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.

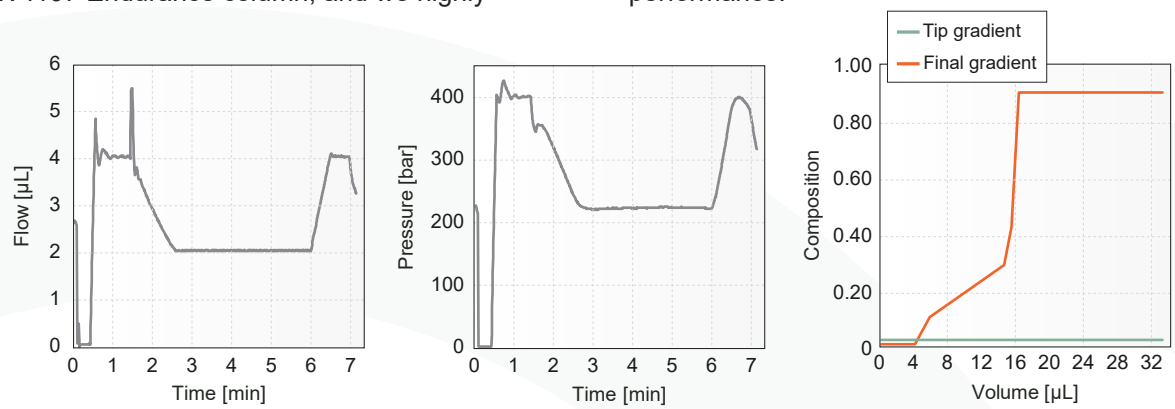

Figure 1: Pump HP flow profile, pump HP pressure profile and gradient of the 200 samples per day method.

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

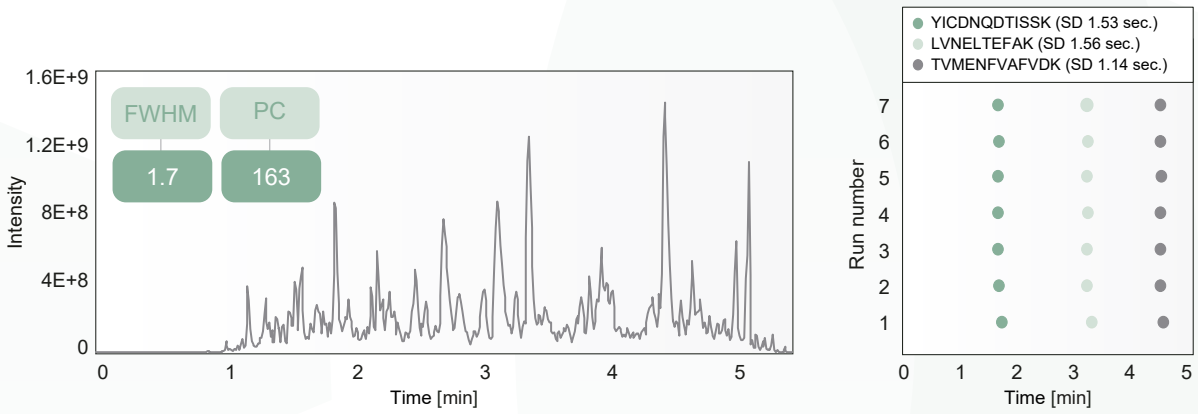

Figure 2: Example base peak chromatogram of 500 ng tryptic HeLa digest and retention time reproducibility of selected peptides across seven consecutive injections.

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.

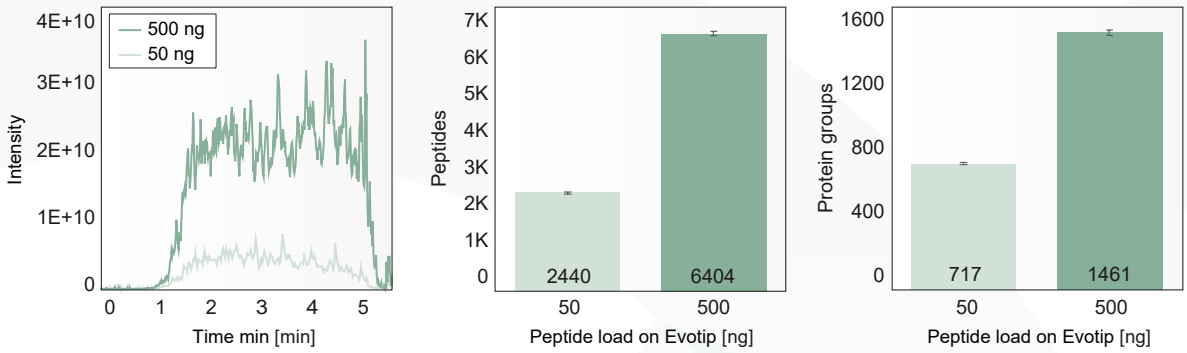

Figure 3: Total ion current chromatogram and identifications of peptides and proteins.

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.