HR/AM Targeted Peptide Quantification on a Q Exactive MS: A Unique Combination of High Selectivity, High Sensitivity, and High Throughput

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Overview

Purpose: The performance of a novel hybrid quadrupole-Orbitrap™ mass spectrometer was evaluated in targeted peptide quantification.

Methods: Two high-resolution, accurate-mass (HR/AM) targeted quantification methods were used to analyze 14 peptide standards spiked into a complex *E. coli* digest background. The data were automatically processed using specialized software.

Results: A detection limit of 10 amol and a linear dynamic range of 4 orders were obtained with msx tSIM methods in a moderately complex background. A detection limit of 10–50 amol and a linear dynamic range of 3–4 orders were obtained with tHCD methods in a highly complex background.

Introduction

Quantitative proteomics enables the identification of a large number of protein candidates, which display biologically interesting dynamics on a global scale in the early discovery phase. A targeted MS approach, in particular selected reaction monitoring (SRM) on a triple quadrupole MS, has become the standard technique for quantitatively analyzing tens to hundreds of peptide candidates across large numbers of samples, either for understanding of signaling regulation or for verification and selection of potential biomarkers. However, the low resolution of triple quadrupole MS limits its ability to achieve high selectivity on the targets from complex backgrounds. It is also difficult to develop SRM method for peptides with high charge states or high mass or for modified peptides.

In this study, a true high-resolution, accurate-mass (HR/AM) mass spectrometer, was evaluated for targeted protein quantification. The dynamic range and LOD/LOQ of two HR/AM methods: spectrum multiplexing targeted selected ion monitoring (msx tSIM) and targeted HCD (tHCD), were investigated.

Methods

Sample Preparation

Thermo Scientific heavy-isotope labeled peptide retention standards were spiked into either 5 ng/µL or 250 ng/µL *E coli*. whole cell tryptic digest to reach final concentrations of 0 amol/µL, 5 amol/µL, 25 amol/µL, 50 amol/µL, 500 amol/µL, 5 fmol/µL, and 50 fmol/µL.

Liquid Chromatography

Peptides from 2 µL of each sample were separated on a Michrom Magic™ C18 nanoLC column (75 µm x 15 cm, 3 µm particle) with a 1 hr gradient at a flow rate of 350 nL/min. LC solvents were 0.1% formic acid in H_2O (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B). The LC gradient was 0-2 min, 3% B; 2-47 min, 3%-30% B; 47-52 min, 30%-95% B; 52-54 min, 95% B; 54-55 min, 95%-5% B; 55-60 min, 5% B.

Mass Spectrometry

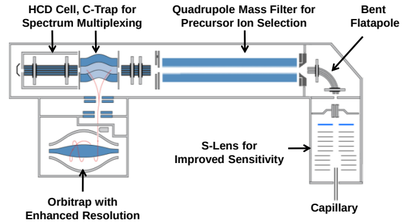
The samples with 5 ng/µL *E coli* digest background were analyzed in triplicate with a Full-msx tSIM method on a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap MS (Figure 1). The resolution was set at 140K for both scan types. The AGC target was 1E6 for full scan and 2E5 for SIM scan. The maximum ion injection time was 120 ms for full scan and 500 ms for SIM scan. The isolation width for SIM scan was 4 amu. The peptide retention standards were monitored over a 3 min window. The multiplexing level was set at 4, which allowed isolation and accumulation of up to four peptide targets in the C-trap before they were transferred to the Orbitrap mass analyzer for detection (Figure 2).

The samples with 250 ng/µL *E coli* digest background were analyzed in triplicate with a tHCD method. The resolution was set at 17.5K. The AGC target was 5E5. The maximum ion injection time was 500 ms. The quadrupole isolation width was 2.0 amu. The normalized collision energy (NCE) was 27%.The peptide retention standards were monitored over a 3 min window.

Data Analysis

The data were automatically processed using Thermo Scientific Pinpoint software. Extracted ion chromatograms for peptide retention standards were obtained with a 5 ppm mass tolerance. Peak areas were calculated with 7-point smoothed complete chromatograms. Linear fitting was performed with a weighing scheme of 1/x.

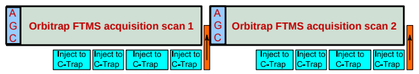
FIGURE 1. Schematic and key features of the Q Exactive MS.



