Application Note

EVOSEP

Evosep Eno - new standards for routine, scalable, and high-performance LC-MS based proteomics

Highlights

- >2,000 proteins ID's per minute using high-performance standardized methods.
- Excellent retention time reproducibility with standard deviation <0.5 seconds.

1. Introduction

Mass spectrometry (MS)-based proteomics is rapidly evolving, offering a powerful suite of technologies with the potential to revolutionize healthcare and enable precision medicine. This momentum is driven by significant improvements in the sensitivity and scan speed of modern mass spectrometers, alongside advancements in adjacent technologies that enhance the overall workflow.
Purpose-built for modern proteomics, Evosep Eno introduces a comprehensive set of standardized, high-performance methods tailored to diverse research and application needs. It includes six standard methods, 500, 300, 200, 100, 60, and 30 samples per day, designed for typical applications and routine workflows.

Together, these methods deliver consistent chromatographic performance, exceptional reliability, and scalable throughput. Whether the goal is ultra-high-throughput analysis at 500 samples per day or comprehensive proteome profiling at 30 samples per day, Evosep Eno ensures flexibility and performance across the full spectrum of proteomics applications.
Here, we describe the relationship between throughput and proteome coverage with the Evosep Eno standard methods.

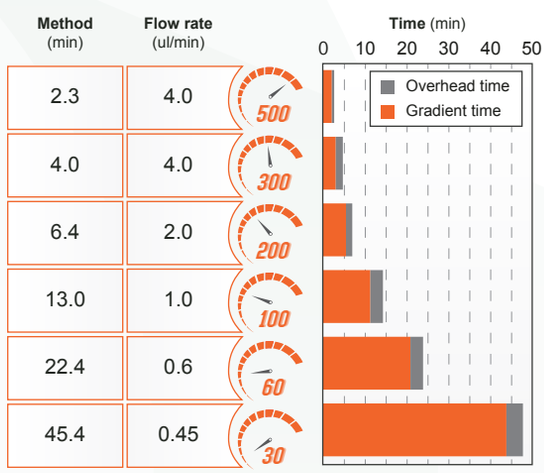

Figure 1: Evosep Eno standard methods.

2. Method details

HeLa digests (Pierce) were loaded onto Evotips and analyzed using the standard Evosep Eno methods. For the assessment of chromatographic stability, 15 injections of 50 ng HeLa digest (Pierce) were carried out. Evosep Performance columns (EV1137 for 30 SPD, EV1109 for 100 SPD and 60 SPD and EV1182 for 200 SPD, 300 SPD and 500 SPD) were used at 40 °C using an Evosep Pod column oven (EV1187) coupled to an EasySpray source (Thermo Scientific).
Samples were analyzed on an Orbitrap Astral mass spectrometer (Thermo Scientific) operating in data-independent acquisition (DIA) mode. The spray voltage was set to 1.9kV, the funnel RF level to 40, and the ion transfer tube temperature to 275 °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. The mass range was divided in 299 windows of 2m/z, and fragmentation was induced using HCD at 25% Normalized Collision Energy (NCE). Fragment spectra were recorded with an IT of 3ms, 500% AGC and a toral loop time of 0.6 secs.
Raw data files were processed using DIA-NN 1.9.2 in library-free mode against the reviewed human proteome (Uniprot, Oct 2020, 20,600 entries) with trypsin/P as the protease. The analysis allowed for carbamidomethylation of cysteine, N-terminal methionine excision, and up to two missed cleavages. "Match between runs" was enabled with default settings. Downstream data analysis was performed using the unique entries from the DIA-NN matrix output.

3. Excellent peak performance

Good chromatographic quality is essential to fully utilize the capacity of modern MS systems. The six standard methods on the Evosep Eno deliver standardized chromatographic performance with sharp, symmetrical peaks, leading to high peak capacity. The median Full Width at Half Maximum (FWHM), ranges from 2.4 to 5 seconds. The methods are desgined for robustness, and the retention time stability is below 1-2 seconds standard deviation (SD), based on 15 replicate injections.

