Quantifying 1000 protein groups per minute of microflow gradient using Zeno SWATH DIA on the ZenoTOF 7600 system

Fast microflow proteomics on the ZenoTOF 7600 system

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High-throughput proteomic analysis has long been the goal of many researchers and this workflow has been shown to work well using the Evosep One HPLC system when coupled to a variety of mass spectrometry systems. Data-independent acquisition (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 quantification 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 5µL/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 (Figure 1).

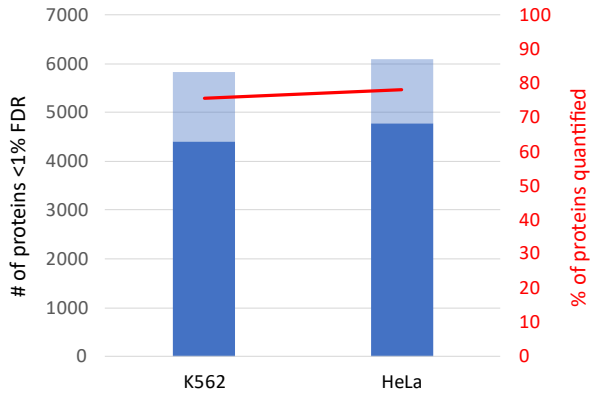

Figure 1. Numbers of protein groups identified with <1% FDR and quantified with <20% CV. These analyses were performed for 200 ng injections of K562 and HeLa human cell lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system with a 5-minute microflow gradient.

Key features of fast gradient microflow proteomics on the ZenoTOF 7600 system

- Zeno SWATH DIA on the ZenoTOF 7600 system combines the speed, sensitivity and quantitative reproducibility needed for robust detection and quantification of proteins with very fast gradients
- Using a 5-minute microflow gradient (14-minute total run time) and Zeno SWATH DIA, approximately 5000 protein groups were quantified with <20% CV from multiple human cell lysates. Approximately 3100 protein groups were quantified from yeast extracts
- These results show that with microflow chromatography and Zeno SWATH DIA, approximately 1000 protein groups can be quantified per minute of active gradient using the ZenoTOF 7600 system

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 0.5mm, P/N 05N-4252-AF) and analytical (Kinetex XB-C18 150 x 0.3mm, 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 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. 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[2]. 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.

Detection and quantification 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 100SPD 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 6000 protein groups could be detected at 1% FDR and nearly 5000 protein groups quantified using Zeno SWATH DIA with a 5-minute gradient, which translated to approximately 1000 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 2).

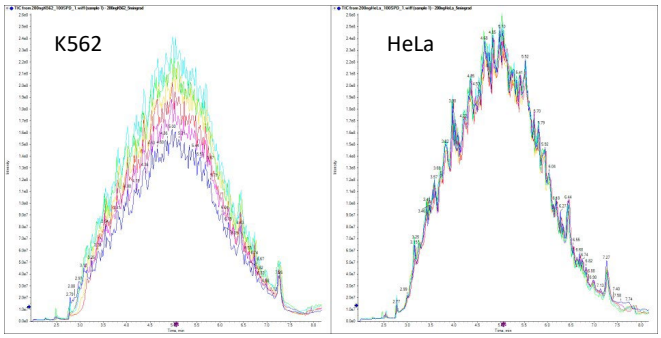

Figure 2. Total ion chromatogram (TIC) overlays for replicates of 200 ng injections of K562 and HeLa human cell lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system using a 5-minute microflow gradient.

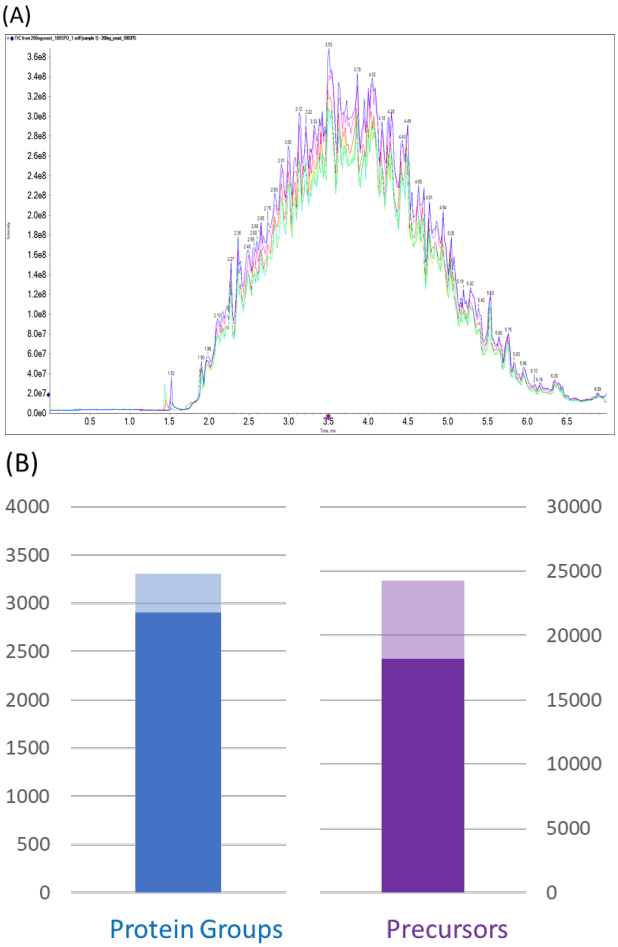

Figure 3. Analysis of yeast extract using a 5-minute microflow gradient. (A) Total ion chromatogram (TIC) overlays for 6 replicates of 200 ng injections of yeast lysate tryptic digests using Zeno SWATH DIA on the ZenoTOF 7600 system. (B) Numbers of protein groups and precursors identified (<1% FDR, light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

These analyses were also successfully applied to a yeast proteome sample. Using a 5-minute microflow gradient method, approximately 3000 protein groups and 18,000 precursors could be quantified with <20% CV (Figure 3).

