PharmaFluidics

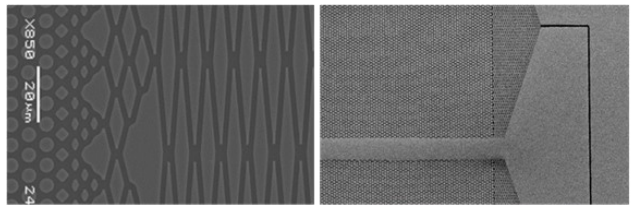
µPAC™ or micro Pillar Array Columns

The next step towards robustness, reproducibility and resolution in low flow LC-MS

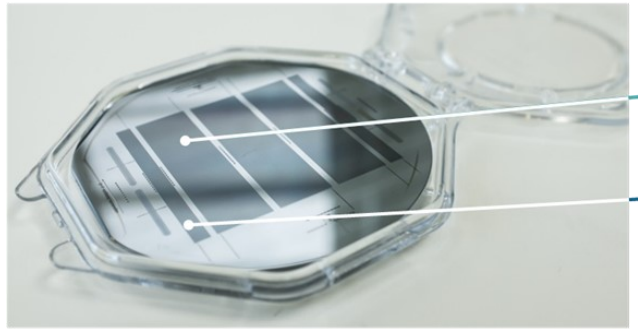
Catherine Stassen & Robert van Ling

X-Omics Festival 2021

