Peptide Quantification using Parallel Reaction Monitoring Analysis Performed on a Quadrupole-Orbitrap Instrument

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Overview

- A novel hybrid tandem mass spectrometer (quadrupole-orbitrap) with high resolution and accurate mass capabilities was evaluated for proteomics quantitative analyses.
- The impact of pertinent instrumental parameters (quadrupole isolation window, orbitrap resolution, and multiplexing degree) on analytical performances was evaluated.
- The study was performed using biological samples.

Introduction

- The novel hybrid quadrupole-orbitrap mass spectrometer offers unique capabilities for high resolution /accurate mass measurements, while enabling isolation of low abundance components by setting a narrow m/z selection range.
- The MS/MS capabilities of this instrument provide an additional stage of selectivity by performing quantification on fragment ions.
- To scale up the number of peptides targeted in one single LC-MS/MS analysis, acquisition parameters need to be adapted to maintain acceptable cycle times. It includes the relaxation of orbitrap resolving power, and the simultaneous measurement of several peptide fragmentation spectra, which implies the selection of wider precursor m/z ranges or several precursor m/z ranges (multiplexed analysis).
- The performance of the Parallel Reaction Monitoring (PRM) method was explored by a systematic evaluation of the pertinent instrumental parameters to estimate (*e.g.* quadrupole isolation window, orbitrap resolution) their impact on selectivity, sensitivity, and the number of peptides targeted in one LC-MS experiment.

Methods

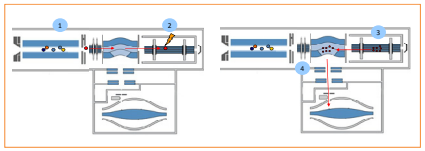
The LC-MS/MS analyses were performed on nano-HPLC system (using 75 micrometer column) coupled to a benchtop orbitrap mass s spectrometer (Q-Exactive, Thermo Scientific, Bremen, Germany), equipped with a quadrupole mass filter at the front-end.

The MS/MS mode was employed to conduct PRM analysis on a set of 150 isotopically labeled reference peptides spiked into urine samples along with their endogenous counterpart. Preparation of urine samples consisted of protein precipitation, trypsin proteolysis, and solid phase clean-up on C_{18} material. Several acquisition methods comprising various combinations of orbitrap resolution (17k-70k) and quadrupole isolation window (2Th-25Th) and including data independent acquisition were employed and the impact on the analytical performances was evaluated. The full set of targeted peptides could be analyzed in one LC-MS/MS analysis or needed to be split in several subsets according to acquisition parameters.

The performances of the multiplexed parallel reaction monitoring mode were assessed using a set of 35 isotopically labeled reference peptides representing either exogenous yeast proteins spiked into urine tryptic digests or endogenous urine proteins. Dilution series of the reference peptides were used to determine the limits of quantification of the method when applied to a complex biological matrix such as urine samples. The approach was benchmarked with the sequential acquisition method.

Large scale multiplexed PRM analysis was conducted on 770 tryptic peptides from yeast digest (representing 436 proteins) targeted in one 60 min LC –MS experiment.

Results

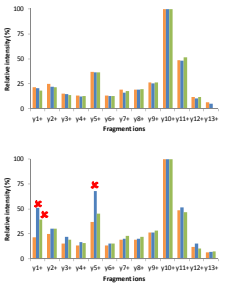


Parallel reaction monitoring (PRM) mode:

relies on fragment ion

1. Selection of targeted precursor ion in the quadrupole
2. Fragmentation in the collision cell (HCD)
3. Accumulation of fragment ions (HCD cell)
4. Transfer of fragment ions to the orbitrap (via C-trap)

Detection of interferences in PRM analysis

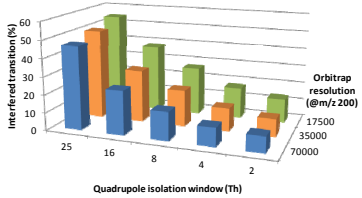


Method

1. Parallel reaction monitoring analysis of neat isotopically labeled reference peptides.
2. Establishment of a reference composite MS/MS spectrum from chromatograms extracted for all y-type fragment ions.
3. Reconstruction of experimental composite MS/MS spectra of H/L peptides in urine samples (500 ng).
4. Semi-automated evaluation of relative differences in normalized fragment ion intensities (H/L peptide in matrix vs. reference)
5. Fragment ions exhibiting a difference > 25% is considered as significantly interfered.

