Application Note

High-Sensitivity and Reproducible DIA Performance of a 15 cm × 75 µm Emitter-Integrated Nanoflow Column on the Orbitrap Astral System

Introduction

High-throughput proteomics increasingly relies on short-gradient, data-independent acquisition (DIA) workflows that demand both chromatographic efficiency and exceptional run-to-run reproducibility. While extended column lengths are often favored for maximal depth, mid-length nanoflow columns must balance separation power, robustness, and speed to support large cohort studies and routine quantitative analyses.

Here, we evaluated the analytical performance of the ProtumLink 15 cm × 75 µm emitter-integrated nanoflow column packed with 1.9 µm C_{18} material, using a HeLa tryptic digest dilution series acquired on a Thermo Scientific™ Orbitrap Astral™ system coupled to a Vanquish™ Neo UHPLC. The study focuses on sensitivity, quantitative reproducibility, and retention time stability under a rapid 24-minute DIA workflow.

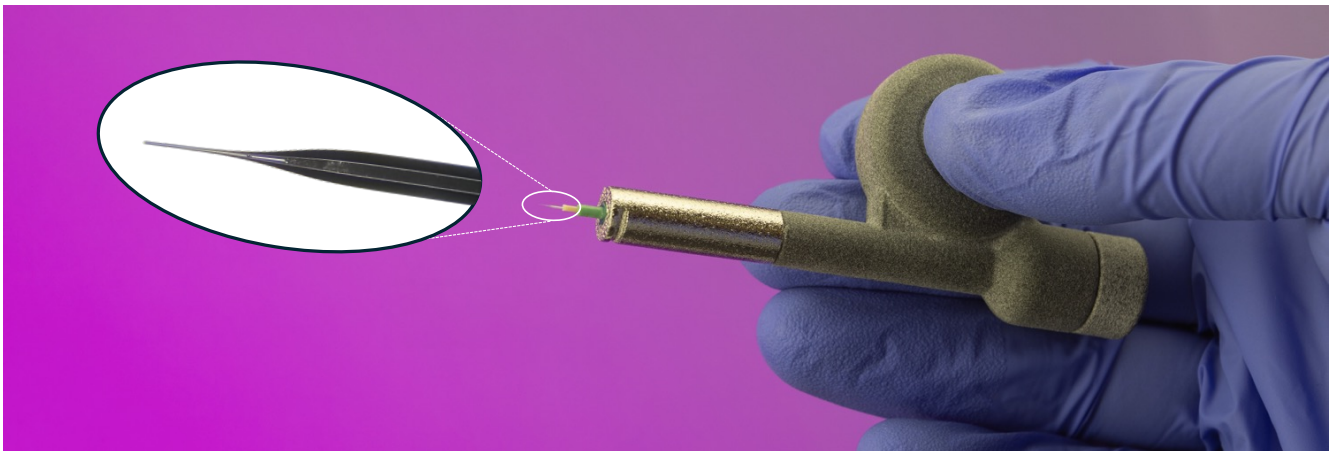

Figure 1: ProtumLink Extend column Cassette for plug-and-play usability at the EasySpray™ and NanoFlex™ source platform. With a highlight focus on the homogeneous emitter, which provides superior flow sensitivity and excellent robustness.

Analytical Performance and Depth of Coverage

To evaluate the chromatographic performance of the ProtumLink emitter-integrated column, a HeLa tryptic digest dilution series was analyzed on a Thermo Scientific™ Orbitrap Astral™ system coupled to a Vanquish™ Neo UHPLC, in collaboration with AG Warscheid (Chair of Biochemistry II), Julius-Maximilians-Universität Würzburg. The column used was a **15 cm × 75 µm capillary packed with 1.9 µm C18 material (120 Å pore size)**, featuring ProtumLink's integrated emitter and adapter cassette compatible with the EasySpray™ and NanoFlex™ source.

Peptide separation was achieved using a **24-minute active gradient** (6–22–30–45% Buffer B) at 300 nL/min, followed by mass spectrometric acquisition in narrow-window DIA mode. Each sample was

analyzed in technical triplicate (n = 3), covering a concentration range from 0.1 ng to 100 ng of total peptide input.

Data processing was performed with DIA-NN v2.1.0 using a predicted human library and 1% precursor-level FDR. Protein groups were inferred from the pg.matrix.tsv output, and precursor-level identifications from the report.parquet file. No match-between-runs (MBR) was applied.