Enjoy the beauty of pillars

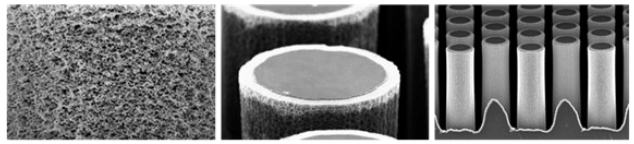


Flow distribution for perfect plug conservation in inlets and turns

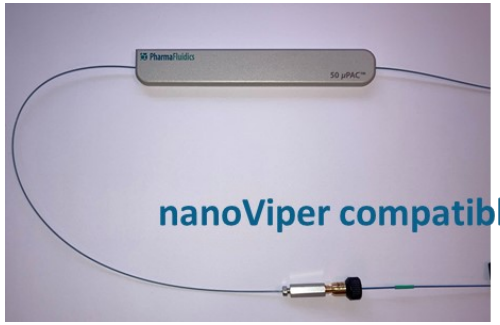


200 cm µPAC™ column

50 cm µPAC™ column



Stationary Phase controlled by design



µPAC™ Performance in deepest diving proteomics

Article

The proteome landscape of the kingdoms of life

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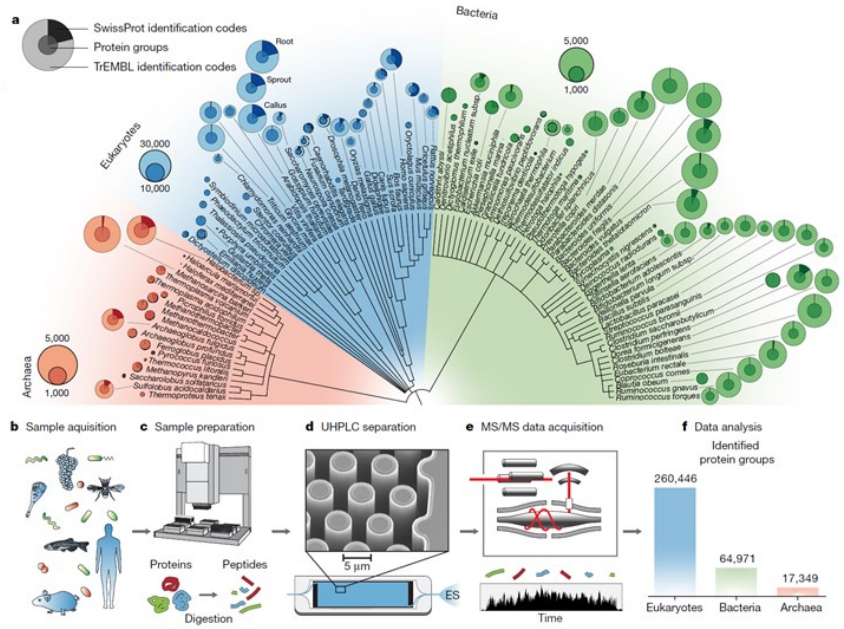
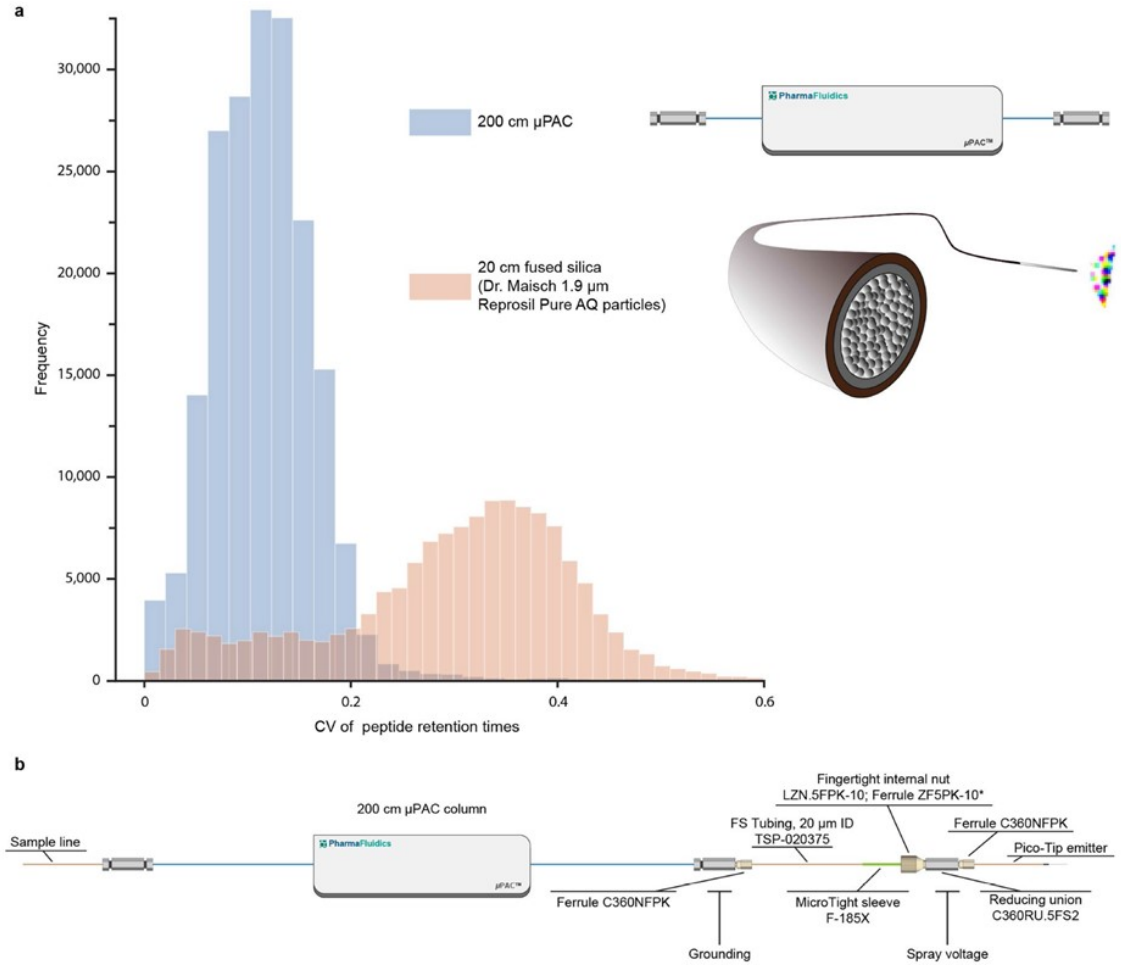
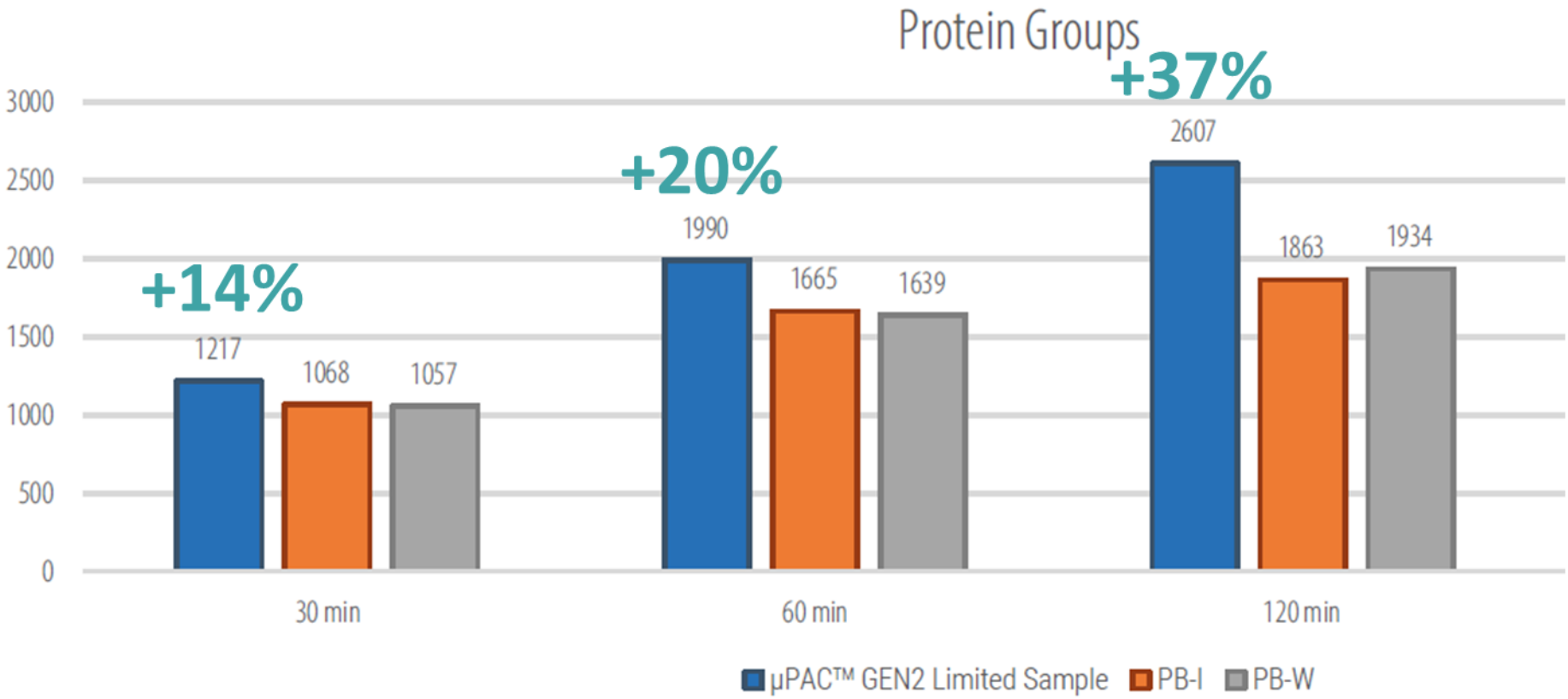
Check for updates

