

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

Reiko Kiyonami¹, Alan Schoen¹, Amol Prakash¹, Huy Nguyen¹, Scott Peterman¹, Nathalie Selevsek², Vlad Zabrouskov¹, Andreas Huhmer¹, Bruno Domon²

¹ Thermo Fisher Scientific, San Jose, CA; ² ETH Zurich, Switzerland

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Overview

Purpose: Develop a new simultaneous qualitative and quantitative method for both rapid SRM assay design/optimization and large scale screening of targeting numerous proteins by using the newly developed intelligent SRM (iSRM) instrument control software on a triple quadrupole mass spectrometer.

Methods: A Thermo Scientific TSO Vantage triple quad equipped with a nanoLC pump (Eksigent) and a nanospray source was used. The new intelligent SRM method was designed to utilize SRM specificity in two ways. The first was compound specific quantification using a time-based SRM acquisition, which monitors several primary transitions for each compound. The second was a data dependent SRM acquisition, which monitors both those primary and additional secondary transitions to generate a composite MS/MS spectrum and was triggered only when the intensities of all primary SRM transitions simultaneously exceed the defined intensity threshold. The composite MS/MS spectrum were used for peptide sequence confirmation and the peak integration results of the primary transitions were used for quantification.

Results: To evaluate the concept of the iSRM, a number of isotopically labeled yeast peptides were added into a yeast cell lysate digest. The endogenous and isotopically labeled peptide pairs were targeted by using iSRM method which includes two primary and six secondary transitions for each peptide. The instrument was able to confirm and quantify each spiked heavy peptide in the sub-attomole range.

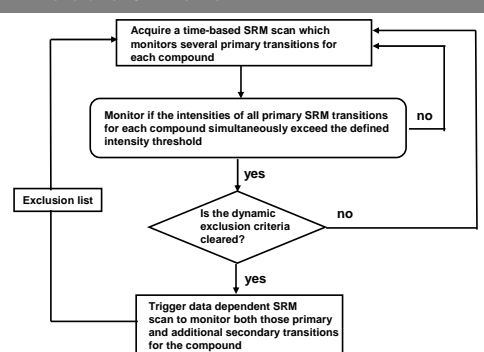
To evaluate the capability of the iSRM method for simultaneously identifying and quantifying hundreds of peptides, a yeast cell lysate digest (1 µg/ul) was used. 372 yeast peptides which were previously identified in house were targeted. The instrument successfully acquired composite MS/MS spectrum for each eluted peptide to unambiguously assign their identity and collected precise quantitative data for each identified peptide.

iSRM method was further employed to a large scale screening experiment for targeting 753 yeast peptides which had been posted on the MRM Atlas (1) by using the same yeast cell lysate digest sample. Among the 757 peptides known from the literature, 673 peptides were precisely quantified by primary SRM scan and 86% of the detected peptides were also confirmed by composite MS/MS spectra.

Introduction

The commonly used SRM technique provides sensitive and precise quantitative results by monitoring one or several primary SRM transitions per targeted compound. Recently this technique was 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. The new iSRM instrument control software can use the specificity of a small subset of SRM transitions to simultaneously quantify and intelligently trigger the full list for confirmation, thereby allowing the analysis of up to 1000 compounds in a single LC-MS run.

FIGURE 1. Flowchart of iSRM workflow



Methods

Samples preparation

For evaluating the performance and sensitivity of iSRM approach, eight heavy labeled peptides were spiked into the yeast digest at different concentrations (25 fmol/µL, 100 amol/µL, 10 amol/µL). An additional yeast cell lysate digest (1 µg/µL) was used for other experiments.

Nano – HPLC

Pump: nanoLC 1D plus, Eksigent
Column: C18 column (75 µm x100 nm, 10 µm tip, New Objective); flow rate: 300 nL/min Injection amount: 1 µL
Buffer A: 0.1% FA; Buffer B: 0.1% FA/100% CAN; Gradient: 5% B to 45% B in 40 min

MS

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

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

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

Results

Proof of the Concept of iSRM workflow

The intelligent SRM (iSRM) instrument control software was newly developed to use instrument time more efficiently thereby increase instrument duty cycle and productivity. The iSRM utilizes SRM specificity in two ways. The first is compound specific quantification using a time-based SRM acquisition, which monitors several primary transitions for each compound. The second is a data dependent SRM acquisition, which monitors both those primary and additional secondary transitions and is triggered only when the intensities of all primary SRM transitions simultaneously exceed the defined intensity threshold. For large scale screening experiments, the dynamic exclusion can be used to trigger secondary acquisition only once for each peak for 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.

In order to evaluate the performances and the benefits of the iSRM, 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).

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

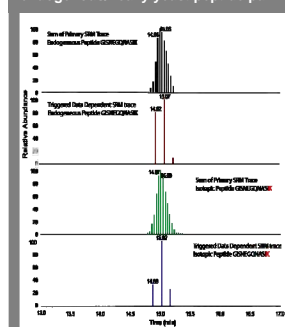
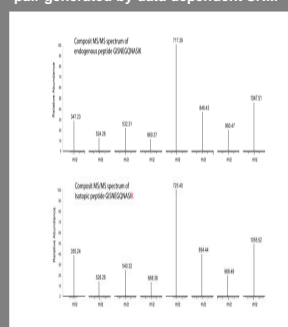


FIGURE 3. The composite MS/MS spectra of the endogenous/heavy yeast pair generated by data dependent SRM



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

One of the most expected benefits of using iSRM is to increase the instrument throughput and selectivity by triggering the data dependent SRM scan only for real eluted peak. As shown in Figure 5, although multiple peaks were detected from each primary transition, the data dependent scan was triggered only for one peak (the real peptide peak) which detected both primary transitions simultaneously.

