Targeted Protein Quantitation Using High-Resolution, Accurate-Mass Selected Ion Monitoring with Scheduled MS/MS on a New Hybrid Ion Trap-FTMS Mass Spectrometer

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Overview

Purpose: To develop a sensitive and robust workflow for simultaneously verifying and quantifying multiple targeted proteins/peptides using hybrid linear ion trap - Orbitrap™ mass spectrometer. Accurate masses of targeted peptides in high-resolution selected ion monitoring (SIM) mode are collected for quantitation and the MS/MS spectrum of each targeted peptide is used for sequence confirmation

Methods: To evaluate quantitative performance of the workflow (limits of detection, quantitation and dynamic range), a peptide mixture containing six isotopically-labeled yeast peptides was spiked into one microgram of yeast digest at different concentration levels. The yeast digest was also used to further evaluate dynamic range, reproducibility and throughput of the workflow by targeting multiple yeast proteins present at different levels (from >10,000 copies/cell to <50 copies/cell). The method development, optimization and data processing were done using Thermo Scientific Pinpoint software.

Results: The LOD for the spiked isotopically labeled peptides was 10 attomole and showed four orders of the linear dynamic range with good analytical precision. All the targeted yeast proteins covering 5 orders of concentration range (most of them were less than 128 copies/cell) were quantified with good analytical precision and unambiguously confirmed by CID MS/MS within the same run.

Introduction

A hybrid ion trap-FTMS mass spectrometer is routinely used in proteomics discovery experiments for both identification and relative quantitation of peptides in complex mixtures. Recently it has been applied for targeted peptide quantitation, taking advantage of lower detection limits of selected ion monitoring[1]. The primary challenge for any targeted assay lies in providing sensitivity, specificity and throughput required to analyze a large number of targeted peptides in a single chromatographic run. In ion trap-FTMS hybrid instruments, the FTMS detection is the largest contributor to the cycle time. The newly developed Thermo Scientific Orbitrap Elite mass spectrometer features next-generation high-field Orbitrap analyzer and advanced signal processing making it possible to produce a high resolution (60K FWHM) scan every 250 ms. Here we describe a targeted high-resolution, accurate-mass (HR/AM) peptide quantitation workflow which uses HR/AM selected ion monitoring (SIM) for quantitation and time-scheduled ion trap MS/MS for simultaneous peptide verification.

Methods

Sample Preparation

Sample 1: A mixture of six isotopically labeled yeast peptides was spiked into 1 µg yeast digest at different concentrations (0.01 fmol/µL, 0.1 fmol/µL, 1 fmol/µL, 10 fmol/µL and 100 fmol/µL).

Sample 2: A yeast digest mixture (1 µg/µL).

Nano-LC

LC: Thermo Scientific EASY n- LC II
Column: PicoFrit Magic C18 column from PCC (75µm x 150mm, 3 µm)
Flow rate: 300 nL/min; Buffer A: 0.1% FA/H2O; Buffer B: 0.1% FA/ACN;
Gradient: 5% B to 45% B in 60 min
Sample loading: Directly loaded on column;
Injection amount: 1µL

MS

Thermo Scientific Orbitrap Elite hybrid mass spectrometer equipped with a nanospray source; Capillary temperature: 220 °C; Spray voltage: 1800 V; S-lens voltage: 50; FT Resolution: 60,000 ; AGC target for FT SIM: 1+E05; Isolation width of FT SIM: 50 amu for sample 1; 200 amu for sample 2; MS/MS: data independent MS/MS lists with scheduled time windows were used for targeted peptide verification. The instrument method was generated using Pinpoint™ software version 1.1.

Generating Targeted HR/AM Assay for Targeting Multiple Yeast Proteins from Sample 2

1. 26 yeast proteins were selected from the literature based on the absolute protein abundances. Cellular abundances ranged from less than 50 copies per cell to more than 1,000,000 copies per cell[2]. The majority of the proteins were at low abundance (<128 copies/cell) in order to demonstrate the sensitivity of the workflow[1].

