Application Note

EVOSEP

Whisper™ Zoom drives scalable workflows for high-sensitivity applications through standardization and ease-of-use

Highlights

- Robust separation and efficient MS utilization for high sensitivity workflows
- Exceptional sensitivity and scalable throughput with up to 120 samples per day

1. Introduction

High-sensitivity proteomics continues to drive advances in biomedical research, with single-cell and low-input analyses pushing the limits of detection and coverage. Achieving robust and reproducible results in these contexts requires optimized sample handling, efficient chromatographic separation, and precise mass spectrometry acquisition. Whisper Zoom methods leverage the Evosep Eno platform to combine optimized gradients, low-flow conditions, and highly synchronized hardware, enabling sharp, symmetrical peaks, consistent retention times, and high peak capacity. This combination minimizes peptide losses, maintains sensitivity even from single-cell–equivalent inputs, and supports reproducible identification and quantification across a wide range of sample loads. The range of methods, from 20 to 120 samples per day (SPD), allows researchers to tailor throughput and depth of coverage to specific experimental goals, balancing proteome coverage, sensitivity, and efficiency. The required robustness is further enhanced by the Evotip, a high-efficiency disposable trap column with no carryover, providing innovative sample introduction and sensitive long-term storage. Collectively, these technologies enable high-sensitivity proteomics accessible to non-experts, supporting workflows ranging from single-cell proteomics to deep visual proteomics and phosphoproteomics.

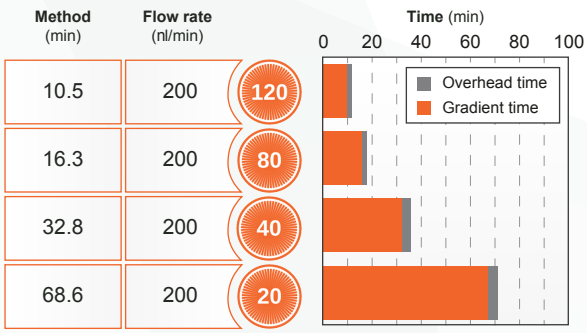

Figure 1: The four Whisper Zoom methods with a throughput of up to 120 samples per day.

2. Method details

HeLa digests (Pierce) were loaded on Evotips (n=5) and analyzed with each of the Whisper Zoom methods. Whisper Zoom 120 and 80 SPD methods used the Aurora Rapid 5x75 C18 column (IonOpticks, operated at 50 °C), while the Aurora Elite 15x75 C18 column (IonOpticks, operated at 50 °C) was used for the Whisper Zoom 40 and 20 SPD methods. Samples were analyzed on a Orbitrap Astral mass spectrometer (Thermo Scientific) operating in data-independent acquisition (DIA) mode. The spray voltage was optimized for the specific columns, the funnel RF level was set to 40, and the ion transfer tube temperature to 280 °C. Full MS scans were acquired at a resolution of 240,000 over a scan range of 380-980 m/z, with an AGC target of 500% and an injection time (IT) of 3 ms. Load-dependent acquisition settings were used for MS2 spectra; 250 pg load used 10 Th DIA isolation windows (m/z) with 20 ms injection time (IT), 5 ng used DIA isolation windows of 5 Th and 10 ms IT, and 50 ng and higher loads used DIA isolation windows of 2 Th and 3 ms IT. Fragmentation was induced using HCD at 25% normalized collision energy (NCE).

Raw data were processed with DIA-NN (v1.9.2) in library-free mode against the reviewed human proteome database (UniProt, October 2020, 20,600 entries), using trypsin/P as the protease. The analysis allowed for carbamidomethylation of cysteines (fixed modification), N-terminal methionine excision, and up to two missed cleavages. The "match between runs" feature was enabled using default settings. Downstream data analysis was performed using the unique entries from the DIA-NN matrix output.

3. Throughput to scale workflows

Whisper Zoom methods benefit significantly from the enhanced chromatographic performance delivered by the Evosep Eno. Faster and more precise hardware synchronization contributes to greater robustness and consistency across runs. Robust chromatography is essential when working with low sample inputs, where even minor peak broadening or sample losses can markedly reduce proteome coverage. Whisper Zoom methods leverage optimized gradients and low-flow conditions to maximize sensitivity, maintain sharp and symmetrical base peak shapes, and ensure consistent retention times. Across the gradient, Whisper Zoom produces narrow, well-defined peaks, as quantified by the full width at half maximum (FWHM) of 2.4 seconds for Whisper Zoom 120 and 4 seconds for Whisper Zoom 20. These tight peaks concentrate peptides into narrow elution windows, reducing co-elution and increasing separation power. The result is high peak capacity, enabling more peptides to be resolved within a single run.