FIGURE 4. The composite MS/MS spectra acquired from the 10 attomole heavy yeast peptide peak

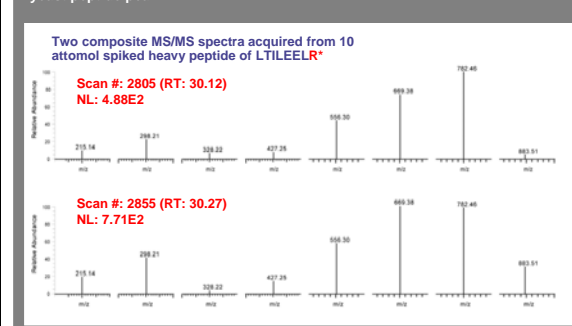
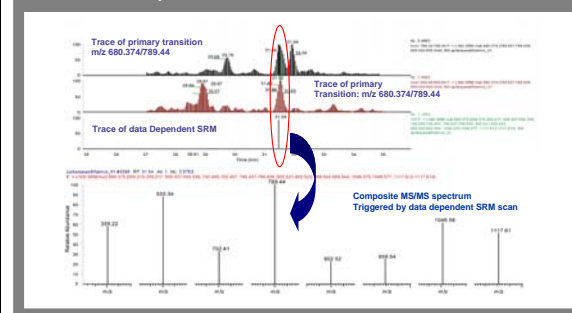


FIGURE 5. An example demonstrating the benefits of iSRM. Only the expected peptide peak triggered the generation of the composite MS/MS spectra. The iSRM method preserves instrument cycle time for precise quantification of the most relevant compounds.



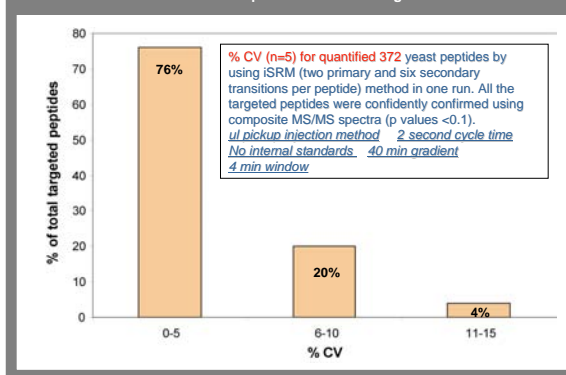
The qualitative and quantitative results of targeting 372 yeast peptides in one single HPLC MS/MS run by using iSRM workflow

To evaluate the capability of the iSRM method for simultaneously identifying and quantifying hundreds of peptides in one HPLC-MS/MS run, 372 known yeast peptides were targeted using the iSRM method. The same sample was analyzed five times testing the analytical precision of the method. All raw files were processed automatically using Thermo Scientific Pinpoint software³. The acquired composite MS/MS spectra were matched to a MS/MS library spectrum for peptide identity confirmation and the integrated peak areas of the primary SRM transitions were used for peptide quantification (Figure 6). The instrument 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 6. Peptide sequence verification and quantification using Pinpoint software



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

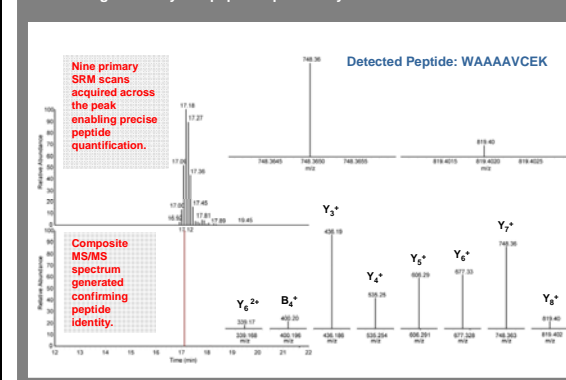


A large scale screening test of targeting 757 literature peptides in a single HPLC-MS/MS run

The iSRM workflow was further employed to a large scale screening test for targeting 757 yeast peptides which were selected from the MRM Atlas. The same run was repeated three times for testing reproducibility. Among the 757 selected yeast peptides that were monitored, 673 peptides were actually detected in our 1 µg yeast digest sample. Although many co-eluting peaks were expected in the 40 minutes gradient run, the instrument was still able to generate enough data points for most primary peaks in order to obtain precise quantitative results (Figure 7). 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™ software³, a new iSRM assay can be automatically generated to only target the peptides which did not trigger a data dependent SRM scans (data not shown).

FIGURE 8. Data examples from a large scale screening iSRM experiment which targeted 757 yeast peptides previously described in literature.



Conclusions

The newly developed intelligent SRM (iSRM) instrument control software provides tremendous benefits in proteomics, environmental and food safety fields for large scale screening experiments on a triple quadrupole instrument.

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

References

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