Application Note

EVOSEP

Evotip Pure simplifies workflows with excellent reproducibility, storage and recovery

Highlights

- Innovative sample introduction and sensitive, long-term storage
- Highly efficient disposable trap column with no carry-over

1. Introduction

The Evosep One enables the analysis of large sample cohorts in a rapid, reproducible, and robust manner. The Evotip is the key feature of this technology, as it simplifies sample preparation by seamlessly integrating desalting - which is typically required in bottom-up proteomics workflows - with LC-MS sample introduction. It is a high precision and well characterized technology with a loading capacity of 1 ug. The Evosep One partially elutes peptides from the Evotip while retaining other large and more hydrophobic molecules that are discarded with the single-use, disposable Evotip. This methodology ensures high system robustness, excellent run-to-run reproducibility, and completely eliminates carryover from the injection system. Altogether, this enables the Evosep to easily switch between different routine analyses without compromising analysis quality. Finally, storage of samples before LC-MS analysis is typically done in tubes, plates, or HPLC vials, resulting in sample losses due to additional sample transfer steps and peptide adsorbance to plastics. In comparison, the Evotip is an integrated part of the workflow, reducing the sample transfer steps, resulting in less exposure of peptides to plastic surfaces once loaded and immobilized on the Evotip. These features reduce losses and improve workflow sensitivity and robustness. Once peptides are loaded on the Evotip, they can be stored and recovered for up to 28 days without lossespaving the way for scalable proteomics. This application note highlights some of these key features of the Evotip, enabling high-throughput robust LC analysis workflows.

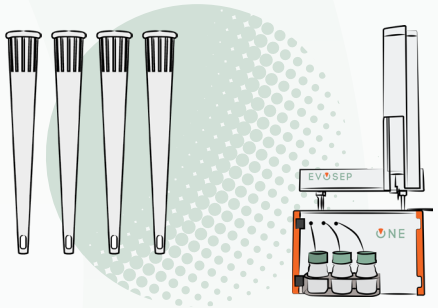

Figure 1: Evotip Pure integrates peptide purification with sample loading and elution.

2. Method details

HeLa and plasma digests were generated using protein aggregation capture assisted digestion. Peptides were desalted on sep-paks and eluate estimated using Nanodrop. Commercially available HeLa digest (Pierce) was used for storage experiments.

For the robustness experiments, 120 µL HeLa digests, plasma digests, and blanks were deposited in a Protein LoBind sample plate (Eppendorf). Five boxes of Evotips were loaded from the same wells with vigorous mixing of the well contents between loadings. The boxes were run sequentially, and the order of analysis was designed to analyze for carryover and instrument robustness as visualized in Figure 2. Samples were analyzed using the 200 SPD method with the EV1107 column (Evosep) at ambient temperature on a ZenoTOF 7600 (SCIEX) system using a 48-variable window DIA method (400-750 m/z) with 11 ms accumulation time (MS/MS) and 25 ms accumulation time (MS) for a cycle time of 0.817 s.

For storage experiments, 50 ng HeLa digest was loaded in quadruplicates submerged in solvent A and stored at 4 °C for up to 28 days. All samples were analyzed back-to-back to minimize variation in MS performance.

The 100 SPD method was used with the EV1109 column (Evosep) operated at 40 °C on a timsTOF pro 2 mass spectrometer using the "dia-PASEF – short gradient" method. For storage experiments, 50 ng Pierce HeLa digests were either loaded directly on Evotips or deposited in a volume of 2 µL in a Protein LoBind sample plate (Eppendorf) and immediately transferred into 18 µL solvent A already present in Evotips during Evotip loading to mimic sample plate-based workflows. Samples were handled with individual pipette tips during all transfer steps. Samples were analyzed with the 100 SPD method using the EV1109 column (Evosep) at 40 °C on a ZenoTOF 7600 system using a 56-window DIA method (400-900 m/z) with 12 ms accumulation time (MS/MS) and 50 ms accumulation time (MS) for a cycle time of 1.023 s.

