

# EVOSEP

## Whisper™ step-by-step guide

### Spray stability is essential for ultra-sensitive proteomics

High spray stability is important for standard proteomics applications, but absolutely crucial for exploring the potential of our Whisper Flow Technology for ultra-sensitive applications. As the electrospray ionization efficiency is concentration dependent, we achieve the highest sensitivity at low flow rates utilized with our EVOSEP+ Whisper methods. The methods are specifically designed for our EV-1112 performance column and EV-1111 fused silica emitters (or a similar 10 µm emitter, such as the 1865691 Captive Spray ZDV Emitter from Bruker). However, the expected chromatographic performance in terms of peak shape and peak width is dependent on correct assembly of these parts.

#### STEP-BY-STEP GUIDE

- 1 Connect the column to the transfer line.
- 2 Run flow to column at 500 nL/min and wait until the solvent is visible on the column end. This is important to ensure a perfect connection.
- 3 Connect the emitter to the column, while flow (500 nL/min) is running and tighten the connection firmly.
- 4 Loosen the connection - without completely disconnecting the connection.
- 5 Re-tighten the connection.
- 6 Set the column temperature to 30 degrees celcius and verify the backpressure to be below 400 bars. Expect the "pump HP pressure profile" to be as shown below during analysis.

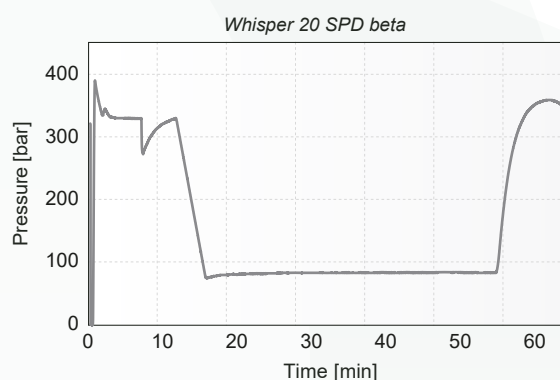
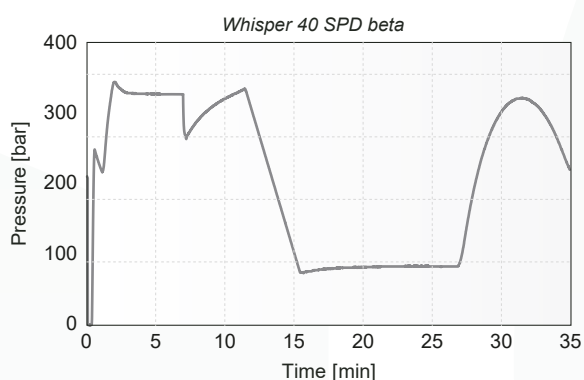


Figure 1: Pump HP pressure profile of the Whisper100 20 SPD beta and Whisper100 40 SPD beta methods