

OVERVIEW**AIM**

- To evaluate the charge state separation capability of a new type of ion mobility spectrometry device which is based on a stacked-ring RF ion guide with a travelling voltage wave.

METHODS

- A modified Q-ToF has been constructed with a travelling wave based collision cell together with enhanced acquisition software to provide arrival time data of the ions at the ToF analyzer.

RESULTS

- The travelling wave based mobility separator exhibits good separation characteristics at an operating pressure of 0.2 mbar with no loss in sensitivity.
- Charge state separation parameters were derived from a peptide mix and efficient singly charged species removal was obtained.
- Application of the charge state separation parameters to a mobility separated protein digest yielded efficient removal of the singly charged ions indicating the approach to be generic.

INTRODUCTION

The improvement in instrumental sensitivity specifically in the field of proteomics is a major driving force for manufacturers. However, quite often the doubly charged peptide ions of interest for MS/MS analysis from a protein digest can be present at analytically useful levels but are swamped by singly charged background, preventing their detection. The utility of mobility separation of ions with similar m/z values but different charge states to enhance detection limits has been shown to be effective when used in a linked scan mode with a quadrupole operating in RF-only mode [1]. In this study it was shown that the observed systematic difference in drift times for ions of different charge state can be used to effect a low-mass cut-off for removal of singly charged species. This is shown schematically in Figure 1. Presented here are the investigations into the charge state separation capabilities of a novel travelling wave based ion mobility separator in conjunction with an oa-ToF.

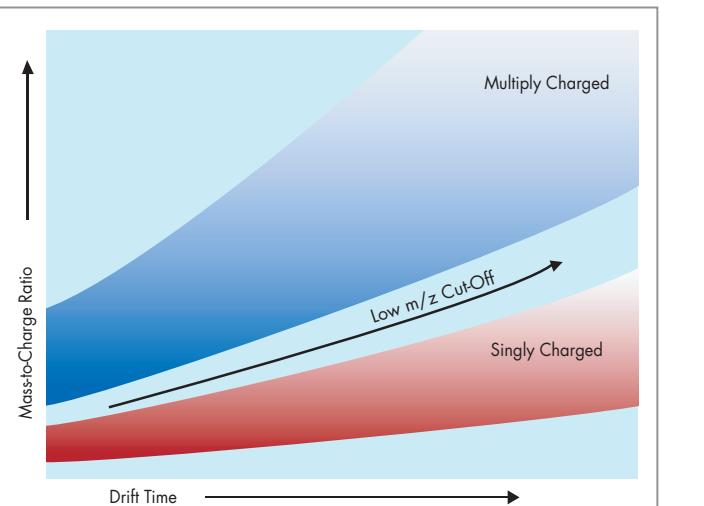


Figure 1. Schematic showing low mass cut-off capability with ion mobility separation.

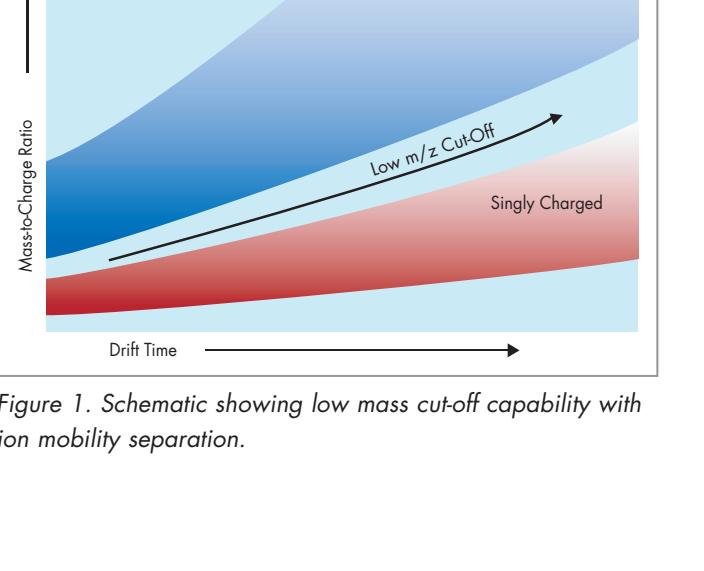


Figure 3. A schematic diagram of the modified hybrid quadrupole oa-ToF instrument.

