Application note

EVOSEP

The proteomics dilemma - High throughput analysis versus proteome depth

1. Introduction

Mass spectrometry (MS)-based proteomics is fast growing and provides a powerful set of technologies, with the potential to revolutionize health care and enable precision medicine. This is among other things possible due to a leap of performance over the last years including improved mass accuracy and significantly increased scan speed and sensitivity of modern mass spectrometers. To further integrate the technology into clinical settings, we launched the Evosep One in 2017, which features a novel and extremely robust way to pre-form and elute one of five different gradients. The corresponding five standard methods ensure throughput, robustness, reproducibility and ease of use. They cover a range of use cases from ultra high throughput analysis with 300 samples per day to more comprehensive proteome analysis with only 30 samples analyzed per day (Figure 1).

Figure 1: The five standard methods with a throughput of up to 300 samples analyzed per day.

2. Minimal overhead time

The Evosep One is designed for throughput and robustness, while maintaining sensitivity for proteomics applications. Low-pressure pumps elute the sample from a disposable trap column, simultaneously forming a chromatographic gradient that is stored in a long storage loop. An auxiliary gradient creates an offset, ensuring efficient re-focusing of the peptides before the separation on the analytical column by a single high-pressure pump. This simplified design enables robust operation over tens of thousands of sample injections. Furthermore, the steps between injections are performed in parallel, reducing overhead time to only 1.6 minutes on the shortest methods and 4 minutes on the longer gradientes allowing for very high MS utilization time (Figure 2).

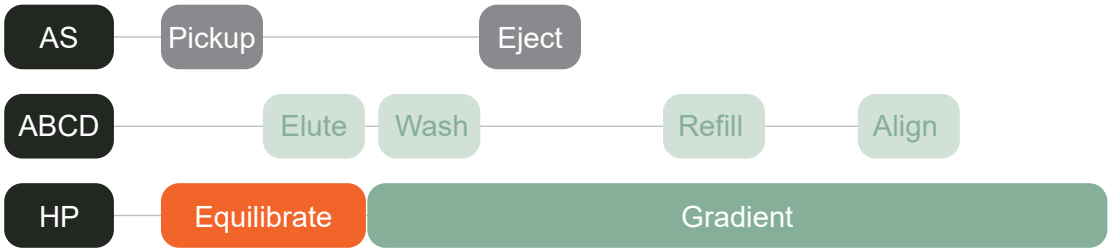
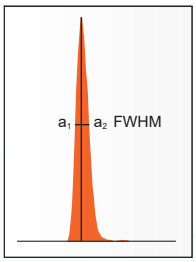


Figure 2: The steps between injections are performed in parallel to reduce overhead time.

3. High separation power

Peak capacity is the most common measure of chromatographic performance and is defined as the number of peaks, that can be resolved over a gradient. Here, we defined the peak capacity as the total number of peaks, that can be separated at full-width-half-maximum (FWHM) within an elution window, which is defined by the retention time window from the first to the last identified precursor. Longer gradients and longer columns are generally used to increase peak capacity (Figure 3), but there is not a linear correlation, and the longer gradient times reduce the sample throughput, which is a crucial factor for clinical analysis. For high throughput analysis of complex samples, highest peak capacity in the shortest time is a relevant measure. This is achieved by ultra fast methods employing short columns and high flow rates providing the best separation power per gradient minute (Figure 3) made affordable by the low overhead time. Importantly, the narrower peaks greatly reduce redundant sampling thus suggesting that the sensitivity of the analysis is boosted by employing the faster standard methods.



$$\text{Peak capacity} = 1 + \frac{\text{retention time window}}{\text{median FWHM}}$$

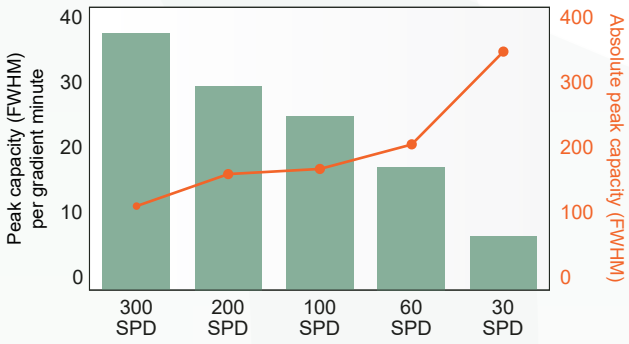


Figure 3: Peak capacity per gradient minute for each method (green) and absolute peak capacity for each method (orange).

5. Method details

HeLa cells were harvested and 500 ng of digested peptides were loaded on Evotips. Technical quadruplicates were analyzed on an Orbitrap Exploris 480 MS (Thermo Scientific). with method details as outlined below (Table 1).

Recommended columns were used at ambient temperature in combination with a stainless steel emitter. Raw data was analyzed with the Spectromine software, version 2.

Samples per Day	300	200	100	60	30
Column	4 cm x 150 µm	4 cm x 150 µm	8 cm x 150 µm	8 cm x 150 µm	15 cm x 150 µm
Packing material	1.9 µm beads	1.9 µm beads	1.5 µm beads	1.5 µm beads	1.9 µm beads
MS method length	3.2 min	4.6 min	11.5 min	21 min	44 min
MS2 resolution	7500	7500	15,000	15,000	15,000
MS2 injection time	11 ms	11 ms	22 ms	22 ms	22 ms

Table 1: Method details for the experiment.

