INTRODUCTION

For quantitation of large numbers of proteins, data independent acquisition (DIA) methods are currently used by many proteomics labs to perform large scale quantitative experiments in thousands of samples in parallel. This method has proven to be a fast and robust strategy for large scale protein quantitation and biomarker discovery efforts when large number of samples are being analyzed. Currently researches have started to use microLC in LC to increase robustness and throughput, while still maintaining good sensitivity. Most labs have been done in 10 µg microLC LC columns with 40 residues include 1 hour per sample. In our previous work, we have demonstrated that with 4x more material, ~85% of proteins were quantified at 400% coverage in less than 2 hours using TripleTOF®. These conditions further improved for 120 min. We now report a series of experiments performed to improve the sensitivity and throughput of these methods.

Figure 1. Protein Quantification by microLC. microLC MS Conditions: Replicate data was processed using SWATH windows (DuoSpray™, Sciex). For preliminary data two liquid chips were used with prior proteolytic proteome quantification covered with a shorter gradient (6 followed by 1 minute of gradient at 10% H₂O). Two different scale was expected and needed some time to have the load increased from 0 to 10% H₂O at a rate. At loadings 10% H₂O at a rate the source (Figure 1). From this load for both gradient lengths ± 2 standard.

Table 1. Quantification Improvement by Three Different Methods for the 2 hour run time. The number of peptides and proteins quantified by SWATH and 4x more material (Table 1). We observed a slight difference instead in the microLC column compared to the previous condition.

Samples Percentage Improvement (Peptides) Percentage Improvement (Proteins)
Plasma 31% 25%
 Yeast 42% 31%
 Human 70% 38%

CONCLUSIONS

- SWATH® acquisition coupled with microLC chromatography provides higher throughput and reproducibility over nanoLC chromatography.
- Previous work demonstrated that with more material—4x of peptides at quantification 85% higher throughput with microLC than nanoLC injection.
- In this work, we investigated the impact of using a longer run time (2 hours per sample) for SWATH® acquisition experiments. Due to the low recovery of the extracted fragmented ions, quantification was performed with a FDR of 0.1%.
- An increased gradient condition (60 min 0-60% H₂O) showed improved peptide/protein quantification.
- A longer column length was not required for this longer gradient time, best results were obtained on a 15 cm column length.
- An increased quantification coverage using 10 µg sample instead of 6 µg (1 sample amount was limited).
- With a total run time of 2 hours per sample, up to 12 samples per day can be analyzed with the conditions explored here. The gains in peptide/protein quantification can be balanced by the decrease in samples through microLC.

REFERENCES

1. Accelerating Data Independent Acquisition with MicroLC-Chromatography: AMG Poster Number TP089
3. SWATH有信心 template 2.0

TRADEMARKS/LICENSING

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