Application Note

Ultra robust performance stability of 25 cm × 75 µm Emitter-Integrated Nanoflow Column with 1.9 µm C_{18} Material over 800 Samples

Introduction

The reliability of chromatographic separation is a critical factor in the success of large-scale proteomics studies. While high sensitivity and depth of coverage are essential, true analytical value is only achieved when this performance is maintained consistently across extended sample cohorts. In this study, we evaluated together with the Proteomics Core Facility Osnabrück the long-term stability and chromatographic robustness of the **ProtumLink Connect 25 cm × 75 µm emitter-integrated nanoflow column**, packed with **1.9 µm C_{18}** material, in an intensive LC-MS workflow exceeding 800 injections using a TimsTOF HT system.

Importantly, the column was not limited to a single experimental setup. It was used with a broad variety of biological sample types, including cell lines, tissues from diverse organisms, and enriched sub-proteome fractions. Despite this matrix diversity, the column maintained exceptional chromatographic resolution, retention time consistency, and sensitivity - demonstrating its robustness across workflows without requiring replacement or regeneration.

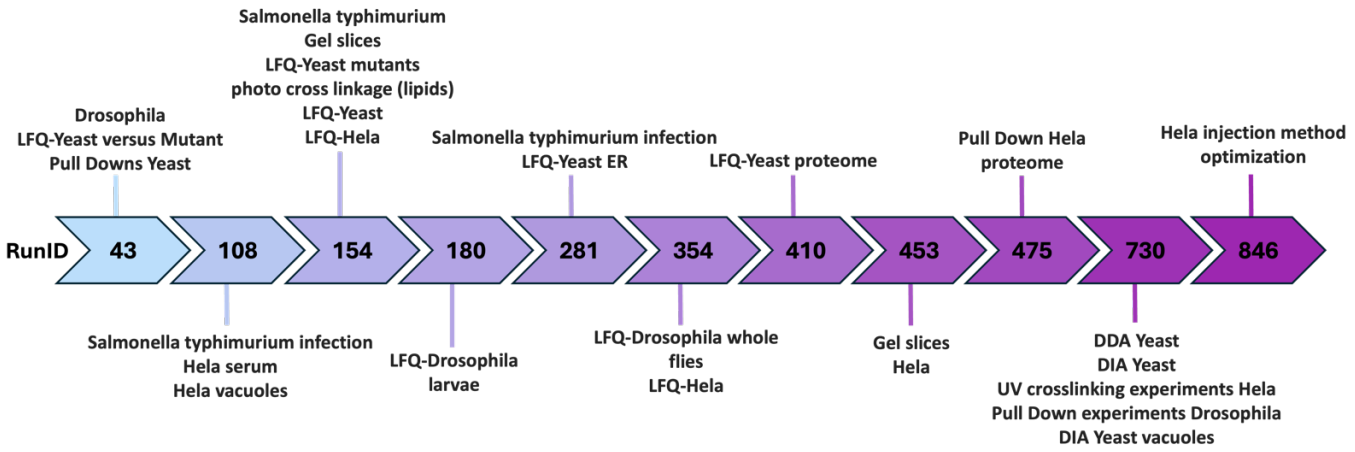

Figure 1: Schematic overview of injected sample types interleaved with HeLa quality control measurements. In total, more than 800 injections were performed over the course of the study.

In dedicated DDA-PASEF benchmarks using 50 ng HeLa digest with iRT peptide standards, the column consistently identified an average of 4,100 protein groups and 31,000 peptides, underscoring its high sensitivity. Retention time stability was excellent throughout the column's use. The coefficient of variation (CV) for precursor retention time across selected HeLa QC injections remained at a median of just 2.04%, confirming robust LC performance suitable for reproducible label-free quantification.