6. Identifications

Good chromatographic quality is essential, and especially to fully employ the high MS/MS scan rates on modern instruments. While the mass spectrometer accounts for mass accuracy and cycle times, the applied chromatographic performance determines the number of peptides available for ionization. Here the number of co-eluting peptides additionally impact the efficiency of the electrospray ionization (ESI) due to competition for space and charge leading to suppressive effects. All these factors directly affect the amount of detectable peptides and, hence, the number of identifiable proteins. Longer gradients increase the number of peptides and proteins with almost 4000 proteins identified from 500 ng of HeLa peptide analyzed with the 30 samples per day method on an Orbitrap Exploris 480 MS (Thermo Scientific) with data-dependent acquisition (Figure 4). Whereas in the past, maximum proteome coverage was usually the goal, faster instrumentation has shifted the priorities toward analysis throughput with more biological replicates.

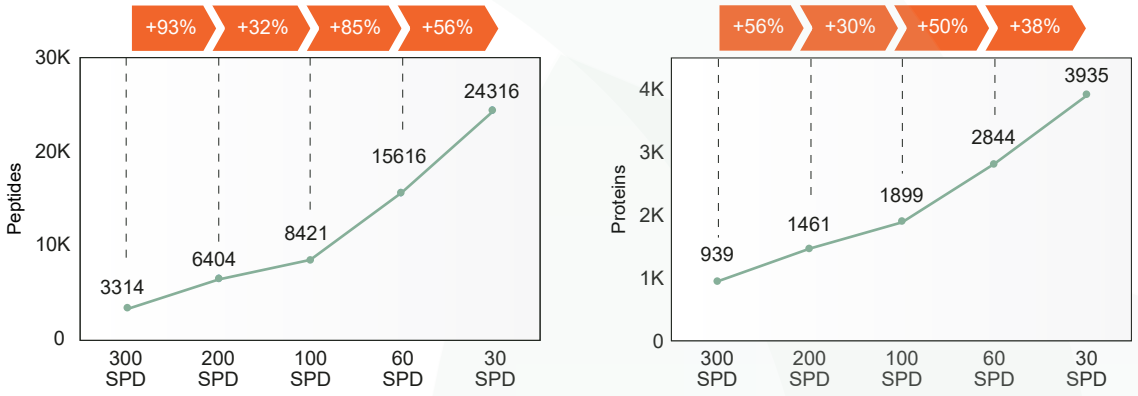


Figure 4: Average peptide and protein identifications from quadruplicate samples loaded with 500 ng peptide. Orange bars indicate percentage increase compared to the faster method before.

This underlines the perspectives of proteome depth versus sample throughput. Almost a thousand proteins are identified in only 3 min gradient time (300 SPD) with an average of three peptides identified per protein. A significant increase in peptide coverage of 93% is observed when decreasing the throughput from 300 to 200 samples per day, whereas only an additional 32% peptides are identified when the throughput is further decreased to 100 samples

per day. A close to linear trend is observed for both peptide and proteome depth throughout the range of standard methods (Figure 4). To achieve the highest protein coverage with short LC-MS time, multidimensional fractionation strategies can be employed with the fastest standard methods and fast scanning MS/MS methods. In general, running many fractions on short gradients is the best compromise between instrument time used and sequencing depth obtained. For high-throughput applications, the 200 samples per day method is an excellent candidate to take advantage of the high sequencing speed, which is reflcted by the number of peptides and proteins identified per minute (Figure 5), whereas the 60 samples per day method provide a good compromise between speed and proteome depth.

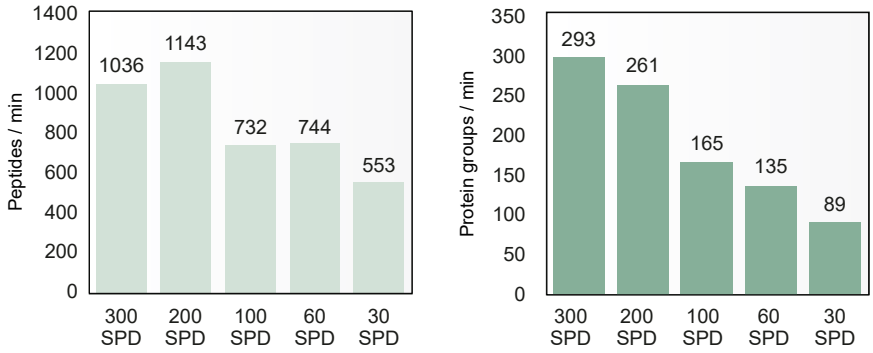


Figure 5: Peptide and protein identifications per minute from samples loaded with 500 ng peptide.

7. Conclusion

With the five standard methods, the Evosep One covers a range of use cases from comprehensive proteome analysis with fractionation strategies to ultra high-throughput single-shot analysis. With each method presented in relation to each other in terms of identifications of peptides and proteins, it is important for the single researcher to carefully review the optimal level of proteome coverage in relation to sample throughput before starting a series of experiments. 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 of the shorter methods.

References

1. Bache N., Geyer PE., Bekker-Jensen DB., Hoerning O., Falkenby L., Treit PV., Doll S., Paron I., Müller JB., Meier F., Olsen JV., Vorm O., Mann M. (2018) A novel LC system embeds analytes in preformed gradients for rapid, ultra-robust proteomics. Mol Cell Proteomics., mcp.TIR118.000853