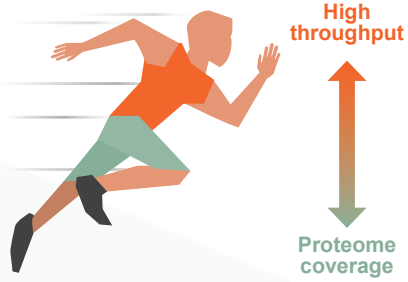

# How far will you go?
## The five standard methods for the Evosep One

### Built for standardization

The Evosep One is designed for throughput and robustness, while maintaining sensitivity for proteomics applications. The five standard methods 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). It provides a standardized solution with excellent reproducibility, which can be fully achieved by using one of our Evosep columns. They deliver sharp symmetrical peaks and each analytical column can routinely analyze thousands of samples. In this booklet, we have gathered an overview of the five standard methods and listed performance and reproducibility to expect from each method, when using the method dependent Endurance columns.




| | Gradient Length (min) | Cycle Time (min) | Flow Rate (µl/min) | |
|---|---|---|---|---|
| 300 | 3.2 | 4.8 | 4.0 | Page 2 |
| 200 | 5.6 | 7.2 | 2.0 | Page 4 |
| 100 | 11.5 | 14.4 | 1.5 | Page 6 |
| 60 | 21.0 | 24.0 | 1.0 | Page 8 |
| 30 | 44.0 | 48.0 | 0.5 | Page 10 |

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

# Towards a Standardized Omics Platform with the **300 samples per day** method

## 1. Introduction

This is the fastest of our standard proteomics methods with a throughput of 300 samples per day. The method has a 3.2 minute gradient and a cycle time of 4.8 minutes. It is designed for our EV1107 Endurance column, and we highly recommend to use this column with our range of emitters and spray adapters for optimal performance.

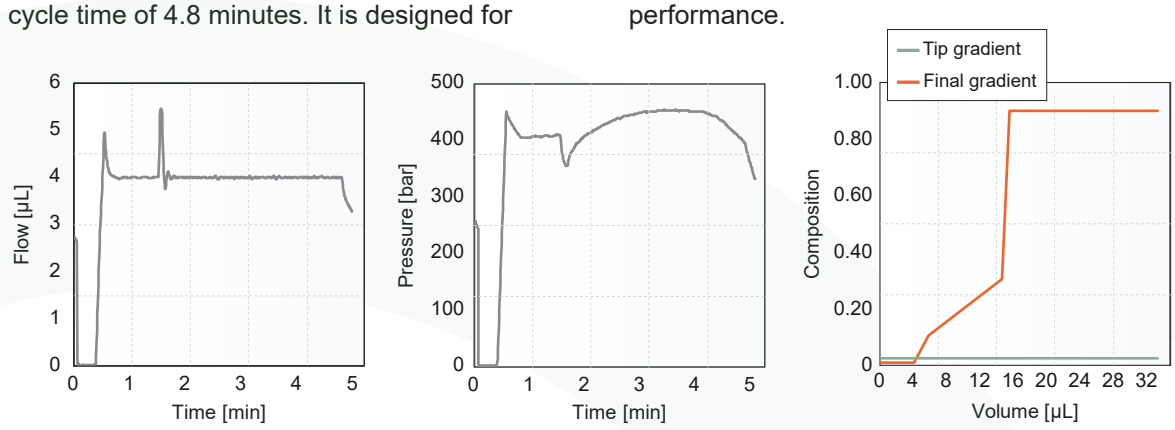



*Figure 1:* Pump HP flow profile, pump HP pressure profile and gradient of the 300 samples per day method.

## 2. Chromatographic performance

By taking advantage of the loading-dilution strategy, we maximize the chromatographic performance with this very short gradient. From a HeLa digest analysis, we calculated peak and