2. Based on either previous discovery data or *in-silico* digestion, peptides with sequences unique to the targeted proteins were selected as putative candidates for quantitation by using Pinpoint software. The 2+ and 3+ precursor *m/z* list of each candidate was exported as global inclusion list. The elution time for each peptide was predicted using linear correlation based on a Thermo Scientific Pierce Peptide Retention Time Calibration kit. Six-minute window was used per peptide.

3. Yeast digest was analyzed on the Orbitrap Elite instrument using full-scan FTMS at 60,000 resolution and rapid CID MS/MS on the linear trap in a targeted data-dependent fashion. Only precursor ions in the global inclusion list were triggered. All unidentified candidate peptides in the first run were exported as an additional global MS/MS list with a larger 10-minute time window using Pinpoint software for the second run.

4. The two raw files were searched against yeast database with Thermo Scientific Proteome Discoverer software. The first and the second search results were compiled together to establish a spectral library using Pinpoint software. Eighty six identified peptides presenting the twenty six targeted proteins were used for the final HR/AM SIM method. These peptides were exported as a global MS/MS list and the actual detected retention time with 4-minute window per peptide was used for scheduling MS/MS acquisition.

Results

Detection Limits, Analytical Precision, and Linear Dynamic Range of the Assay

The six heavy peptides spiked in 1 µg yeast digest were targeted in five different concentration samples . Each sample was run in triplicate for evaluating the analytical precision. All six peptides were detected clearly from 10 amol to 100 fmol on column (Figure 1) and simultaneously verified by CID MS/MS spectra (Figure 2). Four orders of linear dynamic range were observed with good analytical precision (Figure 3). The % CV was less than 25% for the peptides at 10 amol and less than 10% for other four concentration levels. Table 1 shows the summary of the % CV for all five concentration samples.

FIGURE 1. HR/AM extracted ion chromatograms of the targeted peptide SAAGAFGPELSR* spiked into 1 µg yeast matrix at varying levels

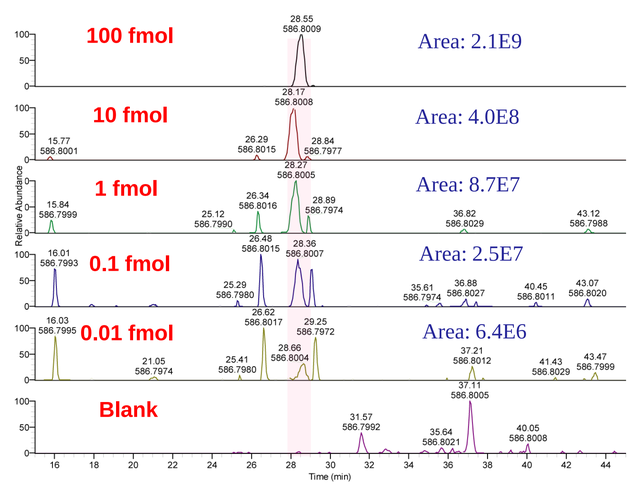


FIGURE 2. MS/MS data acquired from 0.01 fmol and 100 fmol samples.

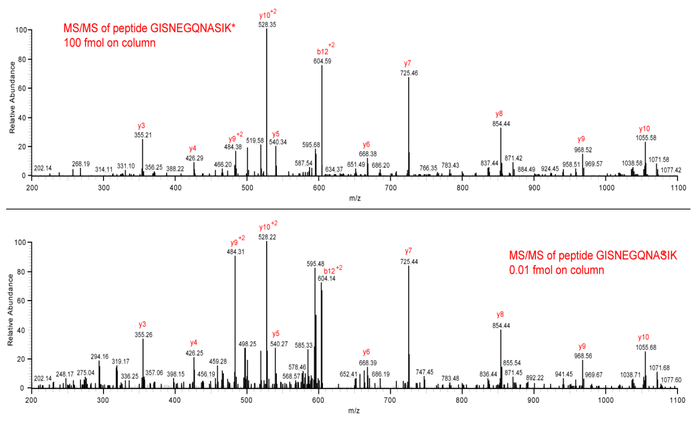


FIGURE 3. Dilution curve of the targeted peptide SAAGAFGPELSR* from 0.01 fmol to 100 fmol on column