Long-Term Consistency in Proteome Coverage

The unique protein group and peptide identifications presented here highlight the exceptional robustness of the **25 cm × 75 µm, 1.9 µm C18 emitter-integrated column**. This configuration provides an optimal balance between **high-throughput capabilities** using fast gradients as short as 20 minutes, and **deep proteome coverage** with extended gradients up to 90 minutes. Its sustained performance across hundreds of injections underscores its reliability for both routine and advanced LC-MS-based proteomic workflows.

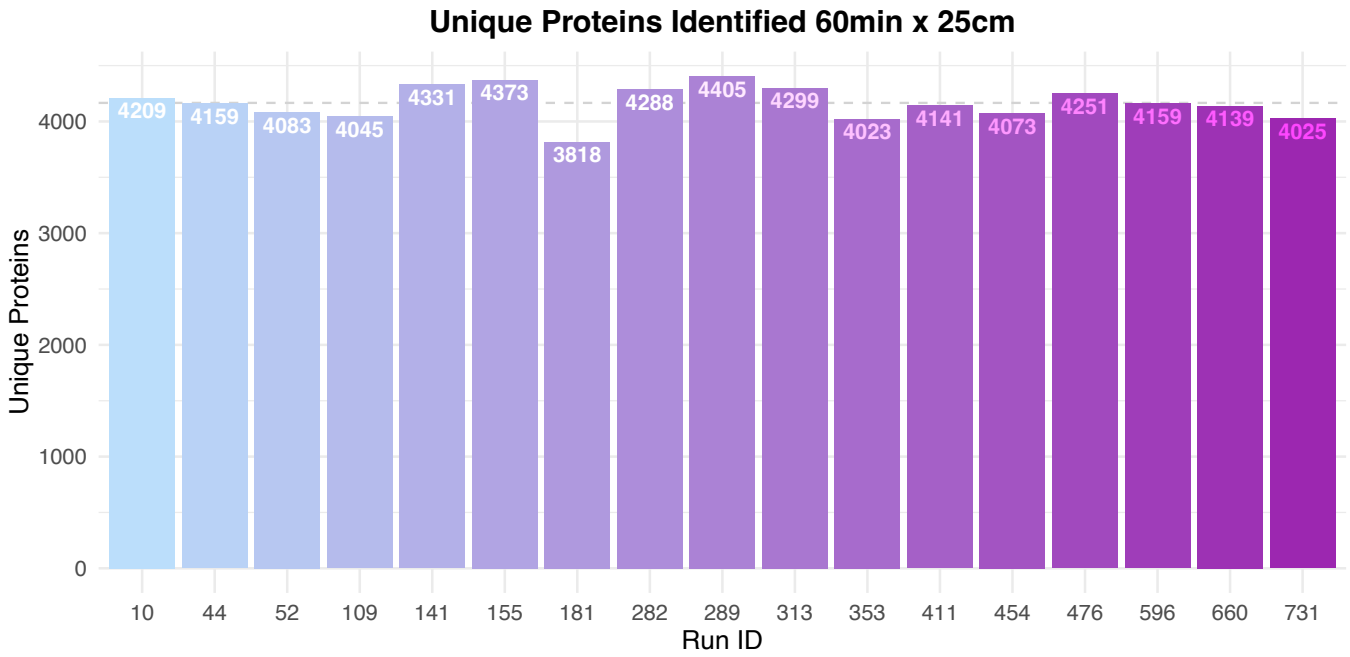

Figure 2: Number of unique protein groups identified in selected 50 ng HeLa digest runs acquired using DDA-PASEF on the timsTOF HT system.