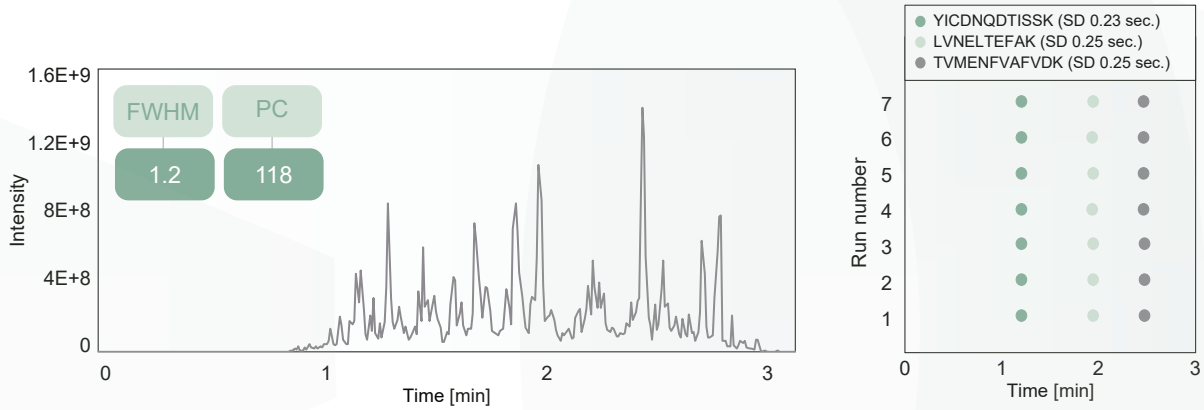



*Figure 2:* Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

retention time properties for the eluting peptides. On average, the full peak width at half maximum is 1.2 seconds, resulting in a peak capacity of 118. We monitored the retention time reproducibility of three different peptides over seven replica injections and find the average standard deviation to be 0.24 seconds indicating highly reproducible retention times (Figure 2).

## 3. Method design

It is important to adjust the MS method to take full advantage of the condensed peptide elution in the fast methods. Therefore, we exploit the fastest scanning mode on an Orbitrap Exploris 480 MS. The MS instrument is operated in data-dependent acquisition mode using a Top18 method. Full MS resolution is set to 60,000 at m/z 200 and full MS AGC target value is 300% with an IT of 45 ms. Mass range is set to 350-1400. AGC target value for fragment spectra is set to 200% and the intensity threshold is kept at 2E5. Isolation width is set to 1.3 m/z and normalized collision energy to 30%. Peptide match is set to off, and isotope exclusion to on. Former target ions are dynamically excluded for selection for 10 seconds. Higher-energy collision dissociation (HCD) fragment scans are acquired at 40 Hz speed with an injection time of 11 ms using an Orbitrap resolution of 7500. Digested HeLa peptides are loaded on Evotips in two dilutions, 50 and 500 ng respectively. Technical quadruplicates are measured and analyzed with the Spectromine 2 software.

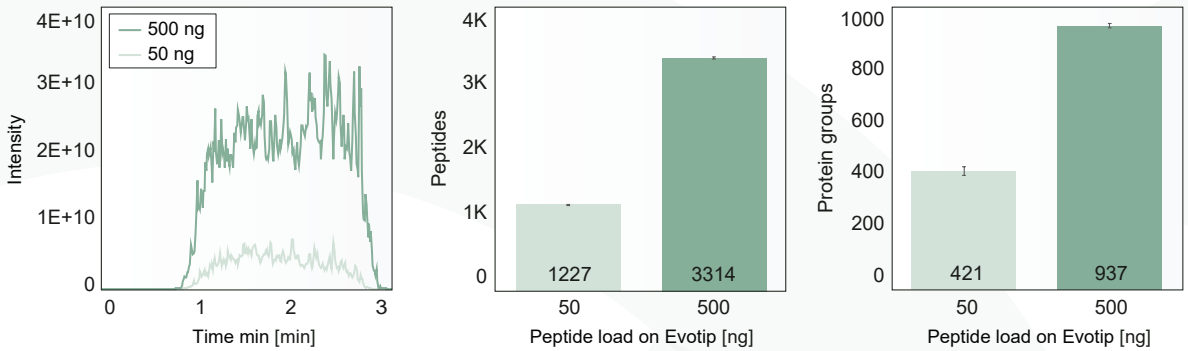



*Figure 3:* Total ion current chromatogram and identifications of peptides and proteins.

## 4. Results and Conclusion

The first peptides elute after 40 seconds, and with a full elution window of 2.3 minutes, the effective gradient elution is 72%. With the highest load, we identify 3300 unique peptides corresponding to nearly 1000 proteins (Figure 3). The short overhead time of only 1.6 minutes allows for an ultra high throughput of 300 samples per day.

# Towards a Standardized Omics Platform with the **200 samples per day** method

## 1. Introduction

This method has a 5.6 minute gradient and a cycle time of 7.2 minutes. It is designed for our EV1107 Endurance column, and we highly recommend to use this column with our range of emitters and spray adapters for optimal performance.

