Method Application Note

EVOSEP 100

Key parameters

- **Method:**
  13.0 minutes MS aquisition time
  14.4 minutes cycle time
- **Flow:**
  2.4 µl/min equilibration flow
  1.0 µl/min gradient flow
- **Column:**
  EV1109 Evosep Performance column, heated to 40 °C

1. Get started with 100 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 100 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.

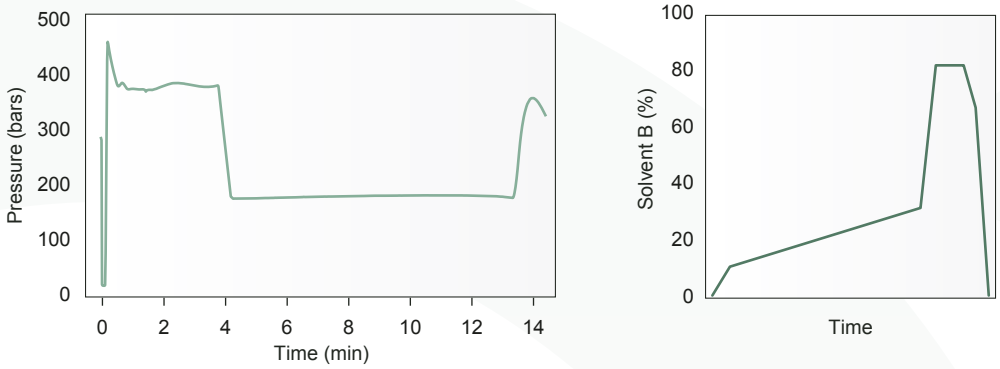

Figure 1: Pump HP pressure profile and gradient representation of the 100 SPD method.

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.

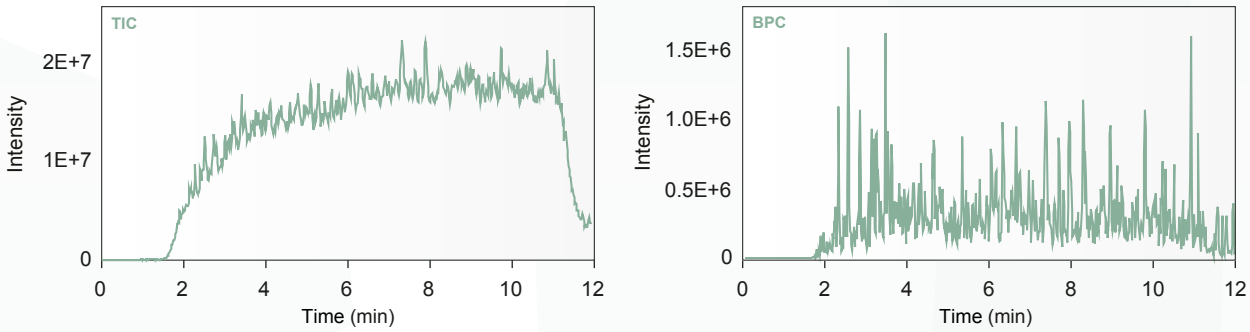

Figure 2: TIC and BPC of 50 ng peptide using the EV1109 column on a timsTOF Pro 2.

EVOSEP

3. Consistent chromatography

A 50 ng HeLa sample was measured on three different Evosep Eno systems and associated EV1109 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.

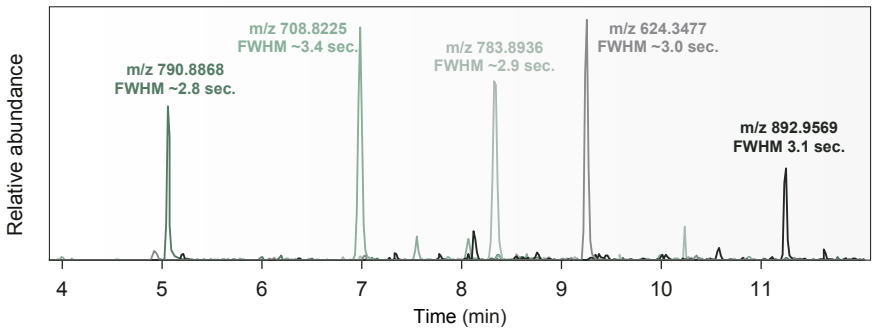

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

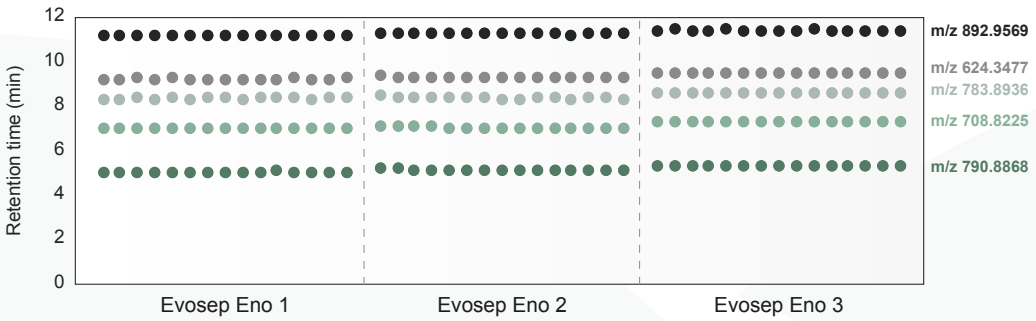

Figure 4: Retention time reproducibility of selected peptides across three different Eno instruments and columns, 50 ng HeLa (n=15).

4. Emitters

Table 1: Overview of emitters and column ovens to use with the EV1109 column.

Source	Emitter	Part no	Column oven
Bruker CaptiveSpray series	Captive Spray 2 Emitter, 20 µm ID	1811107, Bruker	Column Toaster, Bruker
SCIEX OptiFlow Turbo V	SteadySpray Electrode Low micro 1-10 µl/min	5061574, SCIEX	Micro column heater, SCIEX
Thermo Scientific EASY-Spray	Stainless steel emitters, ID 30 µm	EV1086, Evosep	Evosep Pod, Evosep
Thermo Scientific Nanospray	Stainless steel emitters, ID 30 µm	EV1086, Evosep	PRSO-V2-PSUK, Sonation

Evosep Eno is for General Laboratory Use.