

A specialized method using Whisper Flow technology with a throughput of **20 samples per day**

1. Introduction

The Whisper 20 SPD method has a 58 minute gradient and a cycle time of 65 minutes. The analytical column is equilibrated at 450 nl/min. The gradient flow is 100 nl/min and increased to 450 nl/min for washing (Figure 1).

Two columns can be used for the method; the EV1112 Performance column at 40 °C with the appropriate emitter (Table 1), or the IonOpticks Aurora Elite column used at 50 °C when connected to a Bruker or Thermo MS.

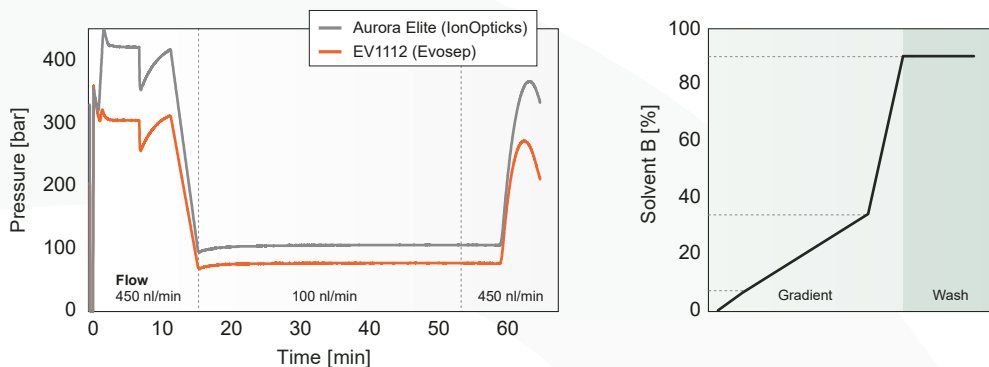


Figure 1: Pump HP pressure profile and representation of gradient in the Whisper 20 SPD method.

2. Chromatographic elution

The performance of the Whisper 40 SPD method is assessed by analyzing 5 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.

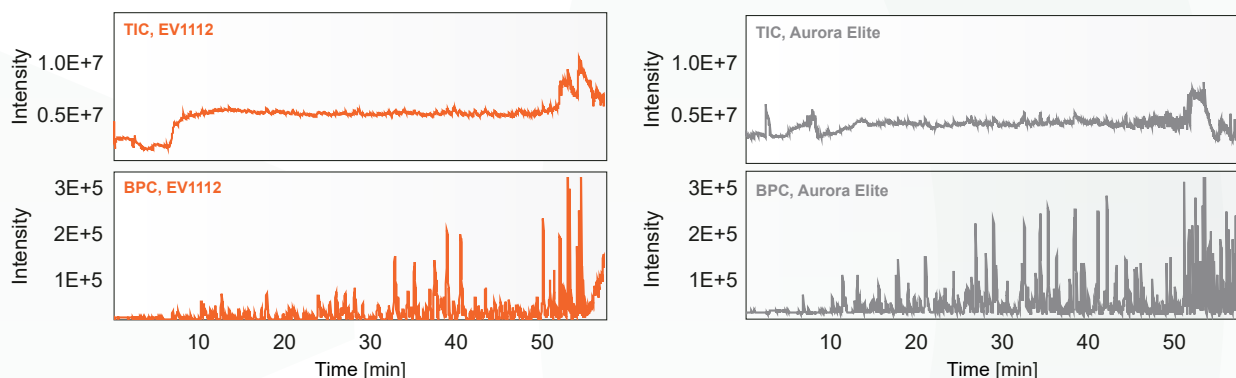


Figure 2: TIC and BPC of 5 ng peptide using the EV1112 and Aurora Elite columns on a timsTOF Pro 2.

3. Reproducible performance

A 5 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.