Key Features of the Q Exactive MS

- Incorporation of the S-lens at the source enhances sensitivity by up to 5 fold.
- Quadrupole mass filter enables precursor selection for data-dependent MS^2 and selected ion monitoring (SIM).
- Advanced signal processing increases resolution by about twofold at the same transient length, which results in a maximum resolution of 140K at *m/z* 200 and a maximum scan speed of 12 Hz at a resolution of 17.5K.
- Spectrum multiplexing (msx) and parallel ion injection/detection significantly improves duty cycle.

FIGURE 2: Spectrum multiplexing and parallel ion injection/detection provide high-throughput analysis.



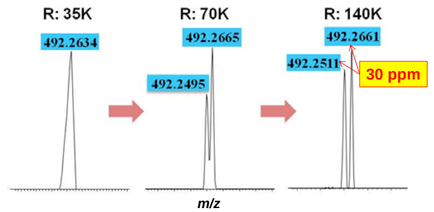
Up to **10** target ions can be isolated by the quadrupoles sequentially, stored in the C-trap, then transferred to the Orbitrap mass analyzer and detected with high resolution simultaneously. The isolation and trapping of the target ions is also concurrent with the Orbitrap mass analyzer detection of the previous ion packet.

Results

Targeted Quantification with msx tSIM

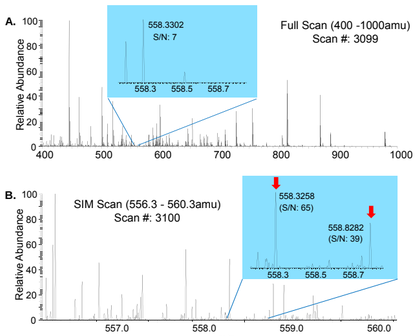
Full or SIM spectra were acquired at three different resolutions: 35K, 70K and 140K. Baseline separation of two ions that are only 30 ppm apart was only achievable at the resolution of 140K. The high mass accuracy (< 5 ppm) and high resolution ensured accurate identification of target peptides from complex background with msx tSIM (Figure 3).

FIGURE 3: High resolution ensures accurate target selection.



Target peptides in 10 ng of *E coli* digest were analyzed with alternating full scans and tSIM scans (Figure 4). The peptide GLILVGGYGTR* (558.3259, 2+) is barely visible in the full scan with a 600 amu isolation window (Figure 4A), but is accurately identified with a **S/N of 65** and mass deviation of **~0.2 ppm** in the SIM scan with a 4 amu isolation window (Figure 4B). The +1 isotope peak is also accurately identified with a S/N of 39 and mass deviation of **~1.4 ppm** in the SIM scan.

FIGURE 4: High sensitivity with quadrupole-based SIM scan.



Extracted ion chromatograms (XIC) of target peptides in SIM scans were obtained with 5 ppm mass tolerance. The XIC of GISNEGQNASIK* (613.3167, 2+) at 100 fmol, 1 fmol and 10 amol is displayed in Figure 5. Although GISNEGQNASIK* was monitored with two other peptides in the same time range, more than 10 SIM scans with a resolution of 140K were obtained across a 12 sec LC peak, even at a sample amount of 10 amol.

As shown in Figure 6, a 4-order linear dynamic range was obtained for most peptides with the msx tSIM method. Table 1 lists the LOD and LOQ of target peptides for the method. The CV% from triplicate analyses is also included for the LOQ.

FIGURE 5. Multiplexing improves throughput and results in accurate quantification.

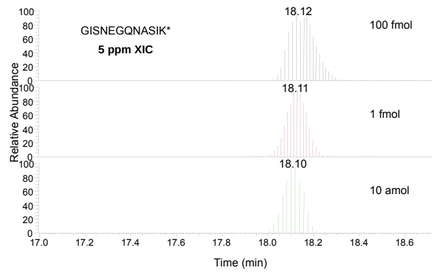


FIGURE 6. Four-order linear dynamic range with msx tSIM method.

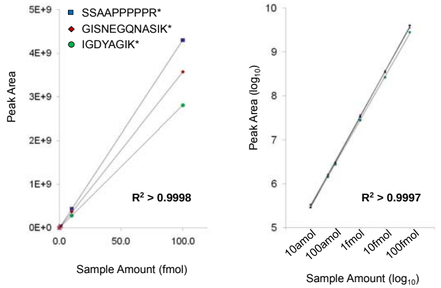


TABLE 1. LOD and LOQ of target peptides for the msx tSIM method.

