



A specialized method for high sensitivity with the **Whisper Zoom 20** samples per day method

1. Introduction

The Whisper 20 SPD method has a 68 minute gradient and a cycle time of 72 minutes. The analytical column is equilibrated at 450 nl/min. The gradient flow is 200 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 15x75 column used at 50 °C when connected to a Bruker or Thermo MS.

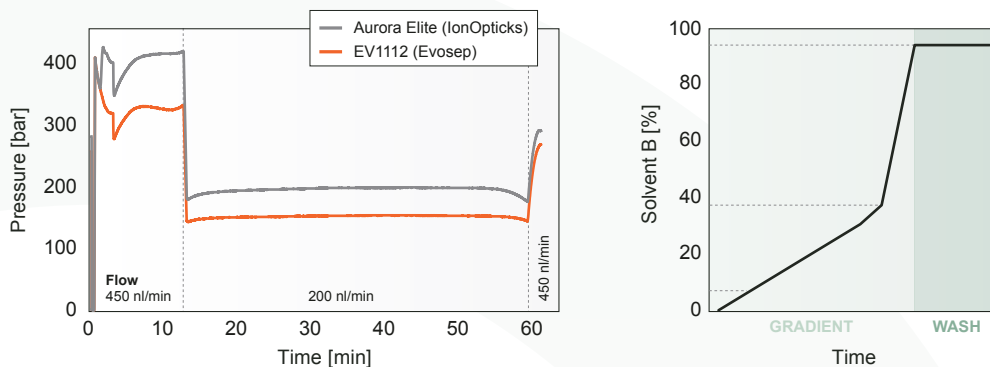


Figure 1: Pump HP pressure profile and representation of gradient in the Whisper 40 SPD method.

2. Chromatographic elution

The performance of the Whisper 20 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.

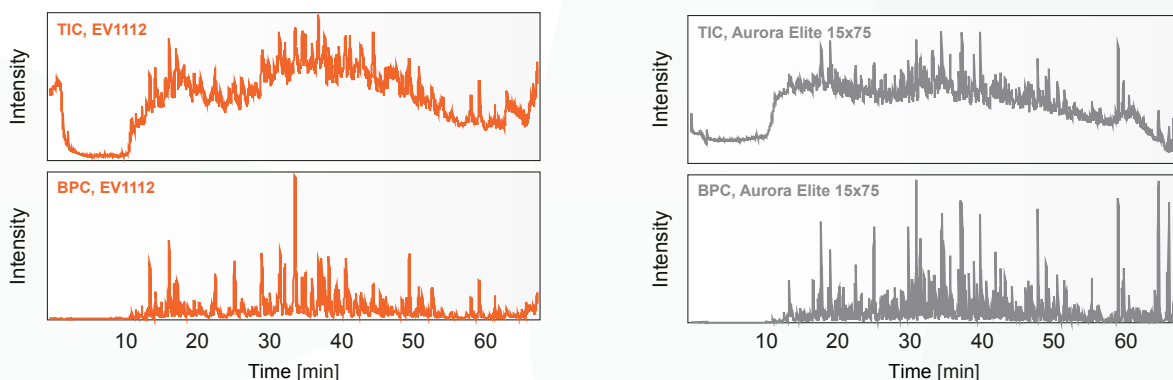


Figure 2: TIC and BPC of 5 ng peptide using the EV1112 and Aurora Elite 15x75 columns.

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.

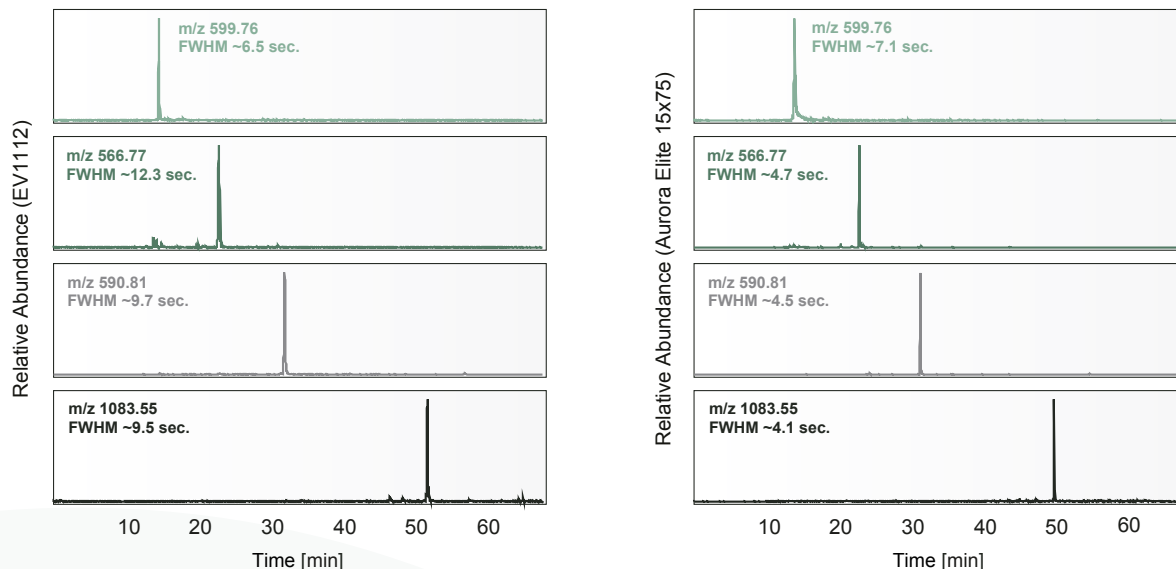


Figure 3: Extracted ion chromatograms and FWHM of selected peptides.

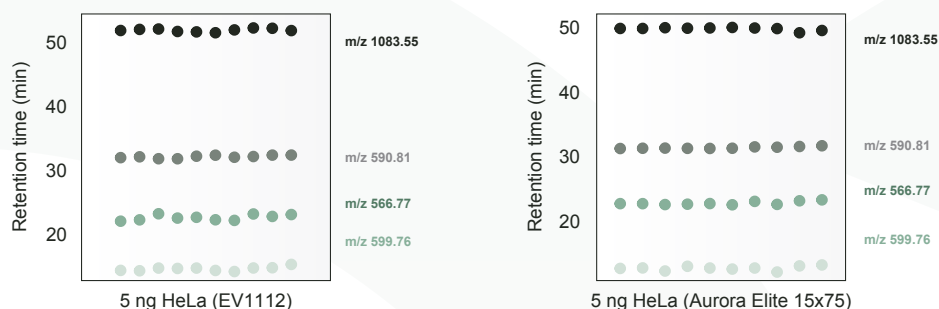


Figure 4: Retention time reproducibility of selected peptides across consecutive runs.

4. Setup and emitters

Prepare the Evosep One to run the Whisper Zoom 20 SPD method using the following guide;

- 1 Select the 'Instrument configuration' method and run the 'Set to Zoom' script.
- 2 Follow the on-screen prompt from the script. The transfer line must be connected in port 3 of the Loop valve.
- 3 Follow the on-screen prompt from the script. The HP flow sensor line must be connected in port 6 of the Loop valve.
- 4 Re-tighten the connections and you are ready to use the method.

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

Mass spec vendor	P/N	Description	Order through
Bruker	1811112	Captive Spray 2 Emitter, 10 µm ID	Bruker
Thermo Scientific	EV1111	Fused silica emitters, ID 10 µm	Evosep