POSTER PRESENTATION

Improved proteome coverage and reproducibility in large-scale analyses using packed emitter columns

Authors: Vincent Albrecht[1], Tim Heymann[1], Greta Briedytė[2], Johannes Bruno Müller-Reif[1].
Affliations: [1]Max Planck Institute of Biochemistry, [2]IonOpticks Pty Ltd.

INTRODUCTION

Proteomic analyses of large cohorts of human patient samples plays a critical role in discovering novel biomarkers for diagnosis and response to treatment. Deep proteome coverage and reproducibility of results is essential for the success of these studies. Here, we introduce a novel packed emitter chromatography column, the IonOpticks Aurora Rapid 8x150 (8 cm x 150 µm inner diameter, 1.7 µm C18), designed to improve the performance of large-scale plasma proteomic analyses. By analysing weekly quality control injections between plasma samples, this study evaluated the efficiency and reproducibility of the column for high-throughput proteomic analysis of large-scale plasma cohorts coupled with an Evosep One and a Thermo Fisher Orbitrap Astral mass spectrometer.

Robust performance across a large patient plasma cohort analysis

IonOpticks' Aurora Rapid 8x150 packed emitter columns feature our revolutionary nanoZero® fitting, allowing for simplified plug-and-play high-throughput proteomics. These columns are capable of analyzing from 50 to more than 100 samples per day while delivering class-leading peptide and protein identifications. The Aurora Rapid 8x150 columns were used to analyse a large patient plasma cohort on an Evosep One and Thermo Fisher Orbitrap Astral. Weekly quality control (QC) of the LC-MS performance and reproducibility was performed by injection of 200ng HeLa tryptic peptides using a 60 SPD method. Plasma samples were analysed using a 100 SPD method with approximately 700 plasma samples run between QC injections.

High-throughput analysis of peptides and proteins:

HeLa cell tryptic peptides, used for LC-MS QC, were injected and separated using a 60 samples per day method on an Evosep One. This method repeatedly identified over 9,300 proteins per run, with an average of 100,000 unique peptide identifications.

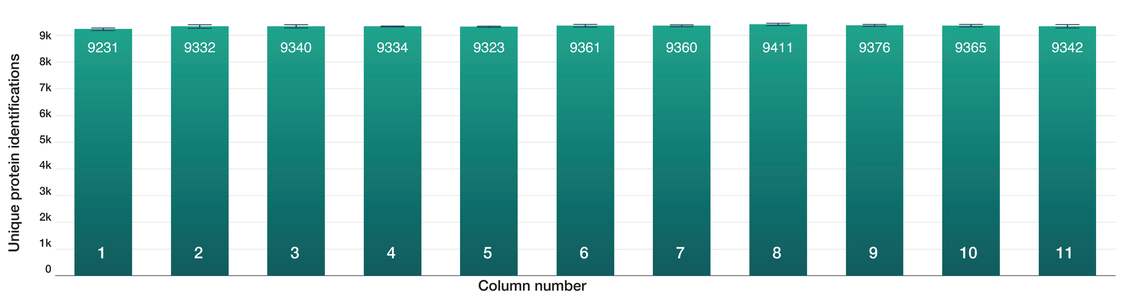

Figure 1: Protein identifications across different columns. A HeLa tryptic digest (200 ng) was separated on an Aurora Rapid 8 cm x 150 µm column on a 60SPD method using Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer.

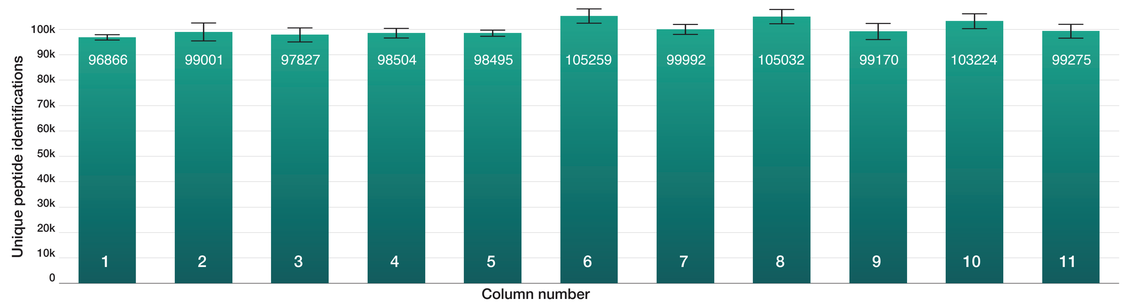

Figure 2: Unique peptide identifications across different columns. A HeLa tryptic digest (200 ng) was separated on an Aurora Rapid 8 cm x 150 µm column on a 60SPD method using Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer.

