Method Application Note

EVOSEP

80

Key parameters

- **Method:**
  16.3 minutes MS aquisition time
  18.0 minutes cycle time
- **Flow:**
  1.0 µl/min equilibration flow
  0.2 µl/min gradient flow
- **Column:**
  IonOpticks Aurora Rapid 5x75 column, heated to 50 °C

1. Get started with Whisper Zoom

This application note outlines key parameters such as expected high-pressure (HP) pump pressure, a gradient representation, chromatographic performance and method reproducibility for the Evosep Eno Whisper Zoom 80 SPD method. Furthermore, it details specified columns, as well as source specific recommended heating solutions. The application note is intended as a reference for the expected chromatographic performance, which collectively serve as the foundation for downstream data processing and results.

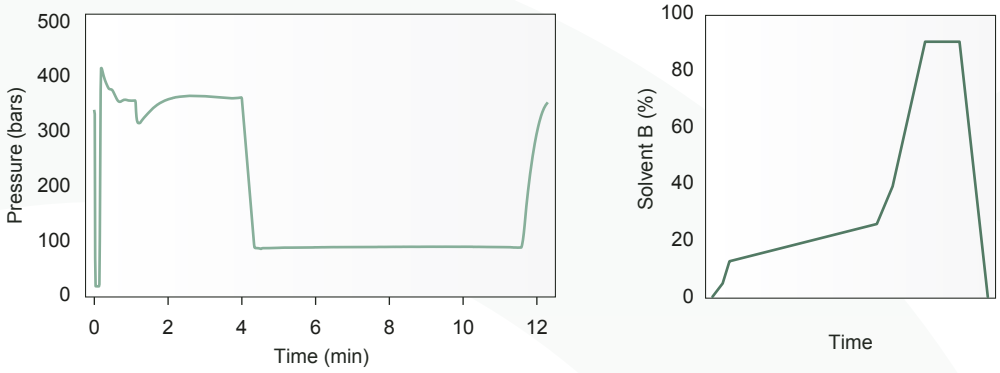

Figure 1: Pump HP pressure profile and gradient representation of the Whisper Zoom 80 SPD method.

2. Chromatographic elution

The performance of the standardized method is assessed from 5 ng tryptic HeLa digest (Pierce). 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.

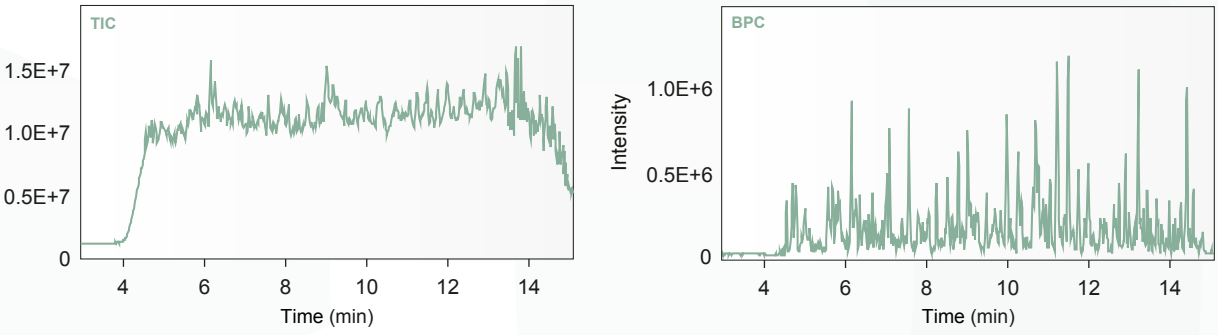

Figure 2: TIC and BPC of 5 ng peptide using the Aurora Rapid 5x75 column on a timsTOF HT.

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3. Consistent chromatography

A 5 ng HeLa sample was measured on three different Evosep Eno systems and associated Aurora Rapid 5x75 columns. Five diagnostic peptides spanning the gradient were extracted and the full width at half maximum (FWHM) for each peak was calculated using the Skyline software. Additionally, the retention time reproducibility was calculated based on 15 injections.

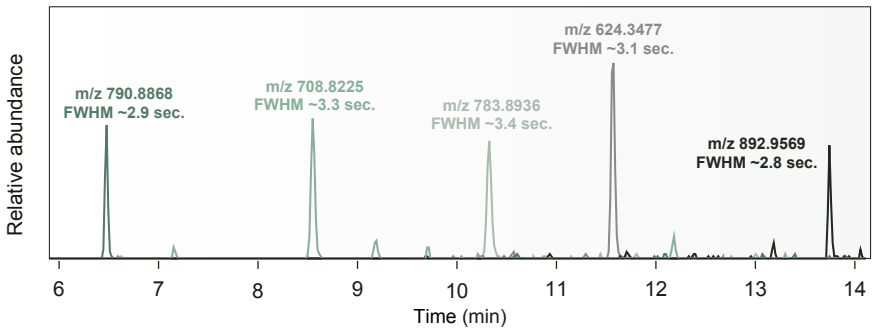

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

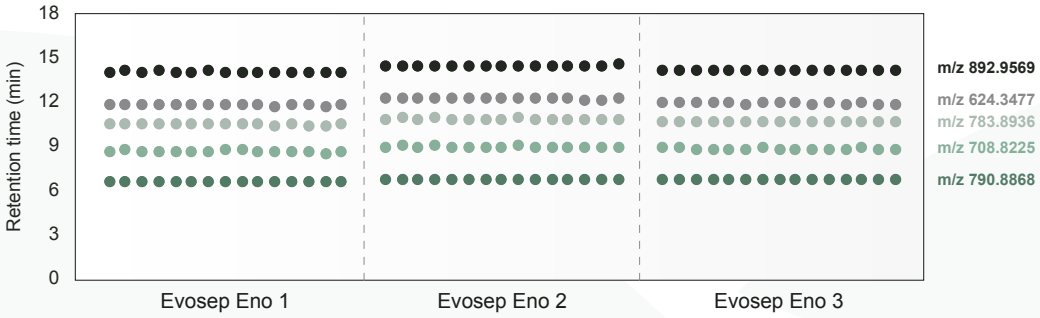

Figure 4: Retention time reproducibility of selected peptides across three different Eno instruments and columns, 5 ng HeLa (n=15).

4. Column ovens

Table 1: Overview of column ovens to use with the Aurora Rapid 5x75 column.

Source	Column oven
Bruker CaptiveSpray series	Column Toaster, Bruker
Thermo Scientific EASY-Spray	HeatSync Column Oven, IonOpticks
Thermo Scientific Nanospray	HeatSync Column Oven, IonOpticks

Evosep Eno is for General Laboratory Use.