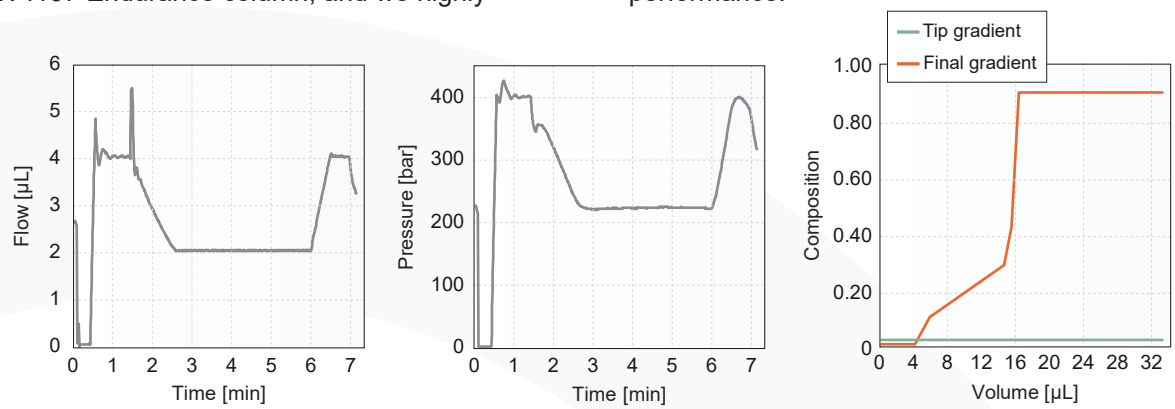



*Figure 1:* Pump HP flow profile, pump HP pressure profile and gradient of the 200 samples per day method.

## 2. Chromatographic performance

By taking advantage of the loading-dilution strategy, we can maximize the chromatographic performance with this very short gradient. From a HeLa digest analysis, we calculated peak and

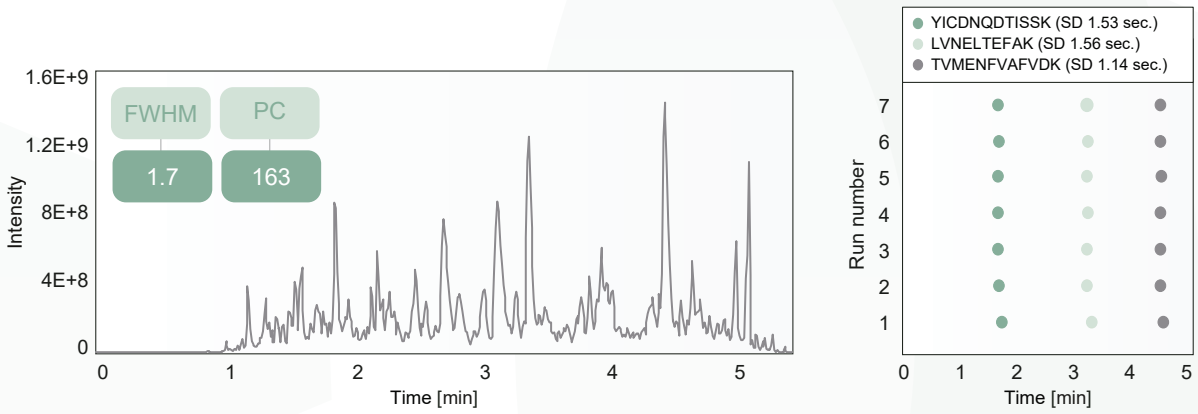



*Figure 2:* Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

retention time properties for the eluting peptides. On average, the full peak width at half maximum is 1.7 seconds, resulting in a peak capacity of 163 within the elution window of 4.6 minutes. We monitored the retention time reproducibility of three different peptides over seven replica injections and find the average standard deviation to be 1.4 seconds indicating highly reproducible retention times (Figure 2).

## 3. Method design

It is important to adjust the MS method to take full advantage of the condensed peptide elution in the fast methods. Therefore, we exploit the fastest scanning mode on an Orbitrap Exploris 480 MS. The MS instrument is operated in data-dependent acquisition mode using a Top18 method. Full MS resolution is set to 60,000 at m/z 200 and full MS AGC target value is 300% with an IT of 45 ms. Mass range is set to 350-1400. AGC target value for fragment spectra is set to 200% and the intensity threshold is kept at 2E5. Isolation width is set to 1.3 m/z and normalized collision energy to 30%. Peptide match is set to off, and isotope exclusion to on. Former target ions are dynamically excluded for selection for 10 seconds. Higher-energy collision dissociation (HCD) fragment scans are acquired at 40 Hz speed with an injection time of 11 ms using an Orbitrap resolution of 7500. Digested HeLa peptides are loaded on Evotips in two dilutions, 50 and 500 ng respectively. Technical quadruplicates are measured and analyzed with the Spectromine 2 software.