OVERVIEW OF TRAVELLING WAVE MOBILITY SEPARATION

Previous work has shown that superposition of a travelling voltage wave on the confining RF of a stacked ring ion guide efficiently propels ions through a collision cell [2], reducing their residence time by ions 'surfing' on the wave as shown in Figure 2(a). However, by reducing the wave amplitude or increasing the velocity ions of lower mobility can no longer keep up with the wave and roll over the top, Figure 2(b).

This process is repeated as successive waves pass through the cell and produces a mobility-based separation of the entrained ions. Through appropriate mass range selection based on ion arrival time at the ToF analyser following mobility separation, discrimination against singly charged species can be achieved.

EXPERIMENTAL

The experiments were performed on a modified Waters hybrid Quadrupole oa-ToF instrument, shown in Figure 3. The Travelling Wave Ion Mobility Separator (TWIMS) is based on a Waters Quattro Premier T-Wave collision cell, a photograph of which is shown in Figure 4. The TWIMS is housed in a separately pumped chamber to allow use of elevated pressures in the cell. The cell has 122 ring electrodes mounted between two printed circuit boards (PCB). Each ring has an internal diameter of 5 mm, a thickness of 0.5 mm and is spaced 1.0 mm from the next. The cell is terminated by DC only lenses with 2 mm diameter apertures. The electrodes in the cell are divided into repeat sections of seven pairs with interconnections made on the supporting PCBs. Each pair of electrodes are connected to separate DC amplifiers which supply both the travelling wave voltage and any DC offset. The confining phase/anti-phase RF voltages