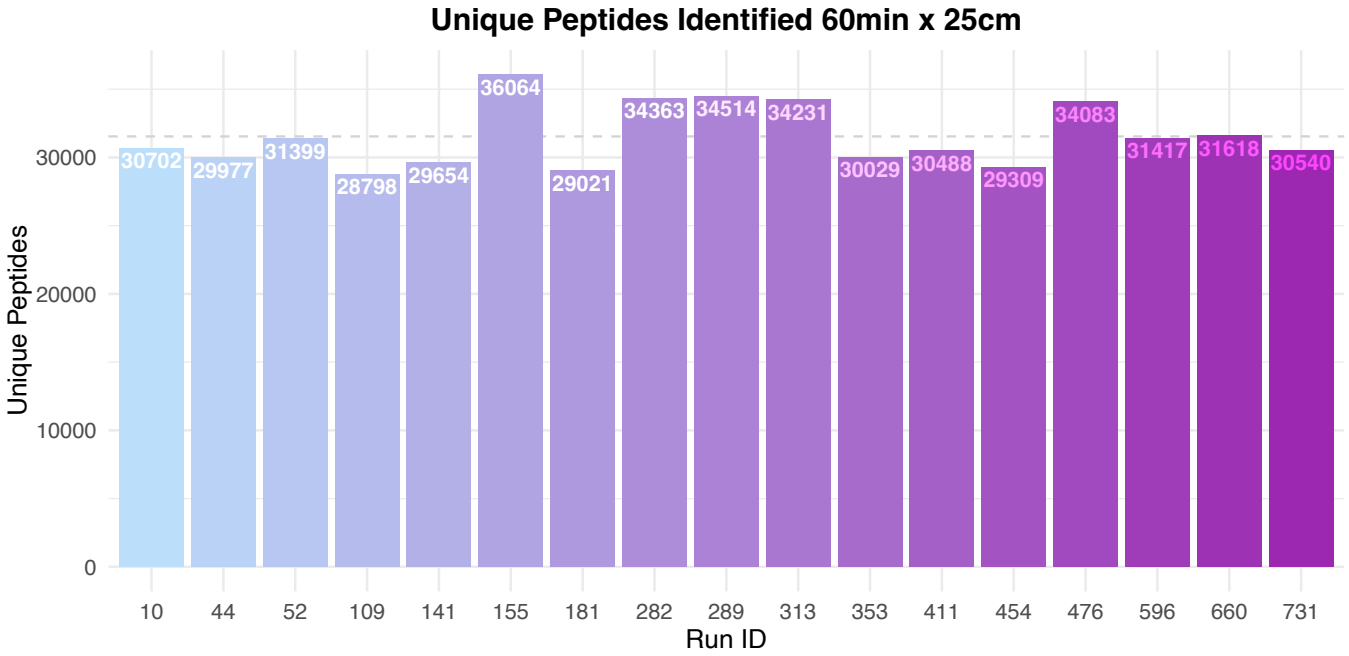

Figure 3: Number of unique peptides identified in selected 50 ng HeLa digest runs acquired using DDA-PASEF on the timsTOF HT system.

Superior Run-to-Run Reproducibility for Label-Free Quantification

Exceptional retention time stability, paired with outstanding chromatographic robustness, makes this column configuration ideally suited for large sample cohorts. Its consistent performance across extended injection series ensures high precision in retention-based alignment and quantification.