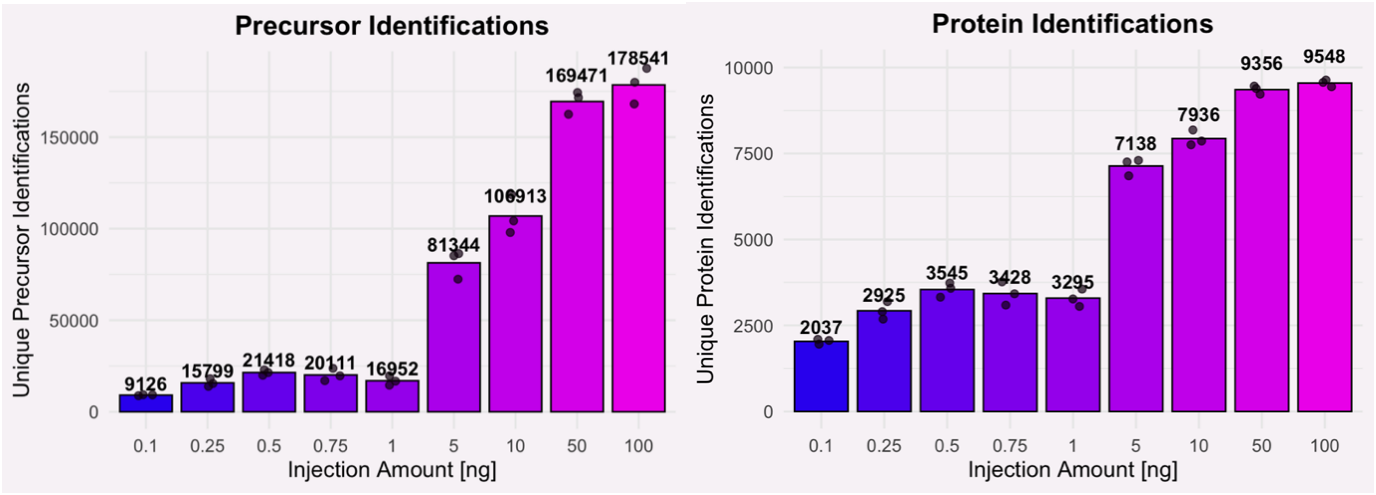

Figure 2: Precursor and protein group identifications across the dilution series. Bar plots show mean values from triplicate analyses (100 pg to 100 ng peptide input); individual replicate values are indicated as black dots.

The column exhibited **exceptional depth of coverage**: at 10 ng input, over **100,000 precursors** and **7,900 protein groups** were identified, increasing to **170,000 precursors** and **9,500 proteins** at 100 ng input. This demonstrates both high sensitivity and excellent scalability across a broad dynamic range.

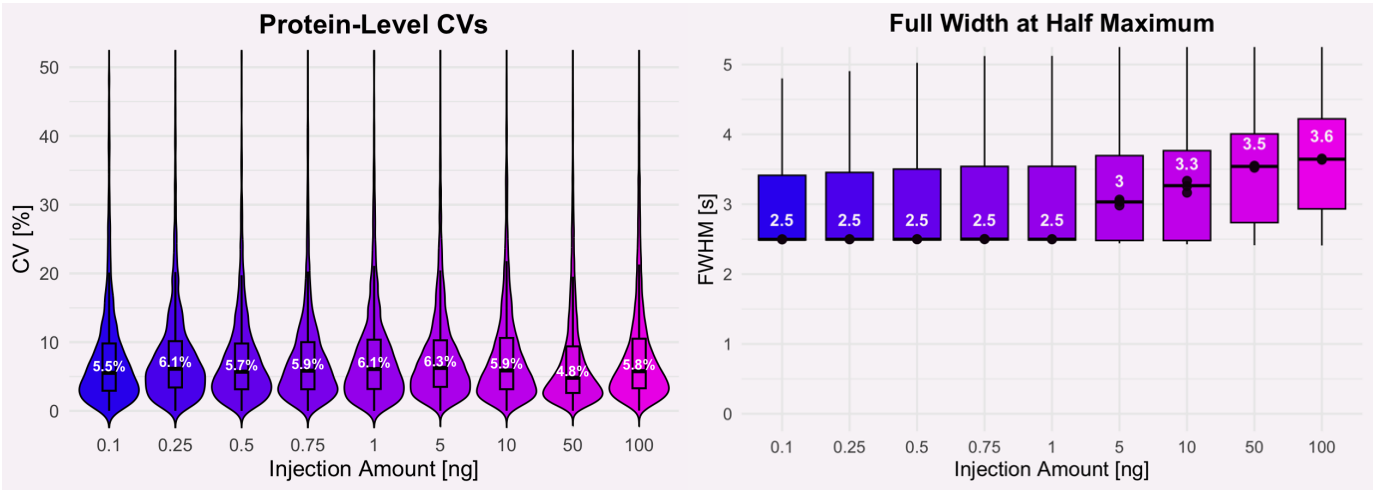

Figure 3: Reproducibility and peak shape stability. Median protein group CVs remain ~6%, with median FWHM ≤3.6 seconds across all conditions on a 30-minute gradient.