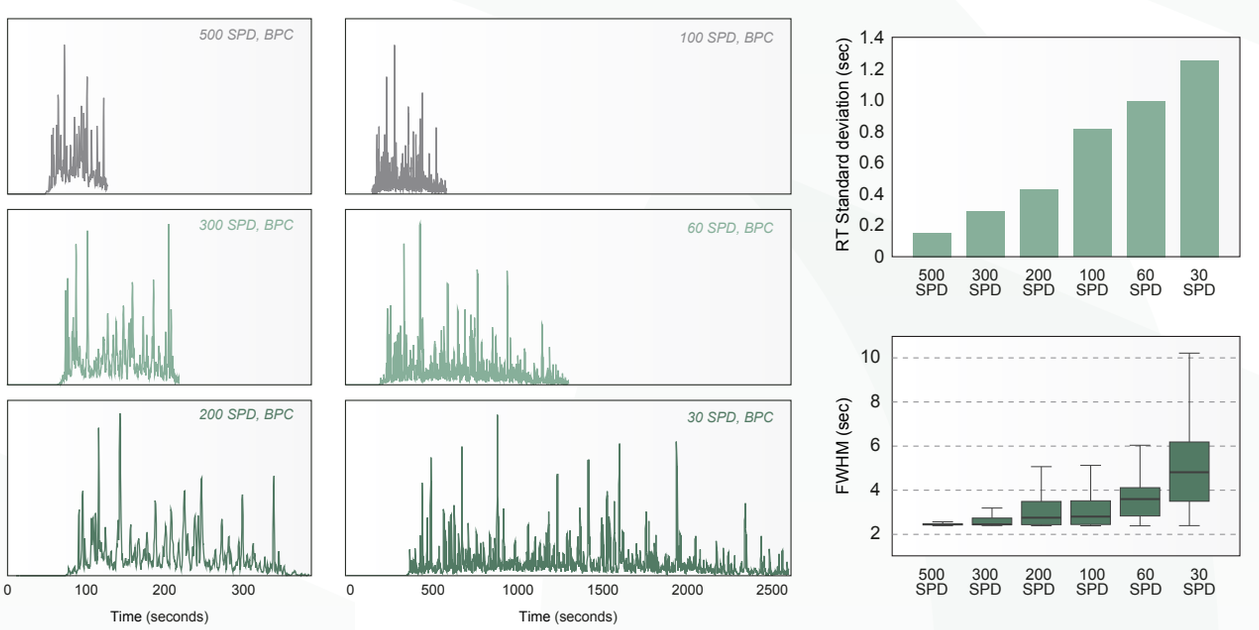

Figure 2: BPC chromatograms from Evosep Eno standard methods, RT SD across these methods using 50 ng HeLa (n=15), and FWHM (n=5).

4. Inter-instrument reproducibility

High inter- and intra-laboratory reproducibility is essential to ensure that results remain consistent, reliable, and comparable across different experiments, users, and laboratory settings. This is particularly critical for large-scale studies, collaborative projects, and applications, where integrity and consistent results directly impacts the confidence and scientific conclusions. The intra-instrument retention time (RT) reproducibility was assessed using the 200 SPD method by evaluating five diagnostic peptides spanning the chromatographic elution window. The SD was ~0.5 seconds, based on 15 injections per instrument across eight instruments. Notably, inter-instrument reproducibility showed only ~2 seconds SD when comparing all eight instruments, underscoring the exceptional instrument consistency and robustness.

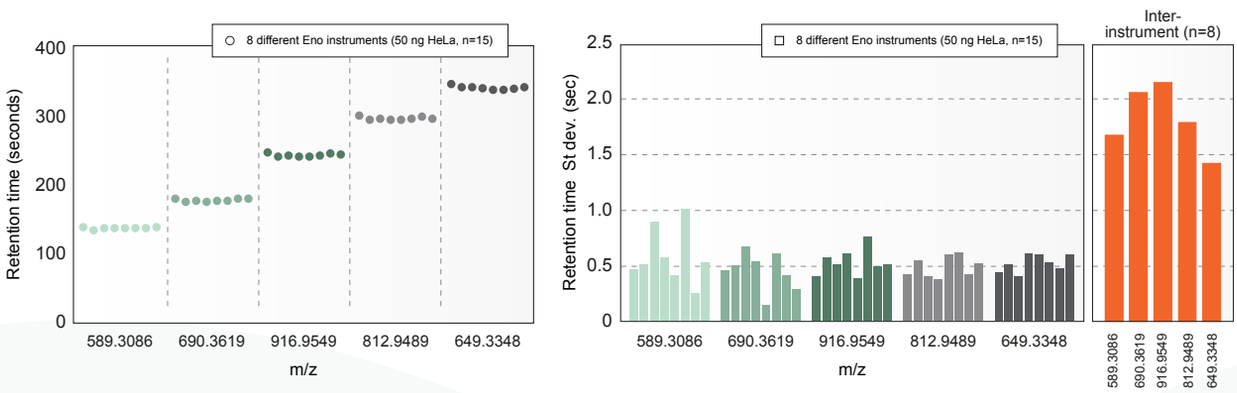

Figure 3: RT reproducibility at 200 SPD using 50 ng HeLa, intra-instrument (n=20), and inter-instrument (n=8).

5. High performance methods

The six Evosep Eno standard methods were evaluated using the Orbitrap Astral mass spectrometer. The exceptional chromatography provided by Evosep Eno coupled to the sensitivity of newer and faster MS analyzers delivers exceptional scalability in proteome coverage without compromising data quality.
Precursor identifications increased linearly with the method length, a trend which remains consistent regardless of the mass spectrometer used, demonstrating the robustness and flexibility of the Evosep Eno. Method selection can therefore be tailored to meet specific experimental needs, allowing to balance speed and depth as required. For example, shifting from 300 to 200 SPD yields a 38% increase in proteome coverage, with a 33% reduction in throughput. Importantly, peptide identifications remain well-distributed across the hydrophobicity spectrum, as measured by the Kyte-Doolittle score, a