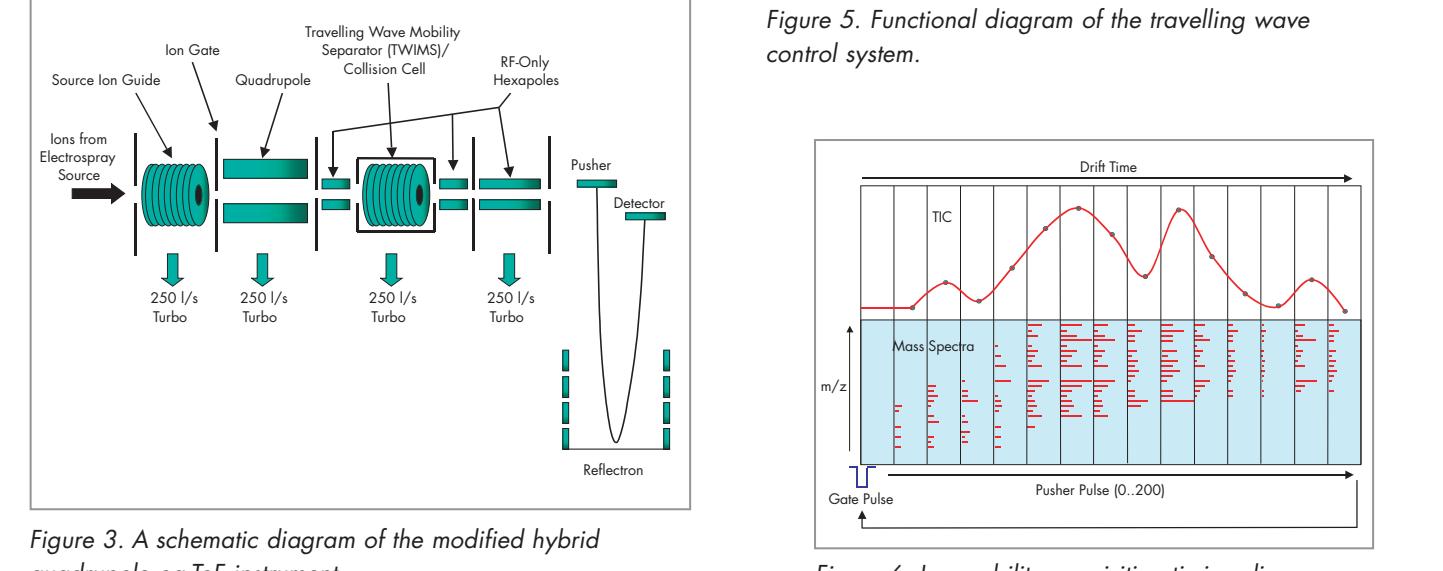
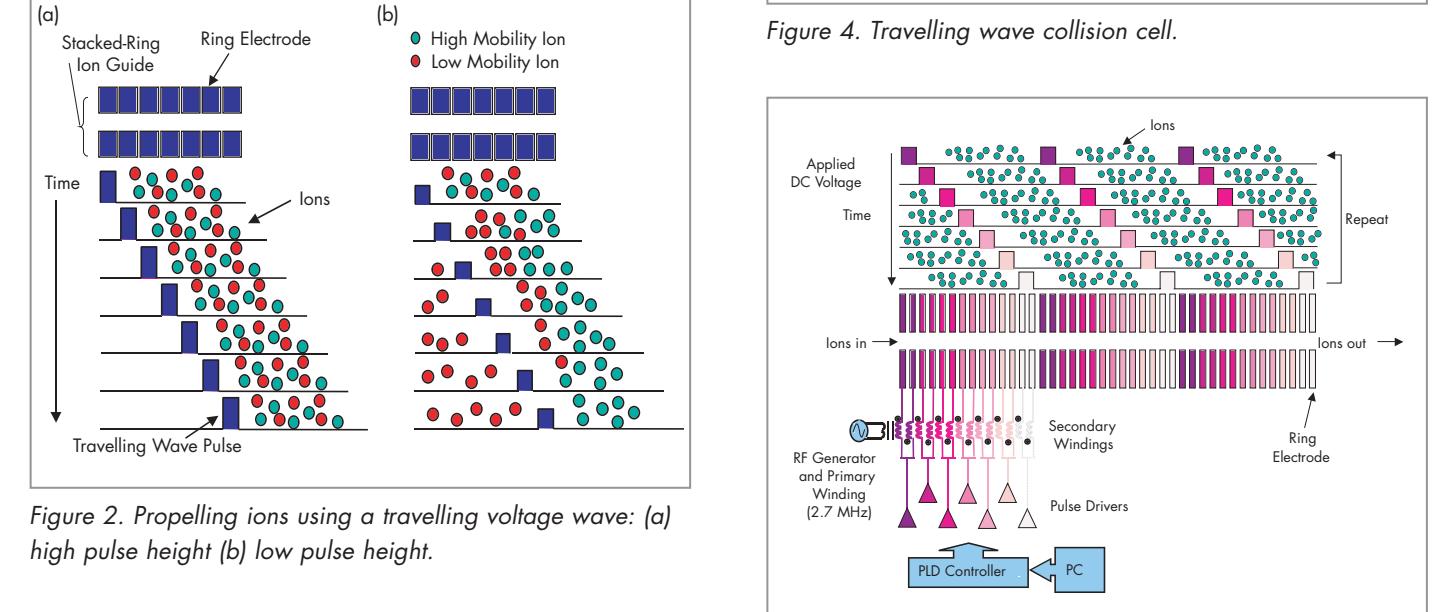
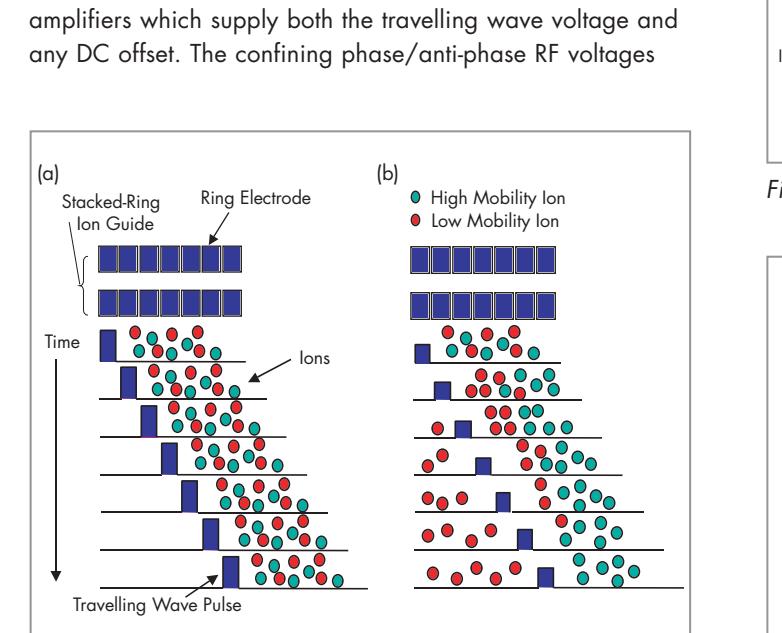


