Key parameters

- **Method:**
  4.0 minutes MS aquisition time
  4.8 minutes cycle time
- **Flow:**
  4.5 µl/min equilibration flow
  4.0 µl/min gradient flow
- **Column:**
  EV1182 Evosep Performance column, heated to 40 °C

1. Get started with 300 SPD

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 300 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.

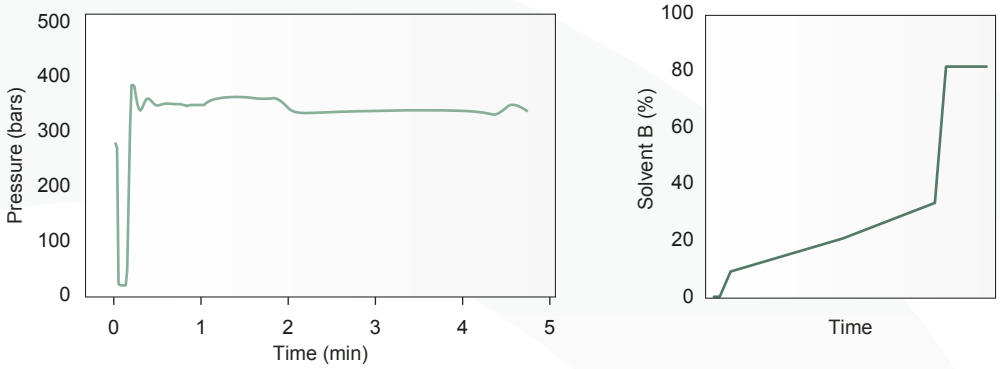

Figure 1: Pump HP pressure profile and gradient representation of the 300 SPD method.

2. Chromatographic elution

The performance of the standardized method is assessed from 50 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.

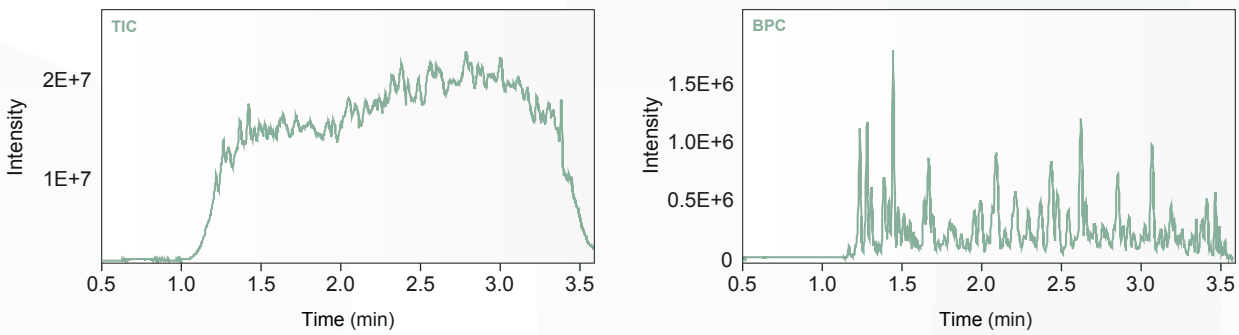

Figure 2: Zoom in on TIC and BPC of 50 ng peptide using the EV1182 column on a timsTOF HT.

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3. Consistent chromatography

A 50 ng HeLa sample was measured on three different Evosep Eno systems and associated EV1182 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.

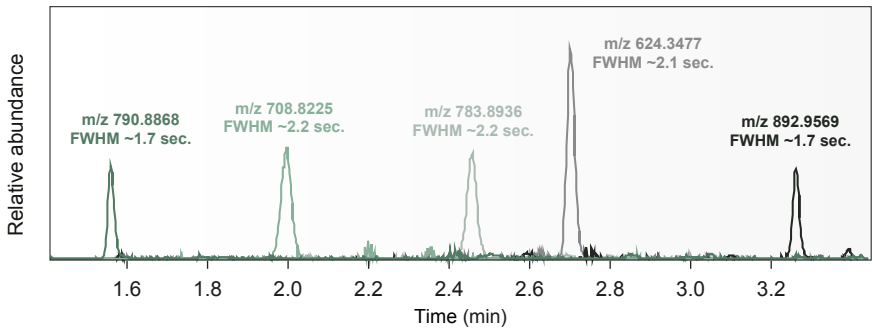

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

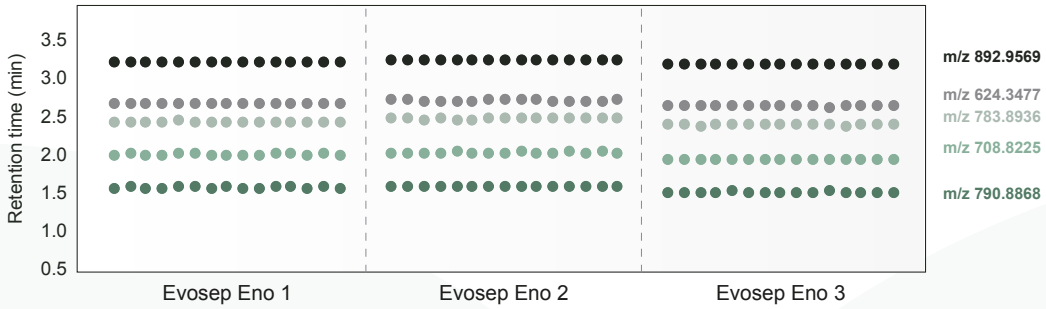

Figure 4: Retention time reproducibility of selected peptides across three different Evosep Eno instruments and columns, 50 ng HeLa (n=15).

4. Emitters

Table 1: Overview of emitters and column ovens to use with the EV1182 column.

Source	Details	
Agilent Nanospray	***Emitter:***	Stainless steel emitters XL, ID 30 µm (EV1117, Evosep)
	Column oven:	Evosep Pod for Agilent Nanospray (EV1191, Evosep)
Bruker CaptiveSpray series	***Emitter:***	Captive Spray 2 Emitter, 20 µm ID (1811107, Bruker)
	Column oven:	Column Toaster (Bruker)
SCIEX OptiFlow Turbo V	***Emitter:***	SteadySpray Electrode Low micro 1-10 µl/min (5061574, SCIEX)
	Column oven:	Micro column heater (SCIEX)
Thermo Scientific EASY-Spray	***Emitter:***	Stainless steel emitters, ID 30 µm (EV1086, Evosep)
	Column oven:	Evosep Pod for Thermo EasySpray (EV1187, Evosep)
Thermo Scientific Nanospray	***Emitter:***	Stainless steel emitters, ID 30 µm (EV1086, Evosep)
	Column oven:	Evosep Pod for Thermo Nanospray Flex (EV1194, Evosep)
Waters	***Emitter:***	Stainless steel emitters, ID 30 µm (EV1086, Evosep)
	Column oven:	Evosep Pod for Waters NanoLockSpray (EV1189, Evosep)

Evosep Eno is for General Laboratory Use.