ionopticks

APPLICATION NOTE

Utilization of the speed, sensitivity and accuracy of the ZenoTOF 7600 system to enhance protein identifications from packed emitter columns.

AUTHORS

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INTRODUCTION

The field of proteomics demands high-performance mass spectrometry tools to achieve comprehensive protein identifications, sensitive analysis, and reliable quantitation. IonOpticks' Aurora Series SX packed emitter columns have been specifically designed to optimize peptide and protein analysis on the ZenoTOF 7600 mass spectrometer, enabling researchers to unlock a new level of sensitivity. This enhanced sensitivity allows for the identification and quantification of proteins from complex samples with unprecedented accuracy, significantly expanding the depth of proteome coverage. Researchers can now confidently explore the complex proteomic landscape and uncover novel biomarkers or protein interactions that were previously challenging to identify.

Furthermore, the combination of IonOpticks' Aurora Series SX columns and the ZenoTOF 7600 mass spectrometer ensures stable quantitation across multiple samples. The Aurora Series SX columns exhibit excellent reproducibility, providing consistent results and minimizing variability in quantitative analyses. The high-sensitivity analysis facilitated by the Aurora Series SX columns on the ZenoTOF 7600 mass spectrometer enables reproducible quantitation and represents a powerful approach for comprehensive proteomics research. From 200 ng of sample, we were able to confidently identify more than 7300 proteins per run on a 45 min gradient with a majority of proteins having a CV <10%. The ability to maximize protein identifications, accurately quantify protein expression, and maintain analytical reproducibility empowers scientists to unravel complex biological processes and gain deeper insights into disease mechanisms, drug responses, and cellular signaling pathways.

7,300+
proteins identified from 200 ng of sample over 45 min.

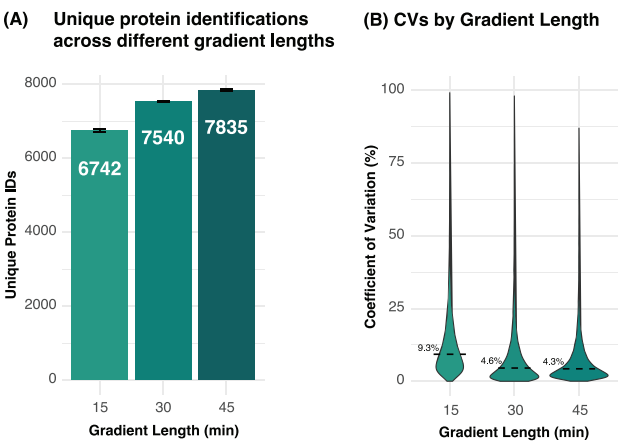


Figure 1. (A) Unique protein identifications using different gradient lengths injecting 200 ng of a K562 cell tryptic digest using the SCIEX ZenoTOF 7600 system in Zeno SWATH DIA mode using the IonOpticks Aurora Elite SX 15 cm x 75 µm column (n = 3). Analysis was performed using a library-free search in DIA-NN 1.8.1. (B) Violin plot showing unique protein CV distributions across the gradient lengths used in (A). The dashed line and associated text indicate the median CV for each group.

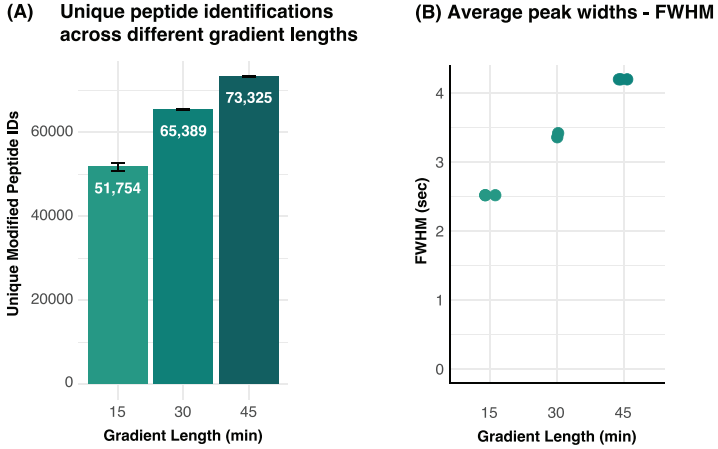


Figure 2: (A) Unique peptide identifications across different gradient lengths injecting 200 ng of a K562 cell tryptic digest using the SCIEX ZenoTOF 7600 system in Zeno SWATH DIA mode using the IonOpticks Aurora Elite SX 15 cm x 75 µm column (n = 3). Analysis was performed using a library-free search in DIA-NN 1.8.1. (B) Summary of average peptide FWHM (seconds) for each input sample. Each replicate is represented by one dot.

METHODS

LC-MS
K562 tryptic digest (Promega) was reconstituted in 2% ACN, 0.1% FA in water. The required amount of sample was injected using a Waters M-Class UPLC. Sample gradients were run at 300 nL/min over a selection of gradient lengths. The UPLC was connected to the IonOpticks Aurora Elite SX column (Figure 2, 15 cm x 75 µm). The ZenoTOF 7600 system was operated in Zeno SWATH DIA using the OptiFlow Turbo V ion source in nanoflow configuration.