Across the entire dilution series, the system showed **remarkable reproducibility**, with protein-level coefficients of variation (CVs) of **4.8% to 6.3%** (calculated from raw intensity values in the protein pivot table). In addition, the chromatographic performance was characterized by **sharp peak profiles**, with full width at half maximum (FWHM) values ranging between **2.5 and 3.6 seconds**.

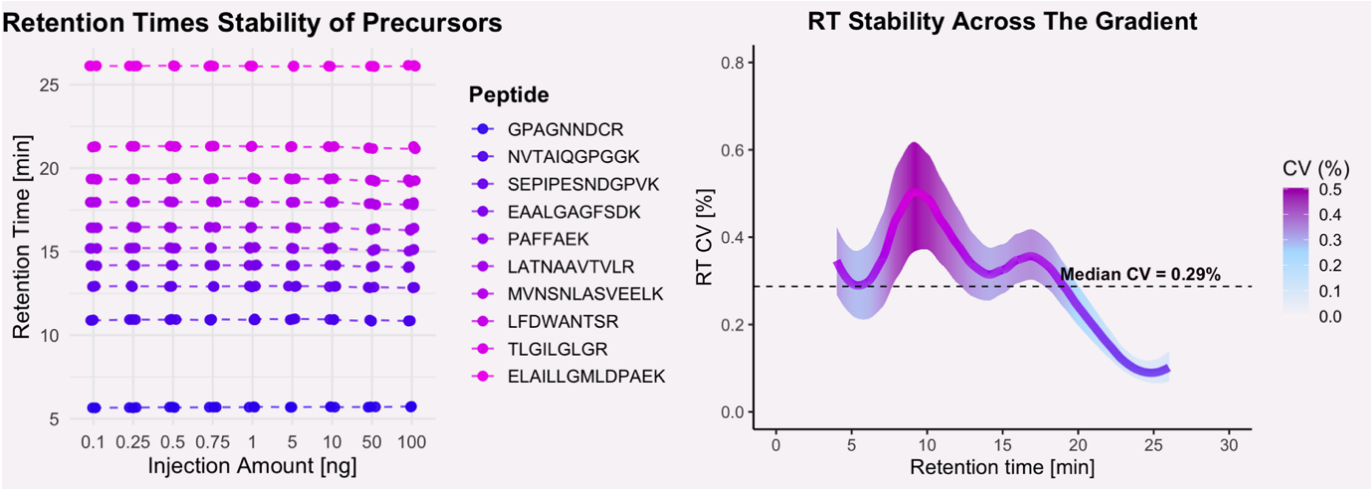

Figure 4: Retention time stability across the dilution series. Scatter plot illustrates consistent retention times of selected peptides across replicates and concentrations. Overall retention time variation for all detected precursors across 27 runs shows a CV of ~0.3%.

Retention time analysis of selected peptides across the gradient further confirmed **exceptional retention time stability**, reinforcing the column's suitability for high-throughput and comparative proteomics.

Together, these data highlight the ProtumLink column's ability to deliver **robust, high-resolution, and reproducible performance**, making it a strong candidate for nanoflow LC-MS workflows where consistency and depth of coverage are critical.

Applied Method

Sample Preparation

HeLa tryptic digest was prepared and diluted to the indicated concentrations in 2% acetonitrile (ACN) with 0.1% formic acid (FA).

Liquid Chromatography

Peptide separation was performed using a Vanquish™ Neo UHPLC system equipped with a ProtumLink 15 cm × 75 µm emitter-integrated column packed with 1.9 µm C_{18} particles (120 Å pore size).
A 24-minute active gradient was applied at a constant flow rate of 300 nL/min.

