Quantifying 1,000 protein groups per minute of LC gradient using Zeno SWATH data-independent acquisition (DIA)

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ABSTRACT

Zeno SWATH DIA on the ZenoTOF 7600 system combines the speed, sensitivity and quantitative reproducibility needed for robust detection and quantitation of proteins with very fast LC gradients, when combined with the selectivity and sensitivity afforded by Zeno trapping in MS/MS. Using a 5-minute microflow LC gradient (14-minute total runtime) and Zeno SWATH DIA with 56 variable-width SWATH DIA windows, approximately 5,000 protein groups were quantified with <20% CV from 200 ng of K562 and HeLa cell lysates. In addition, approximately 3,100 protein groups were quantified from yeast extracts. These results show that with this fast microflow LC chromatography and Zeno SWATH DIA method, approximately 1000 protein groups can be effectively quantified per minute of active gradient using the ZenoTOF 7600 system.

INTRODUCTION

High-throughput, in-depth proteomic analysis has long been the goal of many researchers. DIA has become the standard method of analysis with many investigators. Zeno SWATH DIA on the ZenoTOF 7600 system provides unparalleled speed and sensitivity when performing these types of experiments, in addition to very high quantitative precision, especially when the quantitation is performed on the MS/MS transitions. It has been previously shown that impressive numbers of proteins can be identified and quantified from standard cell lysate digests with Zeno SWATH DIA using a combination of the Evosep One system and the ZenoTOF 7600 system[1]. The workflow presented here utilizes a conventional Waters M-Class UPLC system at a flow rate of 5 µL/min. The numbers of peptides and proteins detected and quantified are shown using these fast gradients on multiple commercially available human cell lysate digests (K562 and HeLa) and in yeast extract digests. The gradients used were designed to approximate the active gradient used on the Evosep One system and cell lysate digests were separated on a Phenomenex Kinetex XB C18 (150 x 0.3 mm) column. The resulting data highlight the speed of the ZenoTOF 7600 system with Zeno SWATH DIA, as approximately 1000 protein groups in human lysates were quantified per minute of active gradient time.

MATERIALS AND METHODS

Sample preparation
Commercial human K562 or HeLa cell lysate tryptic digests (Promega and Thermo Fisher Scientific, respectively) were prepared in buffer containing water with 0.1% formic acid (Honeywell) at a concentration of 100 ng/µL. Yeast extract trypsin digest (Promega) was also prepared at a concentration of 100 ng/µL in the same buffer.

Chromatography
Trap/elute microflow LC methods were performed with a Waters M-Class LC system using Phenomenex trapping (MicroTrap C18, 10 x 0.5 mm, P/N 05N-4252-AF) and analytical (Kinetex XB-C18, 150 x 0.3 mm, P/N: 00F-4496-AC) columns. Mobile phases A and B were water and acetonitrile, each pre-mixed with 0.1% formic acid. Trapping and washing were performed for 2 minutes at 10 µL/min. For the 5-minute gradient method, the flow rate was 5 µL/min and initial conditions were 3-35% mobile phase B over 5 minutes. This was followed by a wash phase in which the gradient ramped to 80% mobile phase B at 6 min and held constant for 2 minutes. At 8.5 minutes, the gradient ramped down to 3% mobile phase B during equilibration and held for 2.5 minutes. Six replicate injections were run for each sample.

Mass spectrometry
The ZenoTOF 7600 system was operated using the OptiFlow TurboV ion source with a vertical microflow LC probe (1-10 µL/min electrode). The Zeno SWATH DIA method consisted of 56 variable-width SWATH DIA windows that spanned the Q1 mass range 400-750 Da. MS/MS accumulation times of 12 ms were used with Zeno trapping over the MS/MS mass range 200-1500 Da.

Data processing
Zeno SWATH DIA data were processed using DIA-NN software, version 1.8.1[2]. Library searches were performed for the data from human extracts against a combined K562/HeLa spectral library generated previously using high-pH fractionation followed by data-dependent acquisition (DDA) analysis on the Zeno TOF 7600 system[3]. The resulting DIA-NN software output files were uploaded to the SCIEX OneOmics software suite for statistical analysis to visualize the reproducibility of the Zeno SWATH DIA runs and differential protein expression between the different human cell lines. For the data acquired from yeast extracts, library-free searches were performed against the Uniprot-SwissProt FASTA file containing yeast protein canonical sequences and isoforms.