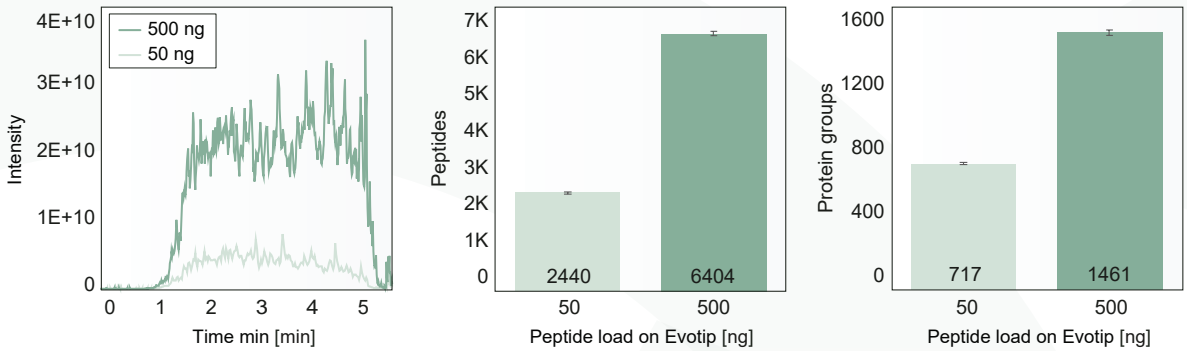



*Figure 3:* Total ion current chromatogram and identifications of peptides and proteins.

## 4. Results and Conclusion

The first peptides elute after 50 seconds, and with a full elution window of 4.6 minutes, the effective gradient elution is 82%. With the highest load, we identify 6400 unique peptides corresponding to 1400 proteins (Figure 3). In conclusion, 200 SPD provides the highest number of peptides per minute with our standard methods when the appropriate MS method is used. The overhead time between injections is only 1.6 minutes.

# Towards a Standardized Omics Platform with the **100 samples per day** method

## 1. Introduction

This method has an 11.5 minute gradient and a cycle time of 14.4 minutes. The method is designed for our EV1064 Endurance column, and we highly recommend to use this column with our range of emitters and spray adapters for optimal performance.

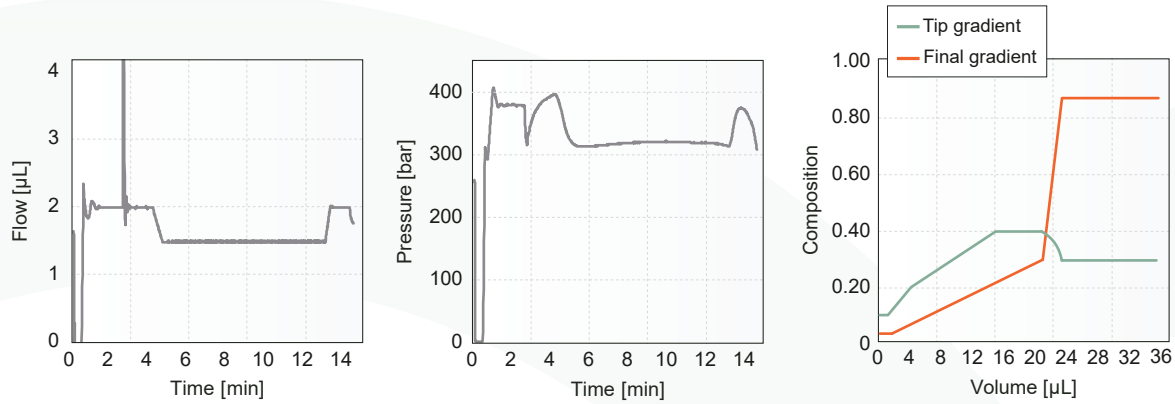



*Figure 1:* Pump HP flow profile, pump HP pressure profile and gradient of the 100 samples per day method.

## 2. Chromatographic performance

By taking advantage of the gradient offset strategy, we maximize the chromatographic performance. From a HeLa digest analysis, we calculated peak and retention time properties

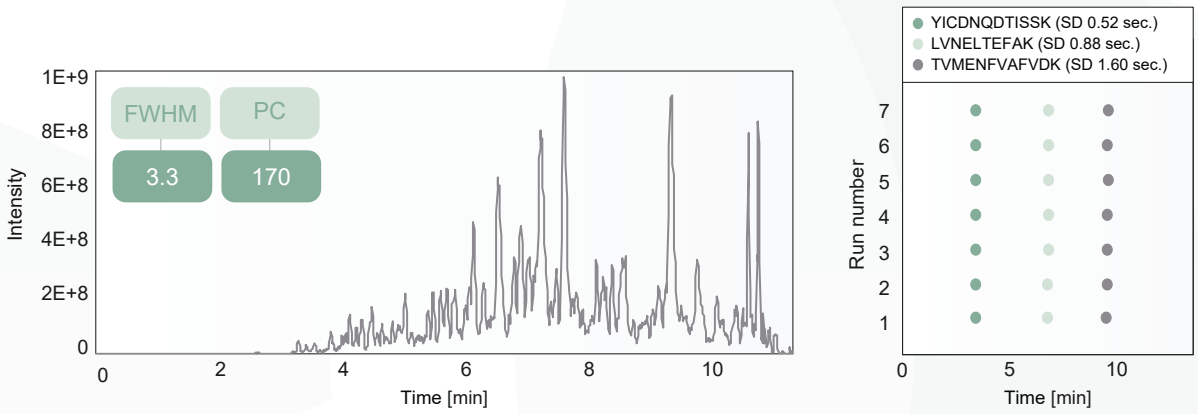



*Figure 2:* Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

for the eluting peptides. On average, the full peak width at half maximum is 3.3 seconds, resulting in a peak capacity of 170 withing the elution window of 9.3 minutes. We monitored the retention time reproducibility of three different peptides over seven replica injections and find the average standard deviation to be only 1 second indicating highly reproducible retention times (Figure 2).