Figure 6. Ion mobility acquisition timing diagram.

for each pair of electrodes are superimposed onto the DC voltages via secondary windings of an RF transformer.

The travelling wave pulse pattern is controlled via a programmable logic device (PLD) connected to a PC. Figure 5 shows a functional diagram of the TWIMS control system with the electrode grouping illustrated using matching colours.

RESULTS**SYSTEM EVALUATION**

Studies were carried out to evaluate the separation capability and transmission efficiency of the TWIMS device. Figure 7 shows the mobility separation of the doubly charged Gramicidin-S ion (m/z 571) and the singly charged Leucine Enkephalin ion (m/z 556) as a function of travelling wave pulse height. Also shown are the mass chromatograms obtained at a pulse height of 7 V. By combining mass spectra separately across each peak, the spectra in Figure 8 are obtained, showing good differentiation between the ion species.

Figure 11 shows the mobility separation of the peptide mix using a travelling wave pulse height of 6 V. The mass spectra obtained by combining over the labelled regions A, B, C and D are shown in Figure 12 where it can be seen that there is a

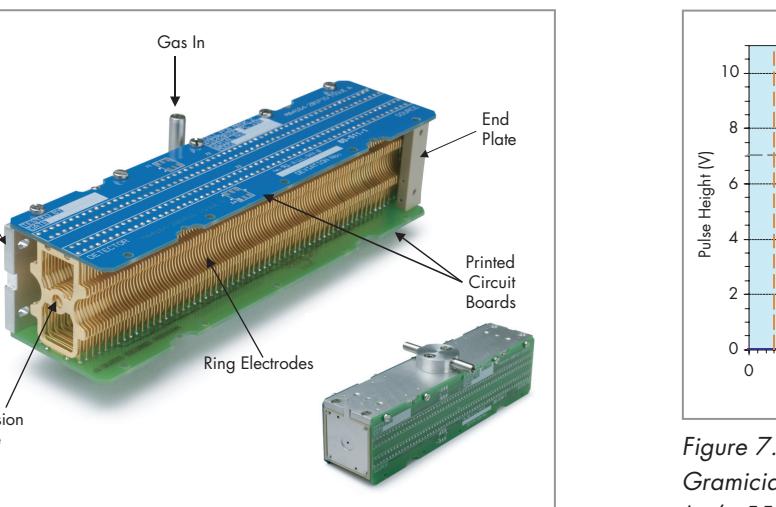


Figure 7. TWIMS separation capability for Gramicidin-S (m/z 571) and Leucine Enkephalin (m/z 556) at 0.2 mbar.