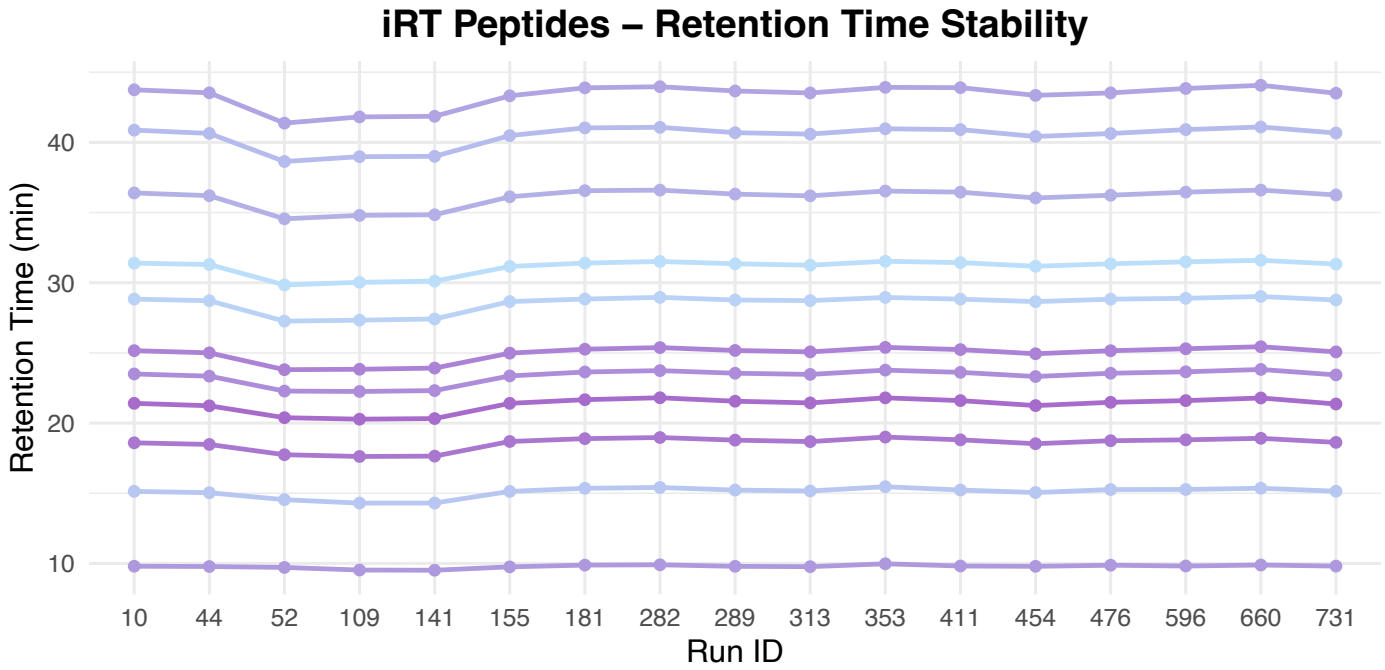

Figure 4: Retention time stability of iRT standard peptides across selected HeLa QC measurements. The data illustrate highly consistent peptide elution, underscoring the column's robustness and suitability for accurate, label-free quantification.