## 3. Method design

It is important to adjust the MS method to take full advantage of the condensed peptide elution. Therefore, we exploit the fastest scanning mode on an Orbitrap Exploris 480 MS. The MS instrument is operated in data-dependent acquisition mode using a Top12 method. Full MS resolution is set to 60,000 at m/z 200 and full MS AGC target value is 300% with an IT of 45 ms. Mass range is set to 350-1400. AGC target value for fragment spectra is set to 200% and the intensity thres- hold is kept at 2E5. Isolation width is set to 1.3 m/z and normalized collision energy to 30%. Peptide match is set to off, and isotope exclusion to on. Former target ions are dynamically excluded for selection for 10 seconds. Higher-energy collision dissociation (HCD) fragment scans are acquired at 28 Hz speed with an injection time of 22 ms using an Orbitrap resolution of 15,000. Digested HeLa peptides are loaded on Evotips in two dilutions, 50 and 500 ng respectively. Technical quadruplicates are measured and analyzed with the Spectromine 2 software.

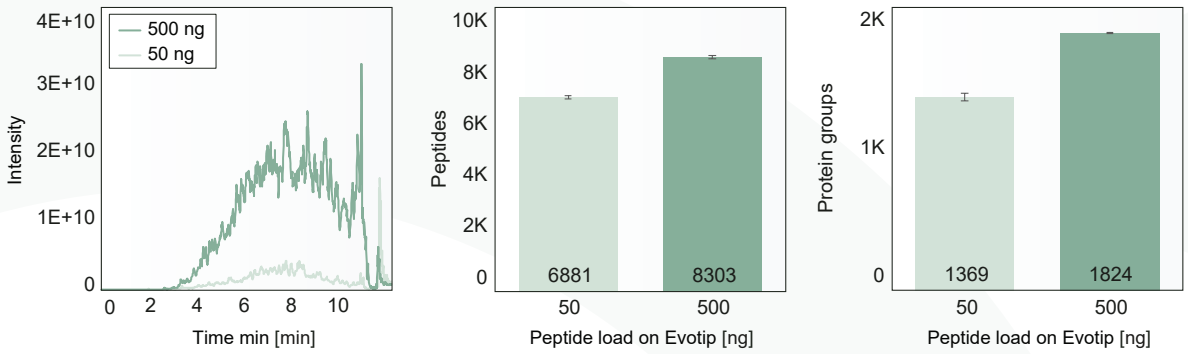



*Figure 3:* Total ion current chromatogram and identifications of peptides and proteins.

## 4. Results and Conclusion

The first peptides elute after 2.4 minutes with the recommended column. With a full peptide elution window of 9.3 minutes, the effective gradient elution is 81%. We identify 8300 peptides with the highest load of 500 ng corresponding to 1800 proteins. (Figure 3). This method is a great “in-between” method, which balances proteome coverage high-throughput with only 2.9 minutes overhead between injections.

EVOSEP

60

# Towards a Standardized Omics Platform with the **60 samples per day** method

## 1. Introduction

This method has a 21 minute gradient and a cycle time of 24 minutes. The method is designed for our EV1064 Endurance column and we highly recommend to use this column with our range of emitters and spray adapters for optimal performance.

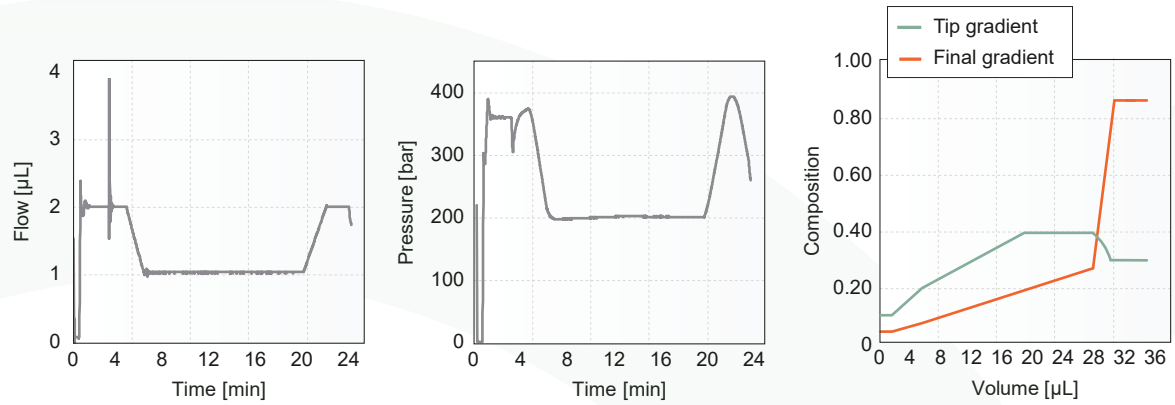



*Figure 1:* Pump HP flow profile, pump HP pressure profile and gradient of the 60 samples per day method.

