Targeted Quantification of Peptides from Complex Biological Sample Using Q Exactive, a Routine Bench Top Quadrupole-Orbitrap Mass Spectrometer

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Overview

Purpose: To evaluate the performance of a hybrid quadrupole-Orbitrap™ mass spectrometer for high resolution and accurate mass (HR/AM) targeted peptide quantification.

Methods: Targeted HR/AM multiplex (MSX) selected ion monitoring (SIM) and targeted HR/AM higher-energy collisional dissociation (HCD)

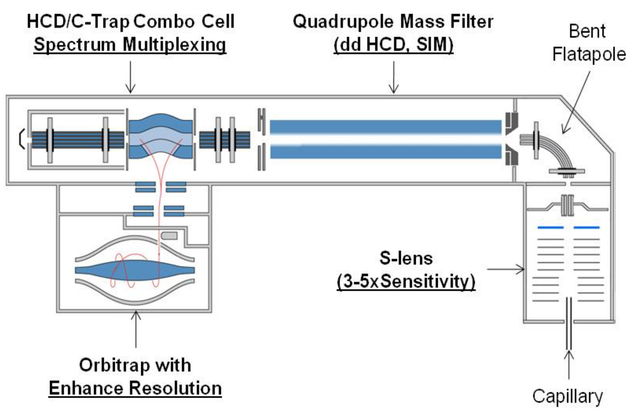
Results: Low attomole detection limit and 4 orders of quantification linearity were routinely achieved.

Introduction

Traditionally, targeted peptide quantification studies use a triple quadrupole (QQQ) mass spectrometer because of the high throughput and high sensitivity, allowing for large numbers of proteins to be quantified in a single LC/MS experiment. However, the transition from discovery to target verification and quantification can be challenging due to the transition between mass spectrometer platforms. The newly developed Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap™ mass spectrometer (Figure 1) enables the seamless transition from discovery to target quantification and verification on a single platform. We describe two high-resolution and accurate mass (HR/AM) targeted peptide quantification approaches. The quadrupole-based high-resolution SIM scan ensures accurate target selection and high sensitivity. Spectrum multiplexing (msx) and concurrent ion injection and detection greatly improve duty cycle (Figure 2). Targeted quantification can also be done using an HCD-based SRM like approach where product ion peak area is integrated for quantification. This approach provides further selectivity from second level of MS (Figure 3). Table 1 lists method parameters for both approaches.

In this study, we evaluated the performance of Q Exactive mass spectrometer on the quantification of peptide targets in complex biological samples. Peptides derived from eicosanoid pathway enzymes in human cerebrospinal fluid (CSF) were quantified using the targeted HCD approach. Eicosanoids exert complex control of inflammation or immunity, and are messengers in the central nervous system. The targeted MSX SIM approach was evaluated using standard peptides spiked into a yeast tryptic digest. Detection limits and linear dynamic range were obtained for these approaches .

FIGURE 1. Schematics of Q Exactive



Methods

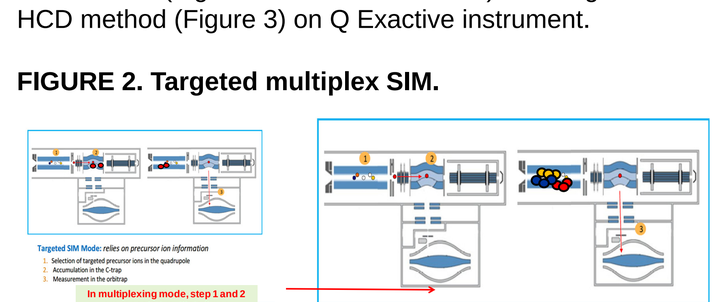
Samples: Thermo Scientific Pierce Heavy Isotope-Labeled Peptide Retention Standards, heavy isotope-labeled peptides from CSF eicosanoid pathway enzyme, yeast whole-cell tryptic digest, CSF tryptic digest.

