Poster Note 64734

Multiplexed Targeted Assays Using Ion Trap Waveform Isolation

Philip M. Remeš
Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose: Explore the use of ion trap multiplexed isolation in quantitative targeted assays of pesticides and peptides.
Methods: Comparison of 1x isolation using a quadrupole mass filter to trap waveform isolation using 1x and 2x multiplexing, for both beam and resonance CID.
Results: When grouped precursors meet a set of criteria, good quantitative results were obtained using 2x multiplexing.

INTRODUCTION

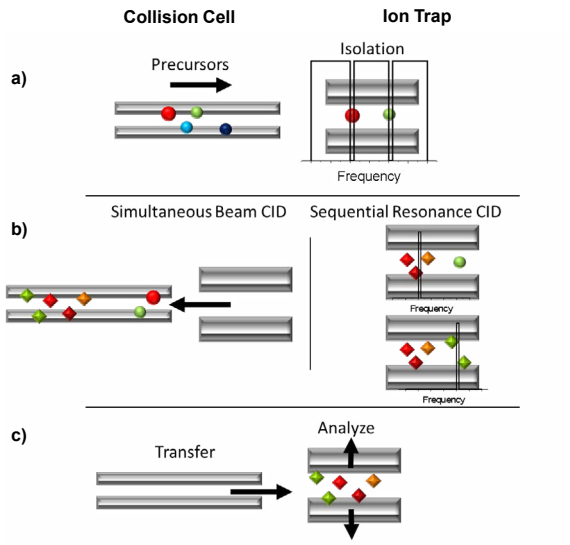
Multiplexing based on ion trap waveform based isolation has the potential to increase sampling efficiency by as much as 2-10x, which would provide a leap in the ability to characterize complex and low abundance samples. Previously, we explored the limits of waveform multiplexing at the MS level of analysis, demonstrating new techniques to maximize mass and dynamic range. However, the analytical utility of these methods have not yet been demonstrated. In this study, waveform multiplexing is applied to real-world LC/MS targeted assays with the goal of characterizing the potential advantages of increased sampling efficiency and the potential disadvantages of decreased selectivity.

MATERIALS AND METHODS

A modified Thermo Scientific™ Orbitrap Fusion™ mass spectrometer was used for these experiments with an Thermo Scientific™ UltiMate™ 3000 LC system. Some experiments used a mixture of 40+ pesticides, having m/z in the range 120 to 700 Da. The sample concentration was 0.1 ng/ul, and analysis was performed with a 15 minute LC gradient at 0.30 ml/min. Additional experiments were performed with the Promega 6x5 peptide mix. This is a QC standard with 6 sets of isotopologues. Each isotopologue set spans a 5 orders of magnitude concentration range and elutes from the LC column at the same time. The most concentrated peptides were 1 pmol/ul, and least concentrated peptides were 100 amol/ul. The LC gradient for the peptides was 30 min with flow rate 0.20 ml/min.

Single precursor isolation was performed with either a quadrupole mass filter, or an ion trap using broadband waveform isolation. Simultaneous precursor isolation was also performed with waveform isolation. Collision induced dissociation (CID) was performed either in a collision cell, or sequentially via resonance excitation in the ion trap.

Figure 1. Multiplexed Experimental Schemes a) Ions are introduced to the ion trap, and multiple precursors isolated at once. b) Fragmentation was performed via CID either in a dedicated collision cell with N2, or via resonance excitation with He in the ion trap. c) Ions were subsequently mass analyzed in the ion trap.



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Cycle Time Analysis

The pesticide assay was used to measure experimental multiplexing benchmarks.

These assays often contain 50-300 species, which represents a great analytical challenge.

15 known pesticides, and 35 unknown species were selected, and methods constructed that acquire spectra to iterate over each target in the list.

The number of scans executed every second was averaged for each experimental condition. The maximum injection time was 10 ms.

Quad isolation, single precursor analysis averaged 53 Hz. This is fast enough for many pesticide assays.

Trap waveform isolation currently incurs a time penalty due to data transfer latency of the waveform. Blue bars are the timings performed without waveform download. Although these scans are not analytically useful, they give a good estimate of future performance.

