Application Note

EVOSEP

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

Highlights

- Democratizing cutting-edge LC-MS performance for high-sensitivity workflows
- Robust and scalable throughput with up to 120 samples per day

1. Cutting-edge performance

High-sensitivity proteomics is required to advance biomedical research, and recent improvements in single cell proteomics (SCP) have shown the most pronounced leap in performance in recent years. The development of new technologies such as loss-less sample preparation workflows and modern high-end mass spectrometers has been a community effort. Currently, the link between sample preparation and MS detection is still a challenge due to sample losses and efficient high performing separation. The Whisper Zoom methods are specifically designed to significantly improve and maximize recovery of the sample with highly optimized flow paths to deliver sensitivity and robustness with unmatched performance. This combined with the easy-to-use and robust Evosep technology have commoditized high-end-LC-MS performance and made it available to all types of research areas. Whisper Zoom methods allow throughputs from 20 to 120 samples per day to scale high-sensitivity proteomics including SCP, deep visual proteomics (DVP) and phosphoproteomics workflows.

The required robustness is accelerated by the Evotip, a high efficiency and disposable trap column with no carryover, providing innovative sample introduction and sensitive long-term storage. Collectively, these technologies enable high-sensitivity proteomics for non-experts.

	Gradient Length (min)	Cycle Time (min)	Flow Rate (nl/min)
120	10.3	12.0	200
80	16.3	18.0	200
40	32.5	36.0	200
20	68.0	72.0	200

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

2. Methods

HeLa digests (Pierce) were loaded on Evotips in quadruplicates and analyzed with each of the Whisper Zoom methods. Samples analyzed using the Whisper Zoom 120 and 80 SPD mehods 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 timsTOF Pro 2 mass spectrometer (Bruker) in dia-PASEF mode. DIA spectra were collected over a mass range of 400-1000 Da and an ion mobility range of 0.64 to 1.37 1/K0, using 8x25 Da windows and 3 TIMS ramps per cycle, with ramp and accumulation times of 100 ms and a cycle time of 0.95 s. Data from each condition were processed independently using DIA-NN (version 1.8.1) in library-free mode against the reviewed human proteome (UniProt, Oct 2020, 20,600 entries excluding isoforms), with MBR (match-between-runs) enabled under default settings. Protein and precursor identifications were derived from unique entries in the DIA-NN matrix outputs.

3. Throughput to scale workflows

High-performing liquid chromatography relies on the separation of individual analytes in an as small and well-defined volume as possible which defines a chromatographic peak. Reducing peak widths, flowrates and improving peak symmetry collectively increases the concentration of individual analytes to increase the sensitivity of LC-MS. In this context, the fluidics and physical constraints of the flow path significantly impacts the overall chromatographic performance and it is critical to minimize these contributions. The Whisper Zoom methods have been specifically designed to reduce the flow path to a bare minimum for best performance and throughput. This enables sharp symmetric peaks of 1.6 seconds at half height (FWHM) with the Whisper Zoom 120 SPD method and ~2.1 seconds at FWHM using the Whisper Zoom 40 SPD method.

The sensitivity of the Whisper Zoom 120, 80, 40, and 20 SPD methods was evaluated with a dilution series of 0.125 pg to 200 ng tryptic HeLa peptides. The Whisper Zoom 120 SPD method shows saturating levels of precursor identifications at 10 ng load where additional peptide input does not improve analysis depth. Using the timsTOF Pro 2 with the Whisper Zoom 40 and 80 SPD methods, saturation occurs at 50 ng, while the Whisper Zoom 20 SPD method is saturated at or above 200 ng input.
Additionally, at single-cell equivalent levels (250 pg) the number of precursors identified is slightly better for Whisper Zoom 40 SPD compared to the other methods, which perform similarly. The Whisper Zoom 120 SPD yields three times higher throughput at a minimal cost of depth compared to the Whisper Zoom 40 SPD method.

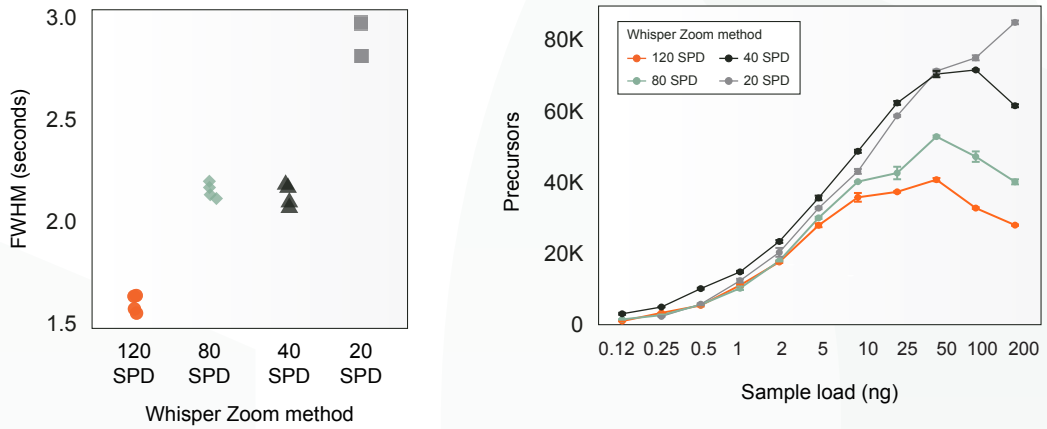

Figure 2: Performance from all four Whisper Zoom methods. FWHM from 5 ng injections (n=4) and HeLa dilution curves from 120 pg to 200 ng (n=4).

