A standardized separation method with a throughput of **60 samples per day**

1. Introduction

The 60 SPD method has a 21 minute gradient and a cycle time of 24 minutes. The analytical column is equilibrated at 2 µl/min. The gradient flow is 1 µl/min and increased to 2 µl/min for washing (Figure 1). Two columns can be used for the method, either the EV1064 Endurance column at ambient temperature (23 °C in this case) or the EV1109 Performance column, which should be used at 40 °C. Both columns should be used in connection with the appropriate emitter (Table 1).

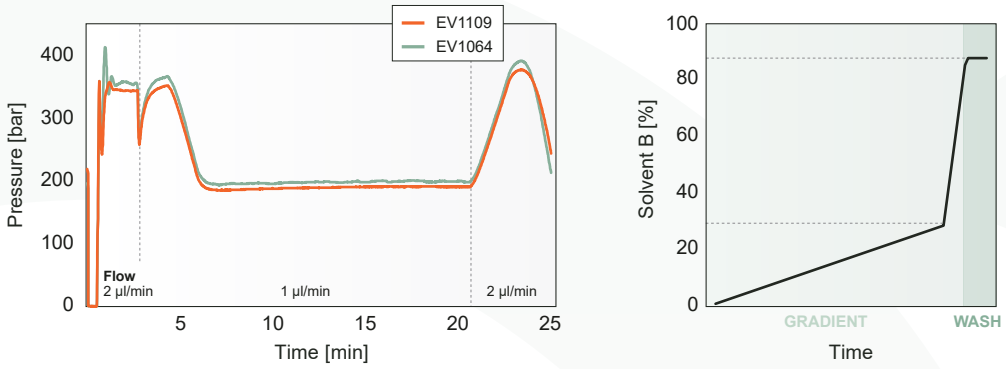

Figure 1: Pump HP pressure profile and representation of gradient in the 60 SPD method.

2. Chromatographic elution

The performance of the 60 SPD method is assessed by analyzing 50 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 down-stream data processing and optimal results.

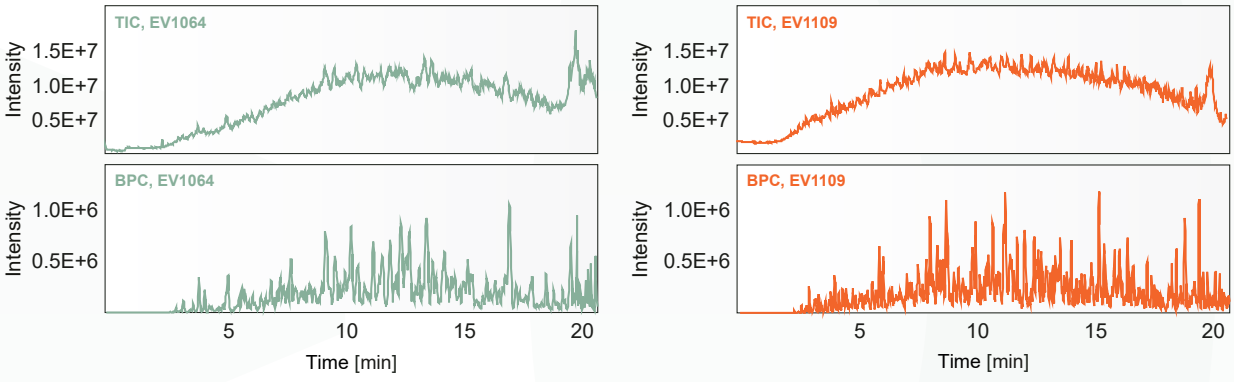

Figure 2: TIC and BPC of 50 ng peptide using the EV1064 and EV1109 columns on a timsTOF Pro 2.

3. Reproducible performance

A 50 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.