Stable retention times ensure confidence in results

The analysis of retention time reproducibility is detailed through a scatter plot and histogram, focusing on the coefficient of variation for precursor retention times. The scatter plot demonstrates a high level of consistency, with most data points clustering below a CV of 6% and a median CV at 2.52%, showing stable retention times across precursors. Additionally, a four-month experiment on 16 selected peptides showed stable average retention times, further proving the reliability and precision of IonOpticks columns.

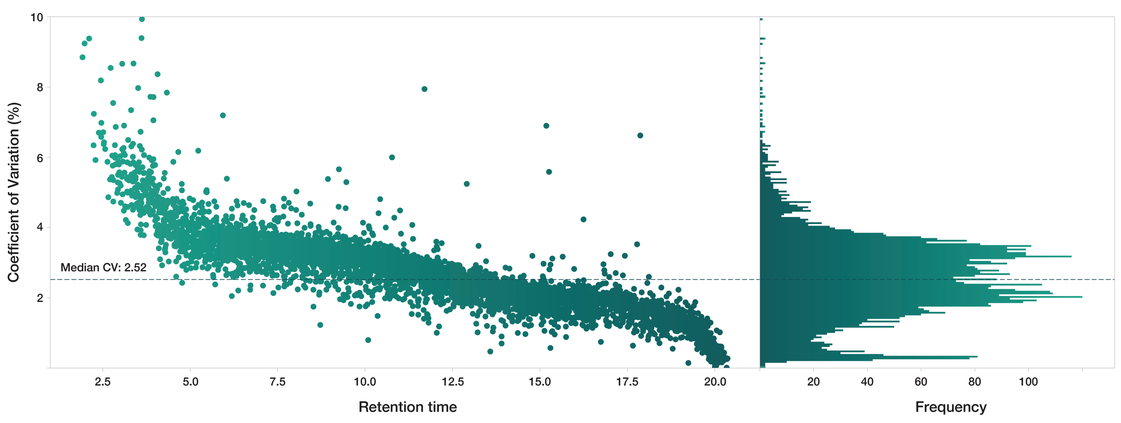

Figure 6: Scatter Plot and histogram analyzing the coefficient of variation of retention times for precursors: each dot represents an individual precursor across all of the 257 HeLa QC runs.The plot shows that most data points cluster below a CV of 6%, with the median CV marked at 2.52, indicating consistent retention times across all precursors.

High reproducibility across multiple columns

To assess the reproducibility of the protein quantification across multiple QC runs and columns, protein intensities were used to calculate a coefficient of variation (CV) and Pearson correlation matrix, revealing uniform performance with low CV values. Pearson correlation matrix visualization further supported this, showing high correlations between runs (mean Pearson correlation coefficient: 0.98).

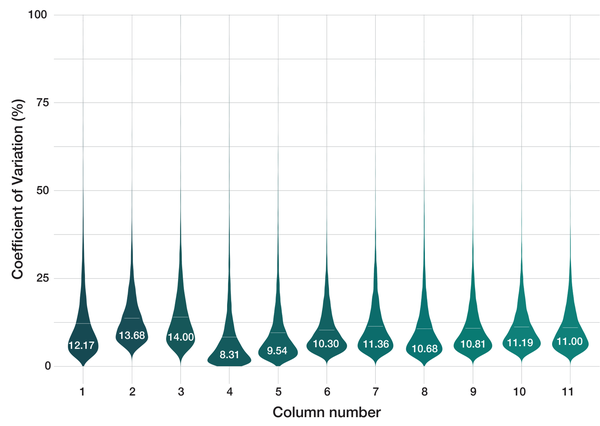

Figure 3: Low CVs across different columns: Violin plot of coefficient of variation for all identified protein intensities from HeLa tryptic digest QC injections on Aurora Rapid 8 cm x 150 µm columns. Samples were run on an Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer. Dashed line represents the median value for each column.