4. Robustness is essential

The Whisper Zoom methods are designed with robustness in mind as they employ higher flowrates that improve spray stability and ease of use. Additionally, the Evosep One is robust by design, leveraging Evotip technology to enhance column longevity by partially eluting the single-use Evotip Pure to retain unwanted material on the tip and protect the analytical system. To demonstrate the Evosep system robustness, we monitored the chromatographic stability across 340 samples with the Whisper Zoom 120 SPD method. The HP pump pressure remained constant throughout the analysis, showing that the analytical column is well-protected by the partial elution and the low carryover enabled by the Evotip Pure. Monitoring five HeLa peptides across the single-cell equivalent samples (250 pg HeLa), demonstrated excellent run-to-run reproducibility for the Whisper Zoom 120 SPD method with an average standard deviation of 2 seconds.

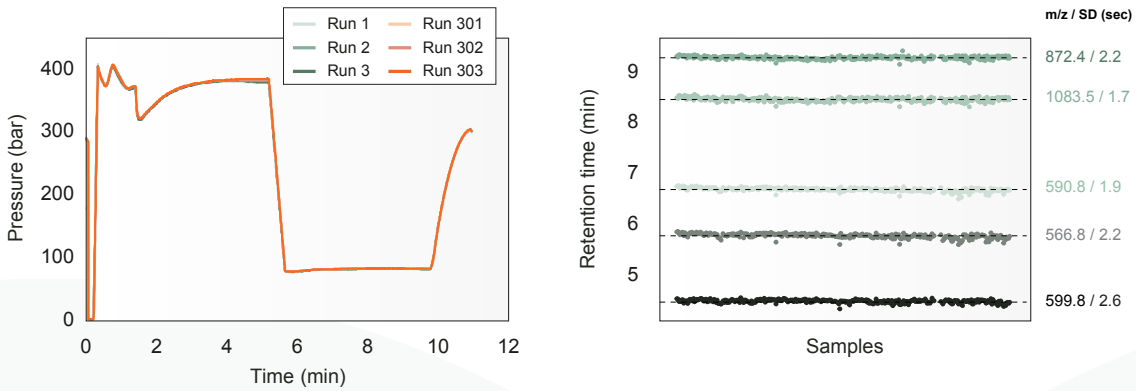

Figure 3: Pressure traces across 340 runs with the Whisper Zoom 120 SPD method and retention times of five monitored HeLa peptides.

5. Reproducibility for comparison

The Whisper Zoom methods use minimal-length flow paths in the Evosep and are designed to increase robustness and reproducibility. To evaluate the robustness and reproducibility of Whisper Zoom, we analyzed 5 ng HeLa samples using the legacy Whisper 80 SPD method on five different Evosep instruments (green) and compared it to the Whisper Zoom 80 SPD on the same five Evosep instruments (orange). The Whisper Zoom methods were also compared across 3 different and external labs (grey). Collectively, the Whisper Zoom method significantly enhances retention time stability and inter-instrument reproducibility across different Evosep instruments. Having high system-to-system reproducibility is essential for comparisons and unlocks multi-laboratory studies across different core-labs.

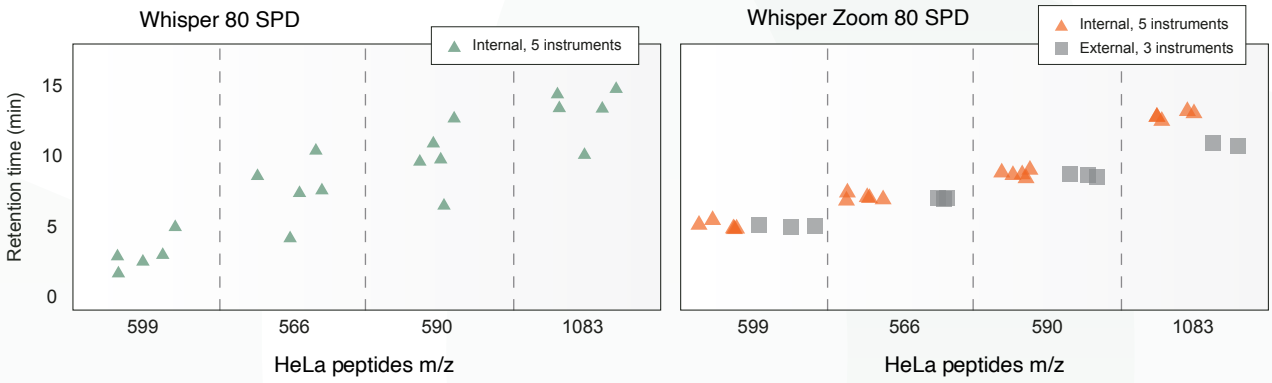

Figure 4: Improvement in inter-instrument retention time (RT) reproducibility of Whisper Zoom 80 SPD, internally (n=5) and externally (n=3). RT from four peptides were monitored from 5 ng injections (n=4).

6. Conclusion

The Whisper Zoom methods provide a significant improvement in performance for high sensitivity and cutting-edge LC-MS proteomics. The sensitivity has been significantly increased due to improved sample recovery and chromatographic performance together with higher throughput of up to 120 samples per day. The intra (less than 5 seconds) and inter (less than 16 seconds) instrument reproducibility and performance will benefit new projects as well as accelerating ongoing collaborations in the proteomics community to advance the field towards new discoveries and technological innovations.

Evosep One is for General Laboratory Use.