A novel nanoflow LCMS limited sample proteomics approach using micro pillar array columns (µPAC™)

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ABSTRACT

Bottom-up proteomics using 50 to 100 ng µC18 packed pillar columns coupled to high resolution mass spectrometry is now established as the common workflow to analyze protein samples from tissues, body fluids or cell lysates. Typically, micrograms of samples are separated in 30 min or 4 hour nano LC gradients resulting in the identification of 4000 to 10000 protein groups [1]. However, ease-of-use and reproducibility of nanoflow LCMS using packed pillars do not allow nosivas and routine use.

PharmaFluidics’ µPAC™ technology (micro Pillar Array Column) is a unique and novel approach to a chromatographic support structure and builds upon microfluidic chromatographic separation beds into silicon. The low pill columns’ dispersion obtained by the resulting perfect order separation bed virtually eliminates axial peak dispersion, resulting in higher gradient plate numbers with sharper peaks and higher concentration of compounds. The trending nature of the pillars also leads to much lower backpressure allowing the use of very long columns (1). These exceptional properties result in excellent chromatographic performance with high-resolution and high-throughput [2].

Here, we are presenting data on testing this novel approach to a nanoflow column in a bottom-up proteomics workflow. Coupling a 2 m long µPAC™ column in a nano source to a Thermo Fisher nLC1200 pump and a Thermo Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer (Figure 2), we used standard proteomics separation and electrospray conditions, e.g. a flow rate of 300 nll/min with a 3 min gradient of 0 to 40% acetonitrile and a 2.4 cm length of a 10 µm silica emitter. The Fusion Lumos™ Orbitrap™ mass spectrometer was operated at a resolution of 120000.

Injecting a dilution series of 1 ng, 100 ng and 10 ng we observed in triplicate runs the highest concentration 5400 protein groups in a 4 hour gradient run (minimizing when injecting only 10 ng HeLa cell digest, corresponding to the content of 50 cells) [3], we still see over 5000 protein groups. Thus, this workflow using µPAC™ columns and a Fusion Lumos™ MS is suitable to proteomics experiments where the sample amount is very limited to a small number of cells and therefore opens up a new tool for biologists.

MATERIALS AND METHODS

Sample Preparation: Lysophilized tryptic HeLa cell digest was purchased from Thermo Fisher and dissolved in 20 µl of solvent A (0.1% formic acid in water) resulting in a concentration of 1000 ng/µl. 1/10 and 1/100 dilution of the stock resulted in samples with a respective peptide concentration of 100 and 10 ng/µl.

Instrumentation: A 250 cm long PharmaFluids µPAC™ C18 nano LC column was operated with a Thermo Fisher nLC1200 pump and coupled via a nano source to a Thermo Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer. Several experiments were performed using standard proteomics separation and electrospray conditions, e.g. a flow rate of 300 nll/min with a 3 min gradient of 0 to 40% acetonitrile and a 2.4 cm length of a 10 µm silica emitter and the Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer was operated at a resolution of 120000.

Data Analysis: Protein and peptide identification was performed using Thermo Scientific™ Proteome Discoverer™ software. Chromatographic characterisation of the raw data was performed using Thermo Scientific™ Excalibur™ software.

RESULTS

Figure 1. Extracted ion chromatograms for 120 min gradient separations of tryptic Hela cell digest. Top: 1000 ng/µl, Center: 100 ng/µl, Bottom: 10 ng/µl. Combined extracted ion chromatograms for the peptides used to evaluate chromatographic performance are shown underneath in red. 1: m/z 255.72; 2: m/z 167.36, 3: m/z 685.12; 4: m/z 446.36, 5: m/z 807.45.

Figure 2. Schematic overview of the setup used for the experiments. Top: Thermo Fisher nLC1200 pump and a Thermo Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer. Bottom: Direct injection configuration used to inject 1 µl.

Figure 3. Baseline chromatograms for 120 min gradient separations of tryptic Hela cell digest. Top: 1000 ng/µl, Center: 100 ng/µl, Bottom: 10 ng/µl. Combined extracted ion chromatograms for the peptides used to evaluate chromatographic performance are shown underneath in red. 1: m/z 255.72; 2: m/z 167.36, 3: m/z 685.12; 4: m/z 446.36, 5: m/z 807.45.

Figure 4. Extracted ion chromatograms for a peptide with m/z 638.32. Triplicates for each concentration (1000 ng/µl, 100 ng/µl and 10 ng/µl) are compared.

Figure 5. Peak capacity values calculated according to equation 1 using the average peak width of 5 peptides distributed over the elution window. Extracted ion chromatograms of these peptides shown in Figure 3.

Figure 6. Average number of identified protein and peptide groups.

Table 1. Average chromatographic metrics for 120 min gradient separations (n=2).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>n</th>
<th>Average retention time variation [CV]</th>
<th>n</th>
<th>Average m/z variation [CV]</th>
<th>n</th>
<th>Average peak capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ng/µl</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>100 ng/µl</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>10 ng/µl</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>8%</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 7. Schematic overview of the setup used for the experiments. Top: Thermo Fisher nLC1200 pump and a Thermo Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer. Bottom: Direct injection configuration used to inject 1 µl.

CONCLUSIONS

• Highly reproducible chromatographic separation of tryptic digest samples can be achieved using the combination of a 200 cm long PharmaFluidics µPAC™ C18 nano LC column, a Thermo Fisher nLC1200 pump and a Thermo Orbitrap Fusion Lumos™ Tribrid™ mass spectrometer.

• Excellent analytical performance with peak capacities up to 846 to a 240 minute gradient can be obtained using this combination.

• Injecting a dilution series of 1000, 100 and 10 ng tryptic Hela cell digest, over 5400 protein groups were identified in a 240 minute gradient run at the highest sample concentration.

• With injection only tryptic Hela cell digest, roughly corresponding to the content of 50 cells, over 3000 protein groups can be identified in a single 120 minute gradient run.

• Whereas the traditional nano LC/ESI LC/MS approach used to achieve high analytical performance are generally limited in terms of operation flexibility (very high backpressure, low flow rates only, 200 ng long LC/MS analysis can easily be operated in a wide range of flow rates hence enabling chromatographers to optimize separation parameters according to their needs.

REFERENCES


3. R. Milo. What is the total number of protein molecules per cell volume? A call to rethink some published values, Bioseis 15 (2013) 1295-1295

TRADEMARKS/LICENSING

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