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APPLICATION NOTE

Achieve high sensitivity and throughput in low-input proteomics with Aurora® Rapid™ 8x75

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FEATURED PRODUCTS:

Aurora® Rapid™ 8x75 packed emitter column for CaptiveSpray (8 cm x 75µm ID, 1.7µm C18) Part No. AUR4-8075C18A-CSI

Aurora® Rapid™ 8x75 packed emitter column for EASY-Spray and Nanospray Flex (8 cm x 75µm ID, 1.7µm C18) Part No. AUR4-8075C18A-XT

INTRODUCTION

Advances in mass spectrometry-based proteomics have enabled the identification of thousands of proteins from low sample amounts. However, achieving high proteome coverage and reproducibility from low-input samples remains a significant challenge. Here, we evaluate the performance of our LC-MS workflow across a range of sample inputs and using different SPD (samples per day) methods to assess sensitivity, throughput and robustness.

More than 3200 proteins identified from inputs as low as 125 pg.

The number of unique protein groups identified from HeLa digest samples ranging from 125 pg to 10 ng. Even at the lowest input (125 pg), over 3,200 unique protein groups were consistently identified, increasing to more than 6,300 at 10 ng. This highlights the exceptional sensitivity of the workflow, enabling deep proteome coverage.

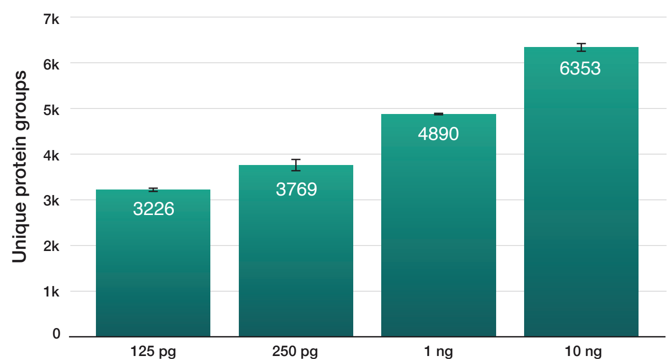

Figure 1: Unique protein group identifications across different sample loads. A HeLa tryptic digest was separated on an Aurora Rapid 8 cm × 75 µm column using an 80 SPD method on a Vanquish Neo system coupled to a Bruker timsTOF Ultra 2 mass spectrometer. Raw data were analyzed using Spectronaut version 19.8, with the "Match between runs" feature disabled.

High reproducibility across multiple columns.

Figure 2 and Figure 3 show the reproducibility of unique protein group and unique peptide identifications across four columns. Unique protein group identifications remained highly consistent, with an average of 3,934 identified across all columns. Peptide identifications followed a similar trend, with an average of 25,308 unique peptides - demonstrating robust performance.

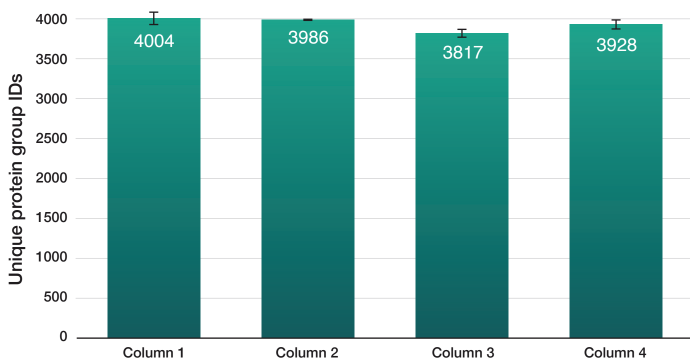

Figure 2: Unique protein group identifications across different columns. A HeLa tryptic digest (250 pg) was separated on an Aurora Rapid 8 cm × 75 µm column using an 80 SPD method on a Vanquish Neo system coupled to a Bruker timsTOF Ultra 2 mass spectrometer. Three runs were performed per column. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