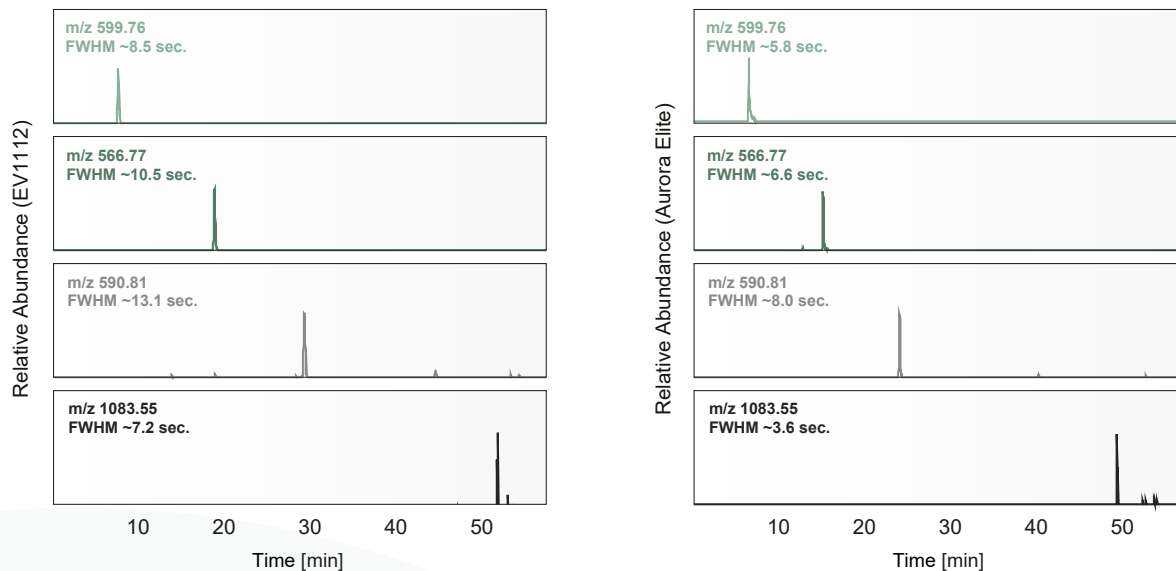


Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

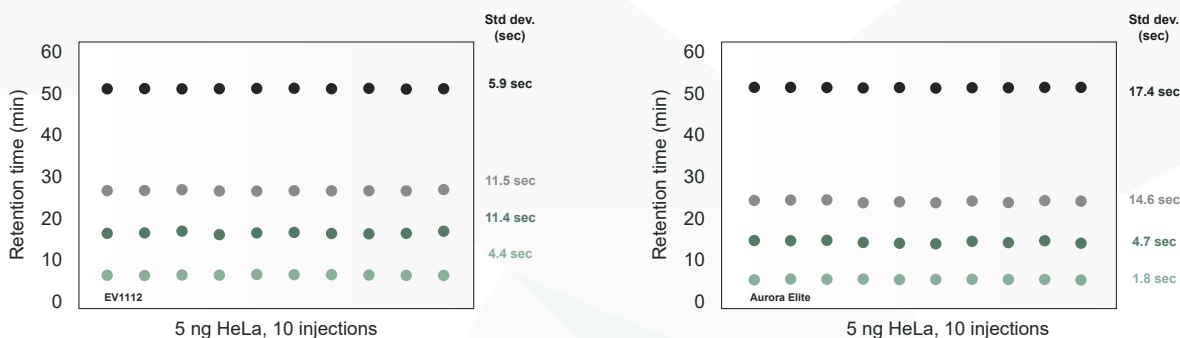


Figure 4: Retention time reproducibility of selected peptides across consecutive runs.

4. Emitters

Table 1: Overview of emitters to use with the EV1112 column across MS platforms.

Mass spec vendor	P/N	Description	Order through
Bruker	1811180	Captive Spray 2 Emitter, 10 µm ID	Bruker
Thermo Scientific	EV1111	Fused silica emitters, ID 10 µm	Evosep