Efficient human plasma proteome profiling at up to 240 samples per day

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Abstract

Purpose: Develop and validate high throughput capillary flow LC-MS methods for use in translational research.

Methods: Neat human plasma protein digests separated using capillary flow LC hyphenated to Orbitrap MS. Data was acquired in data independent acquisition mode.

Results: At a sample throughput rate of 240 samples per day (SPD), 246 protein groups could be identified of which 205 could be quantified at precision levels below 20% CV.

Introduction

LC-MS-based proteomics stands as a vital tool for the analysis of complex biological and clinical samples. Within the separation core, the LC column assumes a pivotal role. The micro pillar array column (µPAC™) emerges as an innovative LC column, offering both highly efficient separation and minimal back pressure. In this study, we used a 5.5 cm long Thermo Scientific™ µPAC™ Neo High Throughput column (figure 1) coupled with HRAM mass spectrometry. The goal was to expedite bottom-up proteomics profiling of neat human plasma samples making use of the unique flow rate flexibility of µPAC.

Materials and methods

Sample preparation

Pooled human blood plasma (Sigma Aldrich) underwent S-Trap™ mini spin column (Protifi) digestion, followed by resuspension in 0.1% TFA to 1000 ng/µL.

Test method(s)

Neat plasma digests were analyzed using a Thermo Scientific™ Orbitrap™ Exploris 240 coupled to a Thermo Scientific™ Vanquish™ Neo UHPLC system, employing a DIA strategy. Separation occurred on a µPAC Neo HT column in direct injection mode. A dilution series (10 to 2000 ng) of neat plasma digests was injected by adjusting the injection volume (10 nL to 2 µL) and separated using various flow rate methods (100, 170, and 240 SPD). The isolation window was 10 Th, with MS1 and MS2 resolutions of 30K and 15K, respectively. Precursor mass ranges were set from 500-700, from 450-700 and from 400-800 m/z, yielding respectively 20, 25 and 40 scan events for the 100, 170 and 240 samples per day method.

Data analysis

LC-MS data were analyzed either using Proteome Discoverer 3.1 with Chimerys or with Spectronaut® 18. Results shown have been filtered to a 1% FDR.

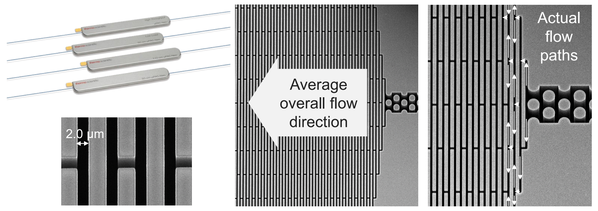

Figure 1. The µPAC Neo HT column

Results

LC method optimization and instrument productivity

Three LC methods for high throughput have been devised to combine sample throughput and sensitivity. With a direct injection approach, just under 2 minutes of the method are allocated to sample loading and column equilibration. Since the initial flow rate of the LC method permits complete column equilibration within half a minute, this procedure can proceed concurrently with the sample uptake phase. Consequently, instrument productivity levels of 57%, 65%, and 76% were respectively attained at sample throughput rates of 100, 170, and 240 samples per day.

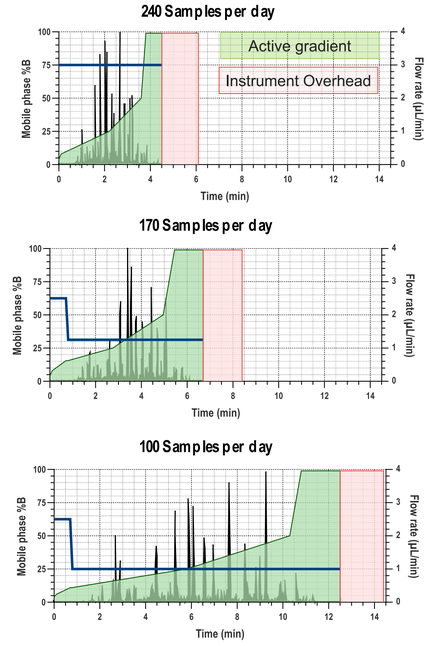

Figure 2. Gradient and flow rate profiles used to achieve 100, 170 and 240 SPD throughput using a direct injection approach. Overlay: Base peak chromatograms for separation of 100 ng crude plasma digest sample.