	LOD (amol)	LOQ (amol)	CV% (LOQ)
IGDYAGIK*	10	50	3
SSAAPPPPPR*	10	50	3
HVLTSIGEK*	10	50	3
LTILEELR*	<10	10	4
GLILVGGYGTR*	10	50	7
NGFILDGFPR*	<10	10	14
SAAGAFGPELSR*	<10	10	4
GISNEGQNASIK*	<10	10	10
ELASGLSFPVGFK*	<10	10	10
TASEFDSAIAQDK*	10	50	1
SFANQPLEVVYSK*	<10	10	8
ELGQSGVDTYLQTK*	10	50	7
LSSEAPALFQFDLK*	<50	50	3
GILFVGSGVSGGEEGAR*	50	100	5

Targeted Quantification with tHCD

With tHCD, high resolution and high mass accuracy allow simultaneous accurate identification of multiple fragment ions from complex mixed MS^2 spectra (Figure 7). As shown in Figure 8, 3-4 orders of linear dynamic range were obtained for most peptides with the tHCD method. Table 2 lists the LOD and LOQ of target peptides for the tHCD method. The CV% from triplicate analyses is also included for the LOQ.

FIGURE 7. HCD spectrum of peptide NGFILDGFPR* (573.305, 2+) at 50 amol in 500 ng *E coli* tryptic digest background.

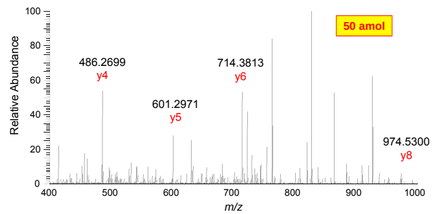


FIGURE 8. Three to four orders of linear dynamic range with the tHCD method.

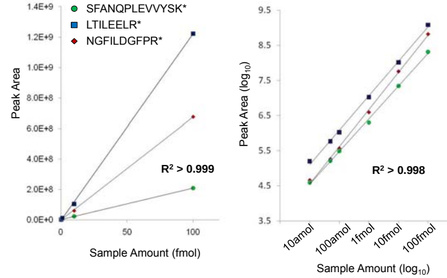


TABLE 2. LOD and LOQ of target peptides for the tHCD method.

	LOD (amol)	LOQ (amol)	CV% (LOQ)
IGDYAGIK*	>10	50	6
SSAAPPPPPR*	10	50	11
HVLTSIGEK*	50	100	11
LTILEELR*	10	50	3
GLILVGGYGTR*	>10	50	8
NGFILDGFPR*	>10	50	6
SAAGAFGPELSR*	10	50	4
GISNEGQNASIK*	10	50	5
ELASGLSFPVGFK*	>10	50	2
TASEFDSAIAQDK	10	50	1
SFANQPLEVVYSK*	<10	10	12
ELGQSGVDTYLQTK*	10	50	6
LSSEAPALFQFDLK*	10	50	6
GILFVGSGVSGGEEGAR*	>10	50	8

Conclusion

The Q Exactive™ MS is well suited for targeted quantification providing two powerful HR/AM approaches: msx tSIM and tHCD. Both approaches demonstrate high selectivity, high sensitivity and high throughput due to several hardware and software innovations.

- For the msx tSIM approach, high mass accuracy (< 5 ppm) and high resolution (140K) ensure accurate identification of target ions in the presence of a complex background, while spectrum multiplexing dramatically increases throughput without sacrificing resolution.
- A LOD of 10 amol and a 4-order linear dynamic range was obtained for most peptides with the msx tSIM method in the presence of a medium complex background.
- Different from triple-quadrupole-based SRM, all fragment ions are simultaneously detected with high mass accuracy and high resolution using the tHCD approach. The HR/AM of fragment ions provides unmatched selectivity. The simultaneous detection of all fragment ions allows more efficient sampling of target peptides and results in improved sensitivity. A scan speed of 12 Hz at a resolution of 17,500 ensures high throughput.
- A LOD of 50 amol and 3–4 orders of linear dynamic range were obtained for most peptides with the tHCD method in the presence of a strong complex background.