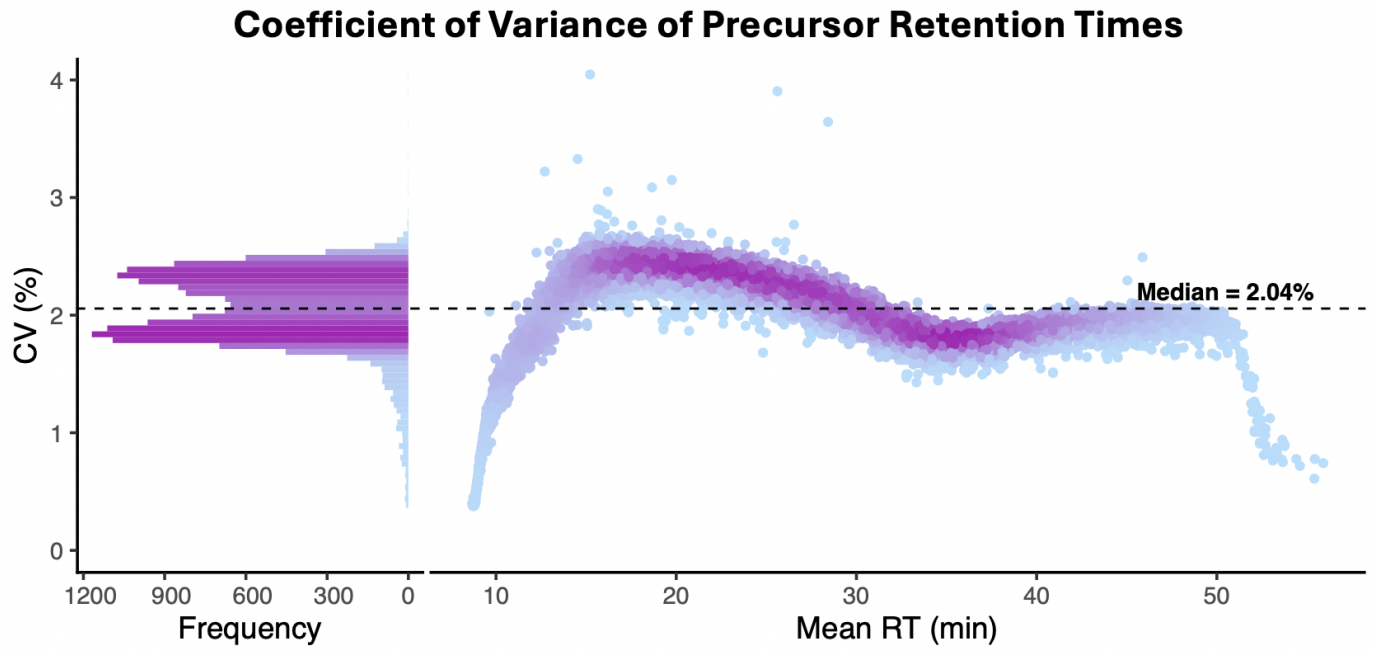

Figure 5: Coefficient of variation (CV) of precursor retention times across 10 selected HeLa QC measurements throughout 800 injections. The median CV was approximately 2% using a 60-minute gradient, indicating excellent retention time stability suitable for large-scale label-free quantification.

Applied Method

HeLa tryptic digest was reconstituted in 2% acetonitrile (ACN) with 0.1% formic acid (FA). A total of 50 ng of peptides was injected using a Dionex Ultimate 3000 HPLC system, employing a 60-minute nonlinear gradient for separation. Peptides were separated using a **25 cm × 75 µm column, packed with 1.9 µm C_{18} particles**, at a constant flow rate of 300 nL/min.

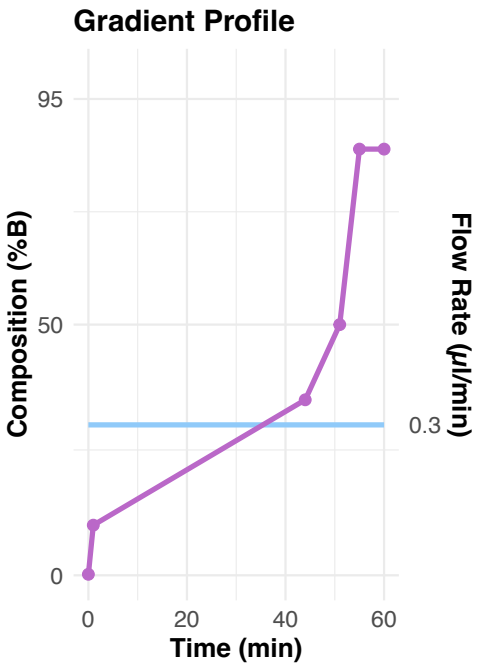

Figure 6: Applied gradient composition and flow rate, illustrating the chromatographic conditions used for peptide separation

Mobile Phase Composition:

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

Mass Spectrometry & Data Analysis

Data acquisition was performed on a **Bruker timsTOF HT** in **PASEF mode**. Raw DDA data were processed using **Fragpipe 21.1** in library-free mode, matched against a UniProt human database with a predicted library, without run-to-run matching. All data were filtered using a 1% false discovery rate (FDR) to ensure high-confidence identifications.

Table 1: Gradient composition and flow rate parameters used for peptide separation.

Time (min)	Composition (%B)	Flow rate (µl/min)
0.00	2	0.3
1.00	10.0	0.3
44.00	35.0	0.3
51.00	50.0	0.3
55.00	85.0	0.3
60.00	85.0	0.3

Conclusion

The **ProtumLink Connect 25 cm emitter-integrated nanoflow column** demonstrates remarkable chromatographic stability, peak shape fidelity, and retention reproducibility across more than 800 injections and diverse sample types. Whether applied to complex tissue lysates, enriched sub-proteomes, or clinical samples, the column delivers the consistent, high-resolution performance required for demanding proteomics workflows. Its robustness and precision make it a valuable tool for any lab aiming to maintain analytical quality over extended acquisition batches.

