Increased Quantitative Throughput and Selectivity for Triple Quadrupole Mass Spectrometer-Based Assays Using Intelligent SRM (iSRM)

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Overview

Purpose: To develop intelligent selected-reaction monitoring (iSRM) methods that will increase throughput and selectivity of large scale, simultaneous qualitative and quantitative LC-MS/MS analysis of targeted proteins.

Methods: Pinpoint software was used to develop three iSRM methods. The first method was to quantify and confirm pairs of endogenous / isotopically labeled yeast peptides. The second method was to quantify and confirm identities of 372 yeast peptides previously identified in house. The third method was to quantify and confirm identities of 757 yeast peptides from the MRM Atlas[1]. Yeast cell lysate digests were used. The experiments were run on a nano-LC and TSQ Vantage triple quadrupole mass spectrometer. Data were analyzed using the Pinpoint software.

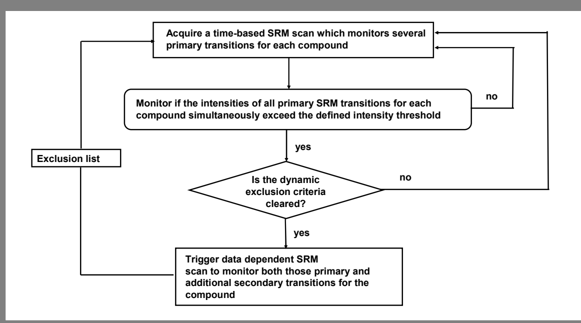
Results: Samples containing the endogenous and isotopically labeled peptides at different concentrations were analyzed. The TSQ Vantage system was able to confirm and quantify each spiked heavy peptide in the sub-femtomole range. A 1μg/uL yeast cell lysate digest was analyzed using the iSRM method targeting 372 yeast peptides previously identified in house. The method successfully confirmed by MS/MS library matching the identities of all 372 peptides and collected precise quantitative data for each peptide. The same 1μg/uL yeast cell lysate digest was analyzed using an iSRM method targeting 757 literature yeast peptides from the MRM Atlas. 673 of the targeted peptides were precisely quantified by primary SRM scan. 86% of the detected peptides were confirmed by composite MS/MS spectra.

Introduction

Traditional SRM achieves sensitive and precise quantitation results by monitoring one or several primary SRM transitions per targeted compound. This technique has been extended to simultaneously confirm the identity and quantify multiple compounds in one HPLC-MS run by monitoring eight or more SRM transitions per compound[2]. The bottleneck of this approach is that only a limited number of compounds can be targeted in one run because of the minimum time required to monitor each transition.

Intelligent SRM (iSRM) was developed to use instrument time more efficiently, increasing instrument duty cycle and productivity, and allowing analysis of up to 1000 compounds in a single LC-MS/MS analysis. iSRM uses SRM specificity in two ways. The first is compound-specific quantification using a time-based SRM acquisition that monitors several primary transitions for each compound. The second is a data-dependent SRM acquisition that monitors the primary transitions and additional secondary transitions. Secondary transitions are triggered only when the intensities of all primary SRM transitions simultaneously exceed the defined intensity threshold. For large-scale screening, secondary acquisition can be limited to once for each peak, providing sufficient structural information to confirm the compound's identity without perturbing the quantification obtained with the primary SRM list. Figure 1 shows the flowchart of the iSRM workflow logic.

FIGURE 1. Flowchart of iSRM workflow



Methods

Samples preparation: Eight heavy labeled peptides were spiked into the yeast digest at different concentrations (25 fmol/μL, 100 amol/μL, 10 amol/μL). Additional yeast cell lysate digests were prepared at 1μg/μL for the other experiments.

Nano-HPLC: ***Pump***: nano-LC 1D plus, Eksigent. ***Column***: C18 column (75 μm x100 mm, 15 μm tip, New Objective). ***Flow rate***: 300 nL/min. ***Injection amount***: 1 μL. ***Buffer A***: water containing 0.1% FA; ***Buffer B***: acetonitril containing 0.1 % FA. ***Gradient***: 5% B to 45% B in 40 min

MS: Thermo Scientific TSQ Vantage triple quadrupole equipped with a nanospray source Capillary temperature: 200 °C; Spray voltage: 1800 V

iSRM set up (primary SRM and data dependent SRM scans): Q1: 0.7 FWHM Da; Q3: 0.7 FWHM Da; Q2:1.2 mTorr;

Time-based SRM (duty cycle time: 2.0 S); CE: 0.034 x precursor mass *m/z* + 3.314; Two primary and additional six secondary fragment ions were used for each targeted peptide.

Experiment 1: 128 transitions (32 primary & 96 secondary) were used for targeting eight endogenous/heavy peptide pairs.

