

Using Discovery To Power Targeted Analysis : From Traps To Triples

Amol Prakash¹, Scott Peterman², Taha Rezai¹, Michael MacCoss³, David Sarracino¹, Michael Athanas¹, Bryan Krastins¹, Mary F. Lopez¹

¹Thermo Fisher Scientific, Cambridge, MA, USA. ²Thermo Fisher Scientific, Somerset, NJ, USA. ³University of Washington, Seattle, WA.

Overview

We present novel software that integrates information from MS/MS spectra generated using on-resonance CID collected on an LTQ-based platform to facilitate the development of SRM methods on a triple quadrupole mass spectrometer. The data presented demonstrate similarity between the rank abundances of y-type product ions obtained by CID on an LTQ ion trap mass spectrometer and the y-ions acquired on a TSQ Quantum™ triple quadrupole LC/MS/MS. The discovery information is used to simplify the selection of peptides and corresponding SRM transitions used in targeted peptide/protein quantitation. The library spectra are also used to verify the correct ion signal based on spectral pattern matching. The complete workflow from discovery to targeted analysis is presented.

Introduction

Targeted SRM assays for proteomics have gained attention because of their robustness and selectivity in complex matrices. However, predicting what peptides to target and the transitions that will produce the greatest S/N for each protein is not trivial. To facilitate this process, algorithms have been developed that determine unique peptides for the targeted protein and predict a unique combination of SRM transitions. Unfortunately, this hypothetical approach provides no assurance that the peptide will actually be detected within the sample or whether the predicted transitions will provide the optimal S/N. High resolution discovery MS/MS spectra such as those that have been acquired in "shotgun" experiments and spectral libraries can be mined to enhance the design of effective SRM assays. The proposed synergy between linear ion trap MS/MS data and the development of SRM assays on a triple quadrupole mass spectrometer depends on the similarity of parent ion fragmentation behavior in a linear trap and a triple quadrupole mass spectrometer. As a proof of concept, in figures 1 and 2, we demonstrate the similarity between CID fragmentation spectra generated on linear ion trap and triple quadrupole mass spectrometers, respectively. The workflow and protocols are then designed based upon these observations.

FIGURE 1. Comparative product ion spectra for two human apolipoprotein E peptides in +2 charge state acquired in the 1A) LTQ via on-resonance CID and 1B) SRM on the TSQ Quantum Ultra™.

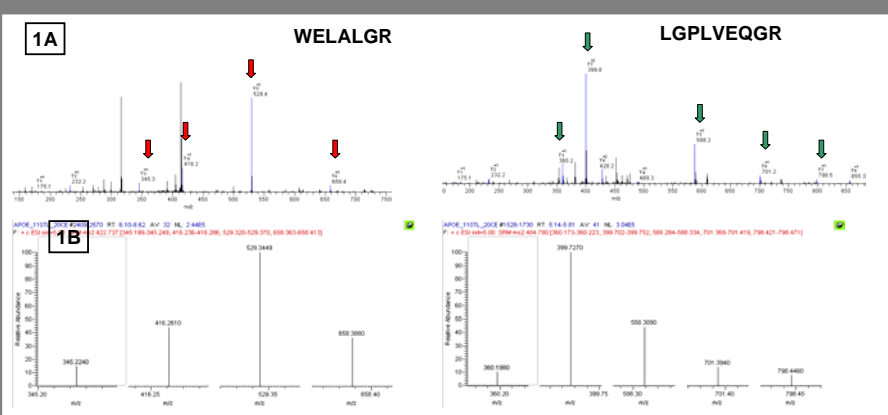
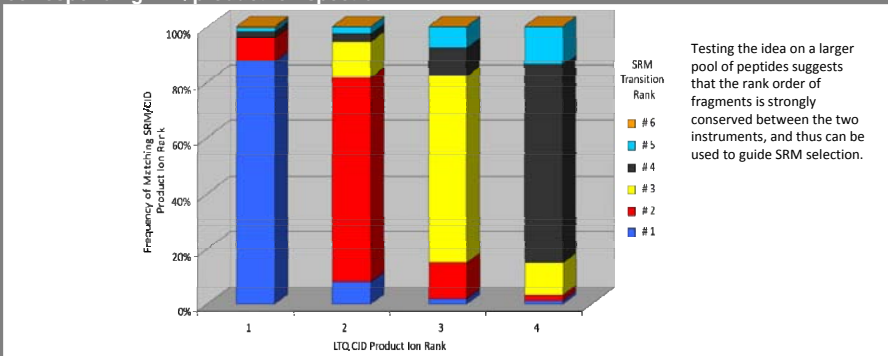


FIGURE 2. Frequency of matching product ion abundance rank between on-resonance CID and SRM for 200 peptides. The SRM analysis was completed by detecting the y3 through yn-1 product ion set. This resulted in over 1600 SRM transitions matched to the corresponding LTQ product ion spectra.



