

- Method:**
10.5 minutes gradient length
12.0 minutes cycle time
- Flow:**
1.0 $\mu\text{L}/\text{min}$ equilibration flow
0.2 $\mu\text{L}/\text{min}$ gradient flow
- Column:**
IonOpticks Aurora Rapid 5x75
column, heated to 50 $^{\circ}\text{C}$

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 120 SPD method. Furthermore, it details specified columns and emitters, 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.

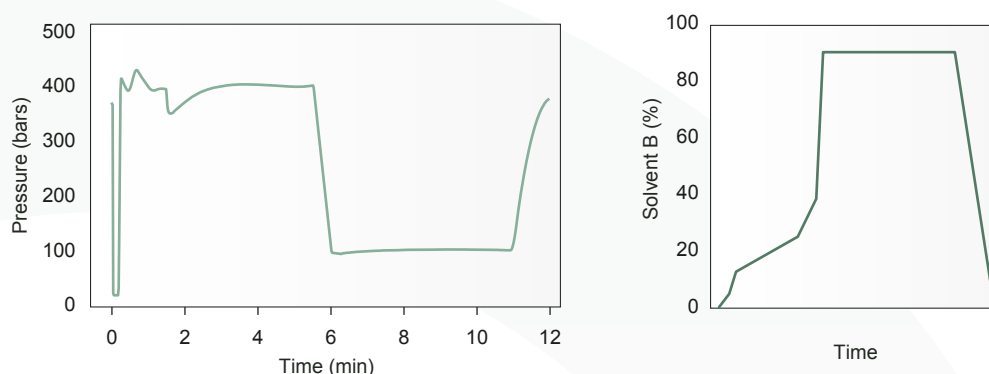


Figure 1: Pump HP pressure profile and gradient representation of the Whisper Zoom 120 SPD method.

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 chromato-

grams (BPC) are monitored and a set of diagnostic peptides are extracted to benchmark expected retention times and peak performance.

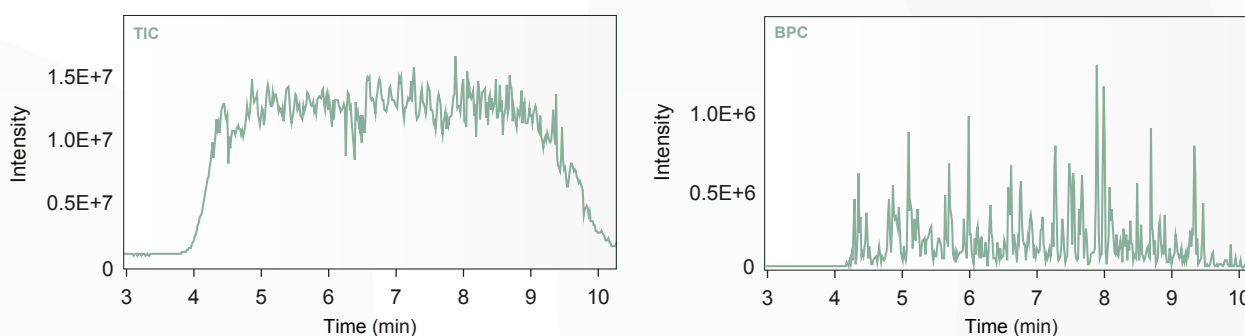


Figure 2: TIC and BPC of 5 ng peptide using the Aurora Rapid 5x75 column on a timsTOF HT.

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.

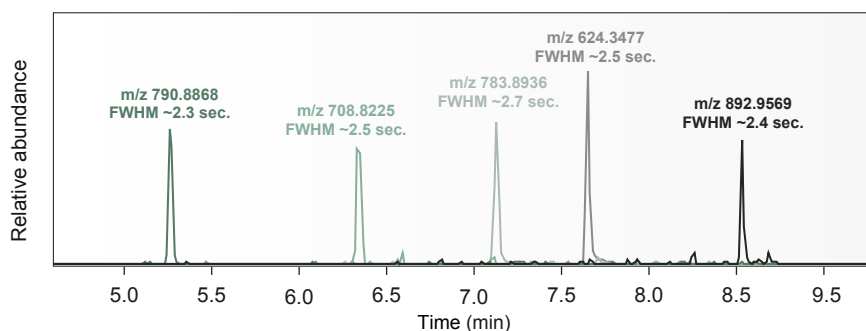


Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

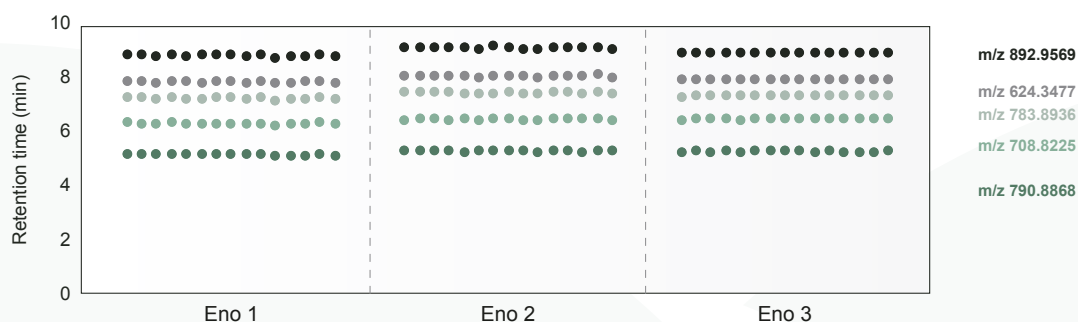


Figure 4: Retention time reproducibility of selected peptides across three different Eno instruments and columns, 5 ng HeLa (n=15).

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.