Experiment 2: 2976 transitions (744 primary & 2232 secondary) were used for simultaneously identify and quantify 372 known peptides.

Experiment 3: 6056 transitions (1514 primary & 4542 secondary) were used for the large-scale screening of targeting 757 literature peptides.

Method Development/Data Analysis: Thermo Scientific Pinpoint software.

Results

Analysis of isotopically labeled peptides

As described, eight isotopically labeled yeast peptides were spiked into a yeast cell lysate digest at different concentration. The eight endogenous and heavy peptide pairs were targeted using the iSRM method. Figure 2 shows extracted chromatograms of one targeted peptide pair. The data dependent SRM scan was triggered for the two co-eluting peaks with good quality and the relative fragment ion ratios of the two composite MS/MS spectra were very comparable (Figure 3).

The sensitivity of iSRM workflow was excellent. Figure 4 shows reproducible composite MS/MS spectrum acquired from one spiked heavy peptide at 10 attomole concentration level.

One benefit of iSRM is increased instrument throughput and selectivity by triggering the data-dependent SRM scan only for real eluted peaks. As shown in Figure 5, although multiple peaks were detected from each primary transition, the data-dependent scan was triggered only for the peptide peak in which both primary transitions were detected simultaneously.

FIGURE 2. Extracted primary and data dependent chromatograms of one endogenous/heavy yeast peptide pair

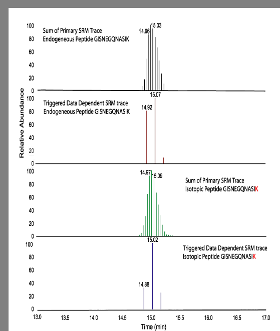


FIGURE 3. The composite MS/MS spectra of the endogenous/heavy yeast pair generated by data dependent SRM

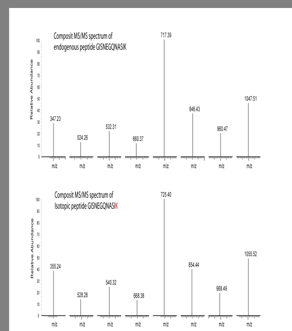


FIGURE 4. The composite MS/MS spectra acquired from the 10 attomole heavy yeast peptide peak which was spiked into 1 μg yeast digest matrix

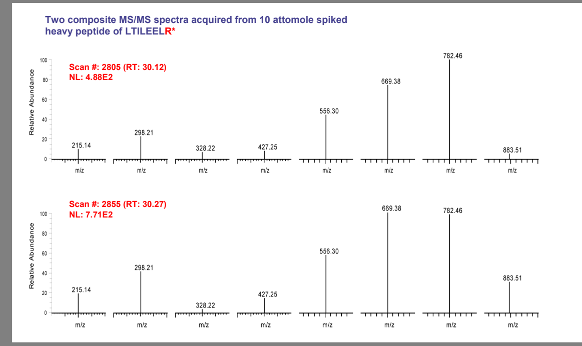


FIGURE 5. Because only expected peptide peaks triggered the generation of the composite MS/MS spectra, iSRM methods preserve instrument cycle time for precise quantification of the most relevant compounds.

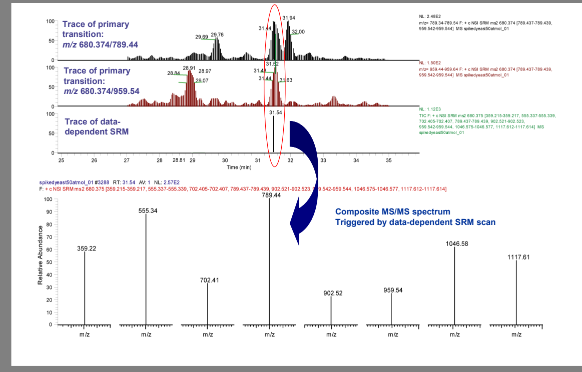
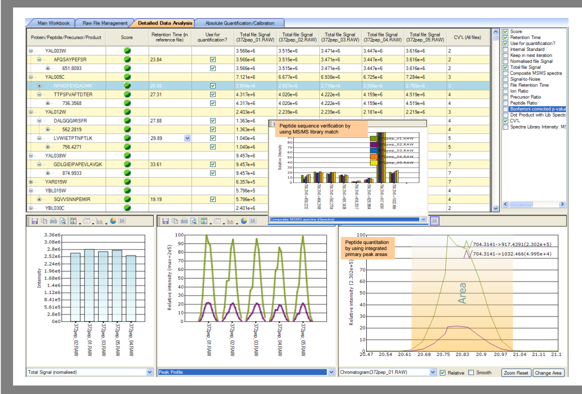


