Maximizing Protein Profiling Using HRAM MS and DDA Methods: Sweeping Through the Proteome Using Wide-Window DDA Parameters

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ABSTRACT

Pinnacle Technologies LLC MS workflow is perform global proteome profiling that enables the greatest breadth, reproducibility and specificity. We created new research requires for the next generation of DDA/DE (data dependent acquisition). Methods: Developing a high mass accuracy-based data analysis method for universal examples of DDA/DE workflows. Results: Thermo Scientific™ Q Exactive Plus and Thermo Scientific™ Orbitrap Eksial 2.0 are the leading systems for high mass accuracy based data analysis. Conclusions: Pinnacle Technologies LLC MS workflow is perform global proteome profiling that enables the greatest breadth, reproducibility and specificity.

INTRODUCTION

Data acquisition strategies for clinical research must maximize proteome coverage and qualitative and quantitative discovery. Traditional data dependent acquisition (DDA) methods use dynamic window selection at both MS1 and MS2 using precursor width settings to narrow the range of precursor mass windows covered at each stage of the analysis. The current workflow is complex and time-consuming, requiring multiple DDA cycles to achieve the necessary proteome coverage. In addition, the isotopic overlap and spectral overlap across cycles introduces new challenges and reduces the overall data quality. Our approach is based on a modified high-resolution version of a high-accuracy method for producing high-mass accuracy spectra. We have developed a novel approach that we call "Wide Window DDA" (wDDA) which is designed to offer improved proteome coverage while retaining high mass accuracy.

MATERIALS AND METHODS (cont.)

All experiments were performed on the Thermo Scientific™ Q Exactive Plus and Thermo Scientific™ Orbitrap Eksial 2.0, respectively. The samples were prepared according to the manufacturer’s recommendations. A total of 100 ng of purified peptides were injected onto the column for each experiment. The following settings were used for the wDDA method: 3 e 5 mass ion fill times for MS and MS/MS, respectively. The overall loop count and cycle times were set to 5e4, 1e5, and 5e5. Mass ion fill times for narrow precursor isolation settings were 50 msec (0.5 Da) and 500 msec (5 Da) for MS and MS/MS, respectively. The overall loop count and cycle times were set to 5e4, 1e5, and 5e5. Mass ion fill times for wide precursor isolation settings were 50 msec (0.5 Da), 500 msec (5 Da), and 5000 msec (50 Da) for MS and MS/MS, respectively. The overall loop count and cycle times were set to 5e4, 1e5, and 5e5.

RESULTS

Figure 1 shows the MS/MS spectra as a function of precursor isolation width for the peptide 634.7426 (Yellow) and 5e5 10 Da (Green). The trend in the mass ion fill times of 50 and 100 msec for MS and MS/MS, respectively. The overall loop count and cycle times were set to 5e4, 1e5, and 5e5.

Figure 2 shows the MS/MS spectra as a function of precursor isolation width for the peptide 634.7426 (Yellow) and 5e5 10 Da (Green). The trend in the mass ion fill times of 50 and 100 msec for MS and MS/MS, respectively. The overall loop count and cycle times were set to 5e4, 1e5, and 5e5.

CONCLUSIONS

The presented research demonstrates a modified wDDA experimental method for evaluation and select high global proteome profiling. The method employs separations with high mass accuracy, allowing for increased discovery and accuracy of the final results. The modified wDDA method resulted in 23% more identifiable peptides and 17% more quantified peptides compared to the original wDDA method.

Table 1: List of MS1 and MS2 peaks with representative fragment intensity and mass accuracy. The columns show the peak intensity, charge state, mass accuracy, and fragment intensity for each of the MS/MS spectra.

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REFERENCES


TRADEMARKS/LICENSING

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