Application Note

EVOSEP

Biological insights at scale with Evosep Eno - 500 samples per day throughput

Highlights

- >1,000 samples processed in ~2.5 days - from protein sample to LC-MS result.
- Robust analytical setup with exceptional performance.

1. Introduction

With faster and more sensitive LC-MS instrumentation, proteomics has become a central tool in both academic research and routine industrial applications. The robustness and high performance of the analytical pipeline enabled by the Evosep Eno, allow researchers to scale studies to larger cohorts and expand screening conditions to gain deeper biological insight. However, LC-MS based proteomics depends on more than the individual technologies – it relies on the integrity of the entire workflow, from sample preparation to data acquisition. To achieve next-generation insights through LC-MS based proteomics, it is essential to maintain sensitivity throughout the workflow and maximize sample recovery. In this context, the Evotip Pure plays a pivotal role in preserving workflow sensitivity, while enabling cost-efficient and scalable analysis of large cohorts.

The Evotip simplifies sample preparation, and simultanously provides seamless integraton with automation allowing for scalable pipelines. Here, we present a fully automated digestion workflow using just 200 ng protein starting amount, featuring in-line sample loading onto Evotips. The protocol is implemented on the Beckman Coulter i7 liquid handler, allowing the routine preparation of 96 samples in 6.5 hours, and up to 1,056 samples in parallel within 9.5 hours. When combined with the 500 SPD method on the Evosep Eno, this end-to-end workflow enables processing - from sample to LC-MS result - in 11 hours for 96 samples, and ~60 hours for 1,056 samples. This complete workflow provides a scalable solution for fully automated LC-MS based proteomics delivering high sensitivity and cost-efficiency.

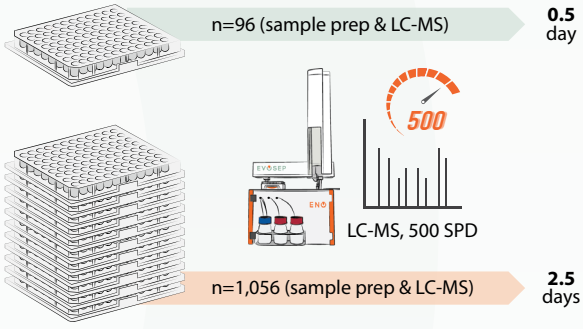

Figure 1: Scalable workflow strategy.

2. Method details

The Biomek i7 MC (Beckman Coulter Life Sciences) liquid handler was used with a 1200 µl multichannel head, the BioShake (D30-T elm, Q-Instruments) and the Magnum FLX magnet plate (Alpaqua) modules. An Evotip loading kit for Biomek i5/i7 1200 µL 96-Channel head (EV1183, Evosep) was used for direct loading. HeLa cells were cultured in DMEM media and harvested in boiling 5% sodium dodecyl sulfate buffer. Protein aggregation capture (PAC) assisted digestion was prepared from 200 ng, 500 ng or 1 µg HeLa lysate respectively, and 5 µl MagReSyn Hydroxyl beads (Resyn Biosciences), both transferred to each well of the sample plate(s) along with isopropanol (IPA) to a final concentration of 80%[1]. Two mixing steps were carried out to facilitate on-bead aggregation for 10 minutes, followed by a single wash in IPA. Digestion was performed at ambient temperature for 6 hours using 10 ng Lys-C (Fujifilm) and 40 ng trypsin (Sigma Aldrich) in 25 mM TEAB. Following digestion, samples were diluted and loaded onto Evotips. The standard methods on the Evosep Eno were used for analysis combined with specified Evosep Performance columns. The EV1137 column was used for the 30 SPD method, the EV1109 for the 60 & 100 SPD methods, and the EV1182 column for the 200-500 SPD methods. All columns were heated to 40 °C, using the Evosep Pod column oven (EV1187), and the samples were analyzed using an Orbitrap Astral mass spectrometer (Thermo Scientific). Spray voltage was set to 1900 V, and heated capillary temperature at 275 °C. The mass spectrometer was operated at a full MS resolution of 240,000 with a full scan range of 380 − 980 m/z. The full MS AGC was set to 500%. MS/MS scans were recorded with 3 Th isolation window, 7 ms maximum ion injection time. MS/MS scan range from 380-980 m/z were used. The isolated ions were fragmented using HCD with 25% NCE. Data was analyzed with DIA-NN (version 1.8.1) in library-free mode against the reviewed human proteome database (Uniprot, Oct 2020, 20,600 entries without isoforms) with trypsin/P as digestion enzyme allowing 2 missed cleavages. All conditions were searched separately with match between runs enabled across replicates within the same condition.

