High-throughput proteome analysis using 50 cm micro Pillar Array Columns (µPAC™)

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Introduction
The practice of bottom-up proteomics relies to a large extent on the separation performance that can be achieved with state-of-the-art nano LC-MS/MS equipment. Depending on the sample complexity or the instrument time that can be dedicated to a certain sample, different LC columns and corresponding LC/MS/MS methods are often required. When aiming for comprehensive proteome analysis with deep coverage, relatively long columns (lengths up to 75 cm) are typically operated with long and shallow solvent gradients, delivering the highest chromatographic performance. This is indeed a good strategy if very complex samples need to be analysed and when as much information as possible needs to be retrieved from these samples. However, daily routine proteome analysis often deals with much less complex samples or demands increased sample throughput, making total analysis times above 120 min undesirable or even impossible. Therefore, a high throughput platform at nanoflow scale was developed that is perfectly suited to perform routine analyses with shorter (30, 60 and 90 minute) gradient solvent times.

A unique approach breaking through the traditional limitations ~ micro Pillar Array Column (µPAC™)

The backbone of the stationary phase of a µPAC™ is defined by design, and reproduced multiple times using a photomask (left figure) using lithography on silicon wafers. The separation bed is machined out of the silicon using advanced etching techniques [1]. Doing so, columns with identical separation beds are obtained.

The control by design of the pillar dimensions and the interpillar distance results in a high permeability, which in turn enables the use of exceptionally long columns at low to moderate system pressures [2].

Channel width: 0.3 mm
Channel depth: 0.018 mm
Total column volume: 3 µl
Flow rate: 0.1 – 2 µl/min

Benchmarking vs packed bed
500 ng of HeLa cell tryptic digest spiked with 50 fmol/µl RTIC mix was injected in full loop mode (1µl) onto the µPAC™ column and on two packed bed alternatives. A Thermo Fischer scientific Orbitrap Elite mass spectrometer operated in 2014 was used to evaluate chromatographic performance as well as the corresponding peptide and protein identification numbers that could be achieved.

Increased throughput
By ramping the flow rate up to 1.5 µl/min, the column void time can be decreased down to 2 minutes. This allows for more time efficient use of the instrument. A cytochrome c tryptic digest (0.3 pmol/µl) was separated with different gradient conditions that yield a sample throughput of 30, 60 and 100 samples per day with respective peak capacity values of 300, 220 and 160.

References

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