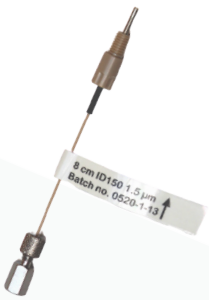
Application Note

EVOSEP

Improve and enhance your proteome coverage with the **Performance column**

1. Introduction

All Evosep One methods are carefully optimized for specific columns to provide the best compromise between performance, robustness and throughput. We have now developed an additional Performance column for the 60 and 100 samples per day methods (SPD) to address applications with high demands. This is packed with smaller 1.5 µm C18 particles compared to the standard column with 3 µm particles. Separation efficiency is inversely proportional to the particle size resulting in increased separation efficiency for the Performance column. However, this higher column efficiency comes at the cost of higher backpressure. By also increasing the internal diameter (ID) to 150 µm compared to the standard column with an ID of 100 µm, the backpressure is again decreased to a reasonable level at ambient temperature (Figure 1). Thus, the Performance column is the ideal choice for applications where excellent chromatographic separation is the goal.



Standard column
(8 cm, 3 µm x 100 µm)
EV-1064

Performance column
(8 cm, 1.5 µm x 150 µm)
EV-1109

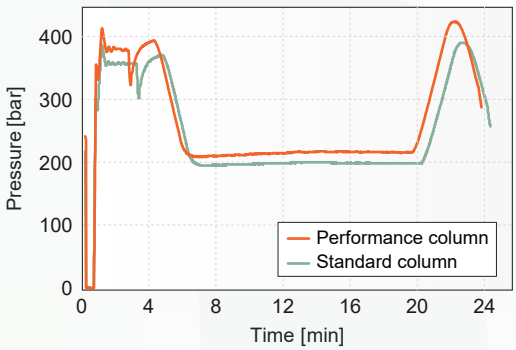


Figure 1: Column specifications for the standard and performance columns and gradient HP pressure profiles for the column using 60 SPD.

2. Method details

Digested HeLa peptides were diluted and loaded on Evotips and analyzed in data-dependent acquisition mode on an Orbitrap Exploris 480 MS (Thermo Scientific) with method details as outlined below (Table 1). Technical quadruplicates were analyzed on both the standard column and performance column with 60 and 100 SPD. Columns were used at ambient temperature in combination with a stainless steel emitter. Raw data was analyzed with the Spectromine software, version 2.

Peptide load [ng]	5	10	25	50	10	25	50
MS2 resolution	45,000	30,000	15,000	15,000	15,000	15,000	15,000
MS2 injection time	86 ms	54 ms	11 ms	11 ms	11 ms	11 ms	11 ms

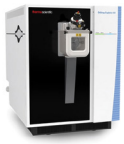


Table 1: Method details for the experiment.

3. Chromatographic improvement

Particle size has a significant impact on efficiency and the width of peaks, which decreases. As peak width becomes narrower, the height of that peak increases proportionally. Narrower peaks that are taller, are easier to detect and differentiate from baseline noise, resulting in higher sensitivity (Figure 2). This is visualized in the example extracted ion chromatogram below from the same peptide with both columns using 60 SPD. The peak widths are significantly decreased with the performance column. The resolving power of a gradient can be calculated by its peak capacity. Thus, peak capacity is simply the theoretical number of peaks that can be separated in a given gradient time. As peak capacity is inversely proportional to peak width, this is increased with 47% and 59% for 100 and 60 SPD respectively (Figure 2).

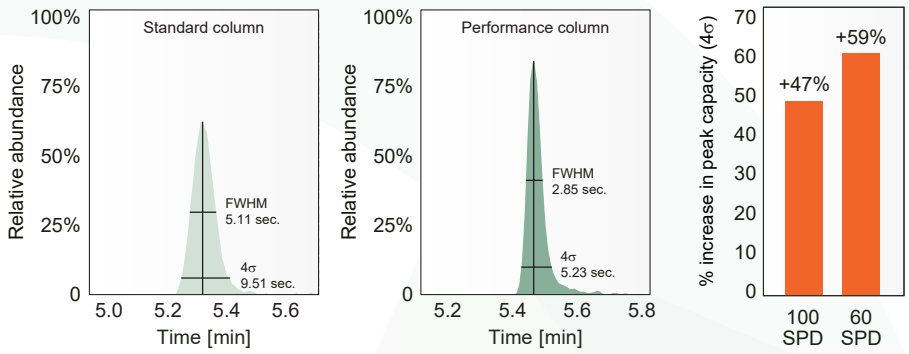


Figure 2: Extracted base peak chromatogram of BSA peptide 722. Relative increase in peak capacity compared to the standard column.