Methods

The described workflow is broadly applicable to a variety of samples and sample preparation techniques. For this study, we used enzymatic digests of human plasma, or *C. elegans* in LC-MS/MS discovery experiments to select proteins for further analysis in SRM assays. The selected protein sequences were submitted to the prototype software for comparison against a spectrum library acquired on an LTQ or LTQ-based hybrid instruments. These libraries range from a simple discovery experiment to one containing >6,000,000 annotated MS/MS spectra. From these proteins, the prototype software created over 1600 individual SRM transitions that were acquired in multiple different injections. The experiments were run using nanospray or microspray. A dwell time setting of 5-20 msec was used for all data collection. Data were processed using SRM workflow software. To capture the rank abundance similarity between the trap and the triple, a Spearman's rank correlation coefficient was calculated to determine a goodness of fit.

FIGURE 3. SRM Workflow software creates a link between traps and triples. It integrates data obtained from shotgun discovery experiments on LTQ/LTQ FT/LTQ Orbitrap and mines the data to those to (1) suggest sensitive transitions for the SRM analysis and (2) confirm the presence of the peptide by comparing the ion ratios to those from the discovery experiment

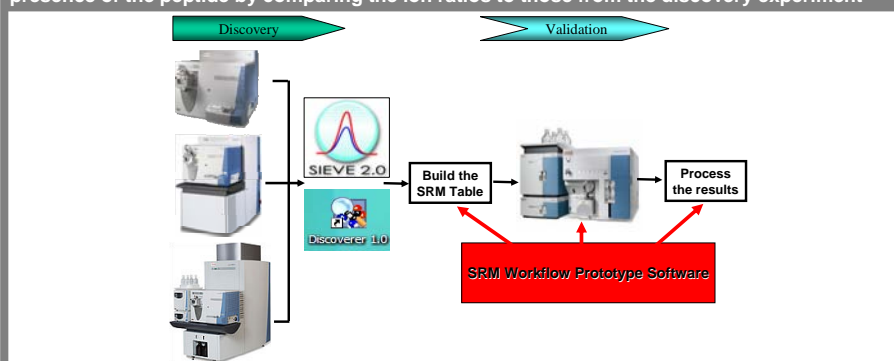
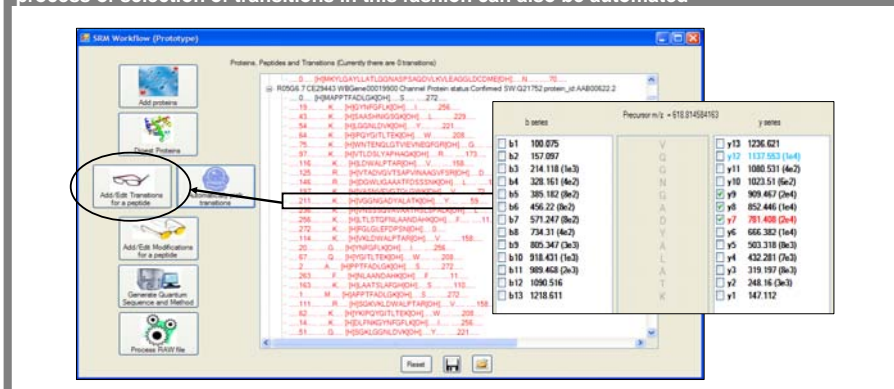


FIGURE 4. A screen shot from the SRM Workflow software. The trap-based library spectra are read in, and the intensities are shown along with the b and y fragment ions. The heuristic based suggestions are shown as the red fragment ion (y7 in this case). The entire process of selection of transitions in this fashion can also be automated



Results

SRM Workflow software facilitates building TSQ method files that contain the most intense ions already observed in discovery experiments. Figure 4 represents a screen capture illustrating this process. The workflow can be automated in batch mode to process thousands of peptides. Currently, we use only y-ions, since these are typically most likely to be observed in a triple quadrupole mass spectrometer. The methodology is independent of the resolution or duty cycle of the LTQ/LTQ FT/LTQ Orbitrap hybrid instruments or the TSQ Quantum Ultra, Access or TSQ Vantage triple quadrupole mass spectrometers. Further information for selectivity can be added if the user provides the background matrix in a FASTA file.

