Zeno SWATH data-independent acquisition (DIA) applied to high-throughput proteomics analysis of human plasma

Plasma proteomics using Zeno SWATH DIA on the ZenoTOF 7600 system

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This technical note describes the analysis of protein digests from nanoparticle-enriched human plasma on the ZenoTOF 7600 system with Zeno SWATH DIA. More than 3,200 protein groups were identified in these samples using 30-minute microflow gradients (Figure 1). With this Zeno SWATH DIA approach, at least 1,500 more proteins were detected in human plasma than were previously reported for data published in the Human Plasma Proteome Project (HPPP) database.[1]

Blood proteome characterization is important for proteome research and biomarker discovery. Due to their wide dynamic range and high complexity, blood samples pose significant challenges to proteomics analysis. To meet these challenges, scientists in this area of research require high-throughput assays with high sensitivity and quantitative robustness that can be used to analyze large sample cohorts.

Zeno SWATH DIA workflows combine the use of the Zeno trap and SWATH DIA. The Zeno trap improves MS/MS sensitivity by increasing the duty cycle to >90% and can also improve MS/MS spectral quality and reproducibility.[2] Zeno SWATH DIA works best when using variable-width Q1 isolation windows. This strategy ensures that the ion intensity distribution in each isolation window is minimized according to the overall ion distribution and therefore acquires all precursor and product ion fragment information within the scanning range. DIA workflows can achieve high protein identification levels and accurate protein quantitative results with fast acquisition rates.[3] This approach allows for new improvements to the high-throughput analysis and in-depth coverage of the plasma proteome.

This work demonstrates the use of Zeno SWATH DIA to analyze trypsin-digested human-derived plasma protein samples with nanoparticle enrichment. This approach was combined with data processing in DIA-NN software to establish a fast, high-throughput analysis workflow and enable deep coverage analysis of the plasma proteome.

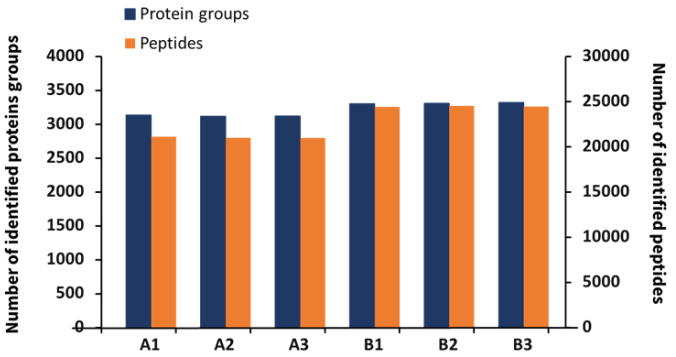

Figure 1. Histogram of identified protein groups and peptides in each human plasma sample. Two groups of human plasma samples were analyzed in triplicate using Zeno SWATH DIA with 30-minute microflow gradients. Data were processed with DIA-NN software using a spectral library approach. The numbers of protein groups and precursors detected are shown.

Key features of plasma proteomics using Zeno SWATH DIA

- The use of the Zeno trap significantly improves MS/MS sensitivity by increasing the duty cycle to >90%, thereby improving MS/MS spectral quality and enhancing qualitative and quantitative reproducibility
- Zeno SWATH DIA with microflow LC provides the sensitivity and reproducibility needed for high-throughput proteomics analysis at high acquisition speeds
- More than 3,200 protein groups were identified in nanoparticle-enriched human plasma digests using Zeno SWATH DIA
- More than 1,500 novel proteins were detected in plasma digests using Zeno SWATH DIA, compared to the HPPP database

Methods

Sample preparation: Two groups of nanoparticle-enriched trypsin-digested human plasma-derived protein samples were provided by ProteinT (Tianjin Biotech Co. Ltd). The final on-column injection amount was 400 ng.

Chromatography: Liquid chromatography separation was performed with a Waters ACQUITY UPLC M-Class system with a Phenomenex Kinetex XB-C18 (2.6 µm, 100 Å, 0.3 mm x 150 mm) analytical column (P/N 00F-4496-AC) and Phenomenex Micro Trap C18 (100Å, 5 µm, 0.3 mm x 10 mm) trapping column (P/N 05N-4252-AC). Mobile phase A was 98% water with 2% acetonitrile and 0.1% formic acid and mobile phase B was 98% acetonitrile with 2% water and 0.1% formic acid. The LC gradient used is described in Table 1. The column was kept at 40°C for all injections.