## 2. Chromatographic performance

The chromatographic performance is maximized with our gradient offset strategy. From a HeLa digest analysis, we calculated peak and retention time properties for the eluting

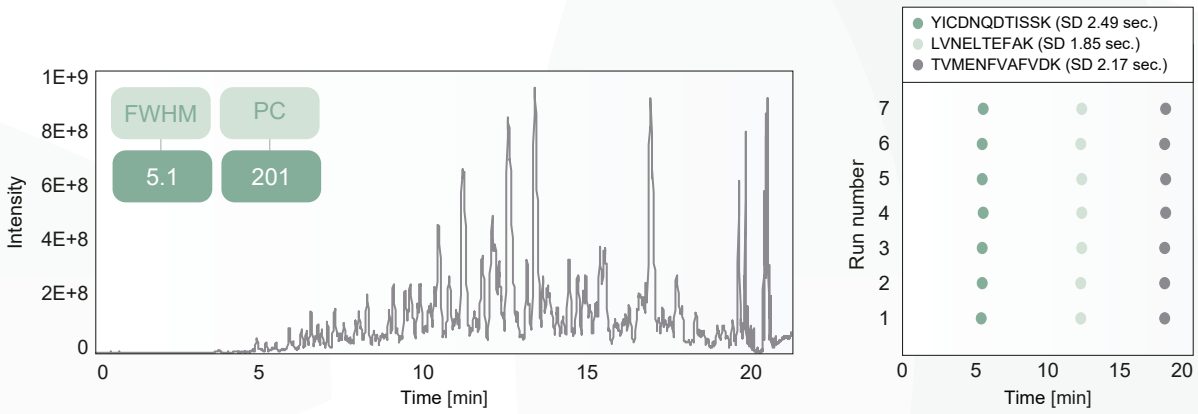



*Figure 2:* Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

peptides. On average, the full peak width at half maximum is 5.1 seconds, resulting in a peak capacity of 201 within the elution window of 17.3 minutes. We monitored the retention time reproducibility of three different peptides over seven replica injections and find the average standard deviation to be 2.2 seconds indicating highly reproducible retention times (Figure 2).

## 3. Method design

It is important to adjust the MS method to take full advantage of the condensed peptide elution. Therefore, we exploit the fastest scanning mode on an Orbitrap Exploris 480 MS. The MS instrument is operated in data-dependent acquisition mode using a Top12 method. Full MS resolution is set to 60,000 at m/z 200 and full MS AGC target value is 300% with an IT of 45 ms. Mass range is set to 350-1400. AGC target value for fragment spectra is set to 200% and the intensity threshold is kept at 2E5. Isolation width is set to 1.3 m/z and normalized collision energy to 30%. Peptide match is set to off, and isotope exclusion to on. Former target ions are dynamically excluded for selection for 10 seconds. Higher-energy collision dissociation (HCD) fragment scans are acquired at 28 Hz speed with an injection time of 22 ms using an Orbitrap resolution of 15,000. Digested HeLa peptides are loaded on Evotips in two dilutions, 50 and 500 ng respectively. Technical quadruplicates are measured and analyzed with the Spectromine 2 software.

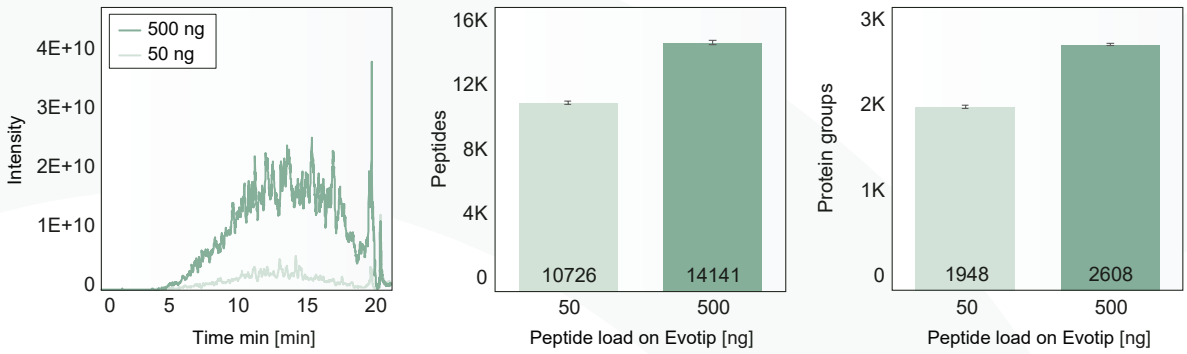



*Figure 3:* Total ion current chromatogram and identifications of peptides and proteins.