3. High efficiency with 500 SPD

The performance of the sample preparation workflow was evaluated across the six standard methods on the Evosep Eno. The 30 SPD method delivered the deepest proteome coverage, identifying 9,000 proteins. Notably, the 500 SPD method excelled in identifying more than 5,000 proteins, corresponding to almost 2,000 unique proteins per minute. As anticipated, the remaining SPD methods, displayed a linear relationship between proteome depth and throughput. Importantly, the LC-MS efficiency, measured as the number of protein groups identified per minute also increases in a linear fashion with throughput. All methods exhibited excellent quantitative precision with

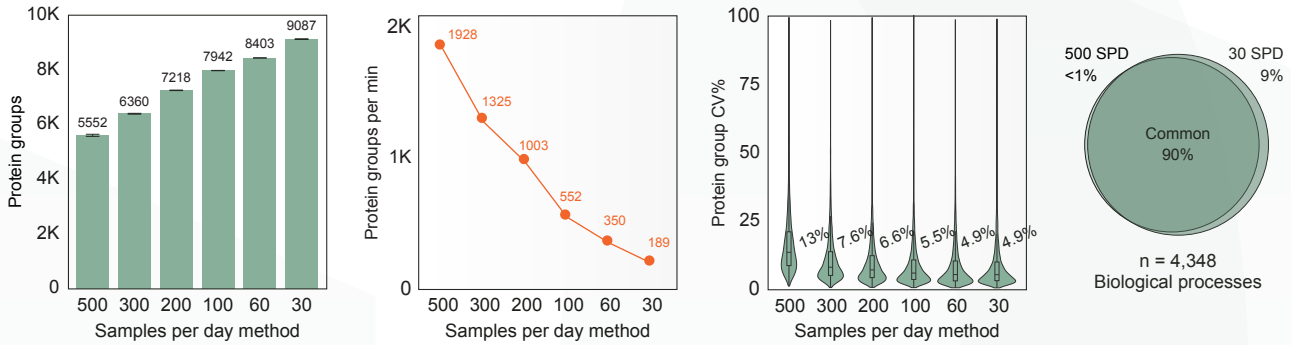

Figure 2: Average protein groups, protein groups per minutes and protein group CV (n=5) across all standard methods, and overlap of biological processes from 30 and 500 SPD methods.

median coefficient of variation (CV) of 5-13%. The data underlines the impressive quantitative performance of the 500 SPD method, even when used in discovery mode, which crucially does not come at the expense of biological insight. This is highlighted by comparable analysis of identified proteins from the 500 and 30 SPD methods, and their involvement in cellular functions and activities. Based on biological processes, the gene ontology analysis revealed an overlap of 90% between the two methods, highligting that throughput can be increased 15-fold, while maintaining functionally relevant proteome coverage.

4. Complete workflow scalability

Achieving LC-MS throughput of 500 samples per day unlocks new opportunities for experimental design. Screening of large number of experimental conditions is now possible and large sample cohorts can be analyzed in short time. To address the need for scalable sample preparation, we designed an experiment, processing 1,056 samples in parallel using the Biomek i7 liquid handler. The total sample preparation time from protein lysate to ready-to-analyze Evotips, was just 9.5 hours, including a 6-hour digestion at ambient temperature corresponding to only 30 seconds per sample. In this experiment, 200, 500 or 1000 ng HeLa protein lysate were used as input materials across the 11 sample plates. Despite the ambient digestion conditions, a consistent digestion efficiency of 75% was achieved across all 11 sample plates. The sensitivity of the workflow was demonstrated by identifying 4,600 protein groups from just 200 ng of protein input and 5,500 protein groups from 1 ug of protein starting amount. Both conditions were analyzed using 500 SPD and with loading 60% of the resulting peptide digest onto Evotips. The reproducibility of the workflow was high, with median (CVs) between 16% and 18% depending on the protein input. Altogether, these results showcase a cost-efficient, scalable analytical pipeline from sample to LC-MS requiring only minute protein input, enabling routine, large-scale proteomics with high sensitivity, precision, robustness and high throughput.