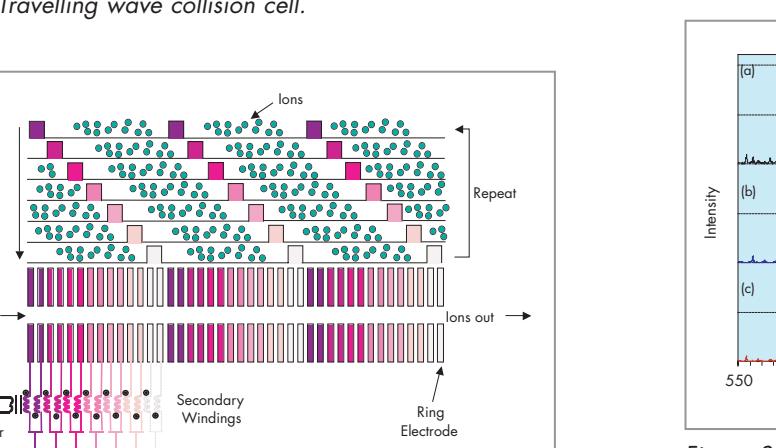


Figure 8. Mass spectra obtained from the mobility separation of Gramicidin-S and Leucine Enkephalin at 0.2 mbar (a) no separation (b) m/z 556 mobility peak and (c) m/z 571 mobility peak from Figure 7.

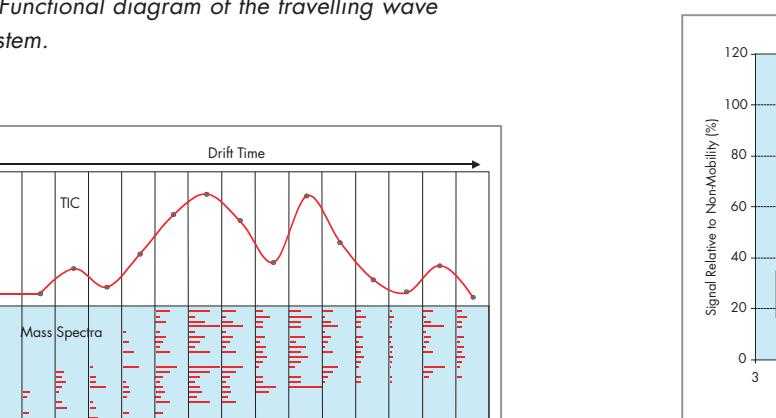


Figure 9. Transmission of the m/z 785 peak of Glu-Fibrinopeptide in mobility mode at 0.2 mbar compared with transmission in non-mobility mode. The corrected data account for dead-time effects in the acquisition system.

In these studies, the TWIMS cell was operated with 0.2 mbar of Argon and with an applied RF of 350 V pk-pk at 2.7 MHz. An ion gate pulse width of 200 μ s was used and a wave velocity of 300 m/sec. All ions were generated using electrospray ionisation with a sample infusion rate of 5 μ L/min. The data presented in Figure 9 shows the transmission of the doubly charged ion of Glu-Fibrinopeptide (m/z 785) (100 fmol/ μ L in 1:1 Methanol:Water 1% acetic acid) in mobility separation mode compared to that with no travelling wave/ion gating. The 'corrected' data account for dead-time losses in the detection system due to multiple ion arrival events per mass spectrum. The data indicate that there is essentially no loss in sensitivity when using the mobility mode compared to standard operation with pulse voltages up to 8 V.

CHARGE STATE SEPARATION OF A PEPTIDE MIX

A mixture of 11 peptides (MW ranges from 555 – 2845 Da) was infused into the TWIMS system and the ToF mass obtained spectrum is shown in Figure 10.