A Zeno SWATH DIA method consisting of 85 variable-width windows was used with MS/MS accumulation times of 18 ms. CID fragmentation was used with dynamic collision energies and Zeno trap pulsing turned on. An ion spray voltage of 2200 V was used.

Data Processing
Zeno SWATH DIA data was processed using DIA-NN software (1.8.1) using a high-pH fractionated spectral library of HeLa and K562 cell lines (11,269 protein groups and 169,395 peptides). The pg.matrix.tsv and pr.matrix.tsv tables were used for calculating unique protein and peptide identifications.

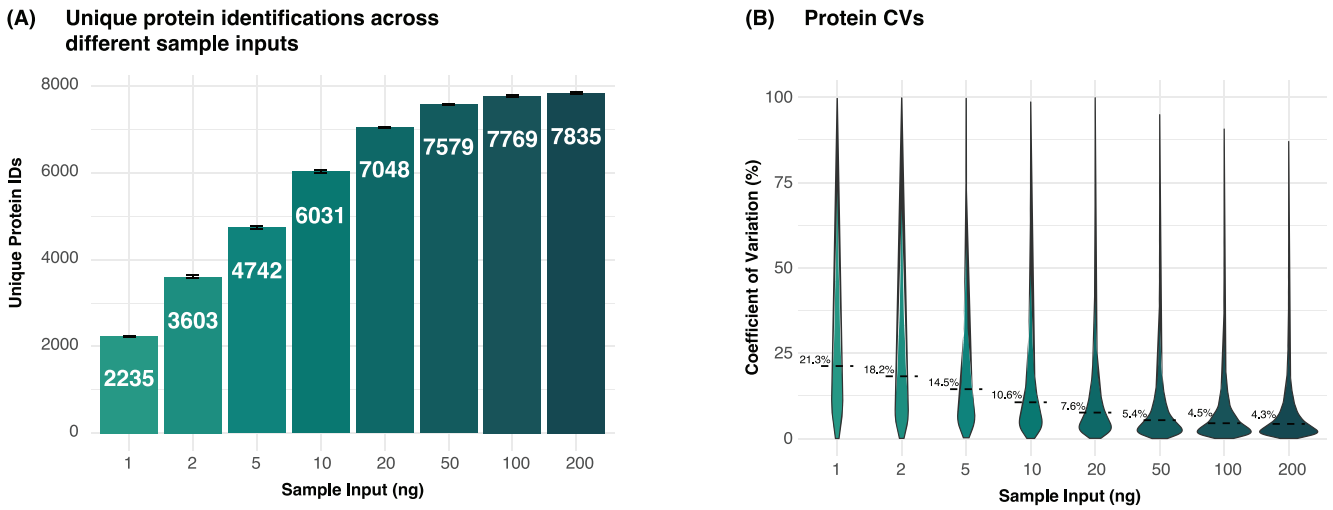


Figure 2. (A) Unique protein identifications across different sample inputs from a K562 cell tryptic digest using the SCIEX ZenoTOF 7600 system in Zeno SWATH DIA mode using the IonOpticks Aurora Elite SX 15 cm x 75 µm column on a 45 minute gradient (n = 3). Analysis was performed using a library-free search in DIA-NN 1.8.1. (B) Violin plot showing unique protein CV distributions across the sample inputs used in (A). The dashed line and associated text indicate the median CV for each group.

