

Implementation of a Progressively Spaced Stacked Ring Ion Guide on a Linear Ion Trap Mass Spectrometer

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Overview

Purpose: To demonstrate the implementation of a novel stacked ring ion guide on a linear ion trap mass spectrometer.

Methods: LC-MS with various linear ion trap mass spectrometers (LTQ Velos™ and LTQ XL™ systems) some of which were equipped with a stacked ring ion guide.

Results: Novel S-lens device mode of operation with ability to observe ions of all masses between 50 and 4,000 m/z with a decreased ion trap injection time.

Introduction

Stacked ring ion guides were introduced in the early 1970s by Bahr, Gerlich, and Telyol⁽¹⁾ to confine ions with radio frequency electric fields. Later, these guides were implemented in LC-MS instruments as an approach to more efficiently transmit ions at high pressure. The best known example is the so called *ion funnel*, in which tapered apertures provide focusing.⁽²⁾

Recently we introduced a novel implementation of the stacked ring ion guide ("S-lens") which uses progressively spaced electrodes⁽³⁾ to more efficiently confine and focus ions with radio frequency electric fields through the high pressure region of a triple quadrupole mass spectrometer. The S-lens device has now been coupled to a linear ion trap and various performance aspects have been evaluated. This work relates to the successful integration of the progressively spaced stacked ring ion guide with a linear ion trap instrument.

Methods

The S-lens as depicted in **FIGURE 1** was incorporated into a linear ion trap mass spectrometer in place of the standard tube lens – skimmer source optics. The transmission profile of the S-lens was studied as a function of various parameters including the S-lens RF amplitude during ion injection for a number of stable ions with mass to charge ratios ranging from 150 to 2,000 Da. Based on these transmission curves an algorithm was developed for optimum transmission of any desired individual mass for SIM or MSⁿ experiments, and for uniform transmission of all masses in full scan MS analysis.

Results

The S-lens RF amplitude tuning curves are shown in **FIGURE 2**. It can be seen that the RF voltage required for optimum ion transmission increases with the mass to charge ratio.

FIGURE 1. Stacked ring ion guide (S-lens) constructed from stainless steel electrodes for ruggedness. The vertical supports also serve to conduct the RF voltage to the electrodes. Opposite phase RF is applied to successive electrodes.

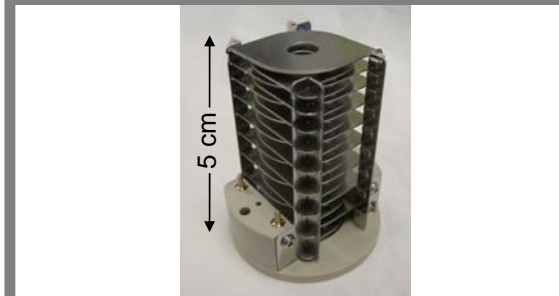


FIGURE 2. Tuning curves of the full scan MS peak intensities for various compounds as a function of RF amplitude applied to the S-lens.

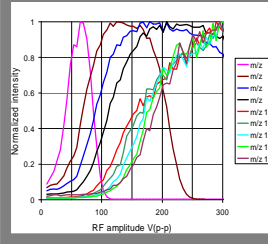
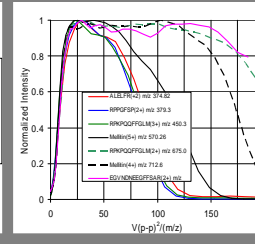


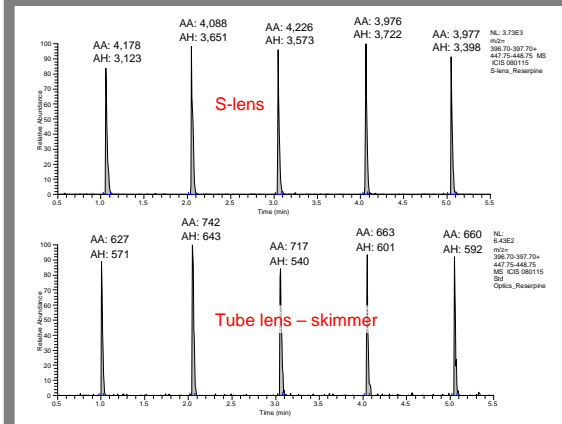
FIGURE 3. Scaled tuning curves of the full scan MS peak intensities of compounds of different mass and different charge states.



This dependency is further explored in **FIGURE 3**, where the transmission is plotted as a function of the square of the RF amplitude scaled with the mass to charge ratio of the ion. This relationship is found to be independent of the charge state of the ion. The scaling has been predicted for the pseudopotential in the adiabatic approximation⁽¹⁾ and it causes the leading edges of the transmission curves to overlap. A scaled squared RF amplitude value of ~25-50 yields optimum transmission for ions of all mass to charge ratios.

FIGURE 4 shows a comparison of the sensitivity of the standard tube lens – skimmer optics versus the S-lens optics, both operated under optimal conditions for Reserpine at m/z 609.3. For various compounds, similar comparisons resulted in a sensitivity increase of 5 fold on average. The brighter source causes a sensitivity improvement which in an ion trap results in an injection time decrease to reach the same target number of ions. At low concentration levels, this corresponds to acquiring an increased number of ions in the trap during the same ion injection time.

