

## A specialized method using Whisper Flow technology with a throughput of **80 samples per day**

### 1. Introduction

The Whisper 80 SPD method has a 15 minute gradient and a cycle time of 18 minutes. The analytical column is equilibrated at 1000 nl/min. The gradient flow is 100 nl/min and increased to

1000 nl/min for washing (Figure 1). The method is designed for the IonOpticks Aurora Rapid75 column at 50 °C. The column and thereby the method is exclusive to a Bruker MS.

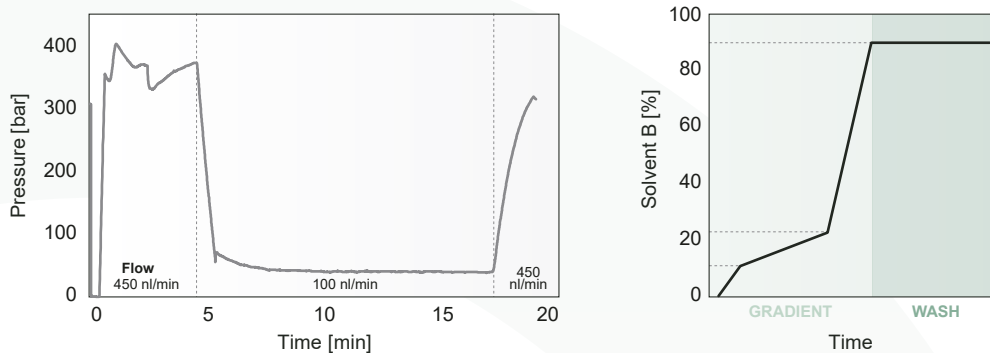


Figure 1: Pump HP pressure profile and representation of gradient in the Whisper 80 SPD method.

### 2. Chromatographic elution

The performance of the Whisper 80 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. Collectively, these metrics serve as the foundation for downstream data processing and optimal results.

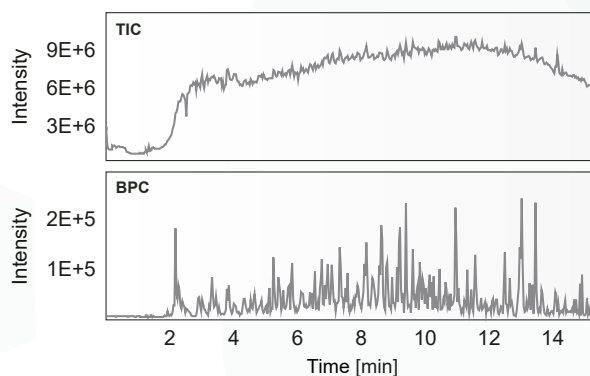


Figure 2: TIC and BPC of 5 ng peptide using the Aurora Rapid75 column on a timsTOF Pro 2.

### 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.

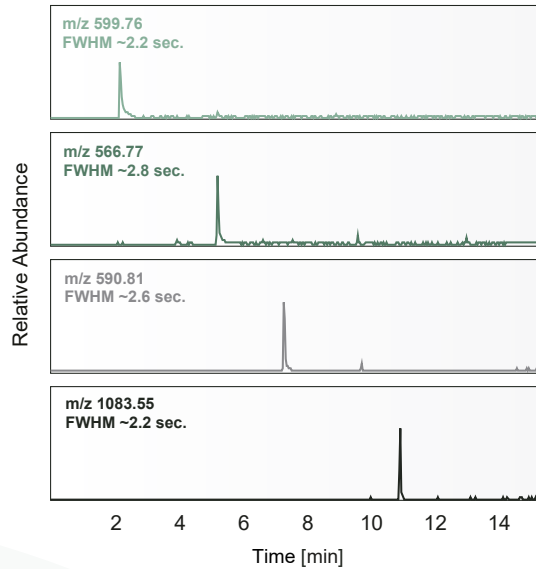


Figure 3: Extracted ion chromatograms and FWHM of selected peptides.



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