

EVOTIP SEPARATOR

Introducing Evosep Eno

Evosep Eno sets new standards for routine and high-performance LC-MS

LC-MS based proteomics has rapidly evolved from a specialized analytical discipline, into a powerful, applied technology to elucidate detailed biological insights at scale. While technological advancements will continue to drive this progress, there is an overarching need for a standardized framework that allows to harness the performance from continuous developments.

The Evosep Eno is the standardized platform, that bridges this gap, and is designed for ease-of-use, industry-leading robustness and reliable chromatographic performance for consistency across instruments and laboratories every single day.

Designed for robustness and performance

- Faster hardware synchronization and better monitoring
- New performance levels from Eno standard methods

Standardized chromatographic improvement

- Peak symmetry improved up to **35%**
- **20%** sharper peaks at base, leading to higher peak capacity
- Up to **80%** better retention time reproducibility
- Significantly improved signal intensity and quantification

Purpose-built, the Evosep Eno sets a new standard for high-performance separation with standardized methods from 30 to 500 samples per day. The unmatched chromatographic performance with significantly sharper peaks and improved peak capacity, ensures the sensitivity required for high-end quantitative proteomics. Additionally, Evosep Eno provides the reliability and robustness needed for both small detailed projects with few samples to very large-scale and ambitious projects where throughput of up to 500 samples per day sets new standards.



Significantly improved LC-MS performance with **Evosep Eno**

Improved chromatographic performance and reproducibility

The Evosep Eno offers a faster and more precise synchronization of hardware providing significantly improved performance especially for the fast methods (ie. 500, 300 and 200 SPD). The peak symmetry is improved up to 35% at 200 SPD, leading to sharper peaks and better peak capacity. The most pronounced Full Width at Base (FWB) improvement was more than 30% for the 500 SPD method. Altogether, this leads to significantly improved intensity and resulting quantification. Moreover, the retention time reproducibility was improved across all the methods on the Evosep Eno compared to Evosep One, with up to 80% improvement with the 100 SPD method. This enables improved reproducibility and significantly benefits targeted assays with more tight method scheduling, enabling the inclusion of more target peptides.

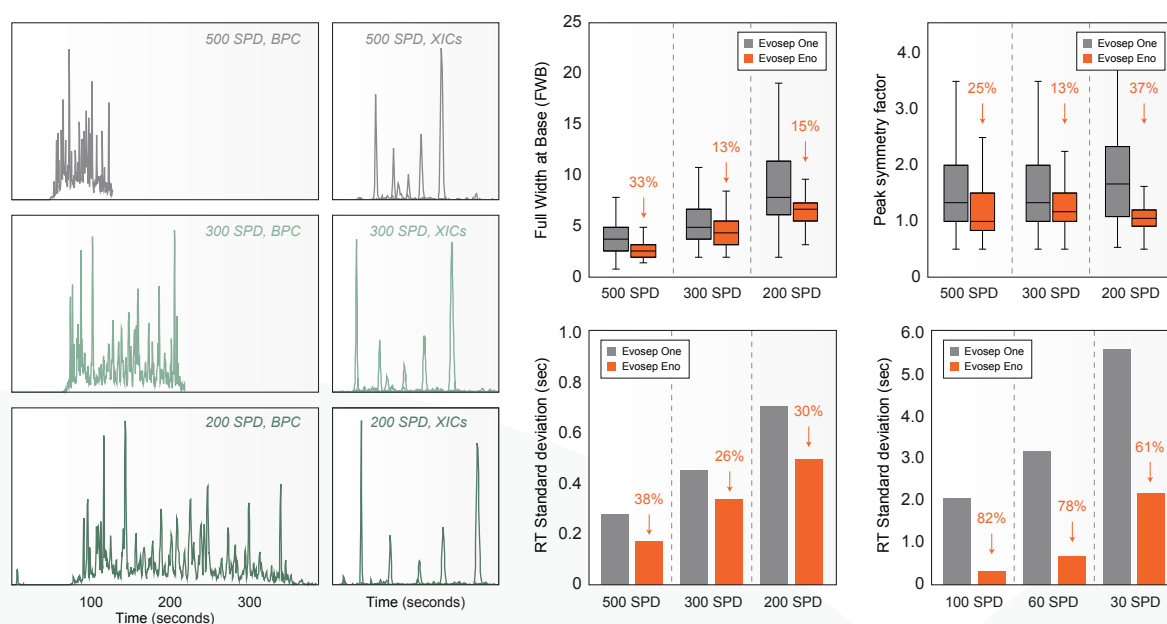


Figure 1: BPC and XIC (m/z 479, 651, 513, 472, 614) chromatograms from Evosep Eno 200, 300 and 500 SPD methods. Improvement in FWB, peak symmetry (n=96) and RT SD across these methods using 50 ng HeLa, intra-instrument (n=15), compared to Evosep One.