Data from each condition was processed independently using DIA-NN (version 1.8.1) in library-free mode against the reviewed human proteome (UniProt, Oct 2020, 20,600 entries without isoforms) without MBR (match-between-runs) using standard settings. Protein and precursor identifications represent unique identifications from the DIA-NN matrix outputs.

3. The power of low carryover

The Evotip Pure was assessed for reproducibility and carryover by sequentially injecting blanks and different loads of plasma and HeLa digests as visualized below. In total, 5 boxes of Evotips (480 samples) were analyzed with this sample order using the 200 SPD method. The summed precursor intensity of the different sample types was extremely reproducible across all LC-MS experiments. Consistent, low carryover of 0.2% following 50 ng plasma injections, and 0.06% following 50 ng HeLa injections across the 480 injections. This is possible as peptides are only partially eluted from the Evotip, which is discarded after every sample. Consequently, this allows users to routinely switch between challenging sample types such as plasma and single cells on the same instrumentation.

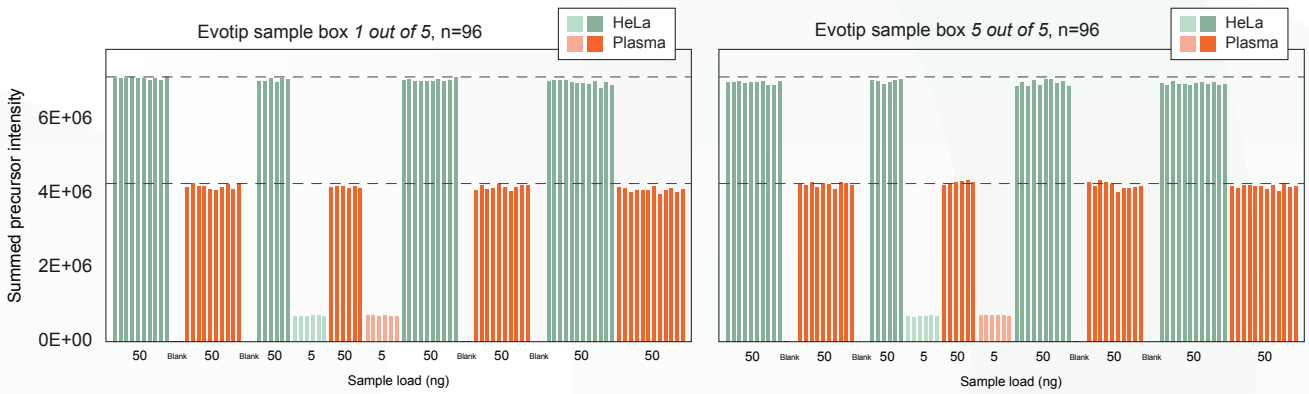

Figure 2: Summed precursor intensity of 50 ng HeLa (n = 190), 50 ng plasma (n = 190), 5 ng HeLa (n = 30), and 5 ng Plasma (n = 30) and blanks (n = 40) injected as visualized.

4. High quantitative precision

Next, we examined the quantitative precision from the previous experiment. Following pairwise log2 correlation of precursor intensities (Pearson, PCC), unsupervised clustering perfectly separates 5 ng and 50 ng injections for both plasma and HeLa samples. The average PCC was larger 0.99 for 50 ng of both HeLa and plasma samples and larger than 0.97 for 5 ng loads. The median and 50 ng plasma were similar when increasing the sample size from a single (n=38) to 5 boxes (n=190) highlighting high quantitative precision within cohorts of hundreds of samples (Figure 3). These results illustrate the reproducibility of the Evosep One workflow based on identification and quantification of diverse sample types.