Figure 2. Pesticide assay with 40+ components

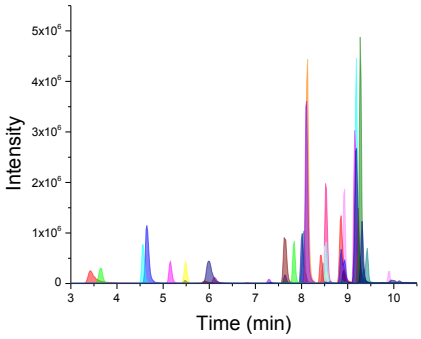
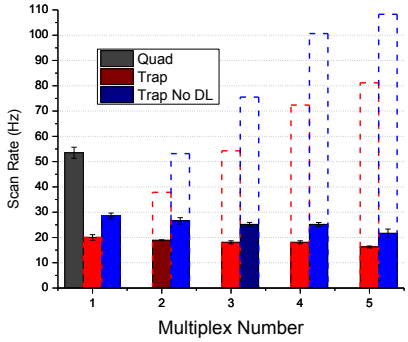


Figure 3. Beam CID cycle time analysis. Dashed lines are scan rate multiplied by multiplex number.



Dashed lines represent the average rate multiplied by the number of precursors. An increase in scan rate over the single precursor quadrupole isolation is achieved at 3 simultaneous precursors.

However, multiplexing allows this faster effective scan rate to occur with longer injection times than the single isolation case, allowing an increase in sampling duty cycle relative to single precursor isolation.

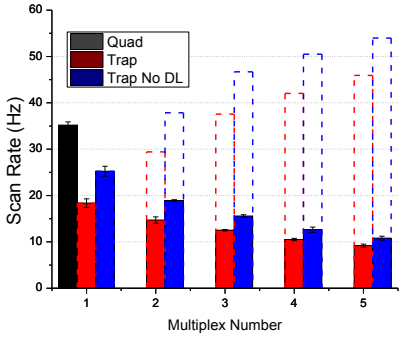
Activation by resonance CID was done in a sequential scheme. Thus each additional precursor incurs an additional period of activation.

The effective cycle time is faster than single precursor quadrupole isolation when the number of precursors is 2-3.

Although resonance CID is slower than beam CID, it can be attractive due to the relatively broad collision energy optima, and sometimes high intensity fragmentation channels.

While in the beam CID case, the precursors must use a consensus collision energy, for sequential resonance CID each precursor can use its optimum collision energy.

Figure 4. Resonance CID cycle time analysis. Dashed lines are scan rate multiplied by multiplex number.



Collision Energy Optimization and MSX Grouping

The structures of pesticides are different enough that a generic collision energy calibration as a function of mass and charge is not sufficient to achieve good sensitivity for all precursors.

For best results, the optimum collision energy for each precursor should be determined, which can be time consuming for large numbers of precursors.

A method of optimizing parameters on an LC time scale was developed, which corrects for the LC peak shape. High throughput optimization of large numbers of compounds can then be performed using LC injections of standard mixtures.

Figure 5. a) Collision energy is varied as a compound elutes from LC column. b) intensities are scaled by the inverse of the LC peak function.

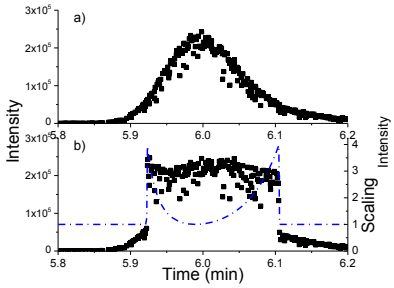
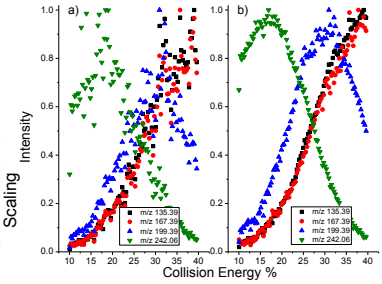


Figure 6. a) Unscaled fragment ion breakdown curves. b) fragment ion intensities after scaling



Once the precursors have been characterized, they should be paired together based on the following criteria:

- Elution times should overlap.
- Ratio of largest to smallest m/z < ~1.5 for isolation process.
- Fragmentation spectra for all precursors should have a minimum of overlapping peaks.
- Intensities should be within about a factor 10.
- For beam-type CID, there should be an overlap in optimal collision energy.
- Sequential resonance CID has an additional mass range constraint on ratio of largest to smallest m/z.

