A specialized method for maximum proteome coverage with the **Extended method**

1. Introduction

The Extended method has an 88 minute gradient and a cycle time of 93 minutes. The analytical column is equilibrated at 1500 nl/min. The gradient flow is 220 nl/min and increased to 1500 nl/min for washing (Figure 1).Two columns can be used for the method; the EV1106 Endurance column at ambient temperature (23 °C in this case) or the EV1137 Performance column at 40 °C. Both columns should be used in combination with the appropriate emitter (Table 1).

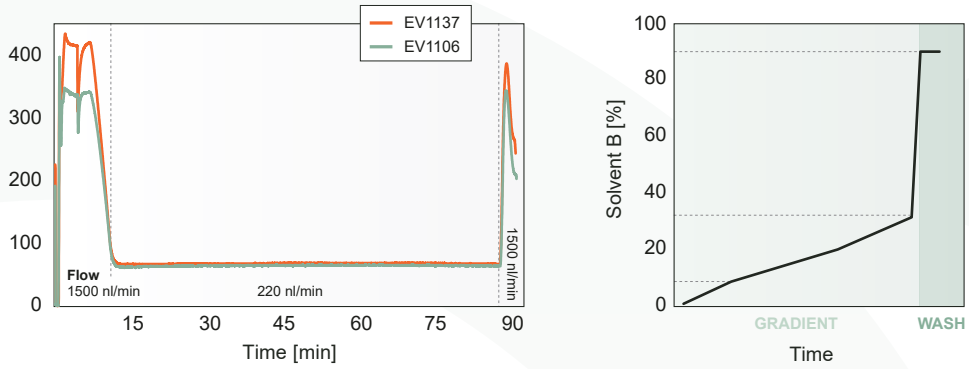

Figure 1: Pump HP pressure profile and representation of gradient in the Extended method.

2. Chromatographic elution

The performance of the Extended 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 downstream data processing and optimal results.

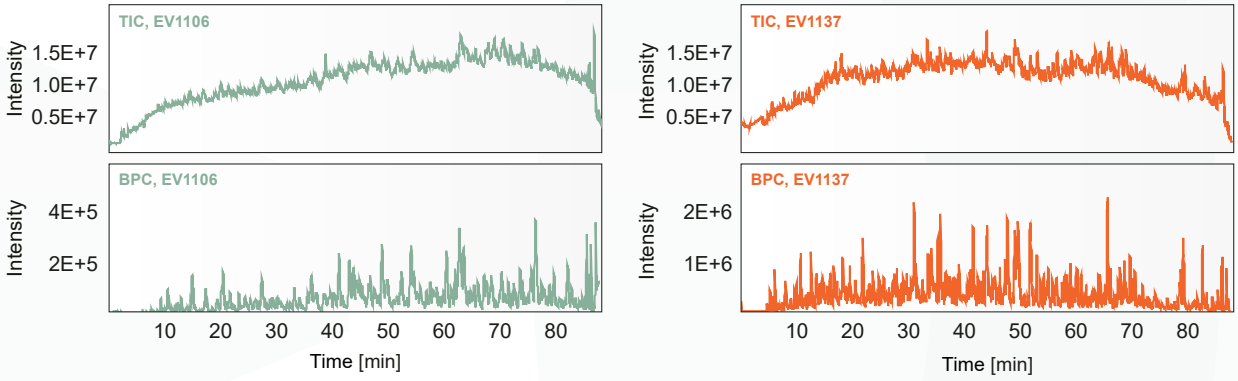

Figure 2: TIC and BPC of 50 ng peptide using the EV1106 and EV1137 columns on a timsTOF Pro 2.

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 eight replicate injections.

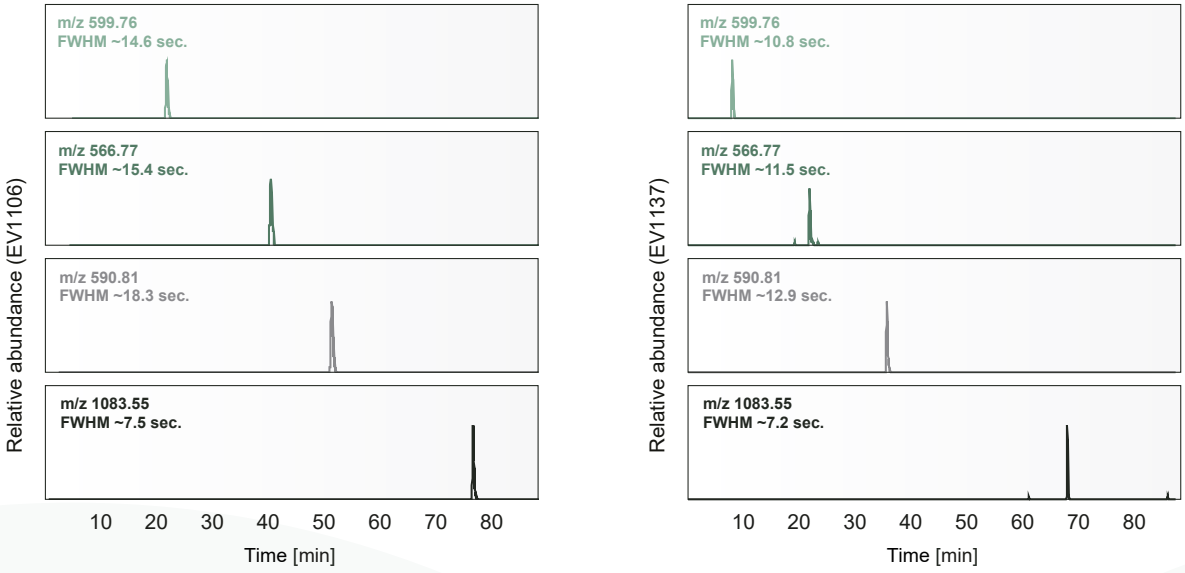

Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

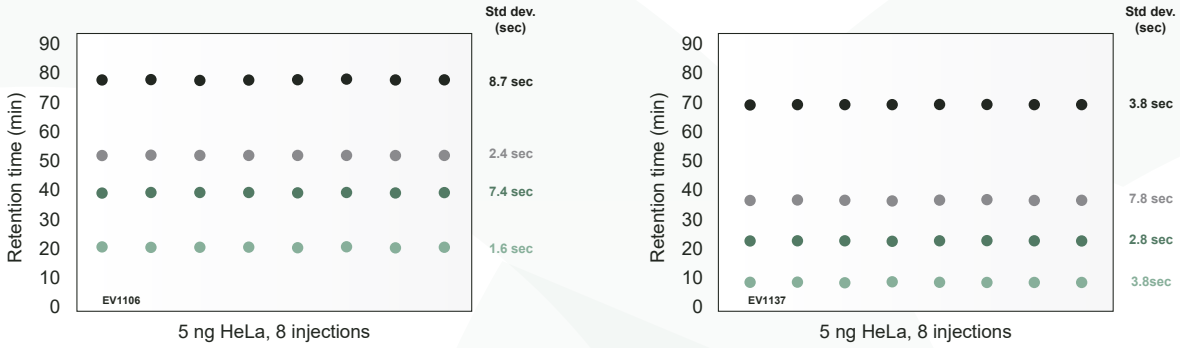

Figure 4: Retention time reproducibility of selected peptides across consecutive runs.

4. Emitters

Table 1: Overview of emitters to use with the EV1106 and EV1137 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