Figure 11 shows the mobility separation of the peptide mix using a travelling wave pulse height of 6 V. The mass spectra obtained by combining over the labelled regions A, B, C and D are shown in Figure 12 where it can be seen that there is a

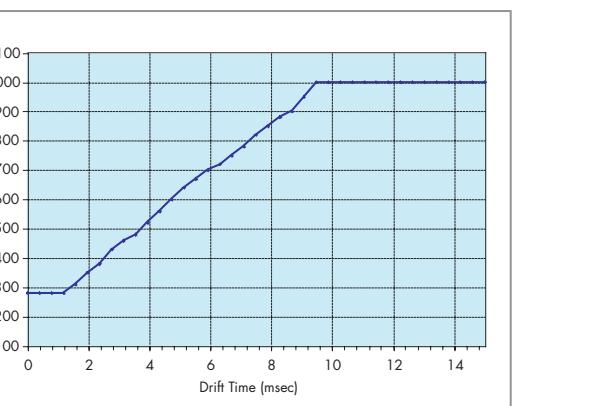


Figure 13. Low mass cut-off curve as a function of drift time for singly charged ion removal.

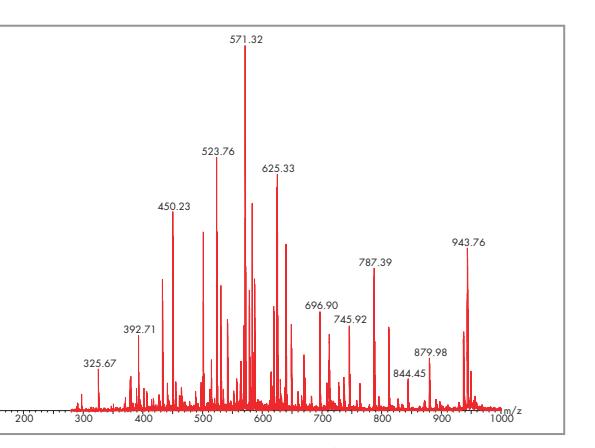


Figure 14. Peptide mix mass spectrum after singly charged ion removal.

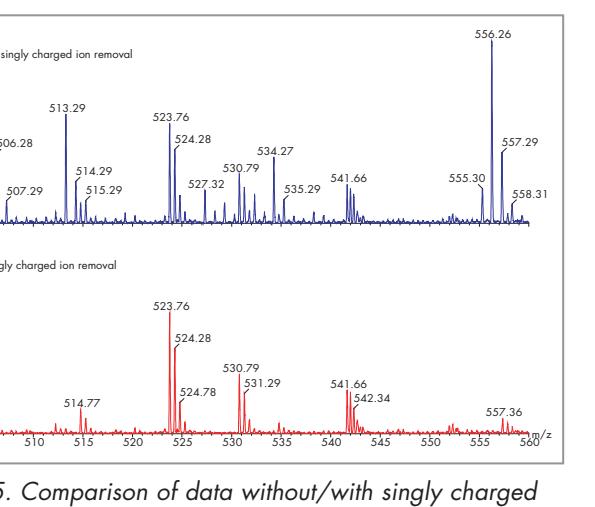


Figure 15. Comparison of data without/with singly charged ion removal illustrating the effectiveness of the technique.

distinct separation between singly and multiply charged ions as a function of drift time.

Through combining groups of five spectra (equivalent to 0.375 msec slices) across the complete mobility spectrum and determining the low mass cut-off point in each, a cut-off curve has been generated and is shown in Figure 13.

Using the data in Figure 13, a visual basic program was written to process the mobility data of Figure 11 and generate a full mass spectrum with singly charged ion removal for comparison with Figure 10. The result is shown in Figure 14. The spectrum is considerably simpler than that of Figure 10. Closer inspection over a reduced mass range, see Figure 15, illustrates that efficient removal of the singly charged species has been achieved.

CONCLUSIONS

A new mobility separation device has been developed which utilises a travelling voltage wave superimposed on the confining RF of a stacked-ring ion guide to provide ion separation.

