The Integration of Nanoscale Separation and Ionization for the Analysis of Complex Proteomes

Tao Yi-Min1, Peter Wang2, Zhou Hu1, Yang Yi-Ming3, Helena Svobodova3, Amanda Berg3, Gary A. Valaskovic3
1Shanghai Institute of Materia Medica, CAS, Shanghai, China; 2New Objective, Inc., Shanghai, China; 3New Objective, Inc., Woburn, MA

Introduction

Nanoflow LC-MS has been used widely in proteomics research. However, the need for specialized training and in-depth knowledge of instrumentation can become a bottleneck to implementing nanoflow LC-MS for high-throughput experiments of complex proteomes. The integration of a nanospray source with chip-based nanoflow columns maximizes performance at low flow rates and ensures reproducibility while simplifying instrument implementation. This novel system facilitates the employment of nanoflow LC-MS in qualitative and quantitative proteomic workflows, delivering efficiency while preserving employment of nanoflow LC-MS in qualitative and quantitative proteomics. The integration of a nanospray source with chip-based nanospray columns enables high-throughput analysis of simple and complex mixtures for qualitative and quantitative workflows.

Chromatographic Performance

Chromatographic Reproducibility Using a Complex Sample

Sequence Coverage

Conclusions

- Integrated chip-based columns and source design provided high performance chromatography.
- Observed symmetrical peak shape and baseline separation for 4-protein digest.
- Self-guided emitter positioning increases the stability of nanospray for improved detection of low abundance peptides.
- Chip-based system enables high-throughput analysis of both simple and complex proteomes through ease-of-use.
- Observed good sequence coverage for 6-protein digest.
- 30% for hirudin.
- 65% for bovine immunotransferrin.
- Demonstrated good retention time reproducibility for three replicate injections of a HeLa cell digest.

Future Work

- Evaluate performance on longer columns in the PosChip column format for:
- Optimized gradient lengths.
- Improved peptide recovery.
- Improved peak shape.
- Identification of more biomarkers.

<table>
<thead>
<tr>
<th>Protein MW (Da)</th>
<th>Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8661</td>
<td>54178</td>
</tr>
<tr>
<td>13630</td>
<td>6966</td>
</tr>
<tr>
<td>1885</td>
<td>HeLa2</td>
</tr>
<tr>
<td>29096</td>
<td>55%</td>
</tr>
<tr>
<td>43196</td>
<td>40%</td>
</tr>
</tbody>
</table>
| 12394 | 50%

Peptide and protein identification results for HeLa cell proteome digest.

<table>
<thead>
<tr>
<th>Peptide Sequences</th>
<th>Identified Peptide</th>
<th>Identified Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>Peptide 2</td>
<td>Peptide 3</td>
</tr>
<tr>
<td>Protein 1</td>
<td>Protein 2</td>
<td>Protein 3</td>
</tr>
</tbody>
</table>

ABOVE: Base peak chromatographic separation of a protease-standard digest on a 10.5 cm PosChip column.
ABOVE: Peptide specific peak capacity, peak width and symmetry data calculated for different 6-protein peptides separated on a 10.5 cm PosChip column.
ABOVE: Extracted ion chromatograms of different 6 protein peptides separated on 10.5 cm PicoChip columns.
ABOVE: Peptide specific peak capacity, peak width and asymmetry data calculated for different 6 protein peptides separated on 10.5 cm PicoChip column.
ABOVE: Extracted ion chromatograms of different 6 protein peptides separated on a 10.5 cm PicoChip column.

**Software**

- MassScope (Bruker Daltonics)
- MaxQuant (MaxPlanck Institute)