COMBINING PROTEOGRAPH TECHNOLOGY WITH ZENO SWATH DIA ACQUISITION ENABLES THE POTENTIAL FOR DEEP, UNBIASED DISCOVERY OF BIOMARKERS IN BLOOD

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ABSTRACT

- Recent instrumental advances have facilitated the ability to execute large-scale proteomics studies with strong quantitative reproducibility and direct impact on clinical research, drug discovery and understanding system biology.[1,2]
- Increasing depth of proteomic measurement and obtaining more complete datasets between samples remains a key challenge in the field even for easily accessible biological samples, such as plasma and serum. In this study, we utilize the newly developed SCIEX ZenoTOF 7600 mass spectrometer equipped with Zeno trap technology for deep proteome profiling of various biological matrices using Zeno SWATH DIA acquisition.[3]
- First, we present the evaluation of SWATH and Zeno SWATH DIA acquisition in K562 standard cell lysate. We observe a significant increase in both precursor and protein identifications using Zeno SWATH DIA acquisition parameters across a loading curve. At the maximum, we identify >70,000 precursors and >7,000 protein groups.
- We also observed the clear advantage of the Zeno trap technology with the observation of 5- to 10- fold increased MS2 area when compared to SWATH technology alone.
- Furthermore, we present using this instrument in tandem with the Seer Proteograph Suite to facilitate deep identifications of the plasma proteome from patient samples with >2,300 proteins being identified in at least one biological sample and >1000 being identified in 25% of all samples. We also demonstrate the complementarity of the Seer nanoparticle (NP) technology with unique proteins being identified in each NP sample.
- This study addresses the unmet need for deep, reproducible identifications from human plasma proteome utilizing advanced sample preparation and LC-MS technology.

EXPERIMENTAL METHODS

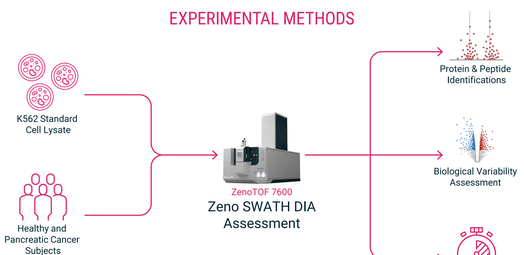


FIGURE 1

- To assess system performance and reproducibility of SWATH and Zeno SWATH DIA, different concentrations of K562 standard cell lysate were injected in triplicate with and without Zeno SWATH DIA acquisition enabled in data independent acquisition (DIA) mode.
- Reconstituted peptides were separated on a Waters ACQUITY M-class system using a 15cm ACQUITY UPLC ® HSS T3 column followed by MS analysis on a ZenoTOF 7600 system from SCIEX.
- Data were analyzed via DIA-NN against SCIEX reviewed K562 spectral library.
- Additionally, a representative subset of 55 patient samples from our internal oncology biobank, comprising of >1,750 different cancer subjects, was used to mimic a cohort study.
- Proteins from patient plasma samples were processed through the Proteograph nanoparticle-based technology and data were analyzed by Proteograph Analysis Suite (PAS) software.

REFERENCES

[1] Blume, J., et al., Nat Commun. 2020 (11) 3662

[2] Ferdosi, F. et al., PNAS 2022; 119(11) 4466

[3] Wang, Z., et al., bioRxiv. 2022 April, doi.org/10.1101/2022.04.14.488299

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RESULTS

- We found that Zeno SWATH DIA acquisition on K562 standard cell lysate led to a minimum increase of 26% in the total number of precursors and an increase of 13 – 83% of identified protein groups compared to traditional SWATH acquisition (Figure 2A and 2B).
- Use of Zeno SWATH DIA technology demonstrates slight increases in overall MS peak area and substantially increased MS/MS peak area for low abundant species leading to improved identifications in both the peptide and protein levels (Figure 3, Figure 4 and Figure 5).
- Zeno SWATH DIA has improved reproducibility to a wider degree when compared to SWATH, even when minimal load mass is introduced to the instrument. We observe a 4-13% decrease in CV(%) of precursor-level intensities with Zeno SWATH DIA when compared to SWATH acquisition (Figure 6).

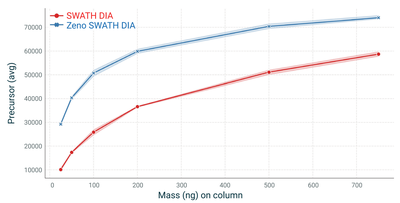


FIGURE 2A

Evaluation of K562 precursor detection with SWATH vs. Zeno SWATH DIA. Minimum increase of 26% in precursor identifications utilizing Zeno SWATH DIA. Generated from pr and pg matrix (all quantified precursors + proteins called identified) from DIA-NN output. All data searched in DIA-NN with "robust LC" and SCIEX K562 spectral library.

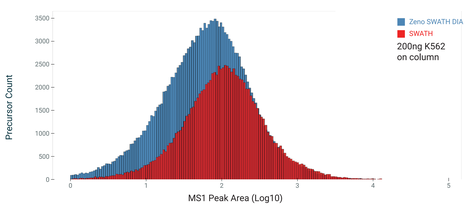


FIGURE 3

Improved sensitivity increases number of low abundant peptides species detected. Detection of low abundant peptides is improved with Zenon SWATH DIA compared to SWATH.

