Assays that are both specific and quantitative for target proteins are critical for preclinical validation of putative biomarkers. Such assays are typically multiplexed, multiple reaction monitoring (MRM) analyses which can provide the high-throughput required. Sensitivity is a key requirement for such assays as protein biomarker concentrations may be quite low in commonly used biofluids such as serum and plasma. Improving the sensitivity of LC/MS can be achieved by using nanoflow LC. and by enhancing the sampling and transmission of ions in the mass spectrometer. This study demonstrates the 5-10x sensitivity gain achieved for peptides using a triple quadrupole mass spectrometer modified with a dual ion funnel. The sensitivity achieved using a microfluidic-based nanoflow LC system compared to a standard LC system will be shown discussed.

### Modified JetStream Ion Source

- **Agilent JetStream** is ESI with thermal gradient ion focusing confinement.
- The standard heat sink was modified to provide additional shielding of the nebulizer to accommodate low flow rates.

### Impact of JetStream on Sensitivity

![Image](https://example.com/image)

**Instrument:** 1290 LC system + 6540 QTOF. Column: Eclipse Plus EC-C18 2.1 x 150 mm. Flow rate: 0.4 mL/min. Sample: BSA digest

Agilent JetStream (AJS) technology has been shown to provide a substantial boost (5-10X) in ion generation and sampling efficiency for a variety of molecules including peptides. The figure below shows the increase in signal obtained for standard flow LC/MS (0.4 mL/min) of a standard protein digest.

<table>
<thead>
<tr>
<th>TIC JetStream</th>
<th>TIC ESI</th>
</tr>
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<tbody>
<tr>
<td>EIC JetStream</td>
<td>EIC ESI</td>
</tr>
<tr>
<td>Ratio of response JetStream/ESI</td>
<td>5.8</td>
</tr>
<tr>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td></td>
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</tbody>
</table>

For capillary flow LC, a new AJS source was used with extended shielding of the nebulizer. This modification allows AJS to be used effectively at lower flow rates with a microflow nebulizer. This source gave a significant improvement in sensitivity to the ESI source (right).

### Ion Funnel Technology

The Agilent 6490 QQQ incorporates ifunnel technology which is a combination of three fundamental innovations:

- **Agilent Jet Stream Technology** – ESI with thermal gradient ion focusing confinement.
- Hexabore sampling capillary with 6 independent parallel bores to enable sampling a much larger fraction of the ions
- Dual-stage ion funnel for efficient removal of large gas volumes and ion transfer to Q1 optics

### Results and Discussion

**Standard, Capillary and Nano Flow LC**

LC System: 1290 Infinity, LC1200 capLC system or HPLC-Chip LC Column: Zorbax SB-C18 columns 2.1 or 0.5 mm id x 150 mm for standard and cap flow. For nanoflow, HPLC-Chip with 40 mL enrichment and 75 µm x 43 mm analytical column.

Flow rate: 300, 17 or 0.6 µL/min. Sample: HSA synthetic peptide standard LVNEYTEFAK spiked into tryptsinized serotransferrin (10 fmol/µL).

- **Results (above)** showed no significant difference between standard flow and capillary flow LC → AJS behaves as a mass detector not a concentration detector.
- **Nanoflow LC** still provides a significant increase in sensitivity most likely due to better sampling of the ions.

**Nanoflow LC/MS Sensitivity**

LC System: HPLC-Chip LC system. Column: HPLC-Chip with 40 mL enrichment and 75 µm x 43 mm analytical column.

Flow rate: 0.6 µL/min. Sample: HSA synthetic peptide standard LVNEYTEFAK spiked into tryptsinized serotransferrin (10 fmol/µL).

The calibration curve below shows results obtained for this simple sample. For replicate injections (n=5), the %RSD for response varied from 4.6% (at 2 amol on-column) to 2.8% (100 fmol on-column). The MRM chromatograms corresponding to those levels are also shown indicating the high quality of data obtained at very low levels.

### Conclusions

- For standard flow LC/MS of peptides, AJS offers a 5x increase in sensitivity compared to ESI.
- The new AJS provides the same increase in sensitivity at capillary flow rates.
- The 6490 QQQ with ifunnel technology showed a 10x improvement in sensitivity compared to the 6460.
- No significant gain in sensitivity was observed from decreasing column id/flow, indicating JetStream behaves in a mass-dependent manner.
- Nanoflow is still significantly more sensitive than capillary or standard flow due to improved ion sampling.