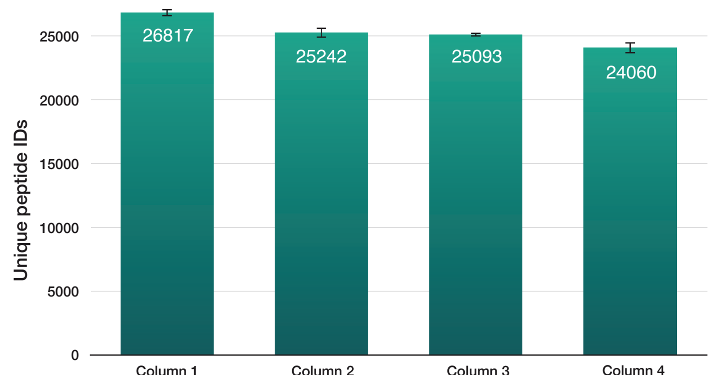

Figure 3: Unique peptide identifications across different columns. A HeLa tryptic digest (250 pg) was separated on an Aurora Rapid 8 cm × 75 µm column using an 80 SPD method on a Vanquish Neo system coupled to a Bruker timsTOF Ultra 2 mass spectrometer. Three runs were performed per column. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

CVs of protein intensities

Figure 4 presents violin plots of the coefficient of variation (CV) for protein quantification at each sample input. Median CVs ranges from 12.9% to 15.8%, indicating excellent quantitative precision across the entire sample input range.

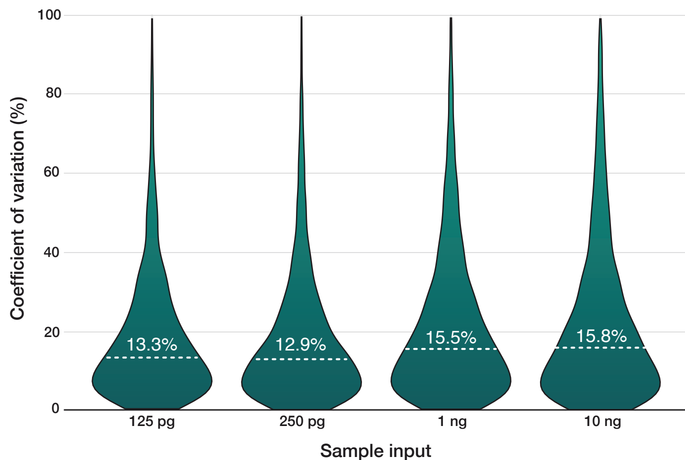

Figure 4: Low CVs across different sample loads. Violin plot of the coefficient of variation for all identified protein intensities from HeLa tryptic digest QC injections on Aurora Rapid 8 cm × 75 µm columns, using an 80 SPD method. For each sample input, three runs were performed. Samples were analyzed on a Vanquish Neo and Bruker timsTOF Ultra 2 mass spectrometer. The dashed line represents the median value for each column. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

Narrow peak widths

The plot shows the average full width at half maximum (FWHM) values at different SPD methods, demonstrating consistently narrow peak widths across all conditions. This stable performance highlights the column's high reproducibility, which is essential for reliable high-throughput proteomics.

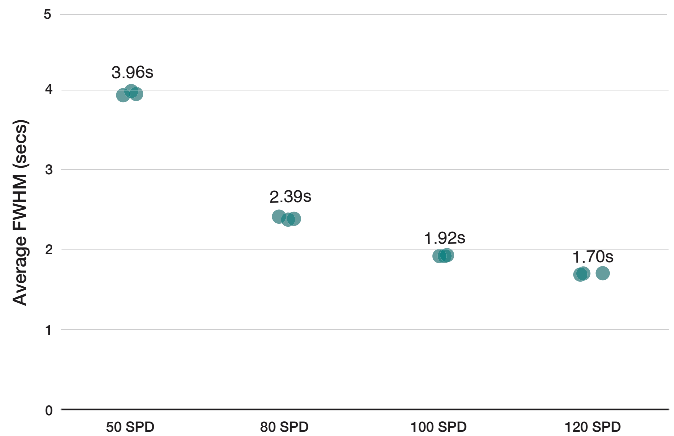

Figure 5: Peak widths across different methods: average full width at half maximum (FWHM) for all identified peptides from HeLa tryptic digest (250 pg) QC injections on Aurora Rapid 8 cm × 75 µm columns. Samples were run on a Vanquish Neo and Bruker timsTOF Ultra 2 mass spectrometer. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

Incredibly stable retention times

Figure 6 illustrates the retention time stability across multiple columns and injections. Retention times were highly consistent, with minimal drift observed - this level of chromatographic reproducibility is critical for large-scale and longitudinal studies.