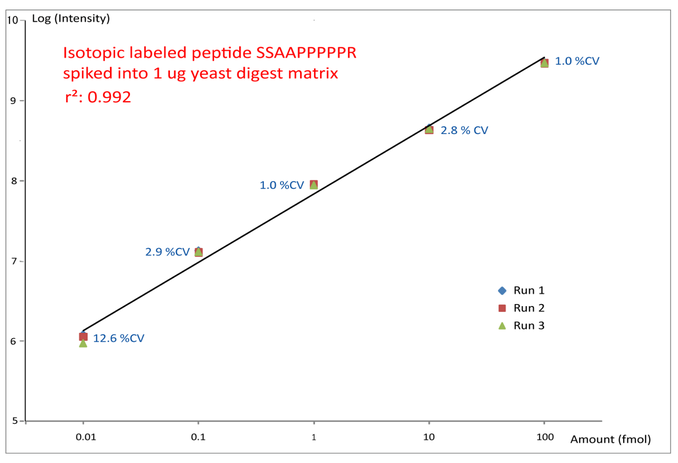


TABLE 1. Analytical precision for the six targeted isotopically labeled peptides spiked into 1 µg yeast matrix at varying levels.

	0.01 fmol	0.1 fmol	1 fmol	10 fmol	100 fmol
			% CV (n=3)		
SSAAPPPPPR*	12.6	2.9	1.0	2.8	1.0
GISNEGQNASIK*	4.1	2.8	3.0	4.3	3.5
HVLTSIGEK*	15.1	1.3	2.2	2.9	1.0
IGDYAGIK*	8.2	4.2	8.2	4.1	5.5
TASEFDSAIAQDK*	12.6	1.3	7.7	8.5	4.0
SAAGAFGPELSR*	20.8	4.5	3.1	6.2	2.0

Detecting and Quantifying Low-Abundance and High-Abundance Yeast Proteins with the Developed Workflow in a Single LC/MS Acquisition

The yeast sample was run in triplicate using the final quantitative assay which targeted 86 peptides as quantitative surrogates of 26 targeted yeast proteins. Four 200 amu wide SIM scans and one additional targeted data independent time scheduled MS/MS scan were used in this analysis. All raw data files were processed using Pinpoint software (Figure 4). The faster FT acquisition offered by the Orbitrap Elite instrument allowed shorter cycle time, yielding more data points across the chromatographic peak for all targeted peptides when compared to the data acquired on a Thermo Scientific LTQ Orbitrap Velos (Figure 5) hybrid mass spectrometer. As a result, higher analytical precision was achieved for the results acquired on the Orbitrap Elite MS compared to that of the LTQ Orbitrap Velos™ MS (Figure 6). All the targeted peptides had CVs below 15% and 96% of the targeted peptides had CVs below 10%.

FIGURE 4. Pinpoint software displays simultaneous identification and quantitation results for the 86 targeted peptides from a single LC/MS run.

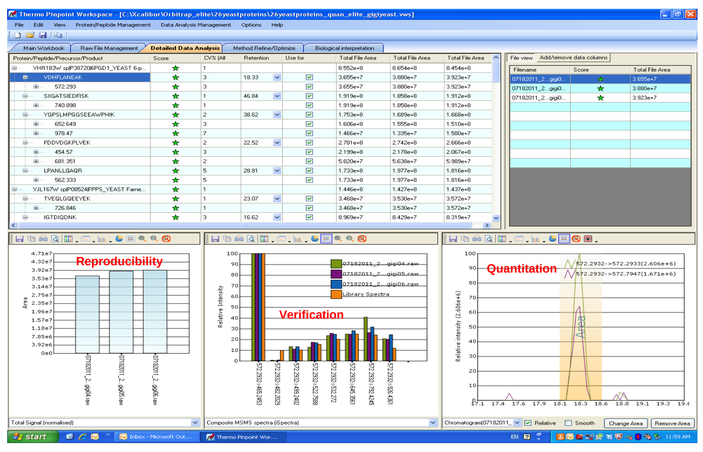


FIGURE 5. More scan points across a peak were observed on the Orbitrap Elite instrument