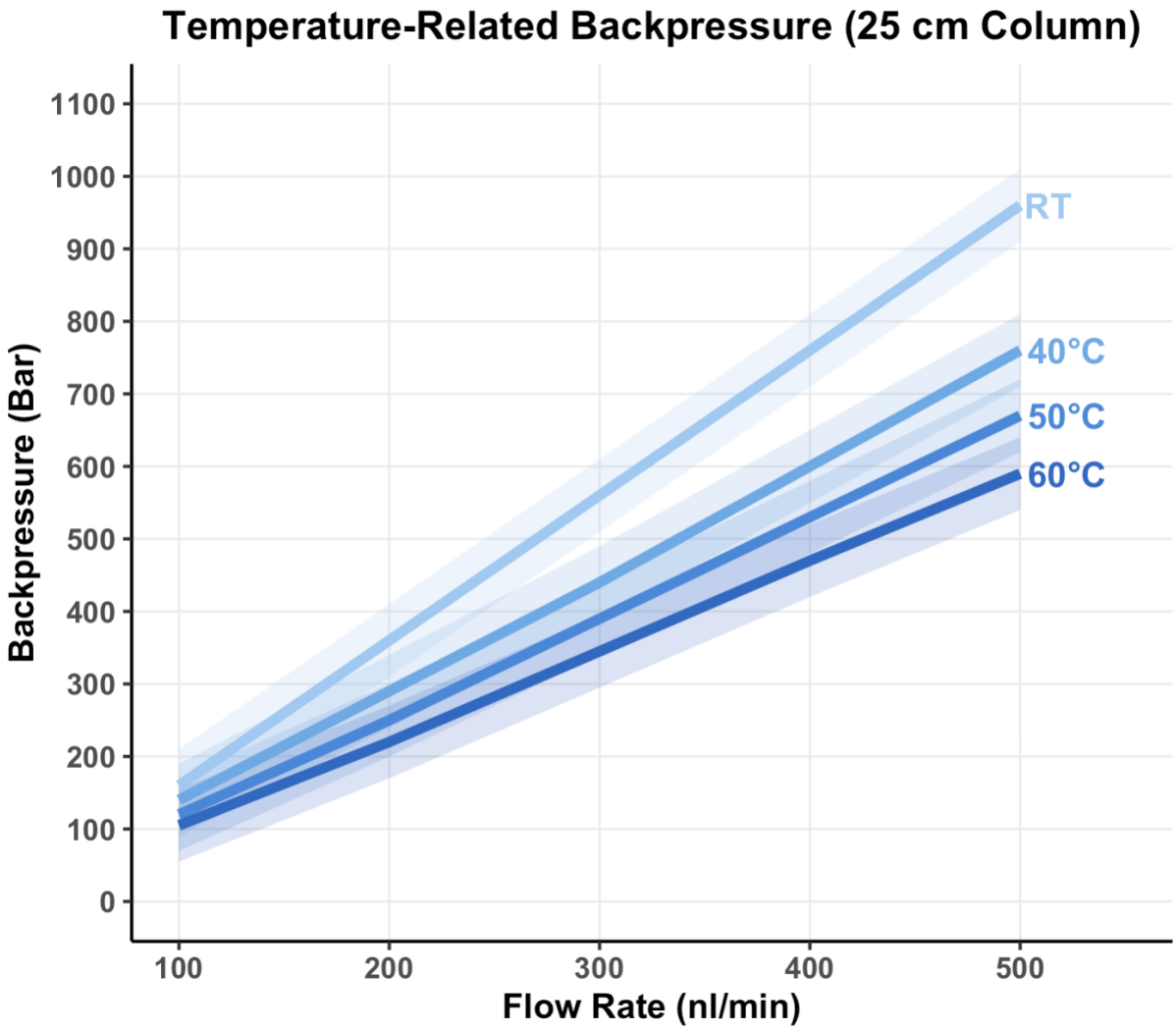

Figure S1: Expected backpressure profile for a 25 cm × 75 µm analytical column at 20% B, shown across relevant flow rates and operating temperatures.

