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Revolutionary Proteome Profiling and Quantitation without Compromising Speed, Sensitivity, and Selectivity

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ABSTRACT

Purpose: A novel high resolution MS1 based DIA method was developed to comprehensively, reproducibly, and precisely quantify proteins in complex matrices.

Methods: Instead of quantifying the peptides on MS2 intensities, MS1 scans offer potentially higher sensitivity since the peptide precursor is not fragmented into multiple fragments.

Results: Separation of the tryptic peptides in complex proteomes was performed with 1-hour total run time on the same capillary LC coupled to both the Thermo Scientific™ Q Exactive ™ HF and the Thermo Scientific™ Q Exactive™ HF-X MS systems. > 4800 protein groups and > 46,000 peptide precursors are identified and quantified on the Q Exactive HF-X system, which produces > 20% more peptide precursors than the standard Q Exactive HF system. High resolution detection removes interferences from analytes of interest, achieving accurate and precise quantitation.

INTRODUCTION

For large cohort studies in translational proteomics research, data independent acquisition (DIA) methods may be used to provide a global view of protein abundance changes among the samples. Here, a novel high resolution MS1 based quantitation DIA (HRMS1) method was developed on a modified Q Exactive HF-X mass spectrometer to optimize usage of scan speed, ion injection time (sensitivity), and high resolution (selectivity). Conventional DIA quantitation is based on the intensities of fragments in MS2 scans. Instead of quantifying the peptides on MS2 intensities, MS1 scans offer potentially higher sensitivity since the peptide precursor is not fragmented into multiple fragments. High resolution detection removes interferences from analytes of interest, achieving accurate and precise quantitation.

MATERIALS AND METHODS

Sample

Thermo Scientific™ Pierce™ HeLa Protein Digest was dissolved into a 1 ug/ul sample solution, and the Biognosys iRT Kit (comprised of synthetic peptides) was spiked into the samples before injection according to the manufacturer's protocol. Different loading amounts ranging between 2 ug to 4 ug were evaluated on the LC-MS systems.

Methods

HELA and 6 HPRF fractions were analyzed using data-dependent acquisition (DDA) methods on the same nanoLC online, coupled to the Q Exactive HF MS to build up the spectral library.

For high-throughput analysis, a Thermo Scientific™ UltiMate™ 3000 RSLC system equipped with capillary flow configuration, coupled to the Q Exactive HF MS and Q Exactive HF-X systems, was used to separate tryptic peptides from HELA using a 1-hour run time (Figure 2). 30mins total run time separations were also evaluated on the Q Exactive HF-X system.

Different MS1 scan resolution settings (120k and 30k) were evaluated to demonstrate the effect of resolution. Full scan mass range cover 4000 -1200 *m/z*, and 10Da width isolation windows and 30k resolution were applied for DIA MS2 scans (Figure 1).

Each experiment is performed with three technical replicates. The results are shown from three technical replicates.

Data Analysis

The DDA data files were searched with Thermo Scientific™ Proteome Discoverer™ software v.2.1 with the SEQUEST® HT engine using the human Uniprot database. The search settings were as follows: trypsin with up to 2 missed cleavages, and mass tolerances set to 10 ppm for precursor mass and 0.02Da for fragment ions. Oxidation of M was chosen as dynamic modification (+15.995 Da) and carbamidomethylation of C as static modification (+57.021 Da). FDR was set to 1% on peptide and protein level. Spectral libraries were generated based on the shotgun runs performed on the Q Exactive HF mass spectrometer.

All DIA data were directly analyzed in Spectronaut™ v10 software (Biognosys, Schlieren). Dynamic score refinement and MS1 scoring were enabled. Total peak areas of the isotopic envelope were chosen for quantification. Interference correction and cross run normalization based on total peak area) were enabled. All results were filtered by a Q value of <0.01 (equals a FDR of 1% on peptide level).

All quantitation analysis are based on MS1 scans.

RESULTS

The high resolution MS1 based DIA method on a modified Q Exactive HF-X mass spectrometer was developed to comprehensively, reproducibly, and precisely quantify the proteins in the complex matrices. Capillary flow LC was utilized to maintain good proteome coverage with increased robustness and throughput (Figure 1).

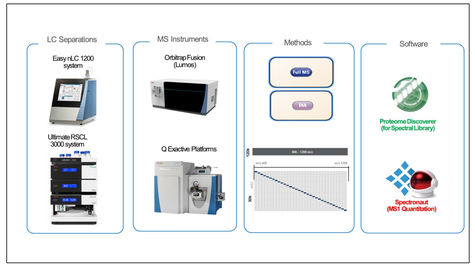

Figure 1. Data Independent Acquisition Solution

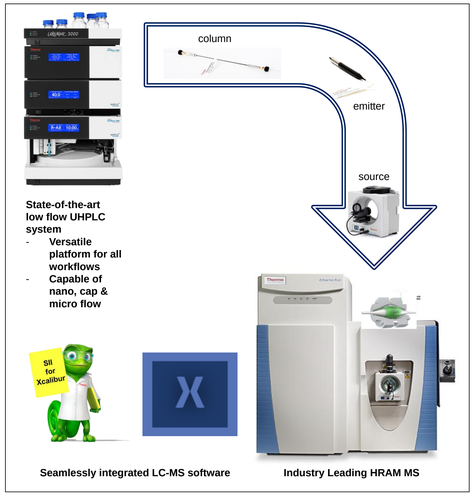

Figure 2. Capillary LC – Q Exactive MS Platform Configuration and Workflow

Comprehensive Proteome Profiling with Doubled Throughput

Separation of the tryptic peptides in HELA was performed with 30mins and 1-hour total run time on the same capillary LC coupled to both Q Exactive HF MS and Q Exactive HF-X MS systems. With the same sample load and same separation time, >20% more peptide precursors are identified with 1% FDR on the Q Exactive HF-X than on the Q Exactive HFMS. With only half of the run time, the Q Exactive HF-X can detect and quantify an equivalent number of protein groups as the QExactive HF MS, thereby doubling the throughtput. Each condition was acquired with 3 technical replicates (Figure 3).