FIGURE 5. Comparison of summed and individual SRM chromatographic traces for the targeted peptide VGGNGADYALATK (+2). (A) The vertical lines represent retention times with abundant signal in four or more SRM transitions. (B) Product ion distribution comparison between the library entry and SRM analysis for the same peptide at the various retention times. (C) Spearman's rank correlation coefficient as a function of retention time for the various retention times. The y3 through y12 product ions were used for determining the correlation coefficient at each retention time.

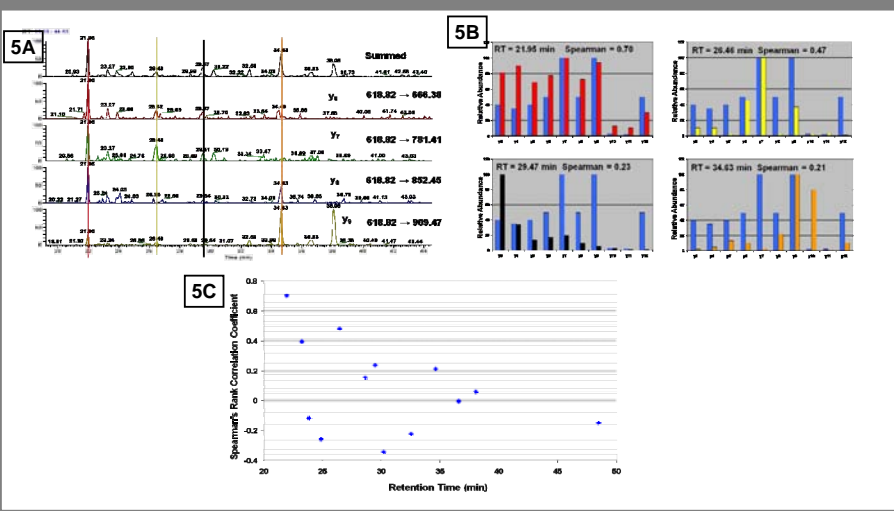
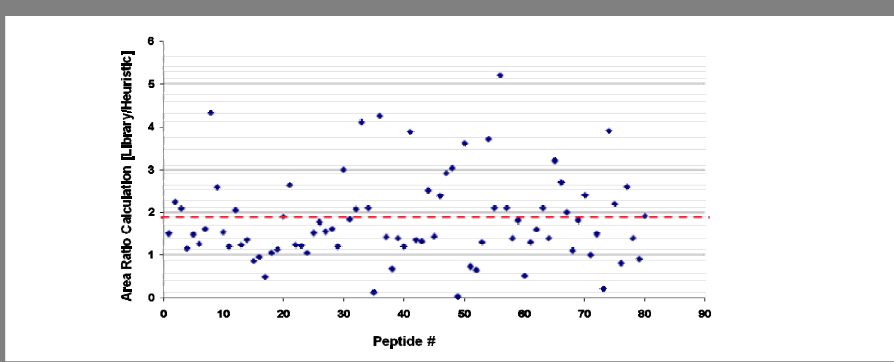


FIGURE 6. Area ratio comparison for library-based product ion selection vs. rules-based selections. Each approach combined the response from three transitions. The dashed red line represents the average area ratio between the two approaches.



Conclusions

1. Targeted protein, peptide, and product ion information is contained in discovery data acquired on an LTQ-based discovery platform: These data are – peptide, charge state, and product ions.
2. Over an 80% matching rate is observed for the top 4 product ions between the LTQ CID spectra and that measured from SRM transition detection on a triple quadrupole mass spectrometer.
3. The integrated SRM Workflow software enables mining discovery libraries for rapidly and efficiently building targeted peptide SRM transition assays.
4. Building SRM assays based on library spectra results in a 2-fold intensity increase over heuristic-based product ion selection. The software enables automated method generation.
5. Verification of targeted peptide detection is performed using a Spearman's rank correlation coefficient analysis contained within the processing aspect of the software.
6. One software package enables a robust method of building SRM assays, instrument methods, data acquisition, processing, and report generation tying protein discovery and quantification together.

All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.