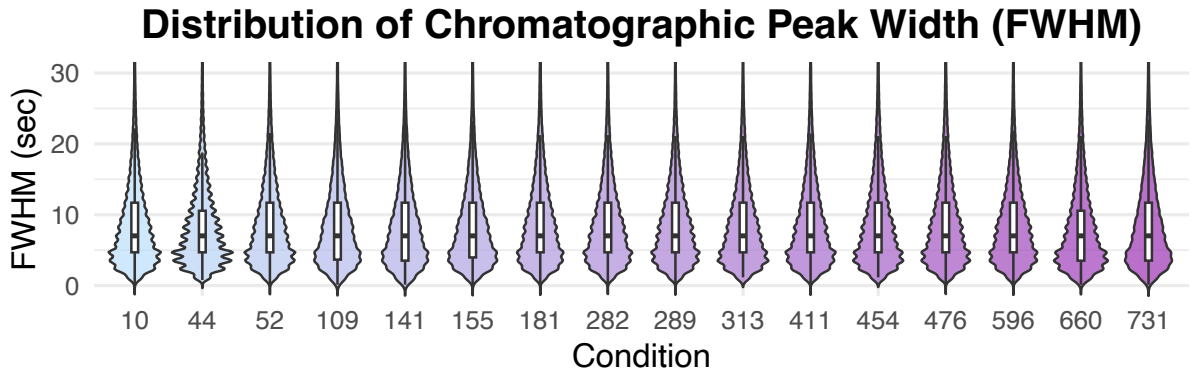

Figure S2: Full width at half maximum (FWHM) values as reported by MaxQuant (version 2.4.2.0). The median FWHM across all peptides is approximately 7 seconds.

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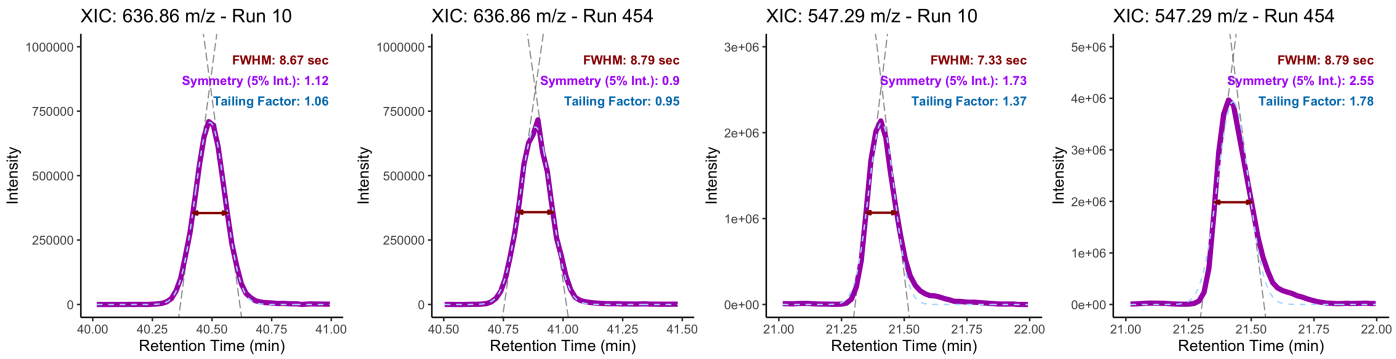

Figure S3. Extracted ion chromatograms (XICs) of selected iRT peptides from run 10 and run 454. Each panel shows the observed peak shape (purple) overlaid with a Gaussian fit (blue). Full width at half maximum (FWHM) and symmetry factors (at 5% peak height) are indicated to assess peak quality and consistency between runs.