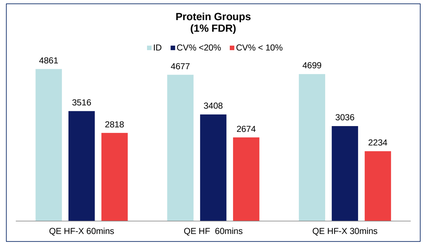

Figure 3 A. Protein groups with total run time 60mins and 30mins Capillary LC – Q Exactive HF MS and Capillary LC- Q Exactive HF-X MS. 4 ug HELA digest was injected.

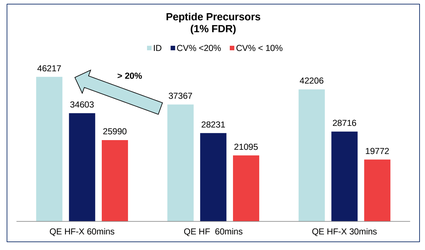

Figure 3 B. Peptide precursors with total run time 60mins and 30mins Capillary LC – Q Exactive HF MS and Capillary LC- Q Exactive HF-X MS. 4 ug HELA digest was injected.

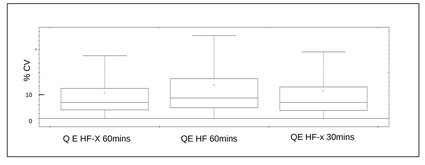

Figure 3 C. Median CV% of all the peptide precursors

Capillary LC – QE HF-X MS Platform twice the Sensitivity

The Q Exactive HF-X system enables collection of a higher number of ions per time facilitated by the improved ion source design. The combination of the HRMS1 method and the Q Exactive HF-X MS enables collection of a high number of ions within several milliseconds, translating into higher efficiency in confident peptide and protein identification with increased sensitivity for precursor-based quantitation. With half of the sample load on the Q Exactive HF-X system, the number of identified protein groups and peptides are almost equal to the Q Exactive HF MS using the same separation time (Figure 4).

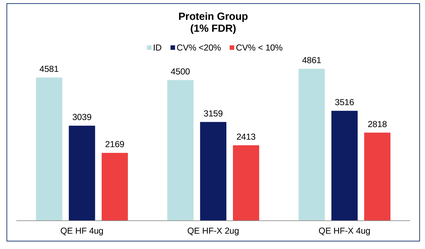

Figure 4 A. Protein Groups with total run time 1h. Capillary LC – QE HF MS and Capillary LC- QE HF-X MS. 2 ug or 4 ug HELA digest were injected.

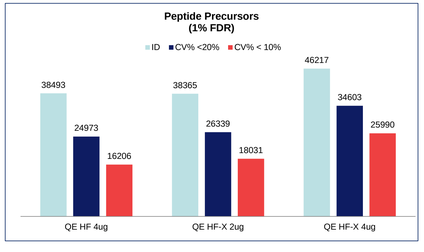

Figure 4 B. Peptide precursors with total run time 1h. Capillary LC – QE HF MS and Capillary LC- QE HF-X MS. 2 ug or 4 ug HELA digest were injected.

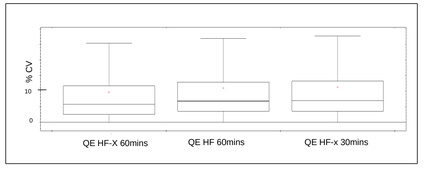

Figure 4 C. Median CV% of all the peptide precursors

Confident Result with High Resolution

Different resolution settings (120 k and 30 k) for data acquisition in MS1 scans were evaluated. With higher resolution, ~ 10% more peptide precursors are identified with 1% FDR, and ~7% more peptide precursors are quantified with CV% < 20%. This result shows that high resolution is beneficial to achieve accurate and precision quantitation by removing interferences (Figure 5).

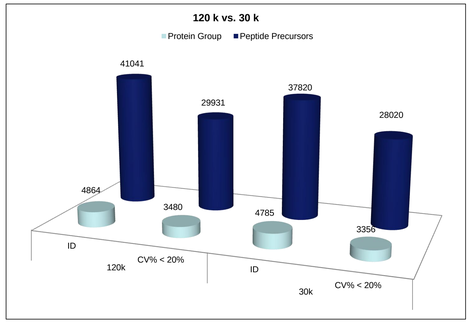

Figure 5. Protein groups and peptide groups identified with 1% FDR. MS1 resolution was set to either 120 k or 30k. 4 ug HELA digest were injected.

CONCLUSIONS

- The high resolution MS1 based quantitation DIA method on the Capillary LC online coupled to the QE HF-X system enables comprehensive proteome profiling with high throughput and robustness, identifying and quantifying > 4800 protein groups and > 46000 peptide precursors with 1% FDR with 1-hour total run time.
- With the higher resolution setting (120k) for the MS1 scans, ~ 10% more peptide precursors are identified with 1% FDR, and ~7% more peptide precursors are quantified with CV% < 20%, comparing to 30k resolution at MS1.
- Median CV% is better than 10% for all the experiments.
- Similar results for the numbers of identified peptides and protein groups are obtained at the same separation time for the Q Exactive HF-X MS and Q Exactive HF MS when the sample loading for the Q Exactive HF-X MS is one-half that of the Q Exactive HF MS, demonstrating the increased sensitivity and improved efficiency of the Q Exactive HF-X instrument.

TRADEMARKS/LICENSING