- Isotopically labeled peptide (neat)
- Isotopically labeled peptide spiked in matrix
- Endogenous peptide (urine matrix)
- ✖ Interference on fragment ion signal (>25%)

Parallel reaction monitoring analyses were conducted in triplicates on 300 peptides (150 pairs of heavy and light peptides) for each of the 15 combinations of quadrupole isolation windows (2, 4, 8, 16 and 25 Th) - orbitrap resolution (17.5 k, 35 k, and 70 k). The same maximum filling time was employed for the different experimental conditions. Ion chromatograms of 2,200 fragments were extracted (+/-10 ppm) and the "purity" of the signal of each transition was evaluated.



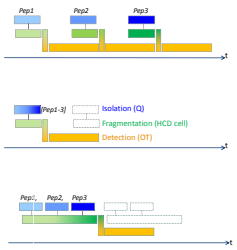
- The selectivity of measurements is dramatically affected by increasing the isolation window
- The benefit of increasing nominal orbitrap resolution from 17.5 k to 70 k is limited.

Quantification in PRM mode

Sequential acquisition method
(narrow isolation windows: 1-2 Th)
- Sequential isolation of target ions
- Sequential fragmentation of ions
- Sequential detection of fragment ions

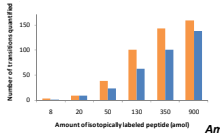
Simultaneous acquisition method
(wide isolation windows: 8- 25 Th)
- Simultaneous isolation of target ions
- Simultaneous fragmentation of ions
- Single detection scan

Multiplexed acquisition method
(multiplexed narrow isolation windows)
- Sequential isolation of target ions
- Sequential fragmentation of ions with intermediate storage of fragments
- Single detection scan



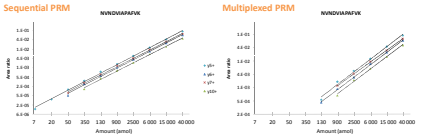
Multiplexed vs. Sequential acquisition methods

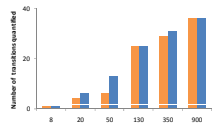
Dilution curves of 35 isotopically labeled peptides spiked in various amounts in 500 ng of urine tryptic digest (0, 8, 20, 50, 130, 350, 900, 2 500, 6 000, 15 000, 40 000 amol).
Sequential method: isolation window of 2 Th; max filling time of 100 ms; target AGC $1^{*}6$.
Multiplexed method: duplex method, isolation window of 2 Th; max filling time of 100 ms; target AGC $1^{*}6$.



➢ Overall decrease in sensitivity when analyses performed in multiplexed mode.

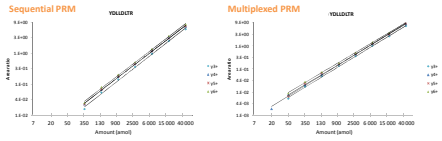
Amount of endogenous peptide (≈ 15 fmol)



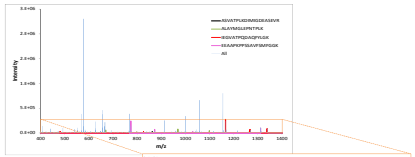


➢ For lower amounts of endogenous peptides (< 4 fmol), multiplexed analysis provided slightly extended linearity range

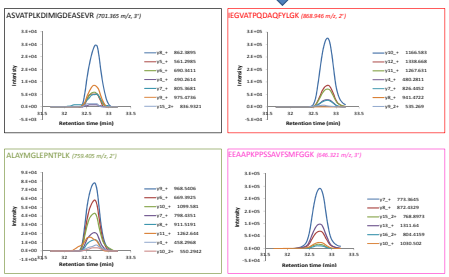
Amount of endogenous peptide (≈ 1 fmol)



Large-scale PRM experiment



- Time scheduled PRM analysis of 770 tryptic peptides from yeast digest (representing 436 proteins).
- Targeted peptides were grouped in subsets of 4 according to their chromatographic elution order.
- The selection of target precursor ions was performed based on the resources of SRM-Atlas .



Conclusion

- The selectivity for each set of experimental parameters provided a baseline for the establishment of acquisition methods generally applicable, including methods well-suited to large-scale targeted quantification.
- Narrow quadrupole isolation window is decisive to ensure high selectivity of measurements. In comparison the benefit of increasing nominal orbitrap resolution (from 17.5 k to 70 k) is more limited.

Acknowledgments

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