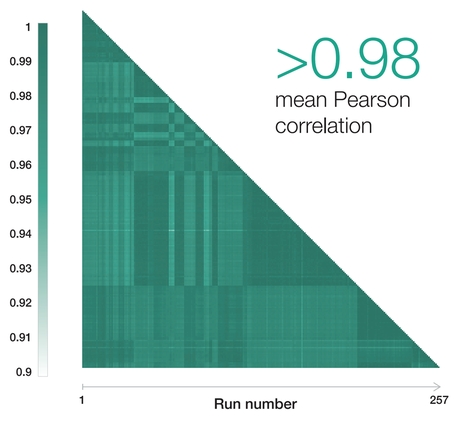

Figure 4: A Pearson correlation matrix of all quantified protein intensities from the 257 QC injections was calculated.

Narrow peak widths

The below plot displays the full width at half maximum (FWHM) values across 257 QC runs, revealing consistently narrow peak widths. This consistent performance indicates good column quality and reproducibility, important for large patient cohort analyses.

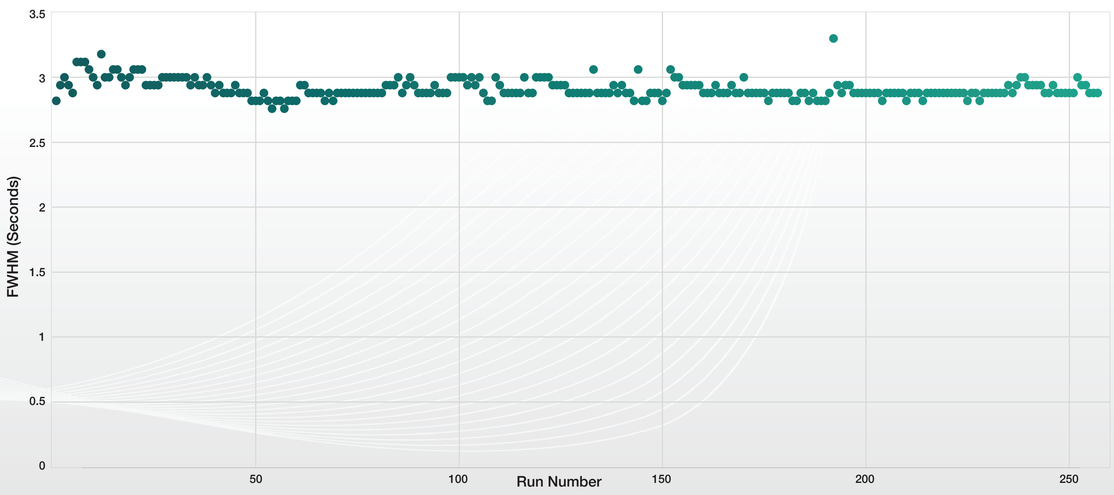

Figure 5: Peak widths across all runs: average full width at half maximum (FWHM) for all identified peptides from Hela Tryptic Digest injections on an Aurora Rapid 8 cm x 150 µm columns. Samples were run on a Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer.

METHODS

LC-MS Analysis: A HeLa tryptic digest (200 ng) was separated on Aurora Rapid 8 cm x 150 µm column on a 60SPD method using Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer.

MS parameters: mass range of MS(Orbi)/MS2(Astral) from 380 to 980, isolation windows of 2Th, a maximum injection time (IT) of 3 ms, FAIMS compensation voltage (CV) set at -40, and a resolution for MS1 set at 120,000.

Data Processing: Data was processed using DIA-NN software (v1.8.1) with match-between-runs enabled. The pg.matrix.tsv, stats.tsv and pr.matrix.tsv tables were used to calculate protein and peptide identifications and metrics.

CONCLUSION

The combination of the IonOpticks Aurora Rapid 8x150 column, the Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer enabled maximum protein and peptide identifications whilst also ensuring robust and reproducible protein quantification across a large patient plasma cohort analysis.

ACKNOWLEDGEMENTS

We thank Professor Matthias Mann and his team at the Max Planck Institute of Biochemistry, where all data was acquired and processed. Special thanks to Dr. Igor Paron for his assistance with the LC-MS setup and operation.

Scan to download the Application Note:
Achieve unmatched high-throughput sample analysis with the Aurora Rapid 8×150