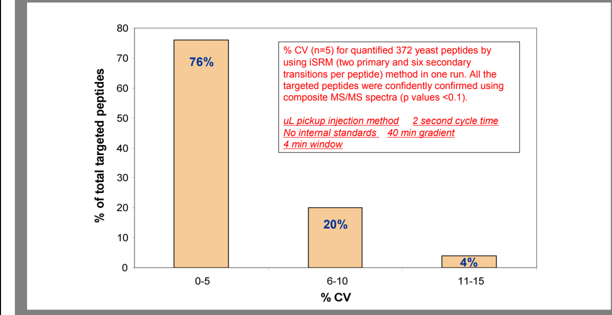
FIGURE 6. Peptide sequence verification and quantification using Pinpoint software



Application of iSRM method to the analysis of 372 known yeast peptides

To evaluate the capability of the iSRM method to simultaneously identify and quantify hundreds of peptides in one HPLC-MS/MS run, 372 known yeast peptides were targeted. Five replicates were analyzed to test the analytical precision of the method. All raw files were processed automatically using Pinpoint™ software[3]. The acquired composite MS/MS spectra were matched to a MS/MS library spectrum for peptide identity confirmation. The integrated peak areas of the primary SRM transitions were used for peptide quantification (Figure 6). The method successfully confirmed the identity of each eluted peptide by the MS/MS library match and collected precise quantitative data for each identified peptide. The analytical precision was excellent. 76% of the targeted peptides gave %CV below 5%. 95% of the targeted peptides gave %CV below 10% and all the peptides gave %CV below 15% (Figure 7).

FIGURE 7. Analytical precision of iSRM for targeting 372 yeast peptides for simultaneous identification and quantification in a single HPLC-MS/MS run



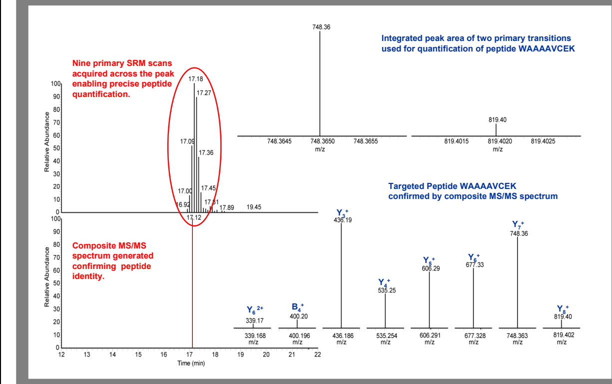
Application of iSRM workflow to the analysis of 757 literature peptides

The iSRM workflow was further employed to target 757 yeast peptides selected from the MRM Atlas. Three replicates were analyzed to test reproducibility. Out of the 757 peptides targeted by the iSRM method, 673 were detected in a 1 μg yeast digest sample. Although many co-eluting peaks were expected in the 40-minute gradient run, the instrument was still able to generate enough data points for most primary peaks in order to obtain precise quantitative results (Figure 8).

91% of the detected peptides gave %CVs below 15%. 98% of the detected peptides gave %CVs below 20%. Only 2% of the peptides gave %CVs between 20-25%.

Regardless the complexity of the experiment, most of the detected peptides (86%) were identified using the composite MS/MS spectra. Using the color control management feature in the Pinpoint software, a new iSRM assay can be automatically generated to only target peptides that did not trigger a data-dependent SRM scan (data not shown).

FIGURE 8. Results from a large-scale screening iSRM experiment that targeted 757 yeast peptides previously described in literature.



Conclusions

Intelligent selected-reaction monitoring and the new Pinpoint software for intelligent SRM (iSRM) provide tremendous benefits for large scale screening experiments on a triple quadrupole mass spectrometer.

- Provides the easiest and most rapid way to develop the robust, accurate and sensitive iSRM assays for targeted protein quantitation.
- Provides increased throughput and selectivity to simultaneously identify and quantify large number of candidate biomarkers generated during discovery experiments.
- Provides a quick way to simultaneously identify and quantify large number of proteins of interest in biological studies.

References

1) David Campbell, Eric Deutsch, Henry Lam, Paola Picotti, and Ruedi Aebersold. (2009) Improved support for targeted proteomics workflows in PeptideAtlas. US HUPO 2009 poster.

2) Reiko Kiyonami, Scott Peterman, Rosa Viner, Amol Parkash, and Vlad Zabrousdov. (2008) A New Methodology for Targeted Peptide Quantitation in Complex Mixtures using a High-Resolution Triple Quadrupole Mass Spectrometer. Application note # 412 published by Thermo Fisher Scientific.

3) Amol Prakash, Reiko Kiyonami, Alan Schoen, Huy Q. Nguyen, Scott Peterman, Andreas Huhmer, Mary F. Lopez, Bruno Domon, Ruedi Aebersold (2009) Integrated workflow to design methods and analyze data in large scale SRM experiments. ASMS 2009 poster.

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