EVOSEP ONE

A Separation Tool Designed for Clinical Omics
Evosep aims to improve quality of life and patient care by radically innovating protein based clinical diagnostics.
Clinical Proteomics must be Sensitive, Fast, and Robust

- More uptime with improved reliability and robustness
- Increased productivity with higher throughput and better duty cycle utilization
- Increased performance with better data quality

“Robust and fast workflows are indispensable for successfully implementing large-cohort clinical plasma proteomics studies”,

Professor Matthias Mann,
Max-Planck Institute of Biochemistry,
Proteomics and Signal Transduction
Optimized standard methods

<table>
<thead>
<tr>
<th>Throughput (samples/day)</th>
<th>Cycle Time (min)</th>
<th>Gradient Length (min)</th>
<th>Flow rate (μl/min)</th>
<th>Column dimensions (length/ID/bead size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>4,8</td>
<td>3,2</td>
<td>4,0</td>
<td>4cm / 150μm / 3μm</td>
</tr>
<tr>
<td>200</td>
<td>7,2</td>
<td>5,6</td>
<td>2,0</td>
<td>4cm / 150μm / 3μm</td>
</tr>
<tr>
<td>100</td>
<td>14,4</td>
<td>11,5</td>
<td>1,5</td>
<td>8cm / 100μm / 3μm</td>
</tr>
<tr>
<td>60</td>
<td>24,0</td>
<td>21,0</td>
<td>1,0</td>
<td>8cm / 100μm / 3μm</td>
</tr>
<tr>
<td>30</td>
<td>48,0</td>
<td>44,0</td>
<td>0,5</td>
<td>15cm / 100μm / 3μm</td>
</tr>
</tbody>
</table>

Disposable trap columns

- The sample is loaded and desalted offline on a C18 pipette tip, the Evotip
- The autosampler picks up the tip and places it in the injection port

High instrumentation efficiency

- Leaves contaminants on the Evotip disposable trap columns
- Elutes analytically relevant peptides
- Minimizes cross-contamination
- Extends column lifetime

Partial elution

*Methods subject to change*
Low maintenance components

All the elution and gradient formation happen at low pressure, ensuring minimal wear and tear

Low pressure pumps

Simplified workflows

Integrating elution with liquid chromatography removes sample handling steps as well as reducing injection cycle overheads
Robust throughput

- 10x reduction in carry-over
- 1,000 of runs on each analytical column
- 30,000 sample service interval

Data courtesy: Dr. Jacob Jaffe, Shawn Egri, and Dr. Steven A. Carr, The Broad Institute, Cambridge, MA

1500 injections of 1ug HeLa on the same column
Chromatographic performance

- Intelligent pump control allows efficient use of acquisition window
- Peptide mix in HeLa background used to calculate peak data
- Overlaid runs show good reproducibility in complex standard

<table>
<thead>
<tr>
<th>Throughput (Samples/day)</th>
<th>FWHM (sec)</th>
<th>Peak capacity (4σ / FWMM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>1.5</td>
<td>35 / 72</td>
</tr>
<tr>
<td>200</td>
<td>2.2</td>
<td>41 / 97</td>
</tr>
<tr>
<td>100</td>
<td>4.0</td>
<td>63 / 129</td>
</tr>
<tr>
<td>60</td>
<td>6.8</td>
<td>79 / 161</td>
</tr>
<tr>
<td>30</td>
<td>15.2</td>
<td>111 / 216</td>
</tr>
</tbody>
</table>
Reproducible plasma performance

Retention time stability of selected peptides over 96 runs

Pearson correlation matrix comparing the 96 runs

Low carry-over between runs (<0.1%)

Data courtesy: Dr. Philipp Geyer, Max Planck Inst., Martinsried
Fast and sensitive HCP screening

- Bottom-up mass spectrometry protocols are established for analyzing host cell proteins (HCPs)
- Detecting low abundance HCPs confidently and with high throughput is now possible with the Evosep One paired with a trapped ion mobility separation TOF instrument

61 HCPs identified in 21 minutes from 25 µg NISTmAb (according to Huang et al., Anal. Chem. 2017, 89, 5436-5444) equivalent to approximately 1 µg load.

- 3 times faster*
- 2 times more sensitive*

*than 1h industry standard

Data courtesy: FN-06 flash note; Bruker Daltonik GmbH, Germany
Protein identification with short gradients

- Stable protein and peptide ID rates over 96 injections with the 200 samples/day methods
- Examples of identification rates possible with different methods.

All measurements were performed on a Bruker Daltonics timsTOF Pro with 50 ng of HeLa digest and at least 15 replicates.

Data courtesy: LCMS-141 app note, Bruker Daltonik GmbH, Germany

Fast generation of DIA libraries

Spectral library of mammalian cell line proteomes made from 46 fractions of HeLa digests.

<table>
<thead>
<tr>
<th>Total time</th>
<th>Gradient time</th>
<th>Peptides</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.4h</td>
<td>16.1h</td>
<td>132,850</td>
<td>9,918</td>
</tr>
</tbody>
</table>

Example ID and quantitation performance with 60 samples/day method

Data courtesy: Prof. Jesper Olsen and Dorte Bekker-Jensen, Novo Nordisk Center for Proteome Research, Copenhagen on a Thermo Scientific Q Exactive HF-X
Large clinical proteomics sample set

Enables large cohort studies in a fast and robust manner with
- Stable retention times
- High data consistency
across hundreds of injections

Data courtesy: Dr. Ben Collins, Dr. Evan Williams and Prof. Ruedi Aebersold, ETH Zürich on a Sciex TripleTOF 6600