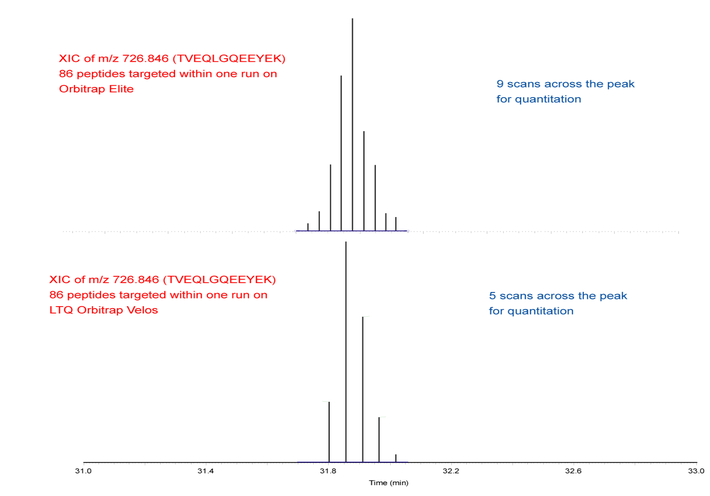
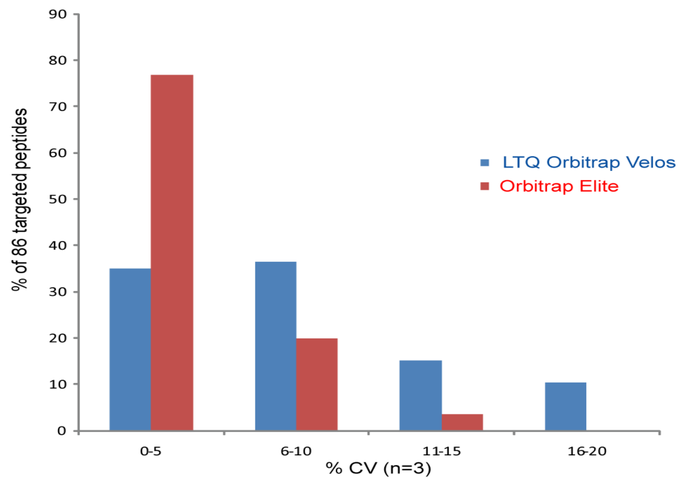


FIGURE 6. Comparison of analytical precision for the developed HR/AM quantitative assay to verify and quantify 86 yeast peptides simultaneously within a single LC-MS run on both the Orbitrap Elite and LTQ Orbitrap Velos instruments.



Conclusion

- A workflow for simultaneous quantification and confirmation of targeted peptides using Orbitrap Elite mass spectrometer was developed and evaluated. The peptides were quantified using selected ion monitoring (SIM), with scheduled MS/MS for sequence confirmation.
- The LOD was as low as 10 amol for six spiked heavy peptides. The linear dilution curves were established across 4 orders of dynamic ranges from 10 amol to 100 fmol with high analytical precision for all six spiked peptides.
- With the established workflow, 5 orders of cellular abundance of targeted yeast proteins were detected and quantified within a single LC MS run. Improved scan rate allowed for more points across chromatographic peak, resulting in excellent analytical precision of the assay. All the 86 targeted peptides had CVs below 15% and ninety-six percent of the targeted peptides had CVs below 10%.
- Throughput (number of targets quantified) was significantly increased by using a wide isolation width (200 amu) for FT SIM and a time-scheduled global MS/MS list.
- The developed workflow can be used for any targeted protein quantitation and delivers highly sensitive and reproducible quantitative results with high throughput.

References

1. Kiyonami et al. et al, Targeted Protein Quantitation of Low-Abundance Yeast Proteins Using High Resolution, Accurate Mass Selected Ion Monitoring. *ASMS poster TP680,* **2011**.
2. Ghaemmaghami,S. et al, Global analysis of protein expression in yeast. *Nature,* **2003** 425, 737-741

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