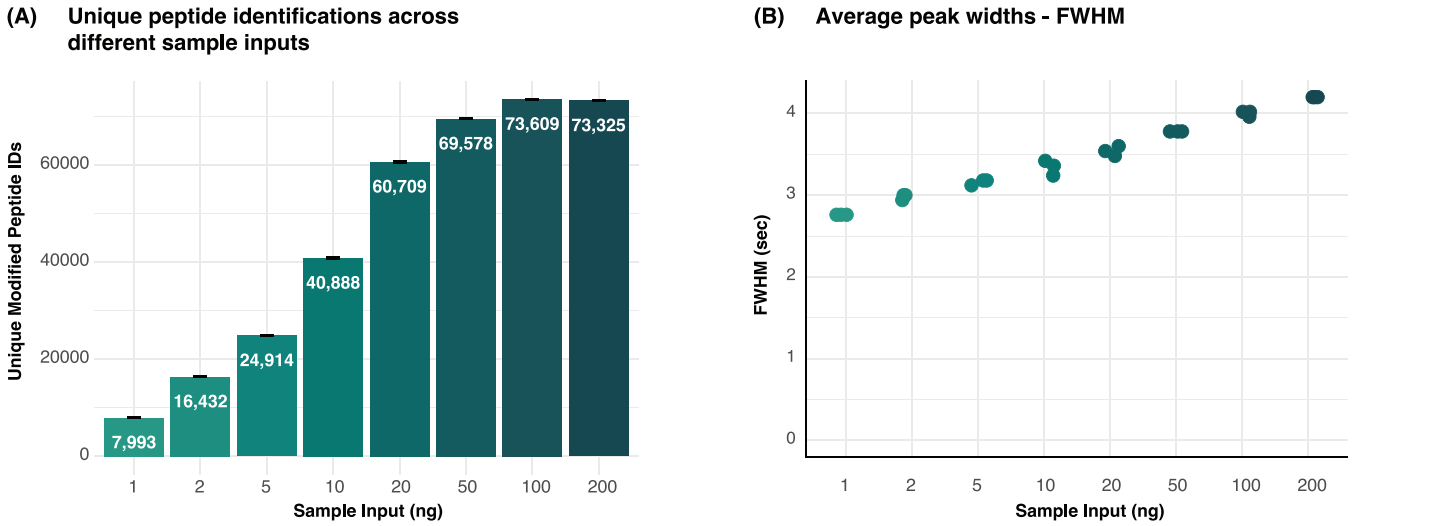
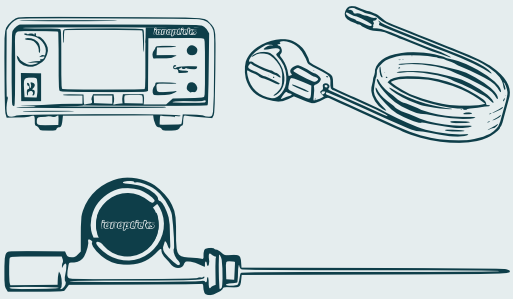
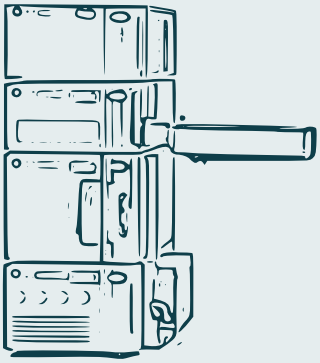


Figure 3 (Above): (A) Unique peptide identifications across different sample inputs from a K562 cell tryptic digest using the SCIEX ZenoTOF 7600 system in Zeno SWATH DIA mode using the IonOpticks Aurora Elite SX 15 cm x 75 µm column on a 45 minute gradient (n = 3). Analysis was performed using a library-free search in DIA-NN 1.8.1. (B) Summary of average peptide FWHM (seconds) for each input sample. Each replicate is represented by one dot.

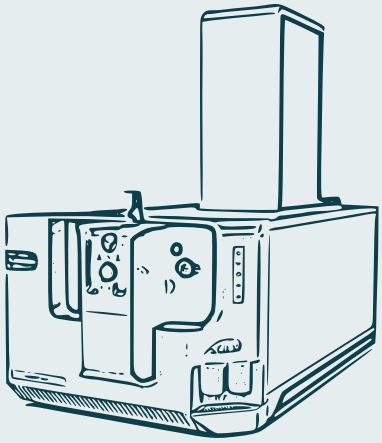
EQUIPMENT USED



Aurora Elite SX, IonOpticks Column Heater and IonOpticks Heater Controller



Waters Acquity M-Class UPLC



SCIEX ZenoTOF 7600
Mass Spectrometer

CONCLUSION

The integration of IonOpticks' Aurora Series SX packed emitter columns with the SCIEX ZenoTOF 7600 mass spectrometer offers a transformative solution for maximizing protein identifications, achieving high-sensitivity analysis, and ensuring stable quantitation in proteomics. This powerful combination enhances the capabilities of mass spectrometry and paves the way for breakthrough discoveries in the field of protein research.

The 'all-in-one' nanoflow solution for the OptiFlow Turbo V ion source.

Effortless installation.

The most user-friendly nanoflow columns on the market are now accessible to SCIEX users. With our included adapter and precision installation tool, the Aurora SX range makes converting your source to nanoflow a breeze.

nanoZero® is built-in.

Eliminate pre and post-column dead volume with our famed nanoZero fitting and integrated emitter. Aurora SX saves time and money, both of which are better spent on your research.

Seamless. Elegant.

Heating at your source couldn't be easier. Combined with our Heater Controller unit, the IonOpticks Column Heater sits atop the Aurora SX column and allows for precision temperature management directly in front of the nanospray probe.

ABOUT THE PURCELL LAB

The Purcell Laboratory, part of the Monash Biomedicine Discovery Institute at Monash University, focuses on targeted and global quantitative proteomics of complex biological samples. Their main interest lies in identifying targets of the immune response and understanding host-pathogen interactions. The lab is involved in various programs related to immunity and cancer and operates under the Department of Biochemistry and Molecular Biology.

Their research combines advanced proteomics with human immunology, molecular virology, and structural and functional immunology. This multidisciplinary approach addresses various questions in fundamental immunology, translational medicine, vaccination, and immunotherapy.

The lab is led by Professor Anthony (Tony) Purcell, who serves as the Head of the Immunoproteomics Laboratory. Professor Purcell's expertise and leadership have been pivotal in steering the lab's research towards groundbreaking discoveries in the proteomics field. His work has significantly impacted our understanding of immune responses, particularly in the context of complex biological systems.