A standardized separation method with a throughput of **500 samples per day**

1. Introduction

The 500 SPD method has a 2.2 minute gradient and a cycle time of 2.9 minutes. The analytical column is equilibrated at 4 µl/min, which is maintained during the gradient (Figure 1).

The method is used with the EV1107 Endurance column, at ambient temperature (23 °C in this case) and with the appropriate emitter (Table 1).
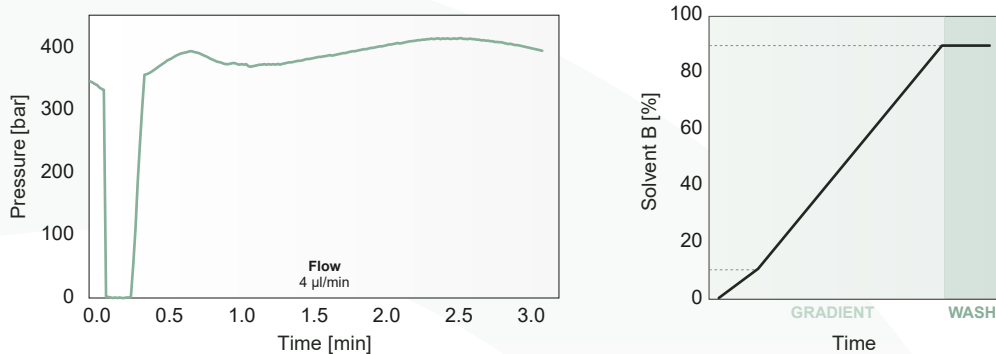

Figure 1: Pump HP pressure profile and representation of gradient in the 500 SPD method.

2. Chromatographic elution

The performance of the standardized 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 bench-mark expected retention times and peak performance. Collectively, these metrics serve as the foundation for downstream data processing and optimal results.
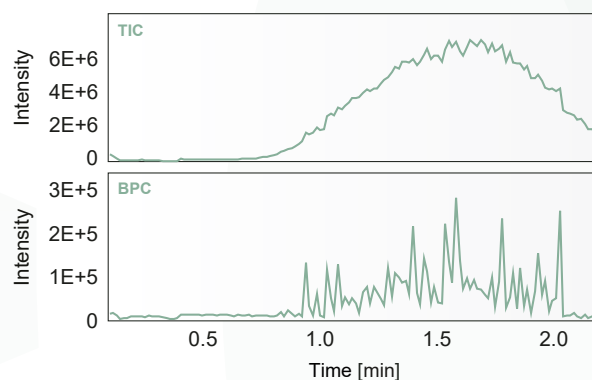

Figure 2: TIC and BPC of 50 ng peptide using the EV1107 column on a timsTOF Pro 2.

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3. Reproducible performance

A 50 ng HeLa sample was measured on a timsTOF Pro 2 mass spectrometer (Bruker). Four diagnostic peptides throughout the gradient were extracted and the full width at half maximum (FWHM) for each peak was calculated using the Compass Data Analysis software. Additionally, the retention time reproducibility was calculated based on ten replicate injections.
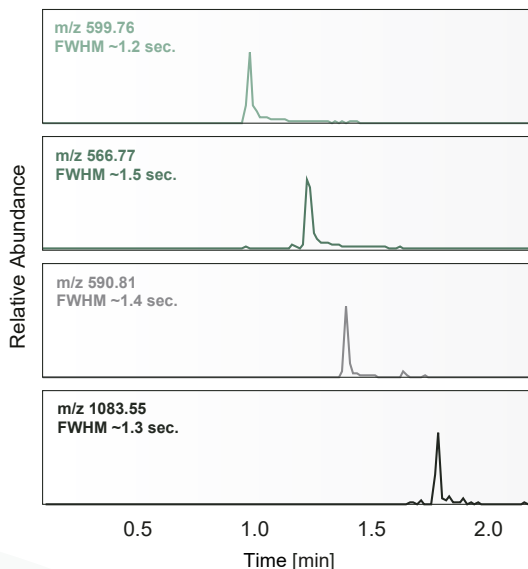

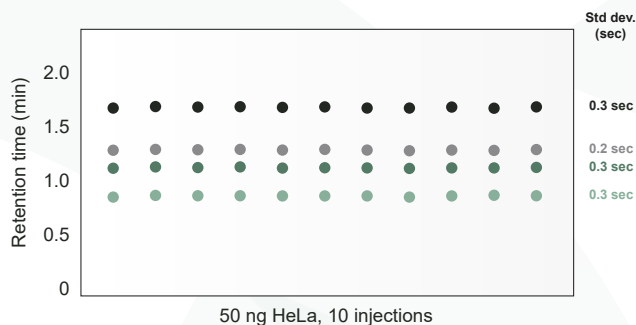*Figure 3:* Extracted ion chromatograms and FWHM of selected peptides.

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

4. Emitters

Table 1: Overview of emitters to use with the EV1107 column 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

EVOSEP