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

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ABSTRACT

Purpose: High-resolution MS/MS based DIA method was developed in collaboration with Biognosys AG to perform high throughput, high sensitivity, and high selectivity protein profiling. The method is designed for precise and accurate quantitation of proteins in complex samples.

Methods: Instead of quantifying the peptides on MS2 injection, MS2 data are potentially highest resolution data that is possible to detect and quantify a peptide. High resolution and high sensitivity data are obtained in the DIA experiment.

Results: Different high resolution MS1 based DIA method on the Capillary LC online coupled to the Versatile Q Exactive HF-X system with CV% < 10% were evaluated. QE HF-X 30mins and QE HF 60mins systems were evaluated in the DIA experiment. With the same sample, the QE HF-X 60mins system identified more protein groups and peptide precursors than the QE HF 60mins system. With the increased resolution, a number of interferences were removed, improving the quantitation accuracy.

INTRODUCTION

For high throughput shotgun proteomics research, data independent acquisition (DIA) methods may be used to increase the global view of proteome changes among the samples. Here, a high resolution MS/MS based DIA method (DIA-HR) was developed on the Q Exactive HF-X (Thermo Fisher Scientific, Bremen, Germany) system. The method combines high resolution and high sensitivity detection with MS1 scan resolution settings to remove interferences from the peptide precursors than the standard DIA-HR method. The peptide precursors are identified and quantified without using MS2 scans. For large cohort studies in translational proteomics research, data independent acquisition (DIA) methods are used to provide a global view of protein abundance changes among the samples. The method was developed to comprehensively, reproducibly, and precisely quantify the proteins in the complex samples.

MATERIALS AND METHODS

Sample

Thermo Scientific™ Proteinase K was used as a Protein Digest was dissolved in 1 ml glass sample vials, and the His-tagged recombinant protein was aliquoted on the samples. The samples were kept on the samples. The samples were kept on 4°C until MS analysis.

Methods

The method requires the injection of a sample into the Q Exactive HF-X MS system with CV% < 10%. QE HF-X 30mins and QE HF 60mins systems were evaluated in the DIA experiment. With the same sample, the QE HF-X 60mins system identified more protein groups and peptide precursors than the QE HF 60mins system. With the increased resolution, a number of interferences were removed, improving the quantitation accuracy.

Data Analysis

The XDA data file was analyzed with "Thermo Scientific™ Proteome Discoverer™ software v2.2 with the SEQUEST algorithm using a human (NCBI 37) database. The sequences of the human proteome were used as the database. The minimum peptide and protein identifications were identified with 1% FDR. The precursor ions were included as the ions that are most abundant. The dynamic range of the method is identified with the dynamic range of the separated peptides.

RESULTS

The high resolution MS1 based DIA method on the Capillary LC online coupled to the Versatile Q Exactive HF-X system was demonstrated to be reliable and accurate. The method was used to detect and quantify a peptide. High resolution and high sensitivity data are obtained in the DIA experiment. With the same sample, the QE HF-X 60mins system identified more protein groups and peptide precursors than the QE HF 60mins system. With the increased resolution, a number of interferences were removed, improving the quantitation accuracy.

CONCLUSIONS

The high resolution MS1 based DIA method on the Capillary LC online coupled to the Versatile Q Exactive HF-X system was identified as a reliable and accurate method for proteome profiling and quantitation. The method was used to detect and quantify a peptide. High resolution and high sensitivity data are obtained in the DIA experiment. With the same sample, the QE HF-X 60mins system identified more protein groups and peptide precursors than the QE HF 60mins system. With the increased resolution, a number of interferences were removed, improving the quantitation accuracy.

TRADEMARKS/LICENSES

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Figures:

Figure 1: Data Independent Acquisition Solution

Figure 2: Protein groups with total run time 60mins and 30mins Capillary LC – QE Exactive HF and Capillary LC – QE Exactive HF 3ug MS1 A digest, both identified.

Figure 3: Peptide precursors with total run time 60mins and 30mins Capillary LC – QE Exactive HF and Capillary LC – QE Exactive HF 3ug MS1 A digest, both identified.

Figure 4: A. Protein groups and peptide groups identified with 1% FDR, QE HF-X was used in each of 30ms or 60ms. 2ug MS1 digest was injected.

Figure 5: A. Protein groups and peptide groups identified with 1% FDR, QE HF-X was used in each of 30ms or 60ms. 2ug MS1 digest was injected.

Figure 6: A. Protein groups and peptide groups identified with 1% FDR, QE HF-X was used in each of 30ms or 60ms. 2ug MS1 digest was injected.

Figure 7: A. Protein groups and peptide groups identified with 1% FDR, QE HF-X was used in each of 30ms or 60ms. 2ug MS1 digest was injected.