RESULTS

Detection and quantitation of peptides and proteins using fast gradients

Microflow LC methods were designed using a 5-minute active gradient (14-minute total run time using trap/elute LC) to mimic the 100 SPD method on the Evosep One system. On-column loadings of 200 ng of human K562 or HeLa cell lysates were analyzed using a Zeno SWATH DIA method consisting of 56 variable-width windows. When processed with DIA-NN software using a spectral library, approximately 6,000 protein groups were detected at 1% FDR and nearly 5,000 protein groups were quantified using Zeno SWATH DIA with a 5-minute gradient, which translated to approximately 1,000 protein groups per minute of active gradient (Figure 1). Approximately 35,000 precursors were detected at 1% FDR and approximately 25,000 precursors quantified at <20% CV. The system running microflow LC was highly reproducible, demonstrated by robust analyses that quantified 80% of protein groups at a CV <20% (Figure 1)
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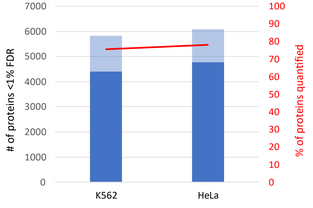
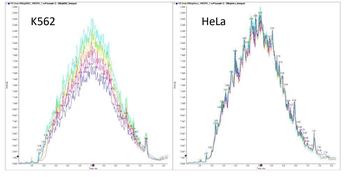
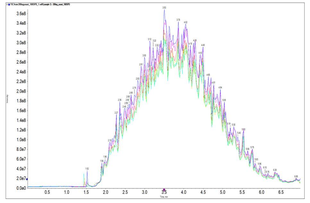


Figure 1. Protein groups identified with <1% FDR and quantified with <20% CV. These analyses were performed for 200 ng injections of K562 and HeLa human cell lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system with a 5-minute microflow LC gradient.

Figure 2. Total ion chromatogram (TIC) overlays for replicates of 200 ng injections of K562 and HeLa human cell lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system using a 5-minute microflow LC gradient.





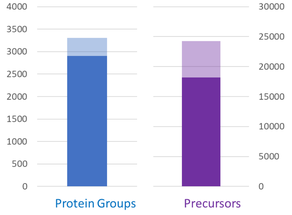


Figure 3. Analysis of yeast extract using a 5-minute microflow LC gradient. (Left) Total ion chromatogram (TIC) overlays for 6 replicates of 200 ng injections of yeast lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system. (Right) Numbers of protein groups and precursors identified (<1% FDR, light shading) and quantified (<20% CV, dark shading).

Further analysis of protein groups quantified in human cell lysates

The protein groupings quantified in the human cell extracts showed a high degree of overlap between the 2 different cell lines. More than 3500 protein groups were shared between the cell lines and 855-1220 protein groups were quantified that were unique to a given cell line (Figure 4).

To highlight the utility of this sensitive and reproducible setup, the DIA-NN software output files from the human lysate dataset analyzed using 5-minute LC gradients were imported into the SCIEX OneOmics software suite for subsequent processing[3]. Following normalization of the results, one can perform statistical analyses such as PCA or t-tests, to profile protein levels across the different experimental groups. In this case, the relative abundance of a representative human protein (ubiquitin-conjugating enzyme, E2 L3 UB2L3_HUMAN) is shown for the K562 and HeLa cell lines (Figure 5).
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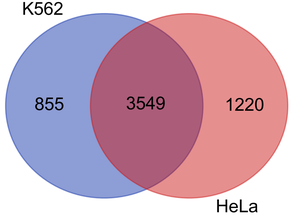
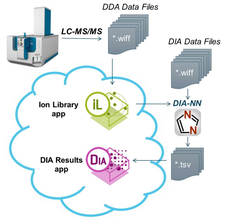


Figure 4. Comparison of protein groups quantified between cell types. The Venn diagram compares the number of protein groups quantified (at <1% FDR and <20% CV) across the 2 different human cell lines analyzed using Zeno SWATH DIA with 5-minute microflow LC gradients on the ZenoTOF 7600 system. The overlap indicates the protein groups that were shared between the 2 types of human cell samples.



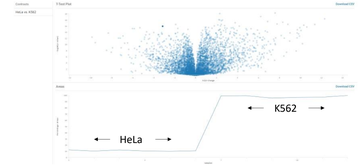


Figure 5. Protein profiling between different cell lines. (Left) DIA-NN software results were imported into the SCIEX OneOmics software suite, data were normalized and protein profiles generated using the MarkerView application in Bioreviews. (Right) This profile shows the differential levels of ubiquitin-conjugating enzyme E2 L3 (UB2L3_HUMAN) across the human K562 and HeLa cell lysate samples. Samples were analyzed using a 5-minute microflow LC gradient and a Zeno SWATH DIA method with 56 variable-width windows. Six replicate injections were acquired for each sample and the data were processed using DIA-NN software to compare against a K562-HeLa spectral library.

CONCLUSIONS

- Approximately 1000 protein groups were quantified per minute of active gradient using a microflow LC method coupled with Zeno SWATH DIA on the ZenoTOF 7600 system
- Variable-window Zeno SWATH DIA was well-suited to a fast separation method due to the selectivity and sensitivity afforded by Zeno trapping in MS/MS
- The high performance of Zeno SWATH DIA was leveraged using a range of LC separation systems, such as the Evosep One system or conventional microflow LC using other devices, such as the Waters M-Class LC system

REFERENCES

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