## 4. Results and Conclusion

The first peptides start elute after 3.6 minutes with the recommended column. Thus, the method provides an effective gradient elution of 83% with a full peptide elution window of 17.3 minutes. With the highest load of 500 ng, 14,000 peptides are identified corresponding to 2600 proteins (Figure 3). This method provides the best compromise between proteome coverage and throughput among our standard methods and is recommended for both single shot analysis and in-depth analysis with fractionated samples with only 3 minutes overhead between injections.

# Towards a Standardized Omics Platform with the **30 samples per day** method

## 1. Introduction

This is the longest of our standard methods with a 44 minute gradient and a cycle time of 48 minutes. The method is designed for our EV1106 Endurance column and we highly recommend to use this with our range of emitters and spray adapters for optimal performance.

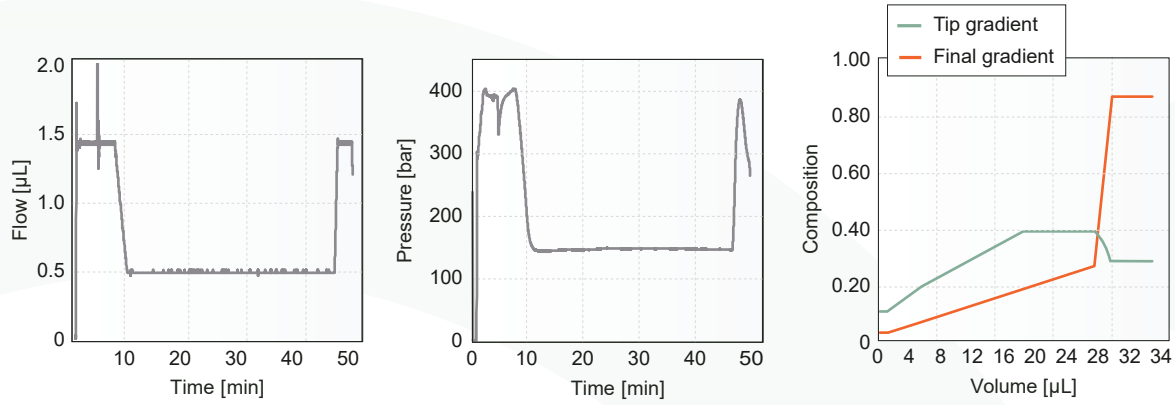



*Figure 1:* Pump HP flow profile, pump HP pressure profile and gradient of the 30 samples per day method.

## 2. Chromatographic performance

The chromatographic performance is maximized with our gradient offset strategy. From a HeLa digest analysis, we calculated peak and retention time properties for the eluting peptides

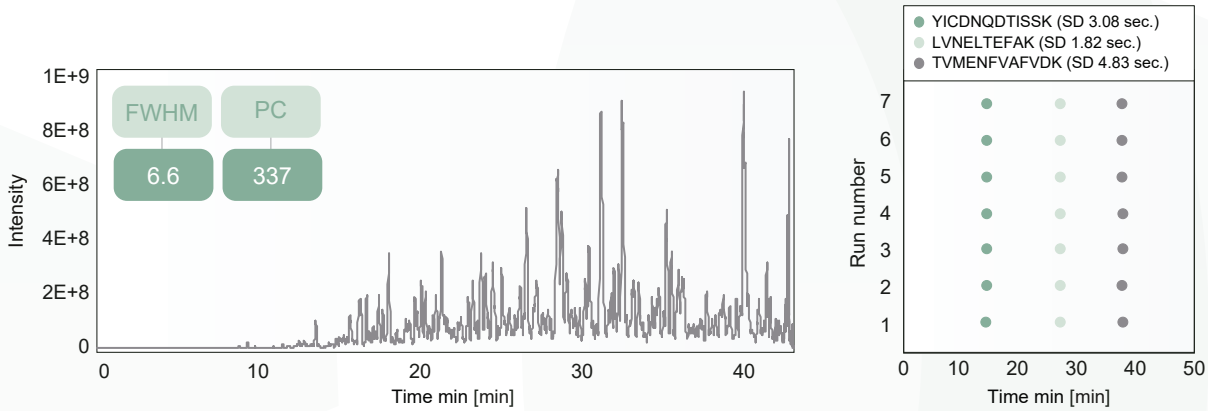



*Figure 2:* Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

On average, the full peak width at half maximum is 6.6 seconds, resulting in a peak capacity of 337 within the elution window of 37 minutes. We monitored the retention time reproducibility of three different peptides over seven replica injections and find the average standard deviation to be 3.3 seconds indicating highly reproducible retention times (Figure 2).