Chromatography: Heavy isotope labeled peptide standards at 0, 0.01, 0.025, 0.05, 0.1, 1, 10, and 100 fmol were spiked into either 10 ng or 1000 ng of yeast tryptic digest (for peptide retention standards), or 250 ng CSF tryptic digest (for CSF peptides). Each sample was analyzed three times with the same 60-min LC gradient over a Michrom Magic C18 nano-LC column (75 µm x 15 cm, 3 µm C18 partial) and a targeted MSX targeted SIM method (Figure 2 shows MSX 3 SIM) or a targeted HCD method (Figure 3) on Q Exactive instrument.

FIGURE 2. Targeted multiplex SIM.

Targeted SIM mode – relies on precursor ion information
1. Selection of targeted precursor ion in the quadrupole
2. Accumulation in c-trap
In multiplexing mode, step 1 and 2 are repeated to accumulate multiple precursors before Orbitrap measurement.
3. Transfer of ions to Orbitrap mass analyzer for detection. In multiplexing mode, multiple precursors isolated separately are measured together.

FIGURE 3. Targeted HCD MS/MS.



Targeted HCD MS/MS mode – relies on product ion information
1. Selection of targeted precursor ion in the quadrupole
2. Fragmentation in collision cell
3. Accumulation of fragment ions
4. Transfer of fragment ions to Orbitrap mass analyzer for detection

TABLE 1. Instrument method parameters

Parameters	Targeted SIM	Targeted HCD
AGC	2e5	1e6
Resolution	140,000	70,000
Max injection time (ms)	500	500
msx	4	1
Isolation width	4	2
CE	NA	27

Data Analysis

All quantification experiments were evaluated and quantified using Thermo Scientific Pinpoint software version 1.1. 5-ppm mass tolerance was used for the extracted ion chromatograms. The sum of the extracted ion chromatogram peak areas for the two most intense fragment ions observed for each heavy peptide was used to generate the quantification curve.

Results

Peptide quantification using targeted MSX SIM

The targeted multiplex SIM methods was used to quantify peptide retention standards from two concentrations of yeast tryptic digest background, 10 ng and 1000 ng.

Accurate quantification is only possible when the target can be correctly identified. As shown in Figure 4, an interference which is 30 ppm apart from the target can be completely separated using high-resolution of 140,000 in the Q Exactive instrument. Improved resolution leads to better selectivity and more accurate quantification

The Q Exactive instrument is the highest duty cycle HR/AM instrument due to its fast scan speed as well as its unique spectrum multiplexing and concurrent ion injection/detection features. Figure 5 shows concurrent ion injection and detection with a multiplex 4 SIM scan where 4 different peptides isolated separately are detected together in a single Orbitrap measurement, which improves duty cycle remarkably. In the Q Exactive instrument, up to 10 multiplexing is allowed for a single detection.

The detection limit and linear dynamic range of heavy peptide standards were evaluated in the presence of either 10 ng or 1 µg of yeast tryptic digest. From the samples with 10 ng background, all eleven heavy peptide targets were detected at 10 amole. Linear regression analysis, both in regular scale and in $\log_{10}$ scale, showed that four orders linear dynamic range, spanning 10 amole to 100 fmol, was achieved for at least six targets with MSX targeted SIM scan. Plots for three peptide standards are shown in Figure 6A. From samples with 1 µg of background, we could detect and quantify two out of eleven heavy peptide targets at 10 amole. The linear regression fitting plots, both in regular scale and in $\log_{10}$ scale, are shown in Figure 6B. The remaining targets could be detected at 50 amole to 1 fmol; well within the concentration range of most biomarkers.

FIGURE 4: High resolution ensures accurate target selection.

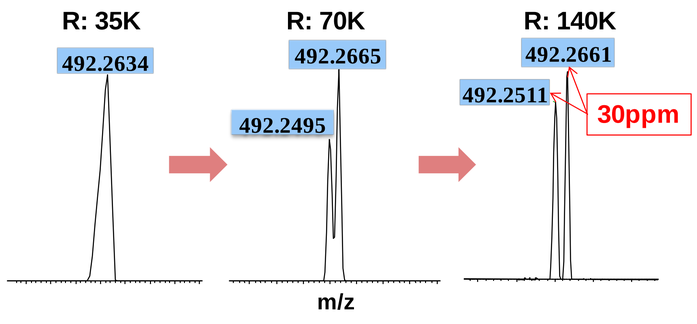


FIGURE 5. Schematic of spectrum multiplexing and concurrent ion injection/detection for high-throughput MSX SIM experiments