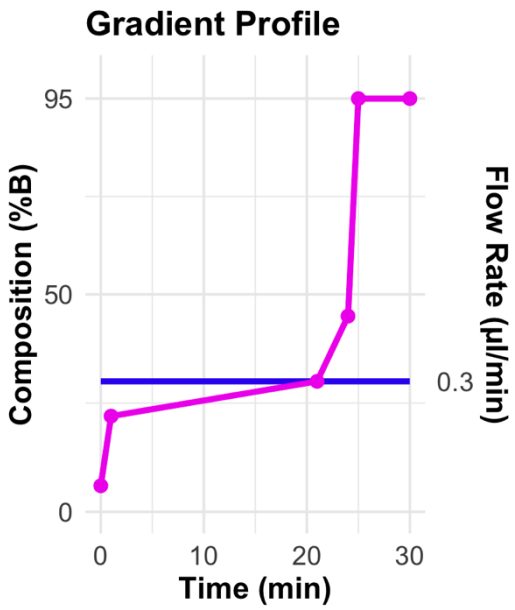

Figure 5: Applied gradient composition and flow rate, illustrating the chromatographic conditions used for peptide separation

Mobile Phase Composition:

- **Buffer A:** 99.9% water with 0.1% FA
- **Buffer B:** 99.9% ACN with 0.1% FA

Mass Spectrometry

Data were acquired on a Thermo Scientific™ Orbitrap Astral™ system in narrow-window DIA mode(MS1 240k, AGC 500%, 2299scans 2m/z, AGC 500%, IT3ms), equipped with a NanoFlex™ Source.

Data Analysis

Raw data were processed using DIA-NN v2.1.0 with a predicted human spectral library. Protein groups were inferred from the pg.matrix.tsv output and precursor-level identifications from the report.parquet file. A 1% precursor-level false discovery rate (FDR) was applied, and no match-between-runs (MBR) was used.

Conclusion

The ProtumLink 15 cm × 75 µm emitter-integrated nanoflow column delivers robust, high-resolution chromatographic performance under short-gradient DIA conditions. Across a wide range of peptide inputs, the column demonstrates strong sensitivity, sharp peak profiles, excellent retention time stability, and highly reproducible quantification.
These characteristics make the 15 cm format particularly well-suited for high-throughput DIA workflows on next-generation mass spectrometry platforms, supporting large-scale quantitative proteomics studies where speed, consistency, and depth of coverage must be balanced effectively.

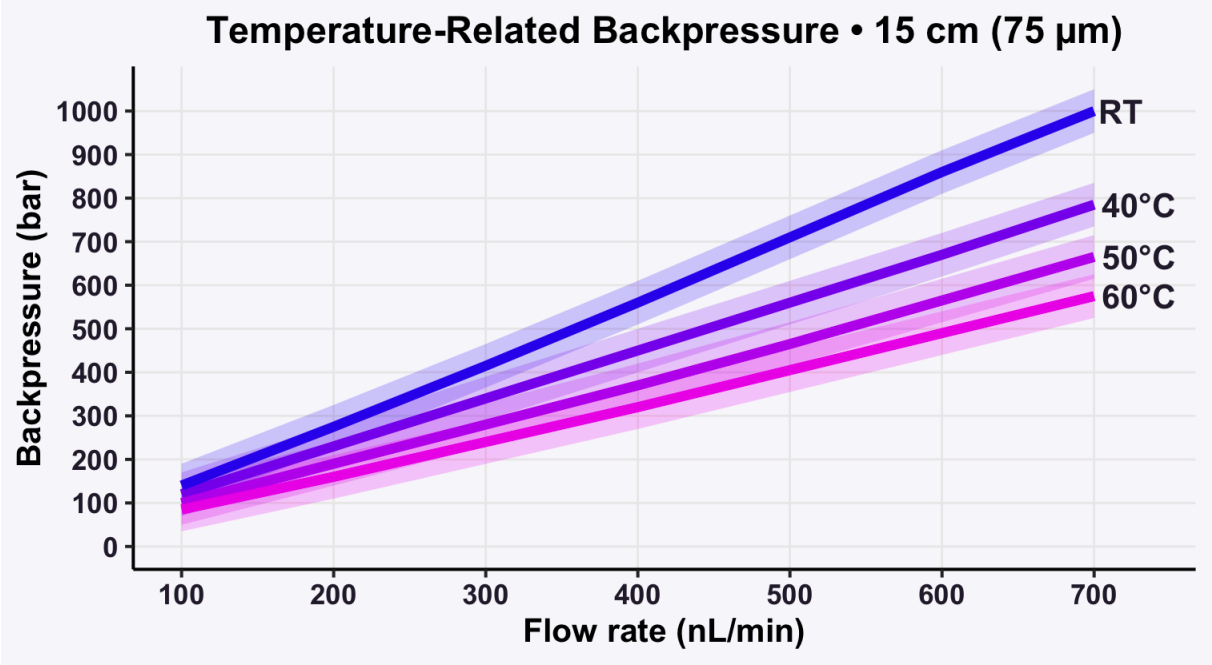

Figure S1: Expected backpressure profile for a 15 cm × 75 µm analytical column at 20% B, shown across relevant flow rates and operating temperatures.

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