Plasma proteome coverage

Using a 3.5-minute gradient 240 SPD method, the amount of protein groups that could be identified from neat human plasma steadily increased according to the sample load, up to a load of 200 ng plasma on column, where a plateau of about 250 protein groups was achieved.

Similar observations were done across different SPD settings (100 and 170), where increasing sample loading beyond 500 ng did not increase proteome depth. Checking the base peak chromatograms confirms 200 ng as the onset of overloading.

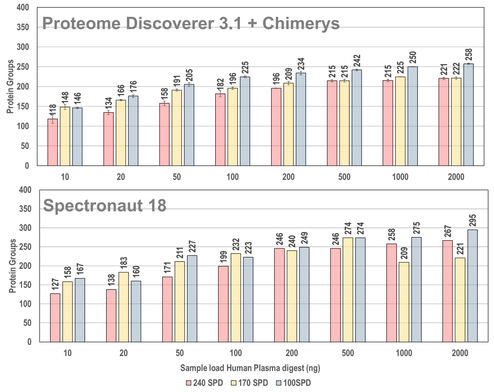

Figure 3. Average number of protein groups identified (1% FDR- technical triplicates) from neat human plasma digest samples (240, 170 and 100 samples per day).

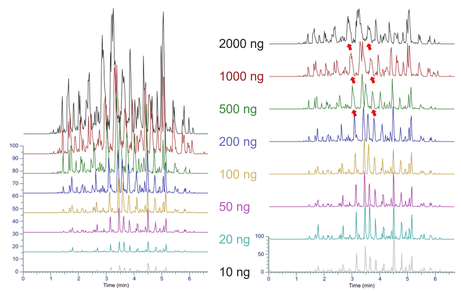

Figure 4. Base peak chromatograms obtained for the separation of neat human plasma digest samples at 170 samples per day (10 to 2000 ng digest on column). Left: no signal normalization. Right: Intensity normalized for each sample load.

By extending the gradient length to respectively 5.5 and 11 min, increased depth and especially an increase in number of quantifiable proteins could be obtained. The µPAC Neo HT column achieved a pragmatic compromise at 170 SPD, quantifying 248 protein groups (below 20% CV). The impact of extending gradients beyond the 5.5 min used for the 170 SPD method appeared to be very limited, as barely any depth could be gained. When comparing the results with those obtained with low capillary flow packed bed alternatives, significantly more proteins could be precisely quantified from technical triplicates (ranging from 60% to 5 % for sample loads below 500 ng on column).

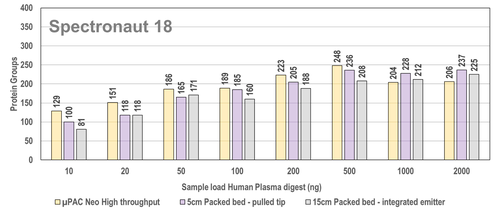

Figure 5. Comparison of µPAC Neo HT to Packed bed column alternatives. Proteins quantified at CV≤ 20% from neat human plasma digest samples using a 170 SPD LC method.

Conclusions

- Three capillary flow LC methods developed for high throughput and sensitivity.
- Sample loading and column equilibration completed in under 2 minutes.
- Optimal protein identification achieved with 200 ng plasma on column.
- Quantitation of up to 248 protein groups at CV below 20% for 500 ng plasma.
- Variable flow rate methods balance ionization efficiency and separation performance for enhanced proteome coverage.

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