Application Note

EVOSEP

Improve and enhance your proteome coverage with the **Performance column** for our long methods

1. Introduction

The Evosep Performance Column product line offers improved peptide identification rates. The 30 samples per day method and the Extended method is supported by, the matched performance column (EV1137) featuring a 15 cm column with an inner diameter of 150 µm packed with 1.5 µm C18 beads, increasing the separation efficiency over the corresponding Endurance column (EV1106). The smaller beads lead to a higher backpressure and it is therefore needed to maintain a column temperature of 40 °C to ensure a reasonable backpressure during the high flow equilibration phase, whereas the Endurance column is operated at ambient temperature (~22 °C) (Figure 1).

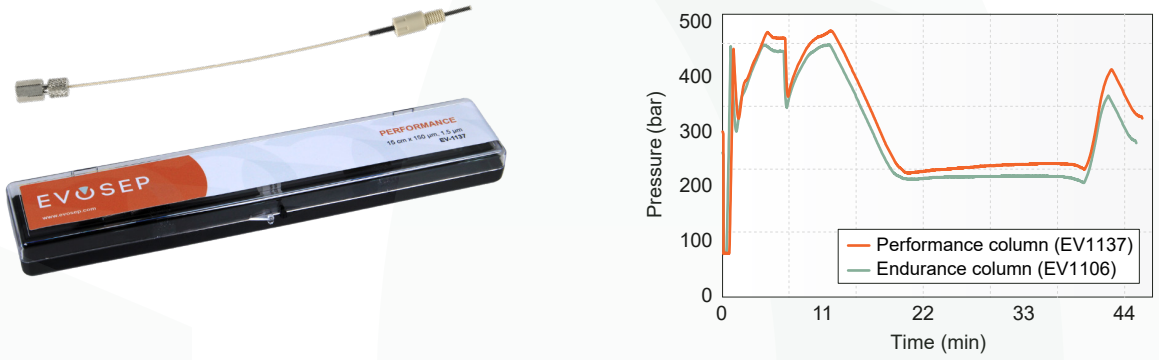

Figure 1: Gradient HP pressure profiles for the Performance column using 30 SPD.

2. Method details

HeLa digest was purchased from Pierce and loaded on Evotip Pure. Samples were analyzed in ddaPASEF mode on an timsTOF Pro 2 mass spectrometer (Bruker) using standard methods. The MS method for short gradients with 0.5 seconds cycle time was used for the 30 SPD method, whereas the cycle time was 1.1 seconds for the Extended method. Technical quintuplicates were analyzed with both the Endurance column (~22 °C) and the Performance column (40 °C) with 30 SPD and the Extended method using a 10 um Captive Spray Emitter ZDV (1865691). Raw data was analyzed with the MaxQuant software, version 2.0.1 with standard settings with LFQ ratio count set to 1. The quintuplicates were searched in batches of five with the “Match between runs” feature enabled.

3. Chromatographic improvement

Smaller beads improve chromatographic performance as peak width becomes narrower and the height of that peak increases proportionally, which results in higher sensitivity. The peak widths are significantly decreased with the performance column, which is shown in the example extracted ion chromatograms below, where BSA digest is analyzed with both columns using the 30 SPD method (Figure 2).

The performance column provides median peak width of 4.5 seconds resulting in 45% slimmer peaks than with the Endurance 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 80% for the 30 SPD method.

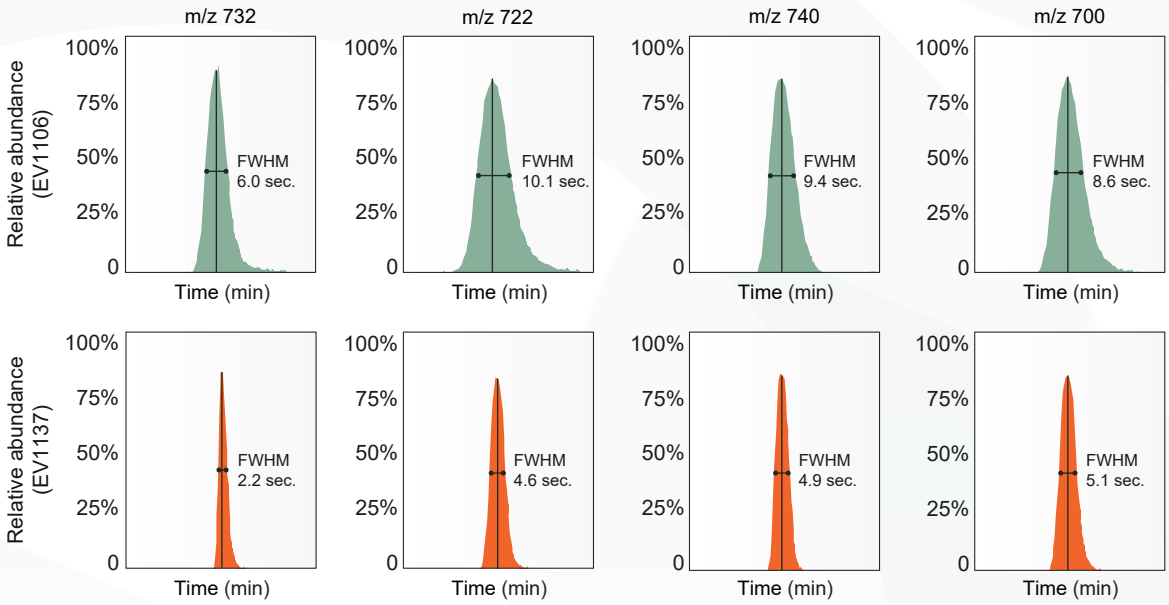

Figure 2: Extracted base peak chromatograms and FWHM of BSA peptides m/z 732, 722, 740 and 700 for Performance and Endurance columns using 30 SPD.

