Performance Improvements in High Mass Range Modes on a Dual Pressure Linear Ion Trap

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Overview

Purpose: The achievement of higher mass range on a dual-pressure linear ion trap with improved scan rate, resolution and sensitivity.

Methods: An LTQ Velos™ linear ion trap mass spectrometer was used for these investigations. This system includes a symmetric dual-pressure linear ion trap design. Several resonance ejection Q values, amplitudes, and phases were investigated and evaluations of scan rate, resolution and ejection efficiency were made.

Results: A resonance ejection Q of 0.391 (Beta 2/7) was found to yield a mass range of 4000 while allowing a scan rate of 10000 amu/sec with peak widths of ~0.60 amu FWHM. Slowing the scanrate to 555 amu/sec results in peak widths <.30 amu FWHM. Ejection efficiency in this mode is improved more than two fold. A mass range of 6000 can also be obtained using a resonance ejection Q of .278 (Beta 1/5), however, a scan rate of 1100 amu/sec is required to achieve peak widths of ~.60 amu FWHM.

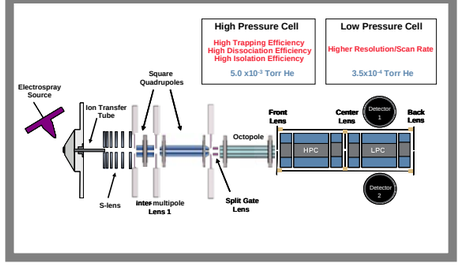
Introduction

The mass range of commercial ion traps is typically extended from a standard mass range by using lower resonance ejection Q values[1]. This method of mass range extension is quite effective and therefore its application has been investigated on a symmetric[2] dual-pressure linear ion trap configuration which performs mass analysis at low pressure[3].

Methods

An LTQ Velos mass spectrometer with a symmetrical dual-pressure linear ion trap was used for these investigations. An instrument schematic is shown in **Figure 1.** This trap configuration utilizes 5 mtorr operating pressures in the first cell, which results in higher trapping efficiencies, fragmentation efficiencies, and isolation resolution than at more conventional operating pressures. Ions are then transferred to the second cell which operates at a lower pressure of 0.35 mtorr and is used for mass analysis. This lower operating pressure yields higher resolution and/or scan rates than at more conventional operating pressures. Various resonance ejection Q values were investigated and its effect on the resulting mass range, resolution, scan rate, and ejection efficiency were evaluated. At each resonance ejection Q value used, the resonance ejection amplitude was optimized. Sufficient spectra were averaged to accurately measure and display peak widths. 3.5 ug/uL solutions of polypropylene glycol (PPG) 2700 and PPG 3500 in sodium acetate MeOH/H20 were utilized for these studies. Solutions of Ubiquitin and Insulin at 1 ug/uL 50:50 ACN/H20 pmol/ul were infused at 1 uL/min.

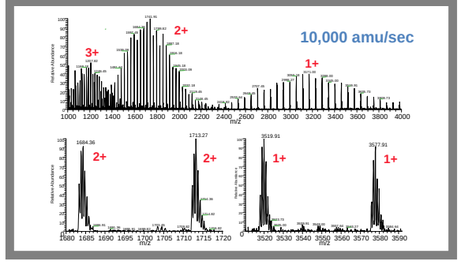
FIGURE 1. Dual-Pressure Linear Ion Trap Configuration



Results

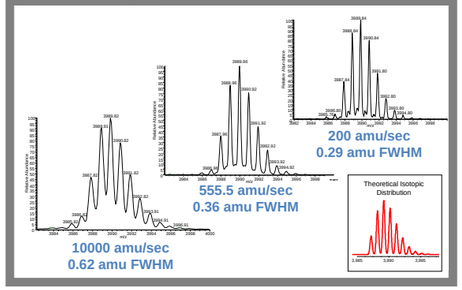
A resonance ejection Q of 0.391 (Beta 2/7) was first considered since it yields a mass range extension to 4000 m/z while allowing phase locking between the resonance ejection and RF voltages. A full scan mass spectrum of PPG up to m/z 4000 is shown in Figure 2 (top). At this Q value, and after optimizing the resonance ejection amplitude, a scan rate of 10,000 amu/sec was found to yield peak widths of better than 0.60 amu FWHM at m/z 3500. This is a four fold increase over the scan rates used in a standard LTQ XL linear ion trap system at this same mass range and resolution. This resolution is sufficient to separate the isotopes and identify the charge states of singly and doubly charged ions as shown in Figure 2 (bottom).