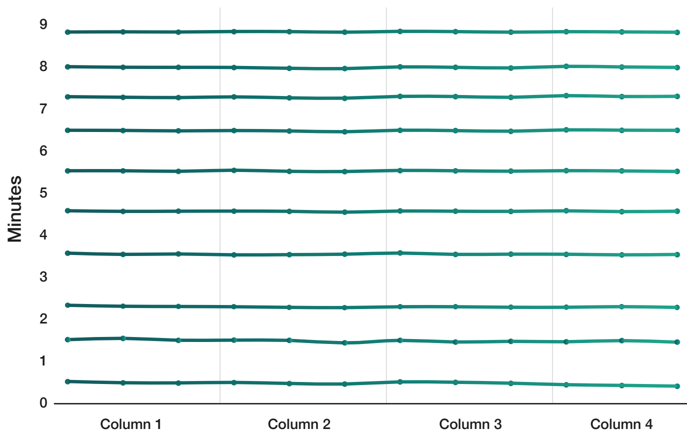

Figure 6: Stable peptide retention times across different columns. Ten peptides were selected and their retention times assessed across all columns from 3 HeLa tryptic digest injections (250 pg) on Aurora Rapid 8 cm × 75 µm columns, using an 80 SPD method. Samples were run on a Vanquish Neo and Bruker timsTOF Ultra 2 mass spectrometer. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

Column backpressure stability and protein group IDs over extended use

Figure 7 shows backpressure at 8 minutes over more than 650 QC runs using 60 SPD method, with only a gradual increase from 130 to 175 bar and consistently high unique protein group IDs. Complex samples were analysed between QC runs, highlighting column's robustness and suitability for high-throughput proteomics.

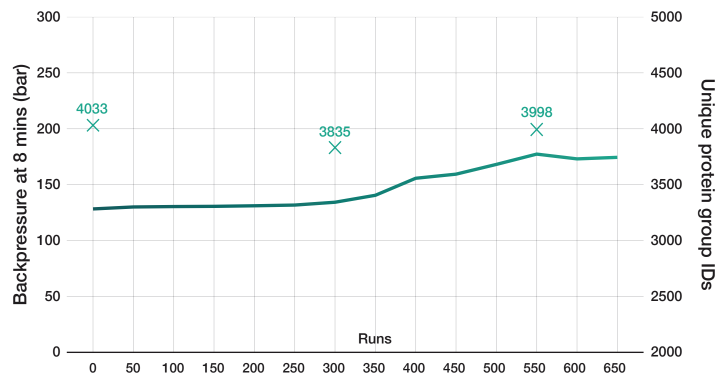

Figure 7: Backpressure stability and protein group IDs over extended column use from HeLa tryptic digest QC injections (250 pg) on Aurora Rapid 8 cm × 75 µm columns, using a 60 SPD method. Samples were run on a Vanquish Neo and Bruker timsTOF Ultra 2 mass spectrometer. Raw data were analyzed in Spectronaut version 19.8 with the "Match between runs" feature disabled.

METHODS

LC-MS Analysis

Different sample inputs of HeLa tryptic digest were separated on an Aurora Rapid 8 cm × 75 µm column using various SPD (samples per day) methods. Chromatographic separation was performed on a Thermo Fisher Vanquish Neo system coupled to a Bruker timsTOF Ultra 2 mass spectrometer.

Data processing

Raw data were analyzed with Spectronaut version 19.8, with the "Match between runs" feature disabled.

CONCLUSION

Together, these results demonstrate that the Aurora Rapid 8 cm × 75 µm columns, operated at different SPD methods, deliver robust and reproducible performance for low-input proteomics. Across hundreds of runs, the columns maintained stable backpressure, narrow peak widths and high protein and peptide identifications - confirming column suitability for large-scale, high-throughput proteomic applications.

FURTHER READING

For futher resources and technical support, visit our Help Centre at **helpcentre.ionopticks.com**. To view other application notes, read the latest publications featuring Aurora Series columns, or view the full range of IonOpticks products, visit our website at **www.ionopticks.com**