Table 1. LC gradient profile.

Time (min)	Flow rate (µL/min)	%A	%B
0	*5*	*95*	*5*
30	*5*	*55*	*45*
34	*5*	*20*	*80*
37	*5*	*20*	*80*
39	*5*	*95*	*5*

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Mass spectrometry: Analysis was performed using the SCIEX ZenoTOF 7600 system. The ion source parameters used included GS1 = 20 psi, GS2 = 60 psi, CUR = 35 psi, ISV = 5000 V, TEM = 200°C and DP = 80. The Zeno SWATH DIA acquisition method consisted of 65 variable-width windows. TOF MS scans were done using a mass range of 350-1500 m/z with an accumulation time of 100 ms. Zeno SWATH DIA MS/MS was performed over the mass range of 200-1500 m/z with accumulation times of 13 ms.

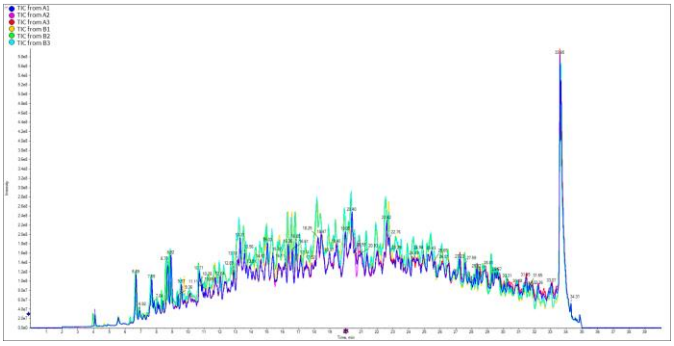

Figure 2. Overlays of the total ion chromatogram (TIC) traces for triplicate injections of both sample groups using Zeno SWATH DIA.

Data processing: Zeno SWATH DIA data was processed using DIA-NN software version 1.8.1.[4] The peptide and protein FDR was set to 1%, and searches were performed against an ion library generated previously with high-pH fractionation of plasma analyzed by data-dependent acquisition (DDA) on the ZenoTOF 7600 system.[5]

Zeno SWATH DIA data acquisition

Two groups of plasma samples were analyzed using Zeno SWATH DIA in triplicate. The reproducibility of the separation and signal intensity is shown by the overlays of the total ion chromatograms for all runs in Figure 2.

Zeno SWATH DIA data analysis

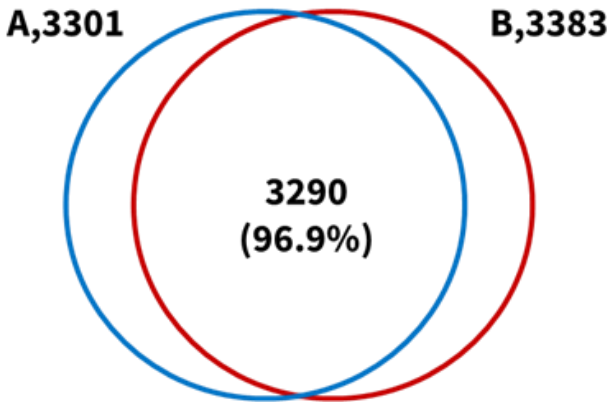

Figure 3. Venn diagram analysis shows the protein identification overlap between the 2 sample groups.

DIA-NN software was used to process the data collected by Zeno SWATH DIA. More than 3,200 protein groups were identified in the 2 groups of samples and more than 21,000 peptides were identified (Figure 1). Venn diagram analysis showed that 3,290 proteins were jointly identified in the 2 groups of samples, and the protein intersection ratio reached 96.9% (Figure 3). Heat map analysis indicated that the repeated acquisition of the same sample had a correlation >0.99 (Figure 4), due to the high reproducibility of Zeno SWATH DIA acquisition. Compared with the traditional DDA methods, Zeno SWATH DIA technology a higher coverage depth of the plasma proteome and better quantitative reproducibility (data not shown).