FIGURE 2. PPG 2700 in 4000 Mass Range - Full Scan MS Spectrum at 10,000 amu/sec.



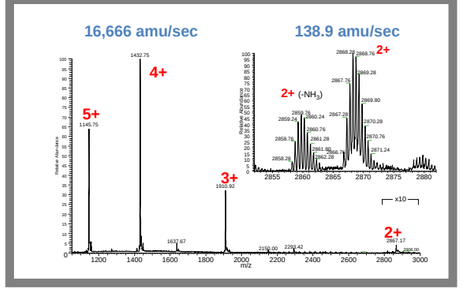
As in normal mass range mode, in order to obtain higher resolution, the scan rate can be reduced and the resonance ejection amplitudes re-optimized. A comparison of the resolution for three different scan rates at m/z 3990 is shown in Figure 3. Reducing the scan rate to 555.5 amu/sec at a mass range of 4000 results in peak widths of 0.36 amu FWHM, allowing charge state determination of higher charge states via isotopic separation. This scan rate is also a significant improvement (factor of four) over the LTQ XL in this same mass range and at this resolution. Slower scans at 200 amu/sec have even better resolution as is shown. At this resolution, there is evidence of a second isobaric component within the isotope cluster. The theoretical isotope distribution is included for comparison, indicating good agreement of the experimental data.

FIGURE 3. PPG 3500 in 4000 Mass Range - Comparison of Peak Widths for Three Different Scan Rates



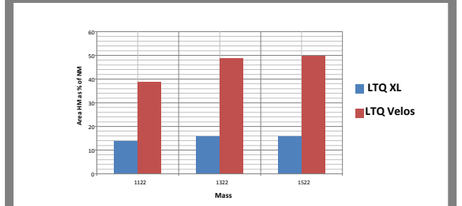
Intact Bovine Insulin (MW 5734) was infused and analyzed in 4000 mass range mode. In this mass range, the charge states of the intact protein between 2+ and 5+ can be observed (Figure 4). The inset shows the 2+ ion at higher resolution using a scan rate of 138.9 amu. Note that the resolution of the molecular ion isotopic distribution is somewhat broadened versus the peak at (M+2H-17)$^{2+}$, due to the presence of an underlying isobaric species.

FIGURE 4. Bovine Insulin in 4000 Mass Range



As the mass range is extended by using lower resonance ejection Q values, it becomes more difficult to get all ions to be ejected out of the trap and to the detectors. A comparison of the ejection efficiency relative to the standard 2000 mass range ejection Q is shown in Figure 5 for both the LTQ XL and the LTQ Velos dual-pressure ion trap. The data indicates that the ion ejection efficiency during mass analysis has been significantly improved by more than a factor of two. This results in higher sensitivity when using the extended mass range mode.

FIGURE 5. Comparison of the Relative Ejection Efficiency between the LTQ XL and LTQ Velos Ion Trap 4000 Mass Range.



A higher mass range of 6000 amu has also been investigated. This mode utilizes a resonance ejection Q of 0.278 (Beta 1/5). **Figure 6** shows a full scan spectrum of PPG 3500 at a scan rate of 16,666 amu/sec. With optimized resonance ejection voltages, near baseline resolution requires a reduction in scan rate to 555 amu/sec. Although useful data can be obtained, the capacity for mass analyzing ions while still avoiding space charge effects was significantly reduced. A comparison of three different scan rates in 6000 mass range is shown **Figure 7 .**

FIGURE 6. PPG 3500 in 6000 Mass Range - Full Scan MS Spectrum at 16,666 amu/sec.