FIGURE 4. Selected ion chromatograms of product ions (397.3 and 448.3) of Reserpine ([M+H]⁺; m/z 609.3) obtained by 250 fg loop injections into a 400 µL/min LC flow of 50% isopropanol, 50% water. Direct comparison between the S-lens interface (top panel), and the tube lens – skimmer interface (bottom panel) on the same prototype linear ion trap mass spectrometer. The S-lens transmission was optimized at RF amplitude 150 V(p-p); the tube lens was optimized at 110V. The peak areas averaged over five injections are 4.1E3 and 6.8E2, respectively, which yields a sensitivity improvement of 6x.



The mass dependent ion transmission efficiency (**FIGURES 2 and 3**) of the S-lens can easily be accommodated in a scanning type mass spectrometer such as a triple quadrupole mass spectrometer. However this aspect makes the use of the S-lens with trapping type mass analyzers more challenging. Based on this observation, an algorithm was devised which yields an optimum balance between a transmission efficiency that is equal across the mass range while also retaining the ion current gain of the device.

The key concept which has been previously used to address analogous dependencies, is to allow the ion injection time window of the ion trap to be split into multiple shorter windows⁽⁴⁾, during each of which the S-lens is operated at a different RF amplitude (see **FIGURE 5**). Besides the number of time windows, other parameters used by the algorithm are the first mass of the scan range of interest, the last mass, and a scaling factor which allows overall amplitude adjustment. The optimum balance was found for three segments, each operated at the following RF voltages:

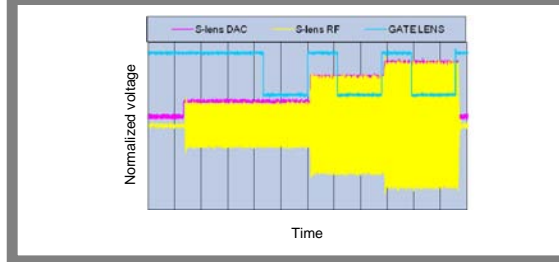
$$V_1 = 0.1 \times S - lensRFlevel(\%) \times \sqrt{(m/z)_{first}}$$

$$V_2 = 0.1 \times S - lensRFlevel(\%) \times \sqrt{(m/z)_{first} + f[(m/z)_{last} - (m/z)_{first}]}$$

$$V_3 = 0.1 \times S - lensRFlevel(\%) \times \sqrt{(m/z)_{last}}$$

where $f = 0.3$, and S-lensRFlevel (%) is the overall scaling factor⁽⁵⁾. Empirically it was found that a factor in the range of 40-60% yields the best compromise for optimum transmission across the entire mass range.

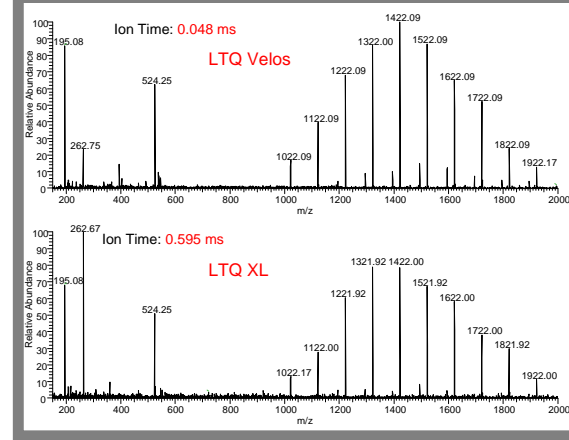
FIGURE 5. Normalized oscilloscope traces of the time dependence of several voltages used in the stacked ring ion guide equipped linear ion trap. The blue trace shows the voltage applied to the gate lens, and thereby when ions are being transmitted into the linear ion trap. The purple trace shows the amplitude control signal to the RF generator for the stacked ring ion guide, the RF output is shown in yellow. The rapid variation of the RF signal is not resolved on the selected time base.



The result of operating the ion trap mass spectrometer with three ion injection segments during each of which the RF amplitude applied to the S-lens is different can be seen in **FIGURE 6**: ions of all masses in the scan range 150-2,000 Da are observed. The relative abundances, as compared to the mass spectrum obtained with the tube lens – skimmer interface, do not show a significant change. Also note that in the case of using the tube lens – skimmer interface, a change of the tube lens voltage would alter the balance between the relative abundance of low and high mass ions.

The two mass spectra in **FIGURE 6** contain the same target number of charges, for which the ion injection times of the trap of the LTQ Velos are reduced by a factor 12. This reduction is not only due to the increased brightness of the source, but also to the increased trapping efficiency of the high pressure cell of the LTQ Velos.⁽⁶⁾

FIGURE 6. Full scan mass spectra for a 3 µL/min infusion of a mixture composed of Caffeine (m/z 195), MRFA (m/z 262, 524), and Ultramark 1621 (m/z 1022, ... , 1922) for a target value of 3x10⁴ elementary charges. The top panel shows the spectrum for the LTQ Velos dual pressure linear ion trap that incorporates the S-lens optics. The S-lens was operated at three RF amplitudes (S-lensRFlevel 60%) during the three segments in which the high pressure cell is filled. The bottom spectrum shows the results obtained on the LTQ XL linear ion trap with tube lens – skimmer optics, tube lens set at 80 V. On the LTQ Velos the ion injection times of the trap are reduced by a factor 12.