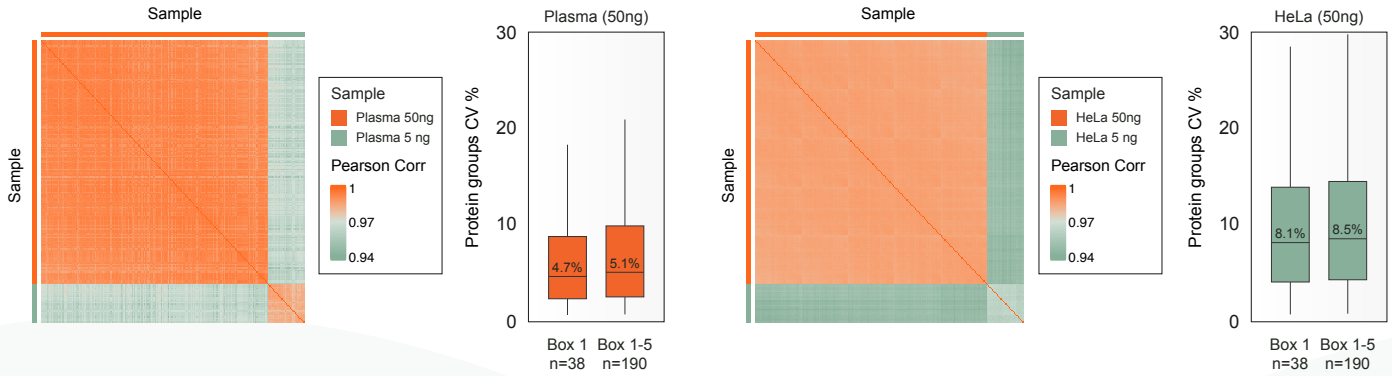

Figure 3: Pairwise log2 Pearson correlation of precursor intensities for all sample types, and CVs of identified protein groups in 50 ng HeLa and plasma samples.

5. System robustness

The Evosep One is robust by design, which can be attributed to the Evotip technology that improves column longevity by partial elution, single-use trap columns, and low carryover. The previous experiment consisted of close to 500 LC-MS experiments using the 200 SPD method at ambient temperature. The backpressure is consistent across the experiments, showing that the analytical column is well protected by partial elution as it eliminates large and hydrophobic molecules to build up over time. 190 of these injections were 50 ng HeLa and by monitoring the retention time of five diagnostic peptides across these runs, we observed excellent run-to-run reproducibility with less than 1.7 seconds standard deviation.

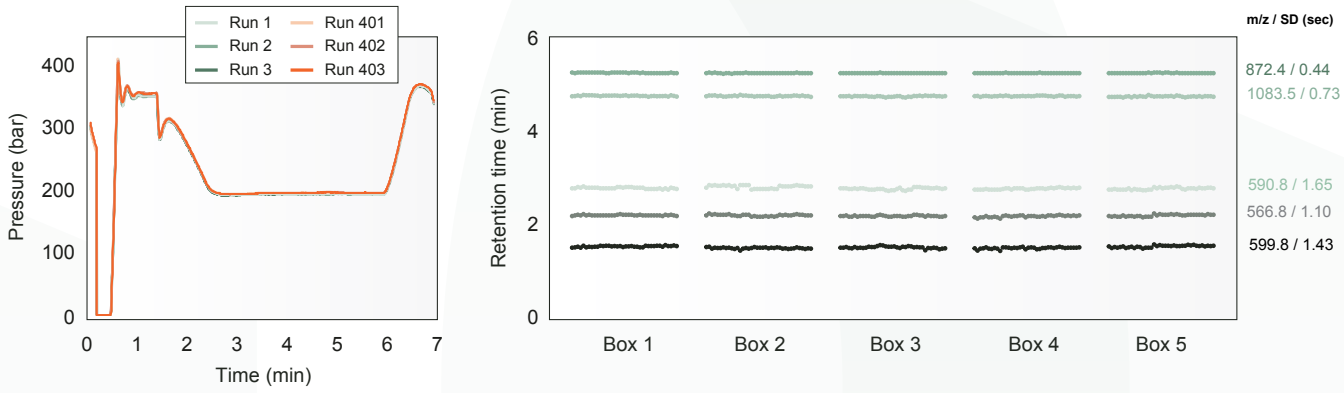

Figure 4: Pressure traces across 400 runs with the 200 SPD method and retention times of 5 monitored HeLa peptides (n = 190) of 50 ng HeLa injections.