New standards in inter- and intra-lab reproducibility - excellent just got better

The Evosep Eno sets a new standard for run-to-run, and instrument-to-instrument reproducibility, enabling customers to setup and maintain multiple instruments in the same lab as well as compare with external laboratories. This is showcased using the 200 SPD method, where the intra-instrument retention time (RT) reproducibility was measured to ~0.5 seconds standard deviation (St dev), based on 20 injections from eight instruments. Impressively, the inter-instrument reproducibility was measured to ~2 seconds st dev, when all eight instruments were compared directly. This provides extremely reproducible data, which can be directly compared and used regardless of origin and it will directly impact the performance of targeted assays due to the precise scheduling of peptide targets.

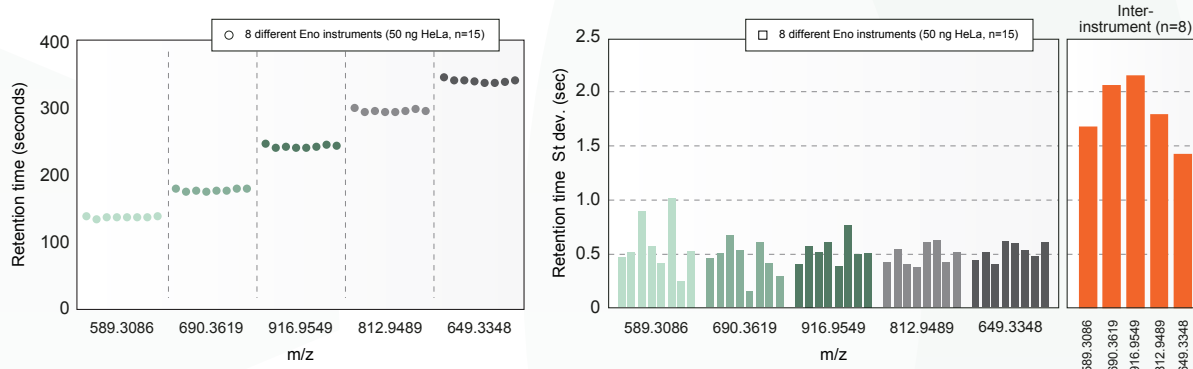


Figure 2: RT reproducibility at 200 SPD using 50 ng HeLa, intra-instrument (n=20) and inter-instrument (n=8)

Significantly improved performance with the fast methods

Chromatography lays the foundation for good MS performance, and improvement in the chromatography will translate directly into improved MS performance as visualized below, where a direct comparison between Evosep One and Evosep Eno shows more than 50% increase in precursor coverage. This gain is mostly pronounced at the faster standard methods, where MS performance is pushed to the limits and thus becomes more sensitive to chromatographic performance. The Whisper Zoom methods have also seen small improvements with Evosep Eno compared to the Evosep One. In both cases, the specific gain will depend on both the sample load and the MS used.

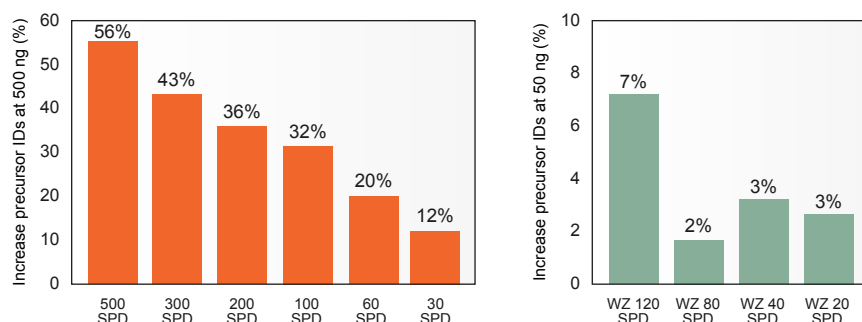


Figure 3: Percentage improvement in precursor identifications with Evosep Eno (n=5) compared to Evosep One (n=5), across standard methods (orange) and Whisper Zoom methods (green).

Outstanding sensitivity and quantitative performance

Enhanced retention time stability enables more precise scheduling of targets in quantitative mass spectrometry (MS) assays, directly improving quantitative performance and increasing the number of peptide targets that can be addressed relative to gradient length. Furthermore, well-defined and symmetric peak shapes contribute to high sensitivity and reduce matrix interference, ensuring robust and reliable quantification.

To illustrate this, we conducted a direct comparison between the Evosep One and Evosep Eno using a scheduled Multiple Reaction Monitoring (MRM) assay. Raw plasma samples were processed using a fully automated workflow targeting clinically relevant plasma proteins. Additionally, matrix-matched calibration curves (MMCCs) were generated by serially diluting human plasma in a chicken plasma background. This approach enables a more realistic assessment of workflow linearity and accuracy, beyond the LC-MS setup alone.