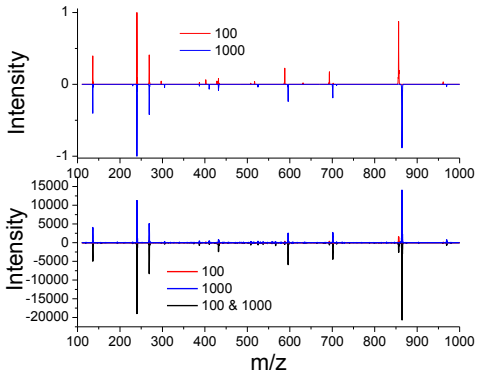
Library spectra for each precursor were generated by averaging spectra across the LC peaks from a 1x quad isolation experiment.

When a set of precursors met criteria derived from the above conditions, the amount of each component could be determined in the multiplexed spectra.

The pesticide MS/MS spectra were in general quite varied, and no peaks were eliminated from the libraries when doing analysis.

The peptide isotopologues had spectral regions of high overlap, and spectral regions of no overlap. The mutually overlapping regions for any group were excluded from the library spectra for analysis.

Figure 7. a) Library spectra for isotopologues of YVYVADVAAK, labeled by relative concentration. b) Example analysis spectra of single precursors (top) and multiplexed precursors (bottom).

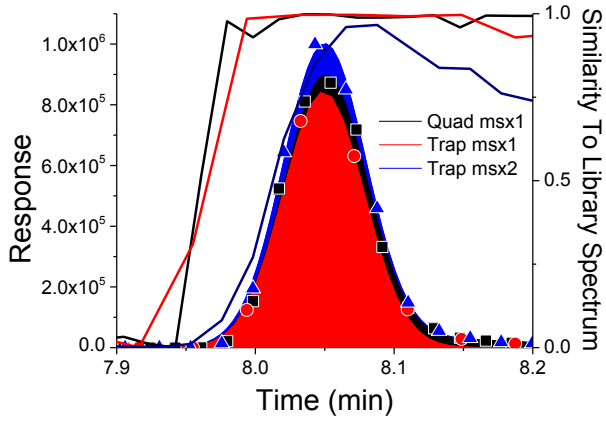


Pesticide Multiplexing Results

For the pesticide sample, only resonance CID was performed, with multiplexing group size of 2, and maximum injection time 20 ms. In total, 40 precursors were targeted.

For most multiplexed groups, the integrated LC peaks were quite comparable to the non multiplexed LC peaks. A few of the groups did not work when multiplexed with resonance CID, due to mass range limitations and overlaps between the second precursor and the first precursor's product ions.

Figure 8. Comparison of LC peaks of the pesticide cycluron, produced by resonance CID with 1x isolation via quad or trap, and 2x multiplexing via trap isolation. Solid lines are similarity of the spectra to the library spectra.



The 2x trap multiplexing yields almost the same number of points across the LC peaks as the 1x quadrupole isolation, which can be seen in Figures 8 and 9. The 1x trap isolation was about twice as slow, due to the latency issue described earlier.

All three techniques gave comparable integrated LC peak areas. However, the sum of responses over the LC peak was twice as small for 1x trap isolation due to the fewer number of points acquired.

Several 2x trap isolation groupings failed to generate significant spectral signal. This was due to limitations of the sequential resonance CID process, which should be accounted for in the multiplex grouping algorithm.

Figure 9. Ratio of number of points across the LC peaks for 1x and 2x trap isolation compared to 1x quad isolation.