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

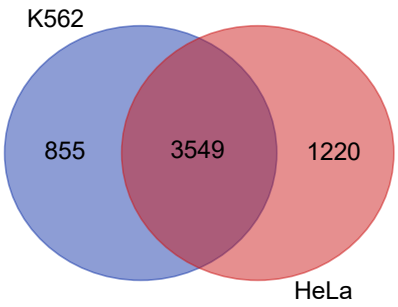

Figure 4. Comparison of protein groups quantified between cell types. Venn diagram compares the number of protein groups quantified (at <1% FDR and <20% CV) across the two different human cell lines analyzed using Zeno SWATH DIA with 5-minute microflow gradients on the ZenoTOF 7600 system. The overlap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

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 analysis 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).

Conclusions

The ZenoTOF 7600 system possesses a unique combination of high acquisition speed, sensitivity and quantitative precision for protein characterization and quantification. The power of a Zeno SWATH DIA method was demonstrated by employing fast gradient separation for the analysis of multiple complex cell lysate digests. This method enabled the high-throughput analysis of complex samples using a wide range of front-end separation schemes.

- 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 using other devices, such as the Waters M-Class LC system

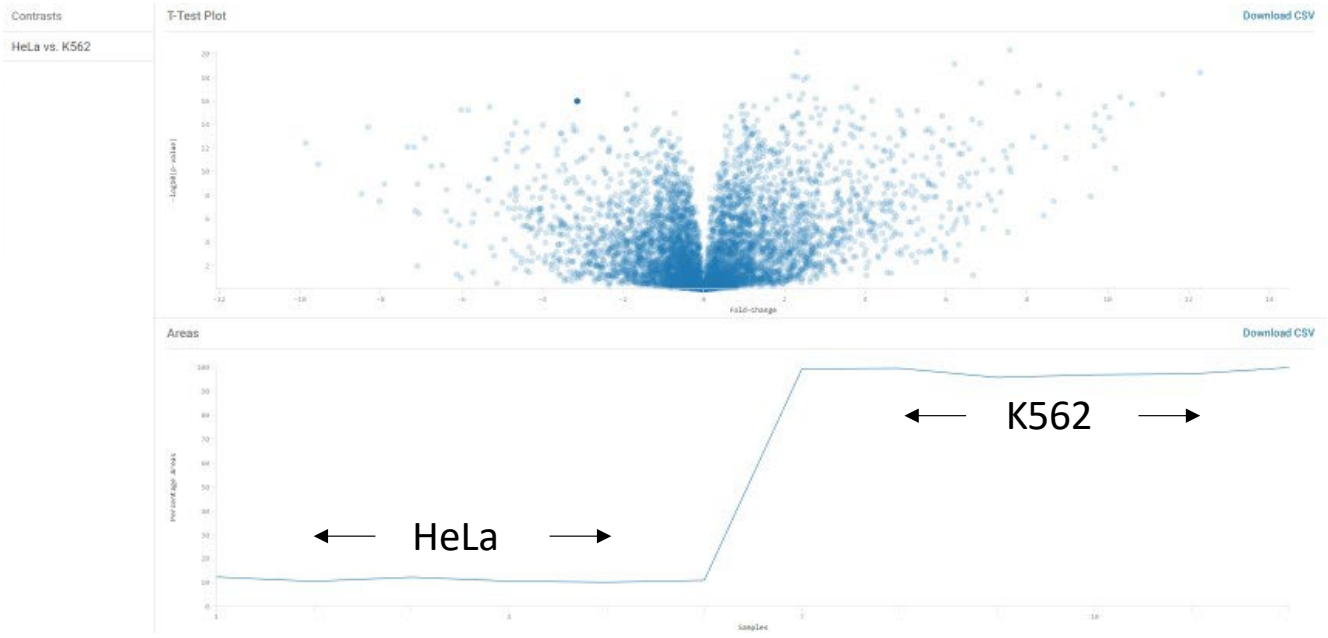

Figure 5. Protein profiling between different cell lines. DIA-NN software results were imported into the SCIEX OneOmics software suite, data were normalized and protein profiles generated using the Markerview application in Bioreviews. This profile shows the differential levels of ubiquitin-conjugating enzyme E2 L3 (UB2L3_HUMAN) across the human K562 and HeLa lysate samples. Samples were analyzed using a 5-minute microflow 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.

References

1. High-throughput quantitative proteomics using Zeno SWATH data-independent analysis (DIA) and the Evosep One system. SCIEX technical note, RUO-MKT-02-14799-A (https://sciex.com/tech-notes/life-science-research/proteomics/-high-throughput-quantitative-proteomics-using-zeno-swath-dia-an).

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3. Integrating DIA-NN software analysis of SWATH DIA data into a cloud processing pipeline. SCIEX technical note, RUO-MKT-02-15125-A (https://sciex.com/tech-notes/life-science-research/multi-omics/integrating-dia-nn-software-analysis-of-swath-dia-data-into-a-cl).

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