Through appropriate choice of operating conditions the mobility separation can be used to provide charge state separation for other mixtures, a tryptic digest of Yeast Enolase (500 fmol/ μ L in 1:1 Acetonitrile:Water 1% Acetic Acid) was

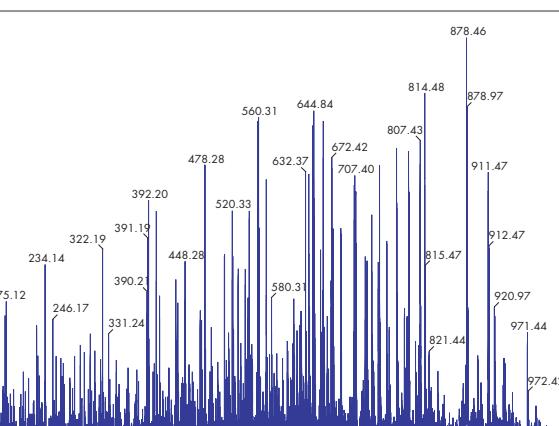


Figure 16. Mass spectrum of Yeast Enolase tryptic digest without mobility separation.

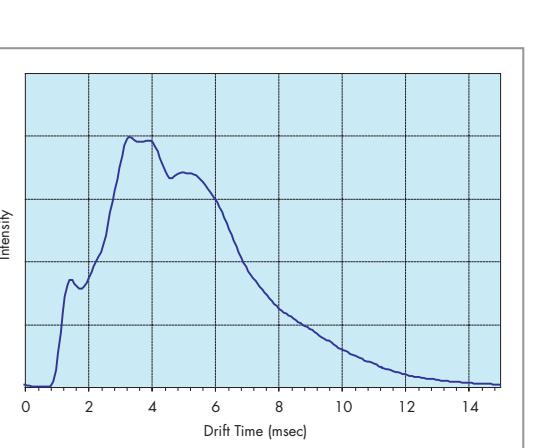


Figure 17. Mobility separation of the Yeast Enolase tryptic digest using a pulse height of 6 V.

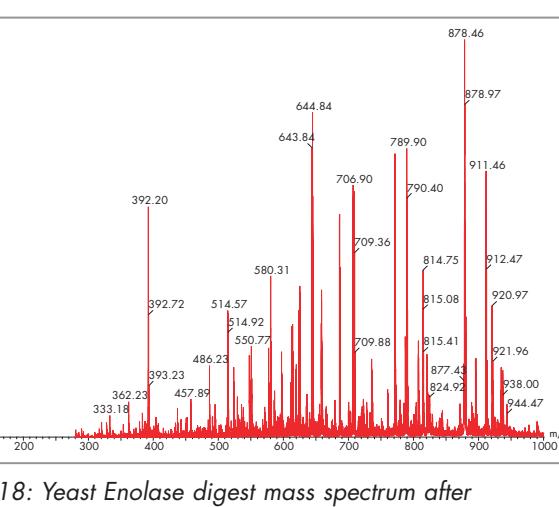


Figure 18. Yeast Enolase digest mass spectrum after singly charged ion removal.

infused into the TWIMS system. The mass spectrum obtained without mobility separation is shown in Figure 16, and the mobility separation shown in Figure 17.

Processing the mobility data as above generated the mass spectrum shown in Figure 18, which, again, is significantly simpler than that in Figure 16.

Comparisons of the spectra in Figures 16 and 18 over reduced mass ranges are shown in Figures 19, 20 and 21. From these it can be seen that efficient singly charged state species removal has been effected over the complete mass range, making identification of multiply charged species easier.

REFERENCES

- "Charge State Separation by Linked Scanning a Mass Filter with Mobility Separator" J. Hayes, J. Wildgoose, K. Giles and S. Pringle—Presented at the 51st ASMS Conference, Montreal, Canada 2003.
- "Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S. Pringle, C. 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).