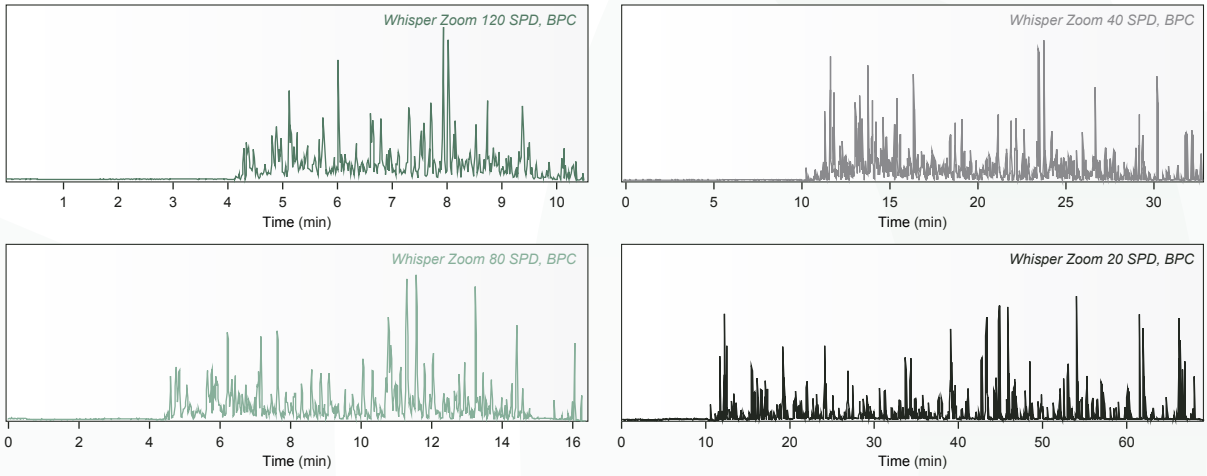

Figure 2: BPC chromatograms from Evosep Eno Whisper Zoom methods.

Whisper Zoom also demonstrates excellent retention time stability with a standard deviation (SD) of ~8 seconds for the 20 SPD method and less than 3 seconds for the rest of the methods, even at low sample inputs, enabling robust and reliable proteomic analysis. This high-quality chromatographic performance provides a strong foundation for sensitive, reproducible, and reliable proteomic analysis.

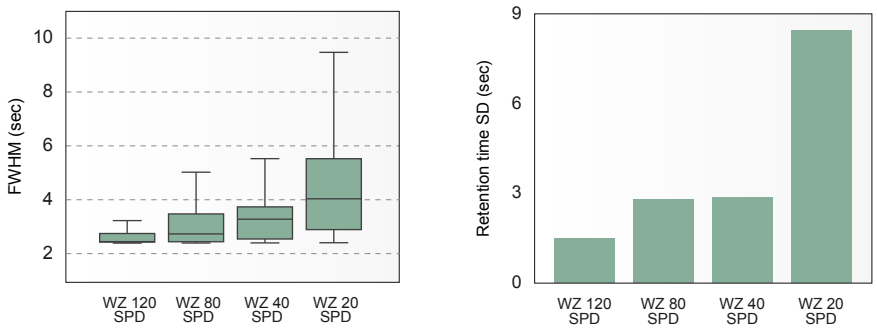

Figure 3: FWHM of all identified precursors (n=5), and RT reproducibility with Whisper Zoom methods using 5 ng HeLa, (n=15), based on five diagnostic peptides.

4. High performance methods

The sensitivity of Whisper Zoom methods was evaluated across sample loads ranging from 0.25 ng to 200 ng of tryptic HeLa digest. As expected, precursor identifications increased with sample load. At lower inputs, the different Whisper Zoom methods deliver comparable performance, highlighting that shorter gradients can be effectively used without compromising sensitivity. When working with single-cell–equivalent amounts, there is no need to sacrifice throughput, as longer gradients provide little additional benefit and can lead to reduced identifications at very low loads.Considering the choice of method is therefore important to avoid working under suboptimal conditions and to ensure efficient utilization of the sample. With higher sample amounts, the slower methods provide additional separation space, which can support further identifications when needed.

Examination of precursor identification density across the gradient further highlights the efficiency of the approach. All methods display a square-like distribution, meaning identifications remain stable and evenly spread across the entire gradient rather than clustering at the beginning or end. This balance ensures optimal MS utilization time and consistent peptide detection throughout the run.