## 3. Method design

It is important to adjust the MS method to take full advantage of the condensed peptide elution. Therefore, we exploit the fastest scanning mode on an Orbitrap Exploris 480 MS. The MS instrument is operated in data-dependent acquisition mode using a Top12 method. Full MS resolution is set to 60,000 at m/z 200 and full MS AGC target value is 300% with an IT of 45 ms. Mass range is set to 350-1400. AGC target value for fragment spectra is set to 200% and the intensity threshold is kept at 2E5. Isolation width is set to 1.3 m/z and normalized collision energy to 30%. Peptide match is set to off, and isotope exclusion to on. Former target ions are dynamically excluded for selection for 10 seconds. Higher-energy collision dissociation (HCD) fragment scans are acquired at 28 Hz speed with an injection time of 22 ms using an Orbitrap resolution of 15,000. Digested HeLa peptides are loaded on Evotips in two dilutions, 50 and 500 ng respectively. Technical quadruplicates are measured and analyzed with the Spectromine 2 software.

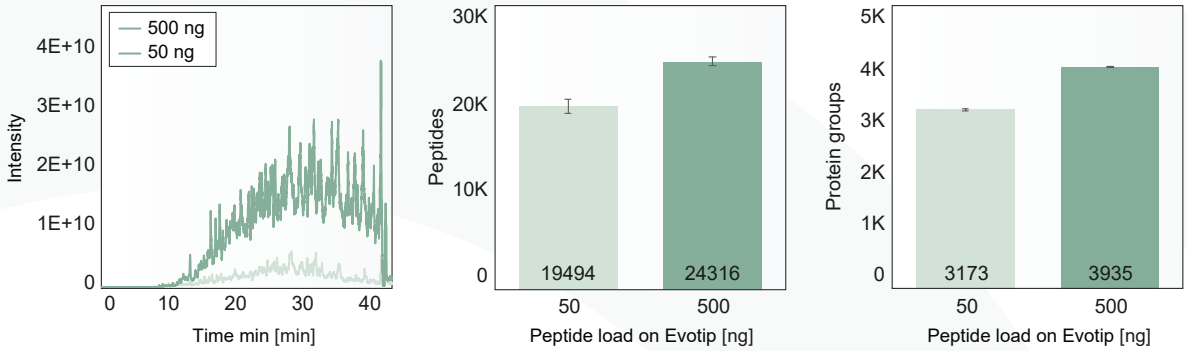



*Figure 3:* Total ion current chromatogram and identifications of peptides and proteins.

## 4. Results and Conclusion

The method provides an effective gradient elution of 84% with the first peptides eluting after 7 minutes enabling a full peptide elution window of 37 minutes with our recommended column. 24,000 peptides are identified corresponding to 4000 proteins (Figure 3). The short overhead of only 4 minutes allows for a throughput of 30 samples per day and results in the highest proteome coverage with our standard methods.

## Enhanced proteome coverage

A Performance column, EV1109 is available for the 60 and 100 samples per day methods and is packed with 1.5 µm C18 beads. This reduces the peak widths with 2 seconds at FWHM and leads to an increase in peak capacity of 60%. Consequently, the proteome coverage is enhanced, and the column provides a robust and competitive commercial option – on the same level as home-pulled and packed options, but with better reproducibility and life-time. The boost in sensitivity is particularly noticeable for lower loads, where peptide and protein coverage is improved with 100%.

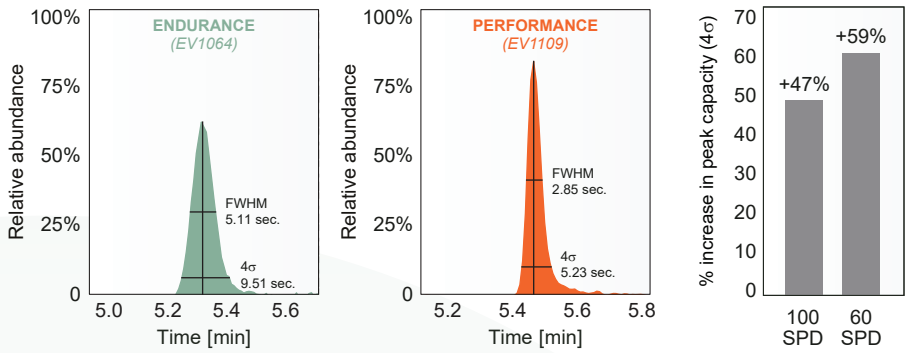



*Figure 2:* Extracted base peak chromatogram of BSA peptide 722. Relative increase in peak capacity compared to the Endurance column.

### 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