To demonstrate the benefits of the LTQ Velos we show the results of the analysis of a complex mixture in **FIGURE 7**. The enhanced performance in terms of cycle time allows for a more frequent sampling of weak precursors by the increased number of data dependent MS/MS spectra, which raises the number of peptide IDs by 1.8x for the 1 µg sample. To further assess the ability to analyze compounds of lower abundance, the sample was diluted 50-fold and analyzed on both instruments. Here, the number of unique peptides was increased by 2.5x, which demonstrates the increased brightness of the source that delivers more ions to the ion trap during the limited maximum injection time, thus increasing MS/MS sensitivity.

FIGURE 7. Number of unique peptides and proteins identified at 1% FDR with Proteome Discoverer software from data dependent MS/MS spectra acquired during a 60 min. reverse phase chromatographic separation of a proteolytic digest of *C. elegans* (average of 3 runs) for two different sample amounts. Comparison of the tube lens – skimmer optics on the LTQ XL versus the S-lens optics on the LTQ Velos.

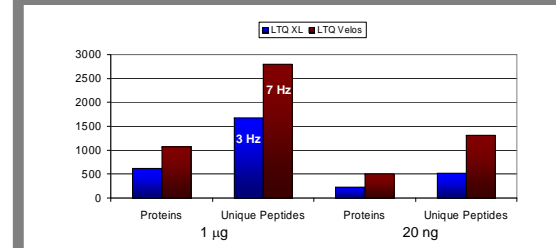
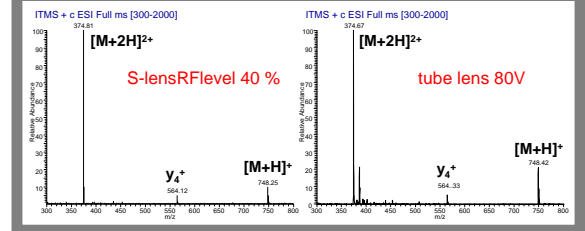


FIGURE 8. Normalized full scan mass spectra of a 3 µL/min infusion of a 10 pmol/µL dilution of the hexapeptide ALELFR with peak assignments to pseudo molecular and fragment ions. The mass range scanned over was 300-2,000 Da. The ion transfer tube was held at 200 °C. The spectrum on the left was obtained on the LTQ Velos dual-pressure linear ion trap with S-lens source optics. The S-lens is operated at S-lensRFlevel of 40%, with an ion injection divided in three segments. The spectrum on the right was obtained with tube lens – skimmer optics on a LTQ XL linear ion trap; the tube lens was set at 80V.



ALELFR is a thermally labile hexapeptide that is often used as a thermometer molecule due to its extreme fragility. We use it to investigate possible heating by the RF electric field because the implementation with three segments exposes the ions to the highest RF level for 33% of the ion injection time. In **FIGURE 8** two mass spectra are shown to compare the S-lens optics with the standard tube lens – skimmer optics. The S-lens and the tube lens each were operated at the optimum in transmission. Fragment y_n⁺ ions are observed in both interfaces and their relative abundance is similar and small. It is therefore clear that RF heating by the S-lens, although present to a certain extent, does not reduce the transmission of intact peptides. More importantly, even for this worst case labile peptide, it is the molecular ion as base peak that will be used for data dependent MSⁿ experiments.

Conclusions

S-lens source optics provide increased sensitivity and shorter ion trap injection times.

An algorithm was developed to calculate the RF voltages required to obtain full scan mass spectra with the S-lens interface on the linear ion trap mass spectrometer.

The algorithm was successfully tested using a calibration mixture to demonstrate the ability to observe all masses within the scan ranges of 50-2,000 m/z and 100-4,000 m/z.

The combination of a brighter source, dual-pressure linear ion trap, and faster scan rates, results in a cycle time decrease on the new LTQ Velos mass spectrometer.

The new LTQ Velos mass spectrometer shows two times more peptide coverage on a complex mixture.

References

- For an in depth introduction to stacked ring ion guides, see D. Gerlich in Advances in Chemical Physics Series, Vol. LXXXII, pp 1-176, 1992, Wiley
- S.A. Shaffer, K. Tang, G.A. Anderson, D.C. Prior, H.R. Udseth, and R.D. Smith, Rapid Communications in Mass Spectrometry, Vol. 11, pp. 1813-17, 1997
- M.W. Senko and V.V. Kovtoun, US Patent 7,514,673, April 7, 2009
- S.T. Quarmby, Ph.D. Thesis, U. Florida, 1997
- M.W. Senko et al., US Patent application publication number US 2009/0045062; Feb 19, 2009
- J.C. Schwartz, D.Taylor, and P.R. Remes, Poster MP195, ASMS 2009

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