EVOSEP

80

A specialized method for high sensitivity with the **Whisper Zoom 80 samples per day** method

1. Introduction

The Whisper 80 SPD method has a 16.3 minute gradient and a cycle time of 18 minutes. The analytical column is equilibrated at 1000 nl/min. The gradient flow is 200 nl/min and increased to 1000 nl/min for washing (Figure 1).The method is designed for the IonOpticks Aurora Rapid 5x75 column used at 50 °C when connected to a Bruker or Thermo MS.

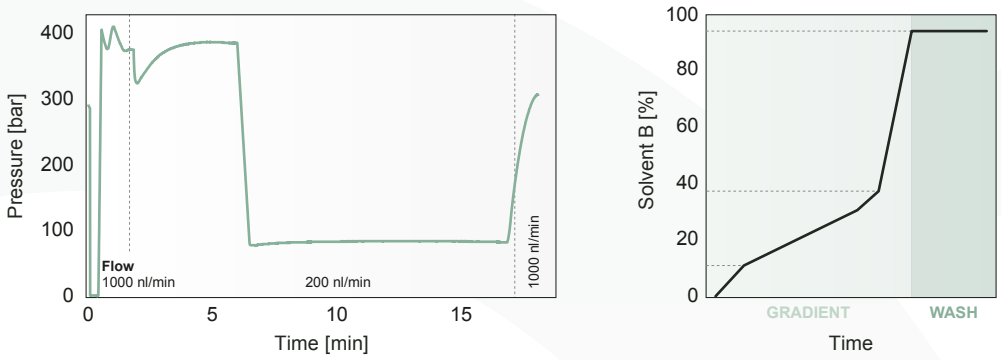

Figure 1: Pump HP pressure profile and representation of gradient in the Whisper 80 SPD method.

2. Chromatographic elution

The performance of the Whisper 80 SPD method is assessed by analyzing 5 ng of tryptic HeLa digest. Total ion current (TIC) and base peak chromatograms (BPC) are monitored and a set of diagnostic peptides are extracted to benchmark expected retention times and peak performance for both columns. Collectively, these metrics serve as the foundation for downstream data processing and optimal results.

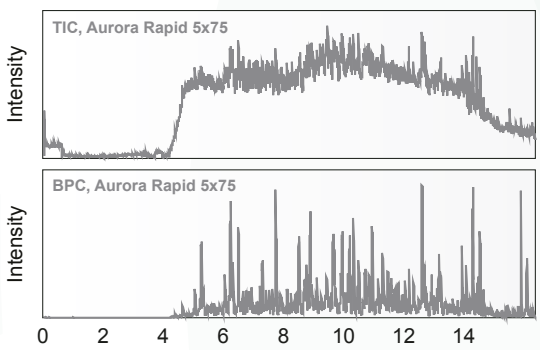

Figure 2: TIC and BPC of 5 ng peptide using the Aurora Rapid 5x75 column.

3. Reproducible performance

A 5 ng HeLa sample was measured on a timsTOF Pro 2 mass spectrometer (Bruker) and Compass Data Analysis software used for analysis. Four diagnostic peptides throughout the gradient were extracted and the full width at half maximum (FWHM) for each peak was calculated by the software. Additionally, the retention time reproducibility was calculated based on ten replicate injections.

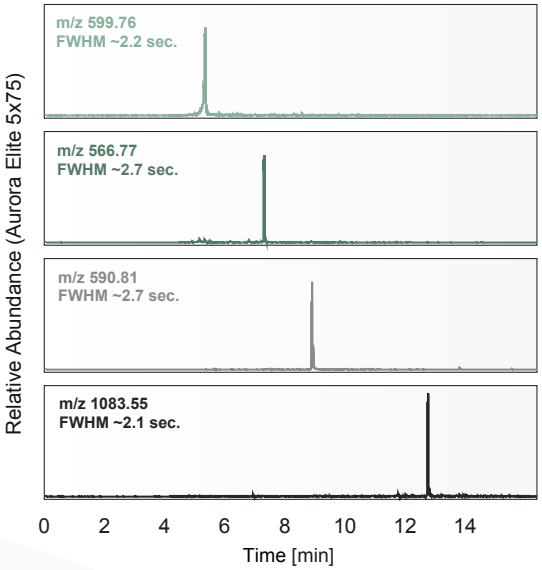

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

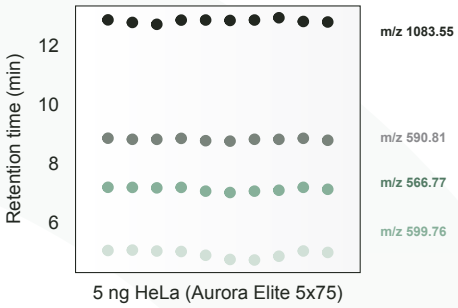

Figure 4: Retention time reproducibility of selected peptides across consecutive runs.

4. Setup

Prepare the Evosep One to run the Whisper Zoom 80 SPD method using the following guide;

1. Select the ‘Instrument configuration’ method and run the ‘Set to Zoom’ script.
2. Follow the on-screen prompt from the script. The transfer line must be connected in port 3 of the Loop valve.
3. Follow the on-screen prompt from the script. The HP flow sensor line must be connected in port 6 of the Loop valve.
4. Re-tighten the connections and you are ready to use the method.