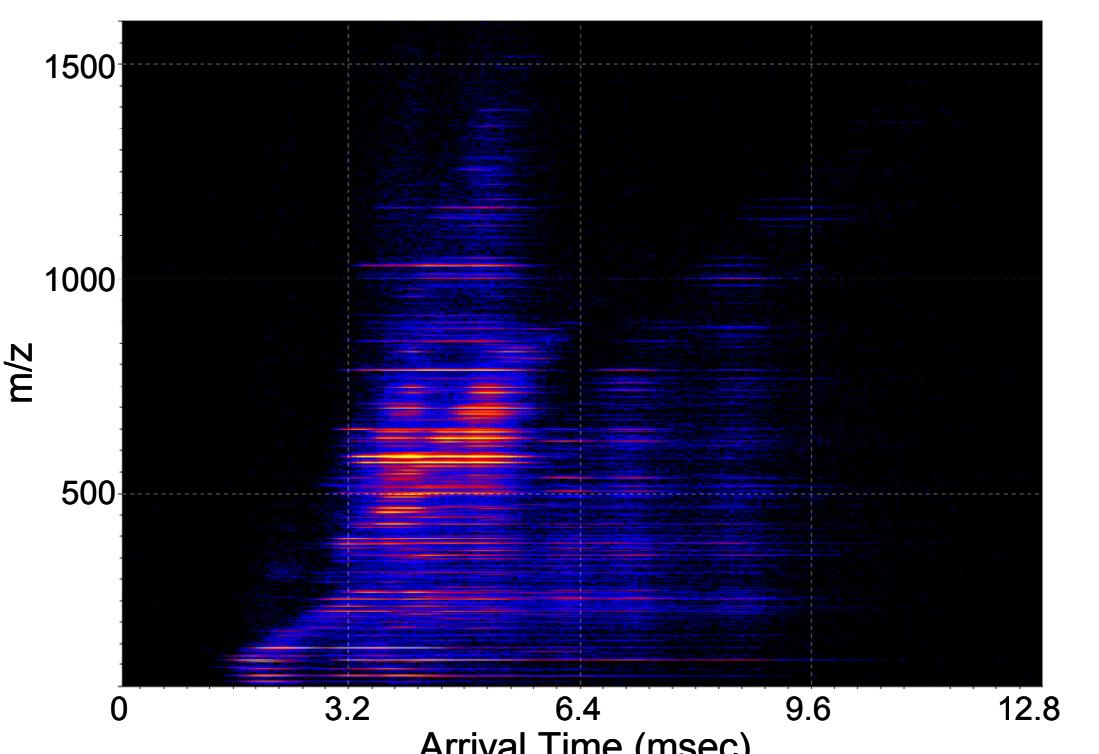


Figure 5 Shows m/z v's arrival time obtained using an IMS linked Transfer CE.

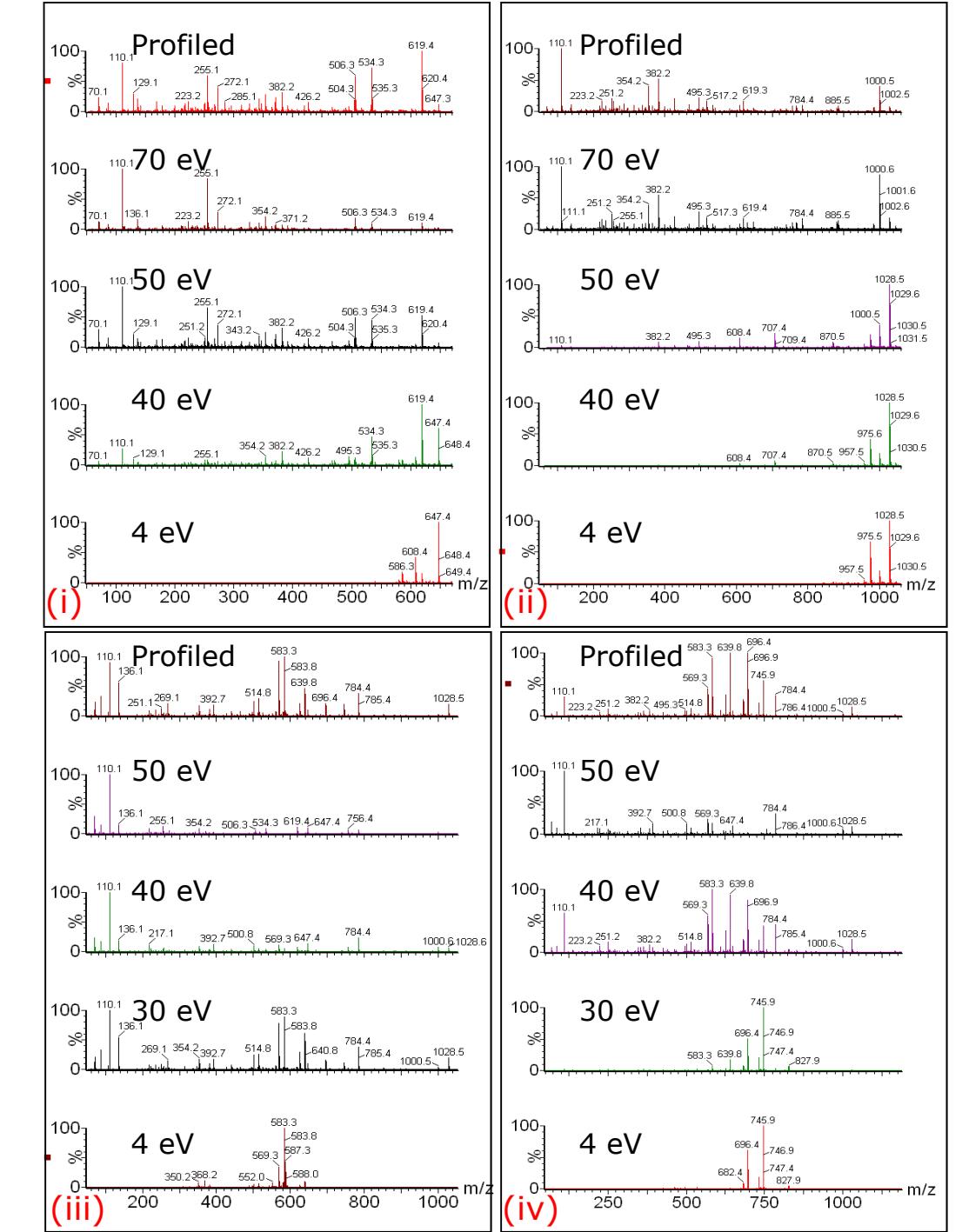


Figure 6 Shows example mass spectra taken from arrival times corresponding to (i) b₅⁺, (ii) b₈⁺, (iii) b₉⁺² and (iv) b₁₂⁺² ions for different Transfer collision conditions.

For example the optimum CE for the b₅⁺ ion is ~ 50 eV, the b₈⁺ ~ 70 eV, the b₉⁺² ~ 30 eV and the b₁₂⁺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 Collision Energy improved the fragmentation efficiency of a wide range of precursor ion masses in a nanoscale 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 23rd March 2006.
[2] Bateman, Giles, Pringle, and Wildgoose, Patent Application GB 2 439 814 published 9th January 2008.