When comparing the 200 SPD methods on both platforms, the Evosep Eno demonstrated superior performance, achieving >95% accuracy with a tighter distribution than the Evosep One. This resulted in improved precision and overall assay accuracy, highlighting the benefits of enhanced chromatographic performance for high-throughput targeted proteomics.

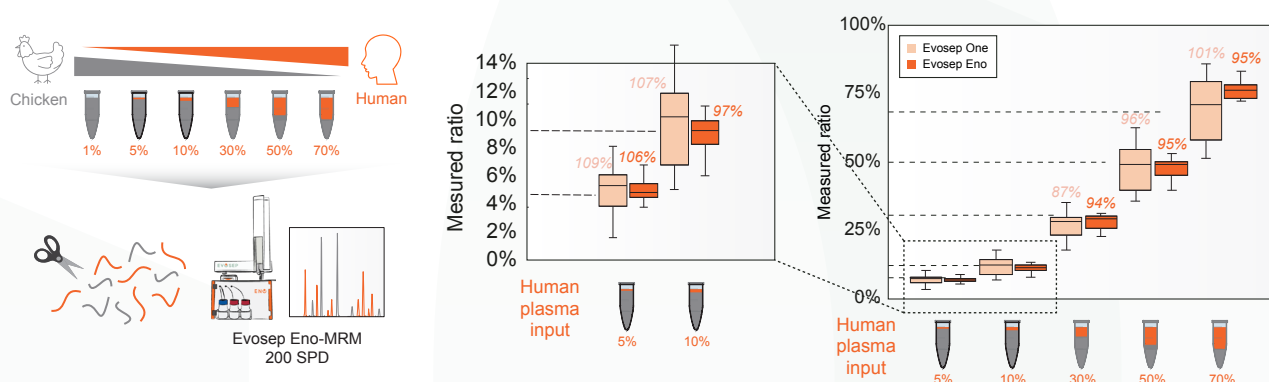


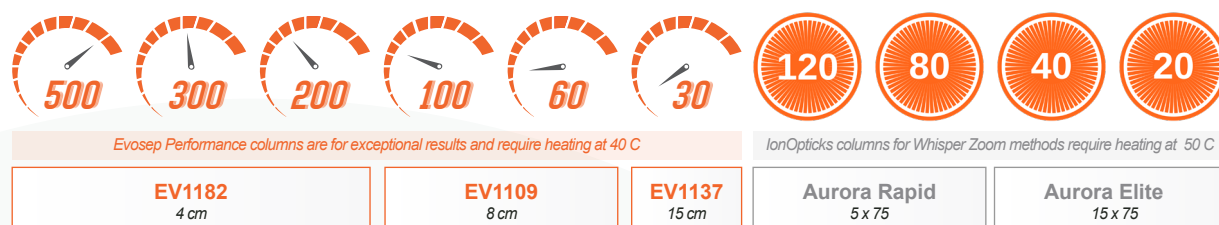
Figure 4: Experimental design for 200 SPD MRM assay monitoring 11 target proteins, Evosep One vs Evosep Eno. Dilution series results of all measured ratios (box plot) against expected (dotted lines). Orange numbers indicate accuracy.

Next generation LC-MS based proteomics - New standards with Evosep Eno

LC-MS performance is usually considered as an isolated measure of MS performance, but sample preparation and chromatographic performance is equally if not more important to utilize the full potential of the MS. The new Evosep Eno is bridging this gap through a standardized, easy-to-use, robust and reliable chromatographic solution that provides consistent high performance across instruments and laboratories every day.

Simplified performance and column specifications

The new standard Evosep Eno methods cover a throughput of 500, 300, 200, 100, 60 and 30 samples per day and are carefully tailored to Evosep Performance columns as specified below and must be heated to 40 °C. The Whisper Zoom methods are specifically designed for ultra-high sensitivity applications and cover a throughput of 120, 80, 40, and 20 SPD, optimized for the IonOpticks columns as specified below and must be heated to 50 °C.



Evosep Pod - Thermo Scientific EASY-Spray a column oven designed for Evosep Performance columns

Evosep Pod™ is a column oven designed for Evosep Performance columns. With the Evosep Pod you get a fully integrated solution for precise temperature control of Evosep analytical columns for LC-MS based proteomics.

Seamless Integration: Designed to work perfectly with Evosep instruments, standard methods and consumables, on the Thermo Scientific™ EASY-Spray™ ionization source.

Optimal Performance: Evosep Pod ensures accurate temperature regulation of the analytical column during LC-MS analysis, for reliable and reproducible results.