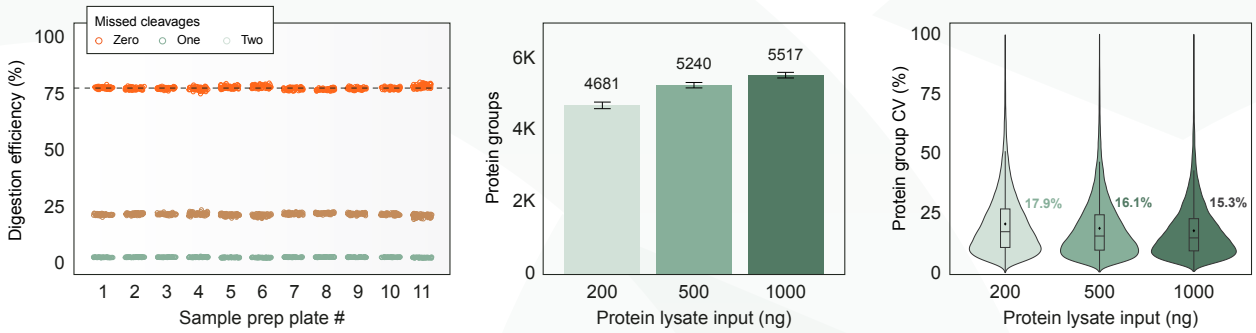

Figure 3: Digestion efficiency across 11 sample plates (n=1,056), identified protein groups and associated inter-plate CVs for 200, 500, and 1000 ng protein lysate input respectively, analyzed with 500 SPD.

5. Robust LC performance

A key requirement for sustaining 500 samples per day throughput is the reliability and robustness of the LC. At this scale, any disruption or instability in the analytical setup will quickly compromise data integrity. To evaluate the robustness of Evosep Eno, we monitored the LC performance across the 1,056 samples, using the 500 SPD method. The system demonstrated excellent retention time stability across the consecutive workflow samples with a standard deviation of 0.75 seconds across all injections. In addition, the high-pressure (HP) pump maintained extremely stable pressure with just 2.5 bars standard deviation throughout the analysis. Overall, this data highlights the robustness of the Evosep Eno for actual workflow samples.

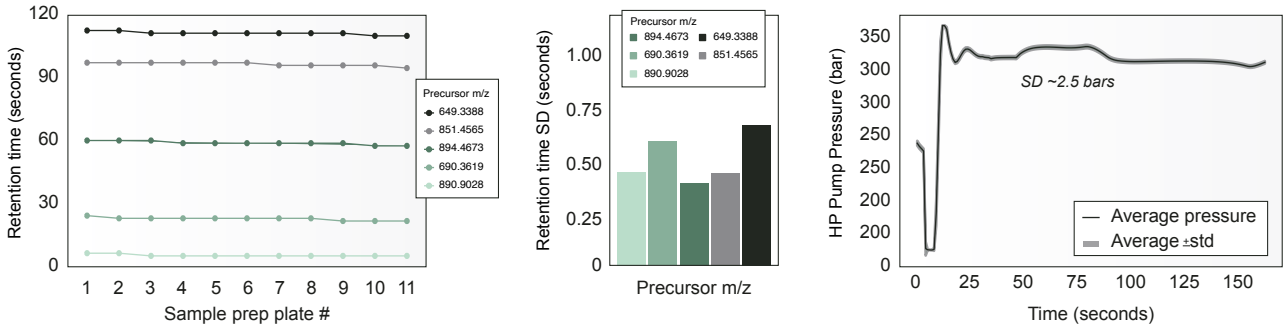

Figure 4: Retention time stability and associated standard deviation, together with the HP pump pressure stability of 1,056 consecutive HeLa workflow sample runs with 500 SPD.

6. Conclusion

Next generation LC-MS based proteomics builds upon a decade of exploratory research and technology developments. The next step is about bridging the gap from technology to application, where scalability, cost-efficiency and ease-of-use are critical to success. This integrated workflow strategy for LC-MS based proteomics addresses these demands by enabling exceptional throughput for processing 1,056 samples, from protein lysate to LC-MS result in just 2.5 days, with minimal hands-on time. This is achieved through parallel sample preparation of 11 plates using the Biomek i7 liquid handler coupled with the 500 SPD method on the Evosep Eno. The obtained results showed high inter- and intraplate reproducibility with consistent digestion efficiency across the 11 sample plates and protein CVs of less than 18% from just 200 ng protein input. Moreover, the Evosep Eno demonstrated exceptional robustness with retention time reproducibility of 0.75 sec standard deviation and robust HP pump pressure with standard deviation of 2.5 bars across >1,000 consecutive runs.

This automation strategy not only ensures scalable, high throughput proteomics on the Evosep Eno, but also lays the foundation for future expansion into even larger studies. An important point, not addressed here, it is important to consider the downstream data processing pipeline, where scalability and hands-off operation are equally critical parameters to enable ultra high-throughput proteomics.

Evosep Eno and Evosep Pod are for General Laboratory Use.

Evosep assumes no responsibility and shall have no liability for any damage or loss of samples, material and hardware that may arise from use of or in connection with Evosep recommended protocols and SOP's.

References

1. Evosep application note, AN-034 (2024) Scalability for high-throughput proteomics - Evotip Pure integration with the Biomek i5 liquid handler