6. Innovative sample storage

Each sample transfer step entails a source of variation and sample loss through peptide adsorption and loss of sample volume, which should be considered carefully throughout proteomics workflows. The Evotip reduces the number of sample transfer steps compared to alternative strategies by serving as an integrated sample clean-up and introduction device.
We designed an experiment to measure potential losses associated with traditional autosamplers in comparison to the Evotip workflow. The sample was shortly (few seconds) stored in a sample plate before being loaded on an Evotip. This additional sample transfer step resulted in significant losses even at a relatively high load of 50 ng HeLa digest. As expected, the losses in identifications are more pronounced in the middle and hydrophobic part of the gradient due to peptide losses to hydrophobic surfaces. Thus, the Evotip workflow reduces the number of sample manipulation steps required in proteomics workflows and thereby improves the overall sensitivity and reproducibility. Finally, the ability to store samples for several days is crucial when designing experiments and implementing scalable workflows. To evaluate the added benefit of the Evotip Pure as a temporary storage device for peptide digests until LC-MS analysis, we performed an extended stability study. Evotip Pure was loaded with 5 ng and 50 ng HeLa digest and analyzed immediately and up to 28 days after Evotip loading. The precursor identification rates varied slightly across the 28 days of storage for both 5 ng and 50 ng injections, however, the observed variation is likely due to differences in aliquots used for the different days of sample loading as there was no systematic losses over time. This simple feature makes the Evotip a critical component in scalable workflows by allowing the simultaneous preparation of hundreds of samples before subsequent LC-MS analysis to minimize sample preparation bias.

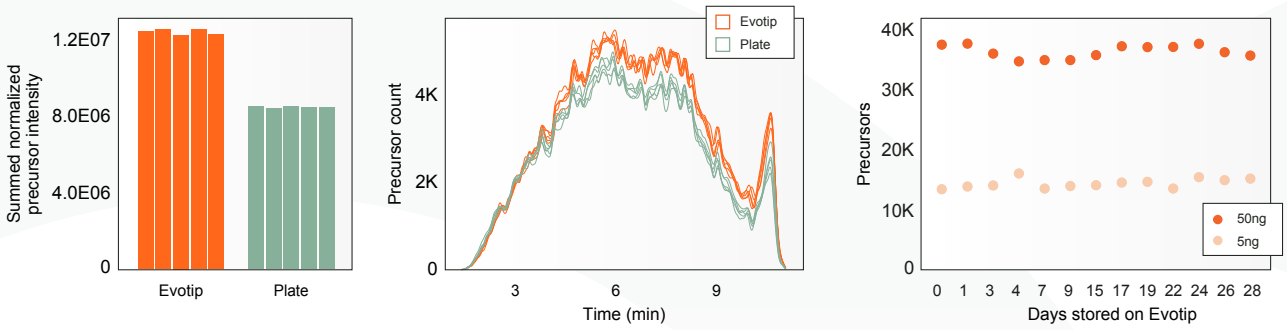

Figure 5: Sample introduction to LC-MS via Evotip Pure and sample plate for 50 ng HeLa and long-term storage data up to 28 days from 5 and 50 ng HeLa, n = 4.

7. Conclusion

The Evotip is the most crucial component of the Evosep One workflow, coupling front-end sample preparation with liquid chromatography and mass spectrometry analysis. During LC-MS analysis, the Evotip is only partially eluted while junk is discarded with the Evotip between each sample. This ensures a clean system and entails consistently low carryover enabling high system robustness and reproducible analyses. The Evosep One can therefore be utilized for a wide range of sample types without compromising data quality and delivers consistent performance across hundreds of replicates. The Evotip workflow reduces sample losses and combined with sensitive long-term storage of samples, the Evotip is essential to scale LC-MS-based proteomics analyses. Finally, Evotip loading can be seamlessly integrated with upstream automated workflows, which is key to the future development of scalable high-throughput proteomics.

Evosep One instrument is for General Laboratory Use.