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 EV-1064 column and we highly recommend to use this column with our range of emitters and spray adapters for optimal performance.

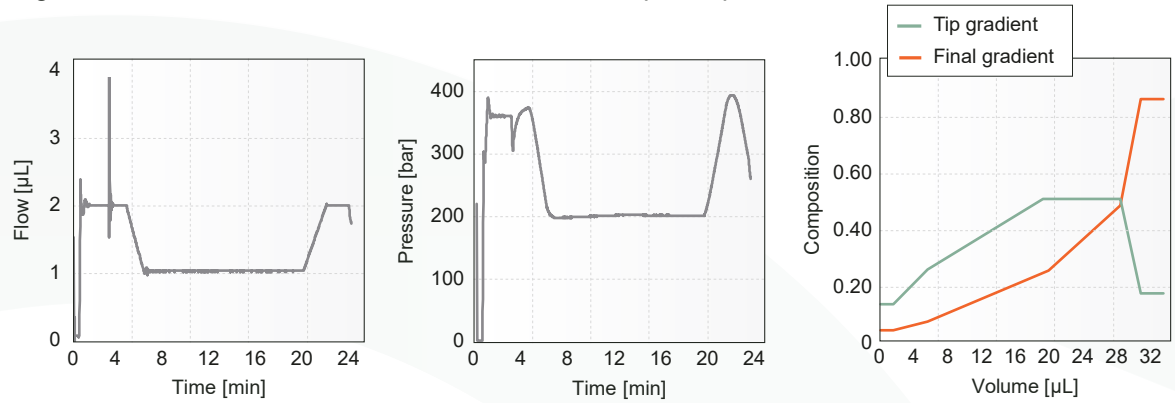

Figure 1: Pump HP flow profile, pump HP pressure profile and gradient of the 60 samples per day method.

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

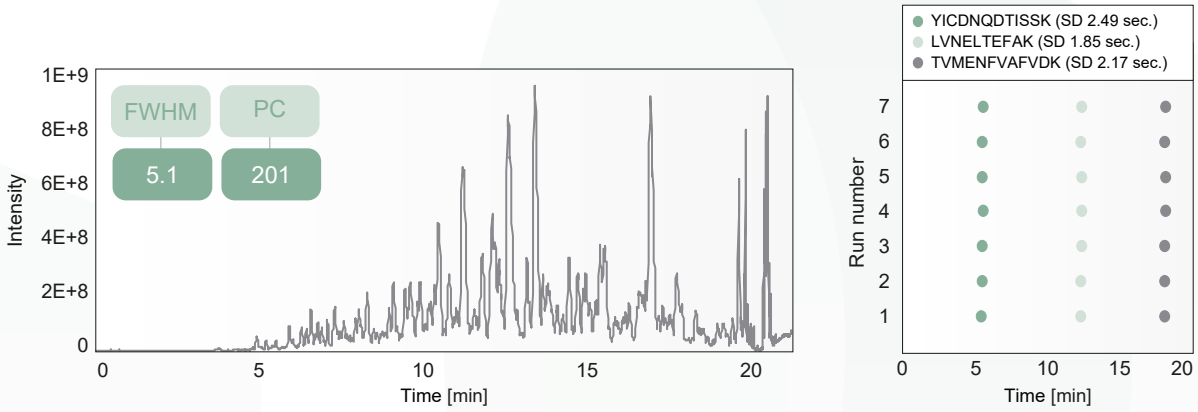

Figure 2: Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

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.

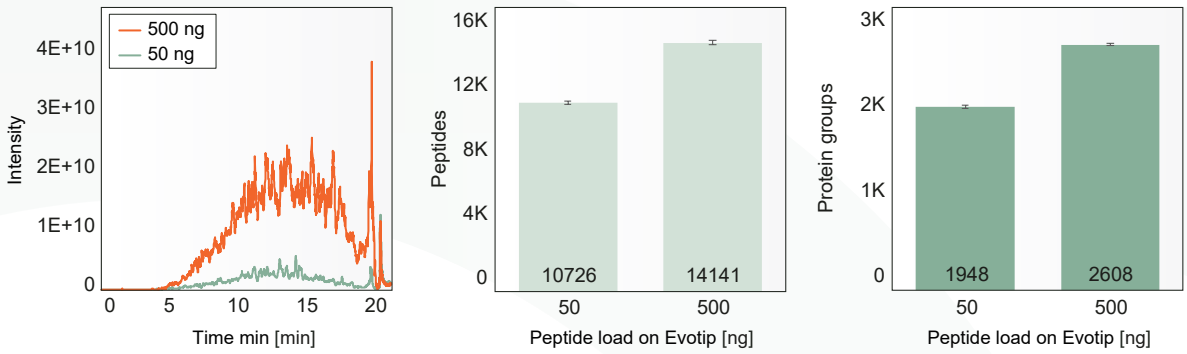

Figure 3: Total ion current chromatogram and identifications of peptides and proteins.

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.