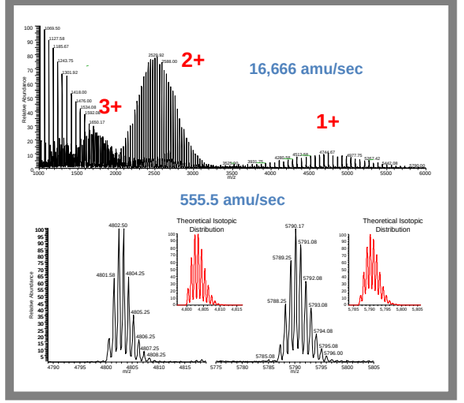
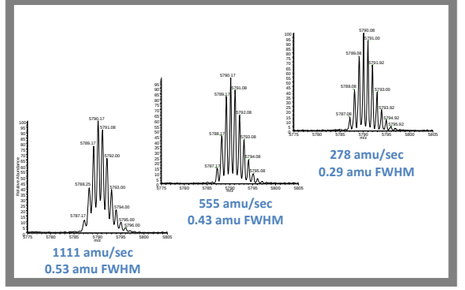
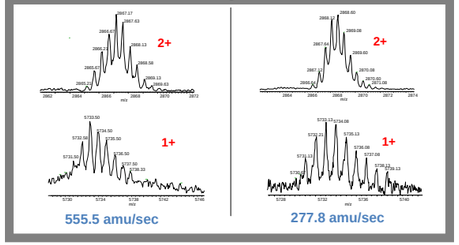


FIGURE 7. PPG 3500 in 6000 Mass Range - Comparison of Peak Widths for Three Different Scan Rates



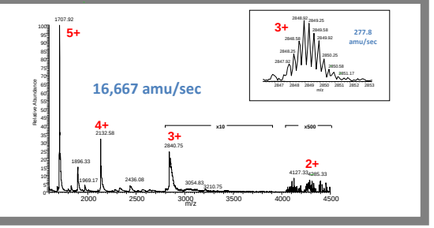
Intact Bovine Insulin as shown in **Figure 4** was analyzed also in 6000 mass range mode. In this mass range, the singly charged ion can be observed. A comparison of two scan rates on both the singly charged and doubly charged molecular ion is shown in **Figure 8** at this mass range. The 2+ ions again shows evidence of an overlapping ion in the molecular ion isotopic distribution.

FIGURE 8. Bovine Insulin in 6000 Mass Range - Comparison of Peak Widths for Two Different Scan Rates and Charge States



Intact Ubiquitin with a molecular weight of 8566 amu was analyzed in 6000 mass range mode and a full scan mass spectrum is shown in **Figure 9.** A scan rate of 277.8 amu/sec allows the isotopes of the triply-charged ion to be resolved demonstrating over 8500 resolution at this mass range and scan rate.

FIGURE 9. Ubiquitin in 6000 Mass Range



Conclusions

- Extended mass range modes show improved performance in a symmetric dual pressure linear trap compared to a conventional LTQ XL.
- A resonance ejection Q of 0.391 (Beta 2/7) was found to yield a mass range of 4000 while allowing a scan rate of 10000 amu/sec with peak widths of ~0.60 amu FWHM.
- Slowing the scan rate to 555 amu/sec results in peak widths <0.30 amu FWHM.
- A mass range of 6000 can be obtained using a resonance ejection Q of 0.278 (Beta 1/5), however lower spectral space charge limits are observed. A scan rate of 1100 amu/sec yields peak widths better than 0.60 amu FWHM.
- Slowing the scan rate to 278 amu/sec results in peak widths <0.30 amu.
- The utility of both mass range modes and the various scan rates have been demonstrated for the analysis of the intact proteins Bovine Insulin (MW 5734) and Ubiquitin (MW 8566).

References

(1) R.E. Kaiser, R.G. Cooks, J.M. Moss, P.H. Hemberger, Rapid Communications in Mass Spectrometry, Vol. 3, pp. 50-53.1989.

(2) P.M. Remes, J.C. Schwartz, MPH 196, Proceedings of the 57th ASMS Conference on Mass Spectrometery and Allied Topics, Philadelphia, PA, May 31-June 4, 2009.

(3) J.C. Schwartz , J.E.P. Syka, P.M. Remes, S.T. Quarmby, WPAA 039, Proceedings of the 56th ASMS Conference on Mass Spectrometery and Allied Topics, Denver, CO, June 1-5, 2008.