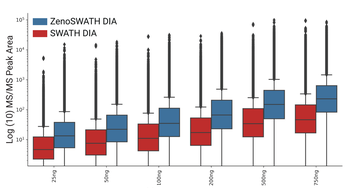


FIGURE 5

Zeno SWATH DIA acquisition results in higher K562 MS2-based precursor quantity compared to SWATH acquisition alone across different peptide injection masses. Generated from all quantified precursors. Data searched in DIA-NN with "robust LC" and SCIEX K562 spectral library.

- We combined the nanoparticle-based Proteograph processing of both subject and pooled control plasma samples with Zeno SWATH DIA acquisition and observed increased depth of protein identification. In nanoparticle-derived samples from pooled control samples, we observed a 53 – 85% increase in peptide identifications with Zeno SWATH DIA acquisition when compared to SWATH (Figure 7).
- In our interim analysis of a subset (55) of 196 control and pancreatic cohort study, we identified an average of 2,357 protein groups thar were found in at least one sample and 1,077 protein groups found in at least 25% of all subject samples (Figure 8).

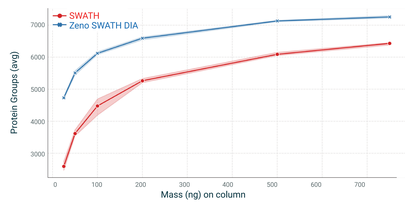


FIGURE 2B

Evaluation of K562 protein group detection with SWATH vs. Zeno SWATH DIA. Minimum increase of 13% in Protein Group identifications utilizing Zeno SWATH DIA. Generated from pr and pg matrix (all quantified precursors + proteins called identified) from DIA-NN output. All data searched in DIA-NN with "robust LC" and SCIEX K562 spectral library.

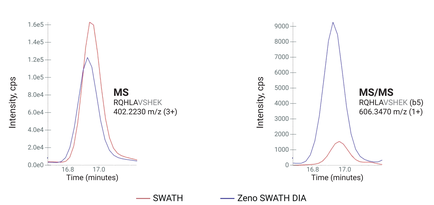


FIGURE 4

Generated from all quantified precursors. Data searched in DIA-NN with "robust LC" and SCIEX K562 spectral library.

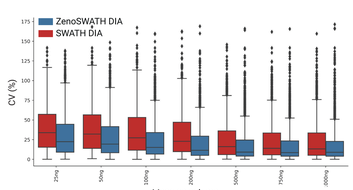


FIGURE 6

Zeno SWATH DIA acquisition results in lower CV(%) for K562 precursor-level quantities compared to SWATH acquisition alone across different peptide injection masses. Generated from all quantified precursors. Data searched in DIA-NN with "robust LC" and SCIEX K562 spectral library.

RESULTS (CONT.)

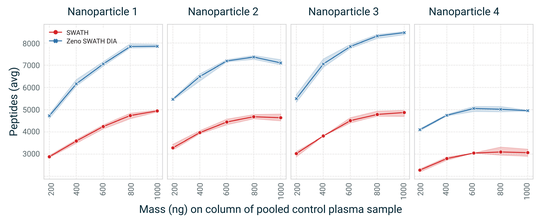


FIGURE 7

Zeno SWATH DIA MS/MS acquisition results in 53 – 85% more peptide identifications from Proteograph generated pooled control samples when compared to SWATH MS/MS DIA acquisition. SWATH vs. Zeno SWATH DIA comparison for NP5 is not displayed due to sample yield limitation for this comparative study. All data was searched in DIA-NN against a proprietary spectral library.

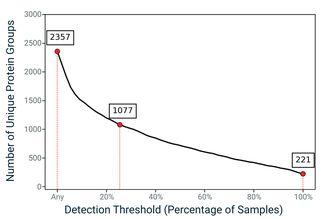


FIGURE 8

In our proteomics workflow, we detected 2,357 protein groups across all 5 nanoparticles in the representative subject cohort. The 1,077 protein groups were identified in at least 25% of the patient samples.

CONCLUSIONS

- In this study, we found that implementation of Zeno SWATH DIA acquisition on the SCIEX ZenoTOF 7600 mass spectrometer equipped with Zeno trap technology outperformed SWATH in both cell lysate and plasma matrices.
- We observed a clear advantage of Zeno SWATH DIA acquisition with an observed 5- to 10- fold increase in MS/MS area compared to SWATH approach alone.
- For subject plasma samples, we found that combining the Seer Proteograph Suite with Zeno SWATH DIA acquisition led to >2,300 protein identifications in at least one biological sample and >1,000 protein identifications in 25% of all samples.
- This study addresses the unmet need for deep, reproducible identifications from human plasma proteome utilizing advanced sample preparation and LC-MS technology.
- Furthermore, our results suggest that the Proteograph and Zeno SWATH DIA acquisition workflow facilitates the ability to identify and quantify thousands of proteins from human plasma without compromising throughput or reproducibility, creating a unique opportunity to detect robust protein biomarkers that translate into viable clinical tests for complex diseases.

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