4. Boost in identifications

The smaller beads in the performance column require heating at 40 °C to maintain a reasonable backpressure. Thus the 30 SPD and Extended methods are optimized for effective peptide separation at both temperatures (Figure 3)

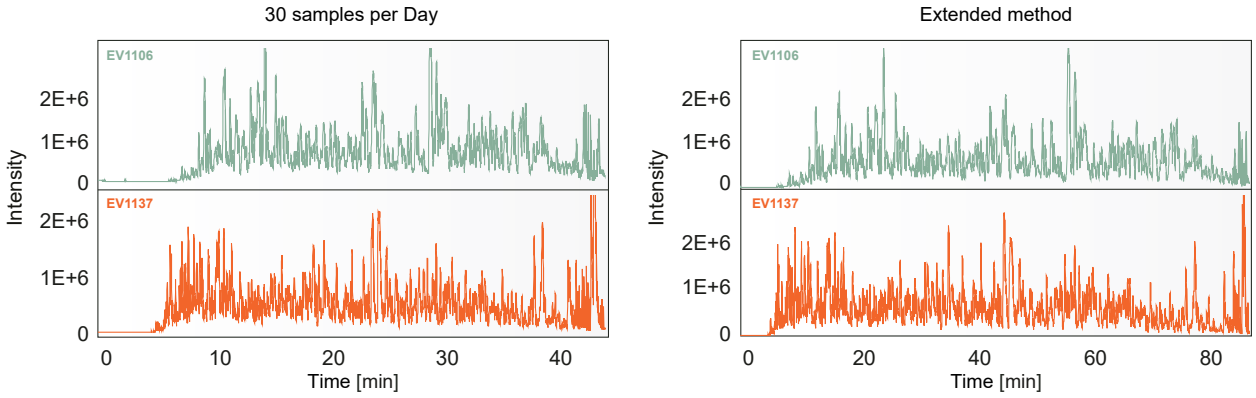

Figure 3: Base peak chromatograms of 400 ng peptide analyzed with the 30 SPD and Extended methods using the Endurance and Performance columns.

We compared the two columns by analyzing 400 ng HeLa peptide loaded on Evotips in quintuplicates with 30 SPD and the Extended method. We observed a relative boost in proteome coverage using the Performance column with both methods. For the 30 SPD method, 33% more peptides were identified with the Performance column, corresponding to 4,200 and 5,200 proteins respectively. Identifications from match-between-runs are indicated in light green (Figure 4). This is further increased by using our longest method, the Extended method with a gradient length of 88 minutes. Again, a relative boost in identifications is observed with nearly 30% more peptides using the Performance column compared to the Endurance column. This results in more than 30,000 peptides corresponding to 6,000 proteins.

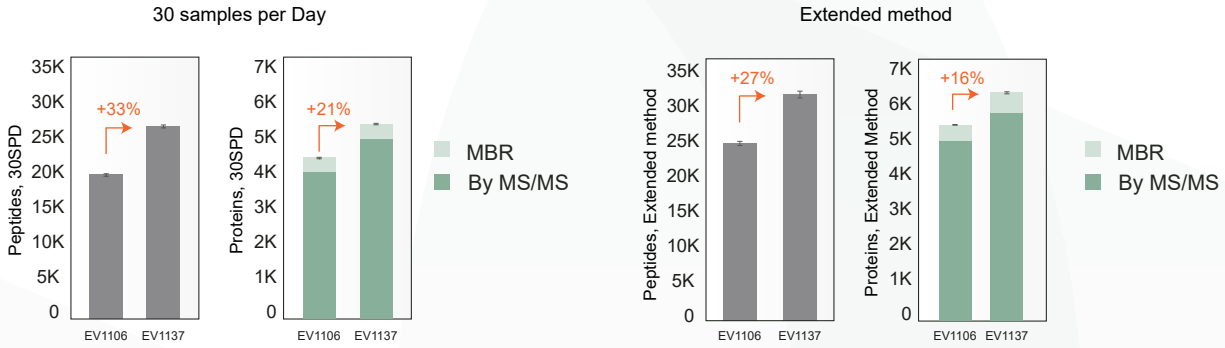

Figure 4: Identification of peptides and proteins and increase in coverage with the Performance column compared to the Endurance column.

5. Conclusion

The Performance column (EV1137) is available for the 30 SPD and Extended method and is packed with 1.5 µm C18 beads. This reduces the peak widths with nearly 50% and leads to an increase in peak capacity. Consequently, the proteome coverage is enhanced leading to 5,000 proteins identified with the 30 SPD method and 6,000 for the Extended method in ddaPASEF mode. These absolute identifications rely on the software used for analysis. We used MaxQuant for this analysis, well aware that other software tools can provide different number of identifications. The performance column described here is recommended for our longer methods, where best peak performance and maximum proteome coverage is desired.

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

Data Courtesy

Marvin Thielert, Andreas-David Brunner and Matthias Mann

Max Planck Insitute for Biochemistry, Munich, Germany

Ordering information

P/N	PART	DESCRIPTION
EV1106	Endurance Column 15 cm x 150 µm ID, 1.9 µm	Used by the 30 SPD & Extended methods. Analytical column with pre- mounted connection fittings. ReproSil-Pur C18, 1.9 µm beads by Dr Maisch.
EV1137	Performance Column 15 cm x 150 µm ID, 1.5 µm	Used by the 30 SPD & Extended methods. Analytical column with pre- mounted connection fittings. ReproSil Saphir C18, 1.5 µm beads by Dr Maisch.