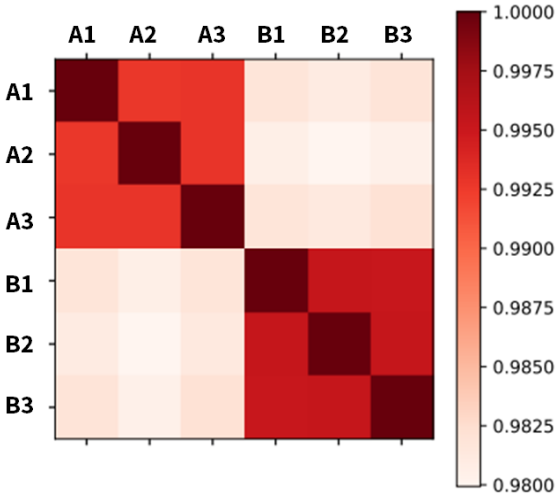

Figure 4. Correlation analysis heatmap. The relationship between protein groups identified among the replicate injections of the 2 plasma sample groups is represented.

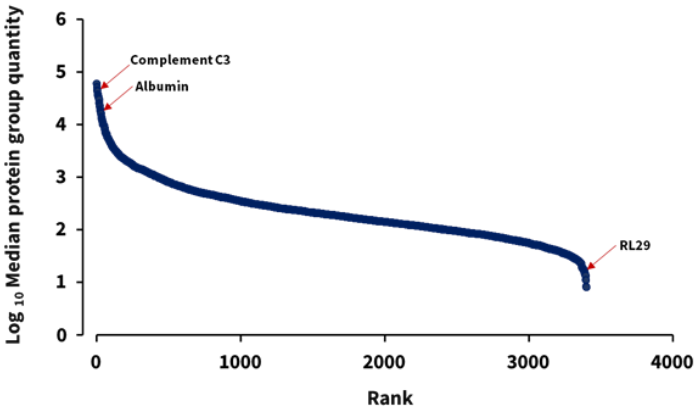

Figure 6. Abundance distribution of identified blood protein levels. The dynamic range of protein identification using Zeno SWATH DIA is highlighted by the detection of both high-abundance proteins, such as complement C3 and albumin, and proteins present at low levels, such as 60S ribosomal protein L29 [RL29], present at 0.2 ng/mL in plasma.

Comparison with the Human Plasma Proteome Project (HPPP) dataset

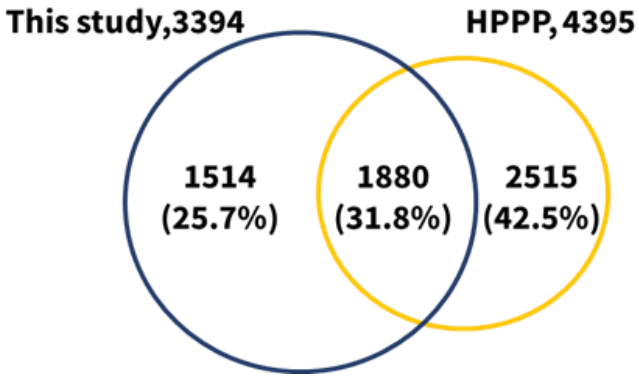

Figure 5. Venn diagram comparison of the protein groups identified in this dataset and those cataloged in the HPPP database.

The protein groups identified in the 2 groups of samples were compared with the HPPP database.[1] A subset of 1,880 proteins were matched to the HPPP database (Figure 5). These proteins included common medium- and high-abundance proteins in blood, such as serum albumin, alpha-2-macroglobulin, complement C3, apolipoprotein B and complement factor H. Low-abundance proteins with a protein concentration ≤10 ng/mL (Figure 6) were also detected, such as protein phosphokinase B1 (PPM1B, 0.1 ng/mL), secreted modular calcium-binding protein 1 (SMOC1, 0.1 ng/mL) and 60S ribosomal protein L29 (RL29, 0.2 ng/mL). Additionally, very low-level proteins, such as E3 ubiquitin ligase RNF213 (RN213, 3.5 pg/mL) were detected. Using Zeno SWATH DIA, more than 1,500 novel proteins were identified in these data that were not present in the HPPP database. Biomarker candidate screening, disease molecular mechanism elucidation, drug target discovery, clinical biotransformation studies and other research applications can benefit from the valuable information derived using this approach.

Conclusions

- The use of the Zeno trap improves MS/MS sensitivity by increasing the duty cycle to >90%, thereby improving MS/MS spectral quality and obtaining better reproducibility
- More than 3,200 protein groups were identified in nanoparticle-enriched human plasma samples with excellent quantitative reproducibility by integrating Zeno SWATH DIA, rapid 30-minute LC gradients and DIA-NN software
- More than 1,500 proteins were identified in human plasma with Zeno SWATH DIA that were not previously detected in prior studies published in the HPPP database

References

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