Novel capillary-flow LC-MS platform for robust proteomics profiling of cell lysates and bio-fluids

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ABSTRACT
Purpose: We present a highly robust novel capillary-flow LC-MS platform that revolutionizes the Thermo Scientific™ capillary-flow UltiMate™ 3000 RSLCnano system (capLC), the new 150 µm ID EASY-Spray column (capLC), and the new Thermo Scientific™ Q Exactive™ HF-X mass-spectrometer.
Methods: We used typical protein shotgun experiments (Full-scan MS, Data-Dependent Acquisition (DDA) to verify the performance and robustness of the novel capLC-MS platform.
Results: We show that the novel capLC-MS platform is a sensitive and reliable solution for shotgun high-resolution accurate-mass (HRAM) proteomics experiments that can be used for routine proteome profiling of complex samples including bio-fluids as well as targeted high-throughput quantification.

INTRODUCTION
Capillary-flow LC-MS (capLC-MS) with 100-300 µm inner diameter (ID) columns and flow rates of 0.1 to 10 µL/min is widely used for high-throughput analysis, high sensitivity, and high-resolution accurate mass (HRAM) analysis. Reduced sample consumption, and reduced MS contamination compared with typical flow LC-MS separations which runs at flow rates of more than 100 µL/min. Additionally, capLC-MS gives higher throughput in comparison to nano-LC, while allowing for simultaneous separation and detection by achieving higher sample amounts. Here we describe a novel capLC-MS platform that can be used for high-throughput analysis, while providing higher MS sensitivity for routine shotgun proteomics due to the brighter ion-source of the Q Exactive™ HF-X mass-spectrometer.

RESULTS

TABLE 1. Chromatographic characteristics for 3 selected CytoC peptides averaged over 150 consecutive injections of CytoC protein digest, separated at 3.2 µL/min. The total analysis time was 60 min.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>PWHM, sec</th>
<th>Ass. PW base, sec</th>
</tr>
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<tbody>
<tr>
<td>KYIPGTK</td>
<td>0.0E+00</td>
<td>0.0E+00</td>
</tr>
<tr>
<td>MIFAGJK</td>
<td>1.0E+00</td>
<td>1.0E+00</td>
</tr>
<tr>
<td>ALRAKPK</td>
<td>2.0E+00</td>
<td>2.0E+00</td>
</tr>
</tbody>
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CRUDE PLASMA PROTEOME
The abundance of proteins in the plasma proteome is above 10 orders of magnitude. Additionally, the 14 most abundant proteins cover more than 94% of the total protein mass. Thus, it is very challenging to perform deep proteome profiling of blood samples without intensive pre-fractionation. However, variations of the 14 most-abundant proteins can be used as targets for plasma proteomics. Furthermore, in several inflammatory markers can be monitored by analysis of crude plasma or serum protein digests. The simple procedure described here utilized EASY-Spray was used to digest plasma samples (Fig. 10) in this work.

Figure 10. Scheme of crude plasma digestion pipeline.

Figure 11. Peptide (red line) and protein (blue bars) identified with 1 % FDR in crude plasma digestion at different MS2 injection times and 60 min total analysis time.

DEPTH OF HUMAN PROTEOME PROFILING
The depth of proteome coverage from a crude plasma digest and a HeLa cell lysate digested achieved by capLC-MS was evaluated based on the logarithmically transformed (enPAl index) and biplot of the proteins identified in the sample. The proteome identified in cell lysate as well as in plasma covered 4 orders of magnitude; however, the difference in protein abundance for those proteins identified in plasma is significantly more pronounced compared to those identified from HeLa cells.

Figure 12. Sorted sequentially modified protein abundance index (enPAl) of proteins identified in HeLa cell lysate digest (red) and crude plasma protein digest (blue).

CONCLUSIONS
The novel capillary-flow LC-MS platform combines the advantages of high-throughput analytical flow LC separations and the high sensitivity of nano-LC-MS analysis. The high robustness and sensitivity it provides, permit routine profiling of proteomics samples and targeted analysis of large sample sets.


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