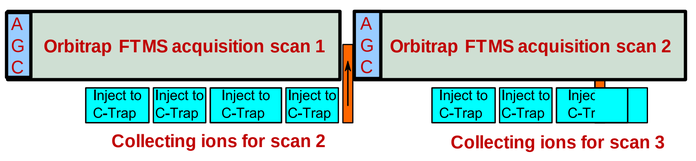
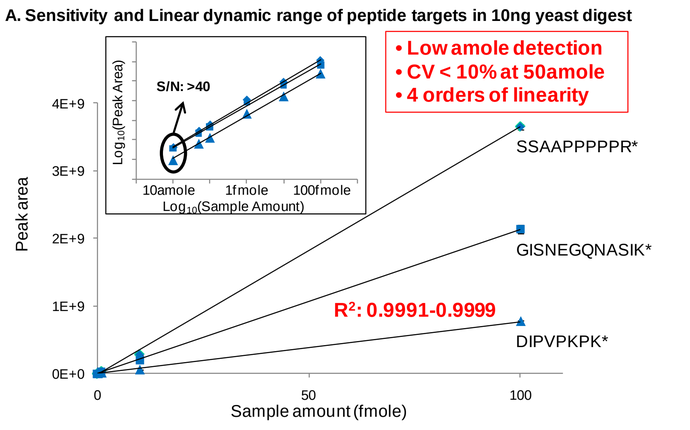
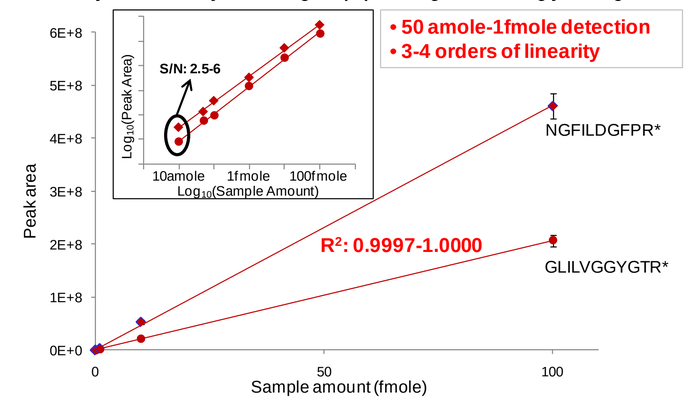


FIGURE 6. LOD of 10 amole or 100 amole is routinely achieved with HR/AM targeted quantification in medium complex to very complex peptide background, respectively.

A. Sensitivity and Linear dynamic range of peptide targets in 10ng yeast digest



B. Sensitivity and Linear dynamic range of peptide targets in 1000ng yeast digest



Quantification of peptides from eicosanoid pathway enzymes using targeted HCD

A target HCD method was used to quantify heavy labeled peptides of eicosanoid pathway enzymes in 250 ng of CSF tryptic digest. Extracted ion chromatograms of fragment ions were used for quantification using 5-ppm mass tolerance.

Fast HCD scanning provided enough data points for quantitative analysis over LC elution peaks. At 70,000 resolution, more than 8 measurements were obtained across LC peaks when 10 amole of peptides with 250 ng of CSF digest was loaded on column (Figure 7).

An LOD of low amole with 4 orders of linearity was routinely achieved using the targeted HCD method with 250 ng CSF digest background. (Figure 8, Table 2).

FIGURE 7: Fast HCD scan in Q Exactive produced more than 8 measurements at 70,000 resolution across LC elution peaks when 10 amole of peptides in 250 ng CSF were loaded on column.

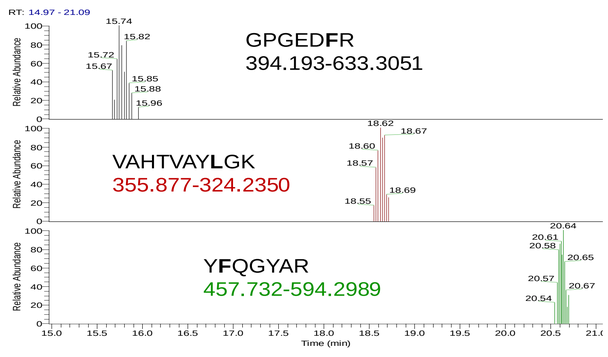


FIGURE 8: Sensitivity and quantification linearity of peptide targets in 250 ng CSF digest. Insets show linearity in log scale.