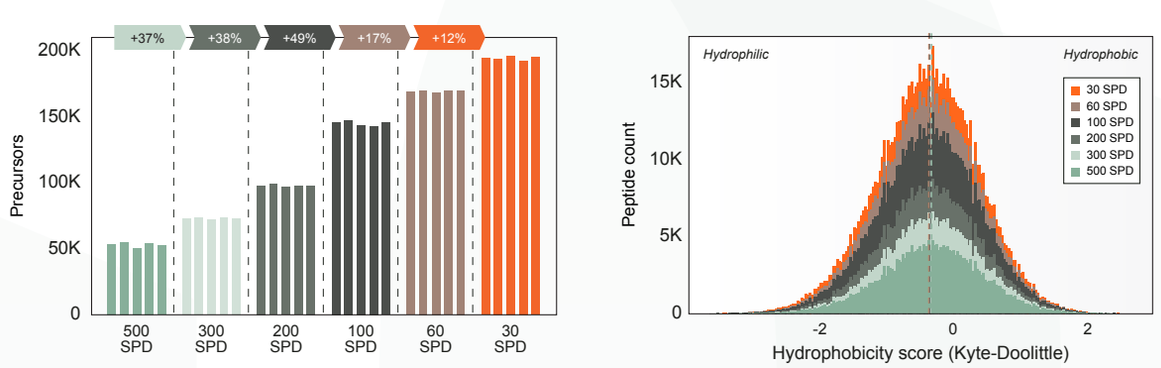

Figure 4: Precursor identifications from 200 ng HeLa peptide (n=5), percentage bars represent increase compare to the faster method before. Histogram represents peptides plotted according to their hydrophobicity. Dotted lines represent median value for each dataset.

commonly used scale, that assigns hydrophobicity values to amino acids to predict peptide behavior in aqueous environments. This even distribution is consistently maintained across all methods, ensuring broad and representative proteome coverage regardless of throughput setting, visualized by the dotted lines, representing the median of hydrophobicity for each SPD method.

6. Quantitative accuracy

The Evosep Eno enables the identification of more than 6,000 proteins with the fastest 500 SPD method with a mean coefficient of variation (CV) of 14.0%, using the Orbitrap Astral MS. The proteome coverage increases linearly across the SPD range, reaching up to 9,240 proteins with the 30 SPD method. Notably, comprehensive proteome coverage of more than 8,000 proteins can be achieved at 100 SPD throughput, with an excellent protein CV of 4.8%, making it a highly attractive option for large-scale screening assays where throughput is a priority. The protein CV remained below 15% across all standard methods at 200 ng peptide input, demonstrating exceptional consistency and data quality. The 30 SPD method achieves a particularly impressive CV of just 3.6%, underlining the analytical performance at low throughput.

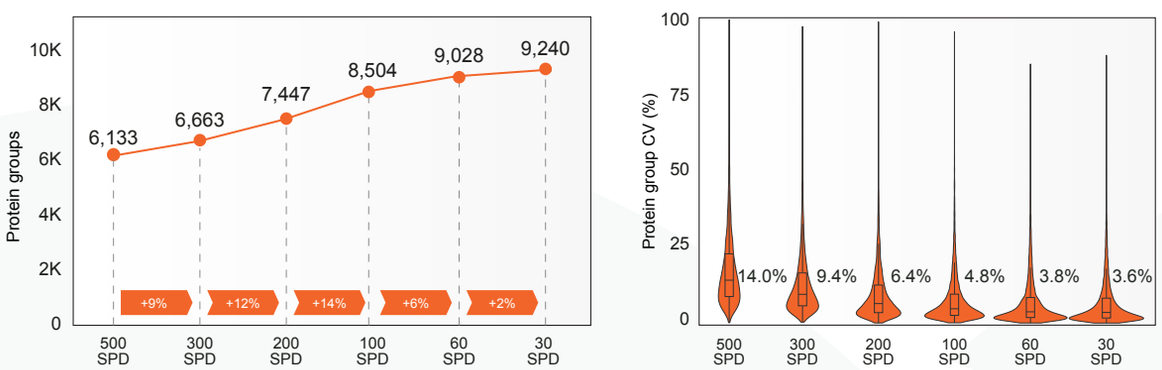

Figure 5: Protein idenfications with Evosep Eno standard methods, and associated CVs, using 200 ng HeLa digest (n=5).

7. Conclusion

With the six standard methods, the Evosep Eno covers a wide range of use cases for LC-MS based proteomics, from comprehensive proteome analysis with close to 10,000 proteins identified to ultra high-throughput single-shot analysis, where the efficiency of the MS is fully utilized with 2,000 proteins identified per minute with the 500 SPD method. Regardless, of the throughput chosen, the standard methods deliver robust chromatography with excellent peak performance as well as high intra- and inter-instrument reproducibility.

As mass spectrometers are likely to become faster and even more sensitive in the future, we expect high-throughput applications to benefit from the higher efficient peak capacity per gradient minute, achieved with the faster methods.

Evosep Eno is for General Laboratory Use.