4. Boost in identifications

Increased peak capacity and better resolving power minimizes ion suppression and improves ionization efficiency. The process leads to better peptide and protein identification, which is especially important for high-end mass spectrometry applications. We compared the two columns with a dilution series representing two orders of magnitude from 5 to 500 ng peptide loaded and analyzed it with 60 and 100 SPD. An increase in peptide and protein identifications with the performance column is observed at all loads for both methods. Interestingly, the

highest increase in identifications are observed with the lower loads, suggesting that these loads benefit significantly more from narrow peaks due to the increase in signal to noise. Notably, more than a 100% improvement was found for the lowest load of 5 ng on both peptide and protein level with 60 SPD. In general, the relative boost in identifications are highest with the lowest load, but a 10% increase in proteins identified for the highest-load of 500 ng with 60 SPD correspond to an additional 200 proteins. With more than 2800 unique protein coding genes, the Performance column provides enhanced proteome coverage on the same level as home-pulled and packed columns, but with better reproducibility and life-time.

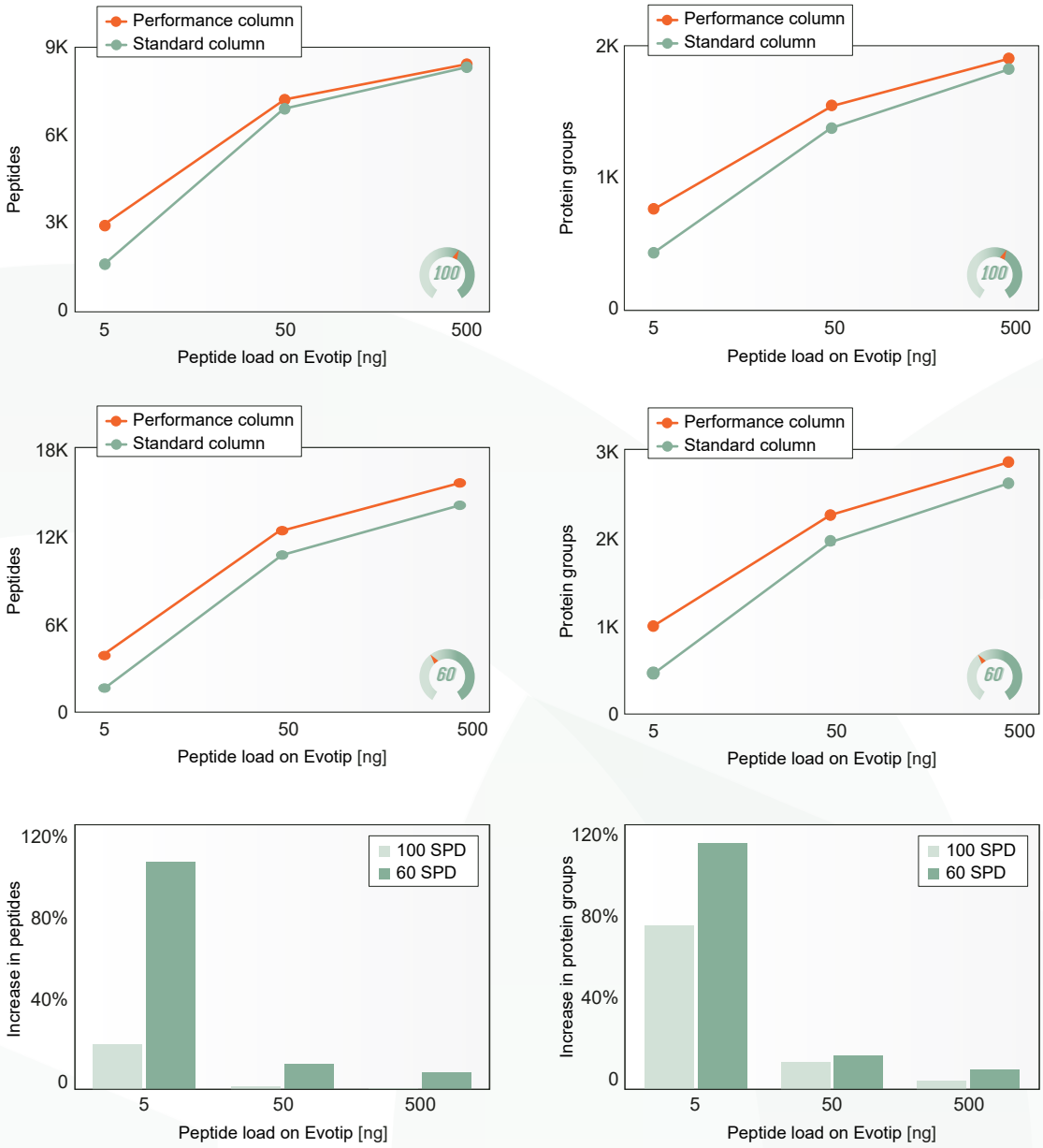


Figure 3: Identification of peptide and proteins and increase in coverage with the Performance column compared to the standard column.

6. Conclusion

The performance column is available for 60 and 100 SPD and is packed with 1.5 µm C18 beads. This reduces the peak widths and leads to an increase in peak capacity. Consequently, the proteome coverage is enhanced, and the column provides a robust and competitive commercial option. The boost in sensitivity is particularly noticeable for lower loads, where peptide and protein coverage is improved with up to 100%. It is important to consider the appropriate column choice for the desired experiment with the characteristics needed to achieve the goal. The Performance column utilizes the speed of modern mass spectrometers, whereas the standard column works well for applications, which benefit from slightly broader peaks such as targeted workflows.

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

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