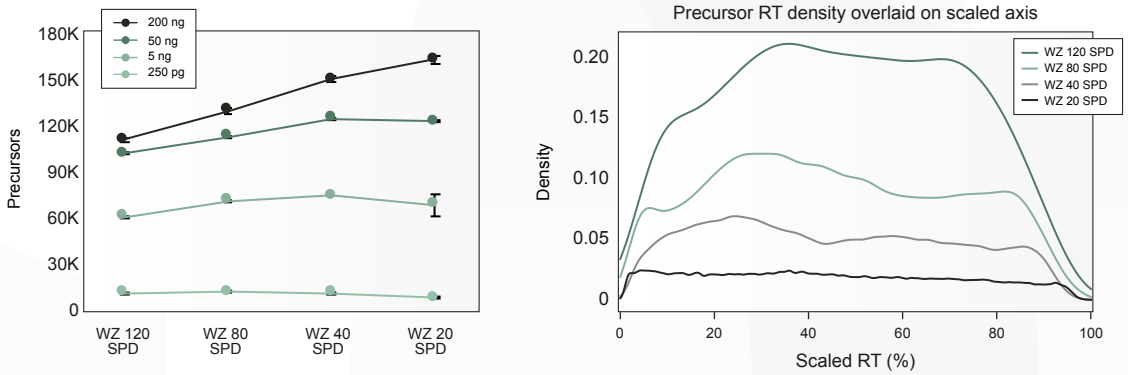

Figure 4: Precursor identifications across Whisper Zoom methods at different sample loads. Overlaid, normalized precursor identification density across the gradient for all methods at 5 ng load (n=5).

5. Quantitative accuracy

Protein identifications across different sample loads were analized for the four Whisper Zoom methods, along with their associated coefficients of variation (CVs). Significant differences in protein identifications were primarily observed at higher sample loads, reflecting that the additional separation capacity of slower methods is only realized under these conditions. At low inputs, the faster methods perform exceptionally well at speed, identifying nearly 7,000 proteins from just 5 ng of HeLa digest with Whisper Zoom 120 SPD. When moving from 20 SPD to 120 SPD, throughput increases by 500% with only a modest reduction in protein identifications, while maintaining strong quantitative performance (CV ~8.5%). These results highlight that faster methods can deliver an exceptional high performance specially when working with limited sample amounts.

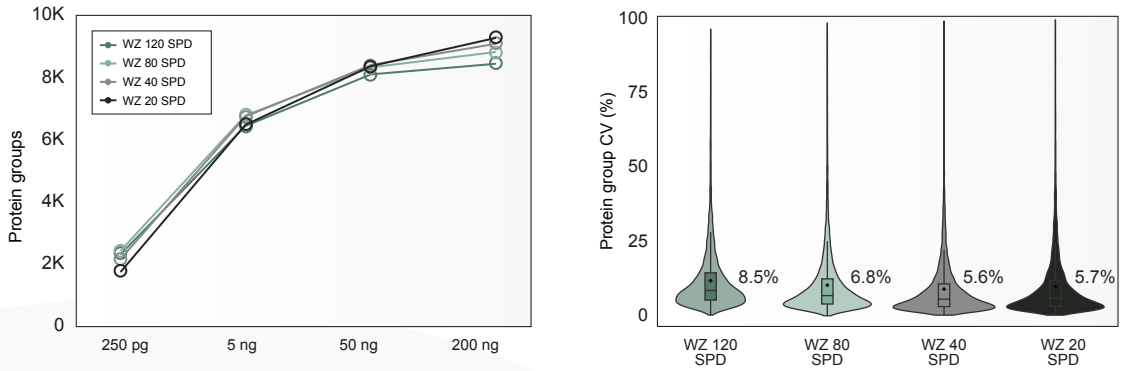

Figure 5: Protein idenfications with Evosep Eno standard methods, and associated CVs, using 200 ng HeLa digest (n=5).

6. Conclusion

Whisper Zoom methods provide robust chromatographic performance, high sensitivity, and reproducible protein identification across a wide range of sample loads. Faster methods excel at low inputs, delivering high proteome coverage while significantly increasing throughput, whereas slower methods offer additional separation capacity that becomes most beneficial at higher sample amounts. The square-like distribution of precursor identifications across the gradient further reflects efficient MS utilization and consistent peptide detection.

These capabilities make Whisper Zoom highly relevant for clinical and biomedical applications, where sample amounts are often limited and reproducibility is critical. By enabling sensitivity and throughput, these methods support the discovery of biomarkers, deep phenotyping, and detailed molecular characterization, accelerating translational research and expanding access to high-quality proteomics for a broad range of laboratories and studies.

Evosep Eno is for General Laboratory Use.