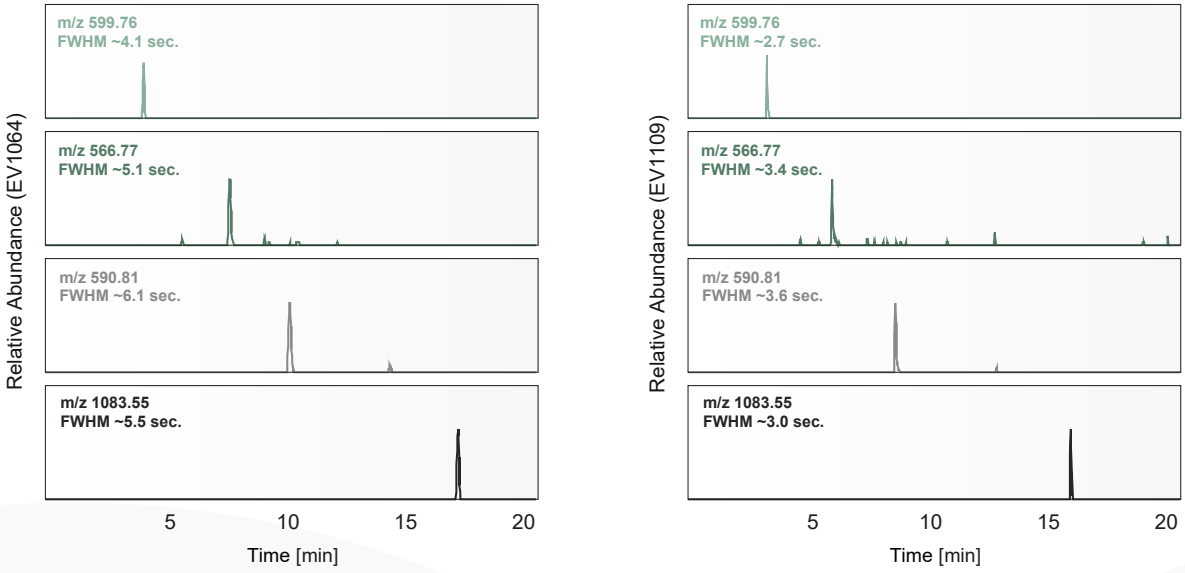

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

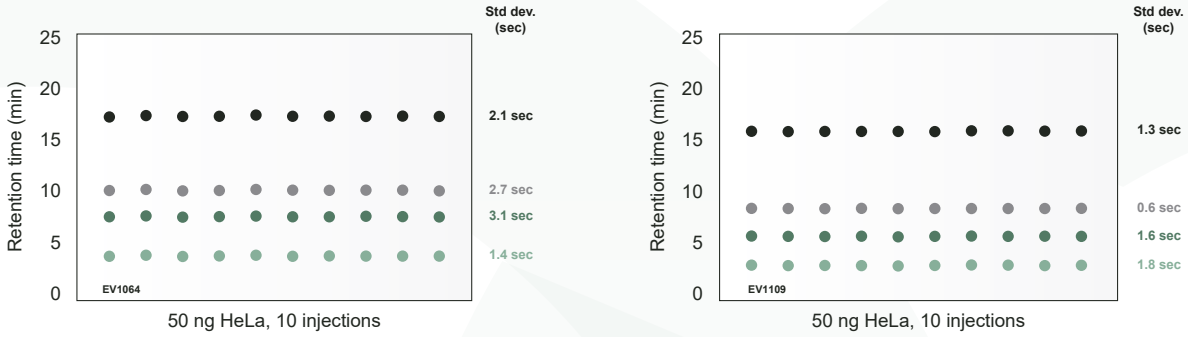

Figure 4: Retention time reproducibility of selected peptides across consecutive runs.

4. Emitters

Table 1: Overview of emitters to use with the EV1064 and EV1109 columns across MS platforms.

Mass spec vendor	P/N	Description	Order through
Agilent	EV1117	Stainless steel emitters XL, ID 30 µm	Evosep
Bruker	1811110	Captive Spray 2 Emitter, 20 µm ID	Bruker
SCIEX	5061574	SteadySpray Electrode Low micro 1-10 µl/min	SCIEX
Thermo Scientific	EV1086	Stainless steel emitters, ID 30 µm	Evosep