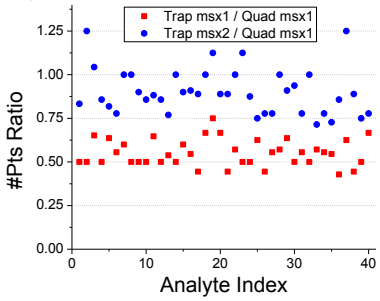
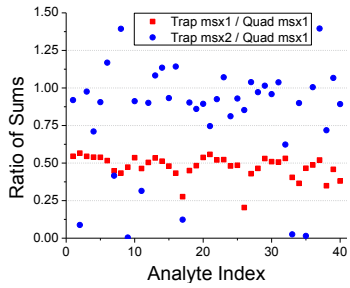


Figure 10. Ratio of responses summed over LC peak for 1x and 2x trap isolation, compared to 1x quad isolation.



Promega Peptide Multiplexing Results

The pesticide LC method was transferred to the peptides without any attempt to optimize the settings. Under these conditions, the lowest 500 amol level couldn't be observed for most peptides.

The remaining 4 levels were grouped together for two sets of experiments: a) groups with concentration ratio 1 to 10, and b) groups with concentration ratio 1 to 100.

For 1 to 10 ratio, 9 of the 12 multiplexed groups had good linearity, while 3 had too much product spectra overlap, resulting in errors for the lowest level.

For the 1 to 100 ratio, only one peptide gave good linear response for all levels.

Figure 11. LC peaks for 6 Promega isotopologues at the 4 response levels that could be observed.

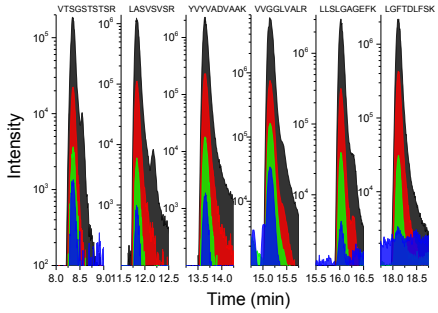


Figure 11. Example quantitation for 2x multiplexing with 1 to 10 concentration ratio.

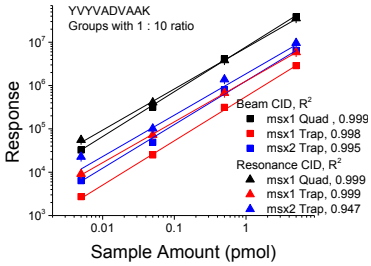


Figure 12. Example quantitation for 2x multiplexing with 1 to 100 concentration ratio.

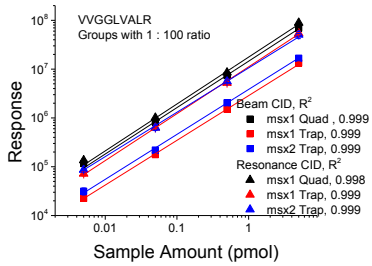


Figure 13. Example RSD's for 2x multiplexing with 1 to 10 concentration ratio.

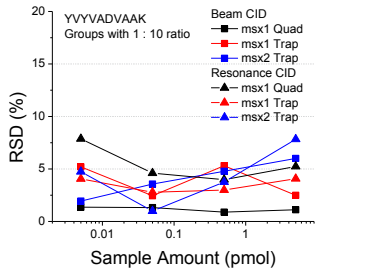
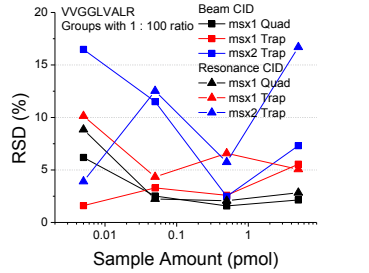


Figure 14. Example RSD's for 2x multiplexing with 1 to 100 concentration ratio.



CONCLUSIONS

- Quantitative ion trap multiplexing was demonstrated for small molecules and peptides
- 4 orders of linear dynamic range was demonstrated for the peptides
- Precursors should only be multiplexed when they meet certain criteria
  * In this study we observed that the ratio of precursor intensities should be <= 1:10
- Currently, speed of the multiplexing is limited by software issues, and msx beam CID sensitivity is limited by a hardware issue
- Future work will fix these issues, improve the multiplexing grouping algorithm, and push the multiplexing number to 5, which would approach 100 Hz sampling rate for the pesticide assay.

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