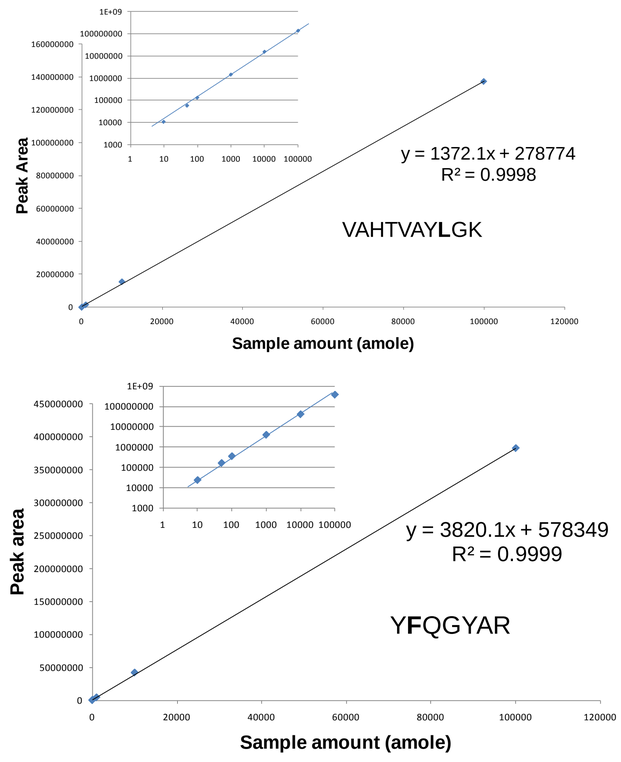
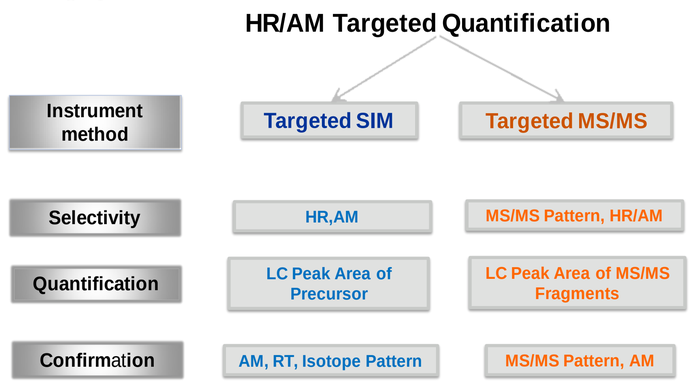


TABLE 2: LOD of low-amole level was routinely achieved using targeted HCD quantification for heavy labeled peptides of eicosanoid pathway enzymes in 250 ng CSF digest.

Protein	Peptide	LOQ (amole)	LOD (amole)
PTGDS	GPGEDFR	25	8
PTGS2	QFQYQNR	25	8
PTGS1	LVLTVR	10	3
HPGDS	STLPFGK	25	8
PTGES	VAHTVAYLGK	50	17
PTGIS	FLNPDGSEK	50	17
TBXA1	SVADSVLFLR	100	33
ALOX15	YTLEINVR	250	83
ALOX12	LWEIIAR	500	167
LTCS4	YFQGYAR	10	3

FIGURE 9: Peptide targeted quantification strategies using Q Exactive instrument.



Conclusions

- At least two different approaches, targeted MSX SIM and targeted HCD, can be used in the Q Exactive MS for HR/AM targeted peptide quantification (Figure 9).
- Low-amole detection limits and 4 orders of quantification linearity were achieved using both methods for quantification in complex biological samples.
- The quadrupole-based high-resolution multiplexing SIM approach provided accurate target selection, high sensitivity and fast duty cycle.
- The HCD-based SRM-like approach provided additional selectivity and sensitivity for targeted quantification in very complex samples. In contrast to traditional SRM approaches, this method provided higher confidence with high-resolution, accurate-mass second-level MS.

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