Johannes B. Müller[1,7], Philipp E. Geyer[1,2,7], Ana R. Colaço[3], Peter V. Treit[1], Maximilian T. Strauss[1,2], Mario Oroshi[1], Sophia Doll[1,2], Sebastian Virreira Winter[1,2], Jakob M. Bader[1], Niklas Köhler[4], Fabian Theis[4,5], Alberto Santos[3,6] & Matthias Mann[1,3]





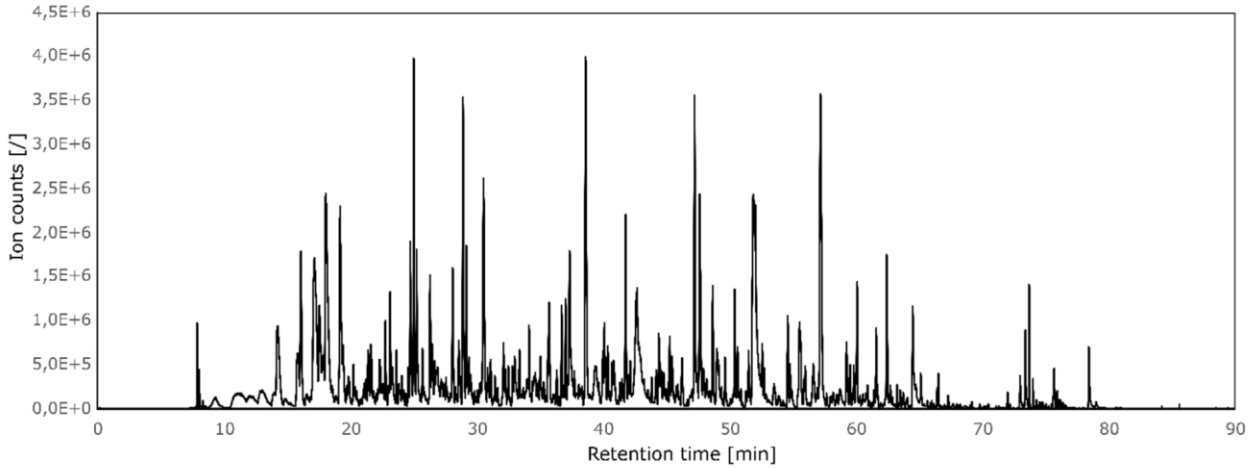
Benchmarking 50 µPAC™ GEN2 Limited Sample (10 ng)



50 µPAC Limited Sample & µPAC Flex iON Connect

- Outstanding results against packed-bed emitter type (PB-I) and packed-bed (W) columns

Pushing towards single cell sensitivity



MS basepeak chromatogram obtained for the separation of 250 pg HeLa tryptic digest using a 60 min non-linear gradient at 250 nL/min

Cold Spring Harbor Laboratory bioRχiv
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New Results

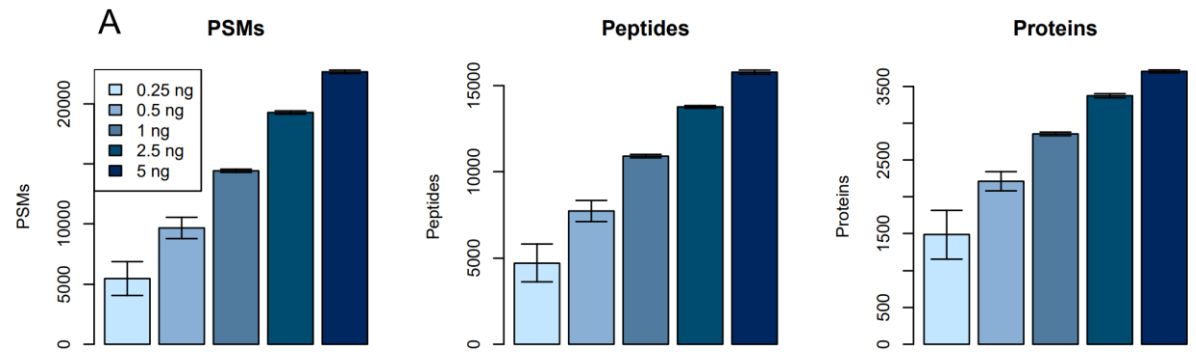
Comment on this paper

Ultra-sensitive nanoLC-MS using second generation micro pillar array LC technology with Orbitrap Exploris 480 and FAIMS PRO to enable single cell proteomics

Karel Stejskal, Jeff Op de Beeck, Gerhard Dürnberger, Paul Jacobs, Karl Mechtler

doi: https://doi.org/10.1101/2021.02.10.430648

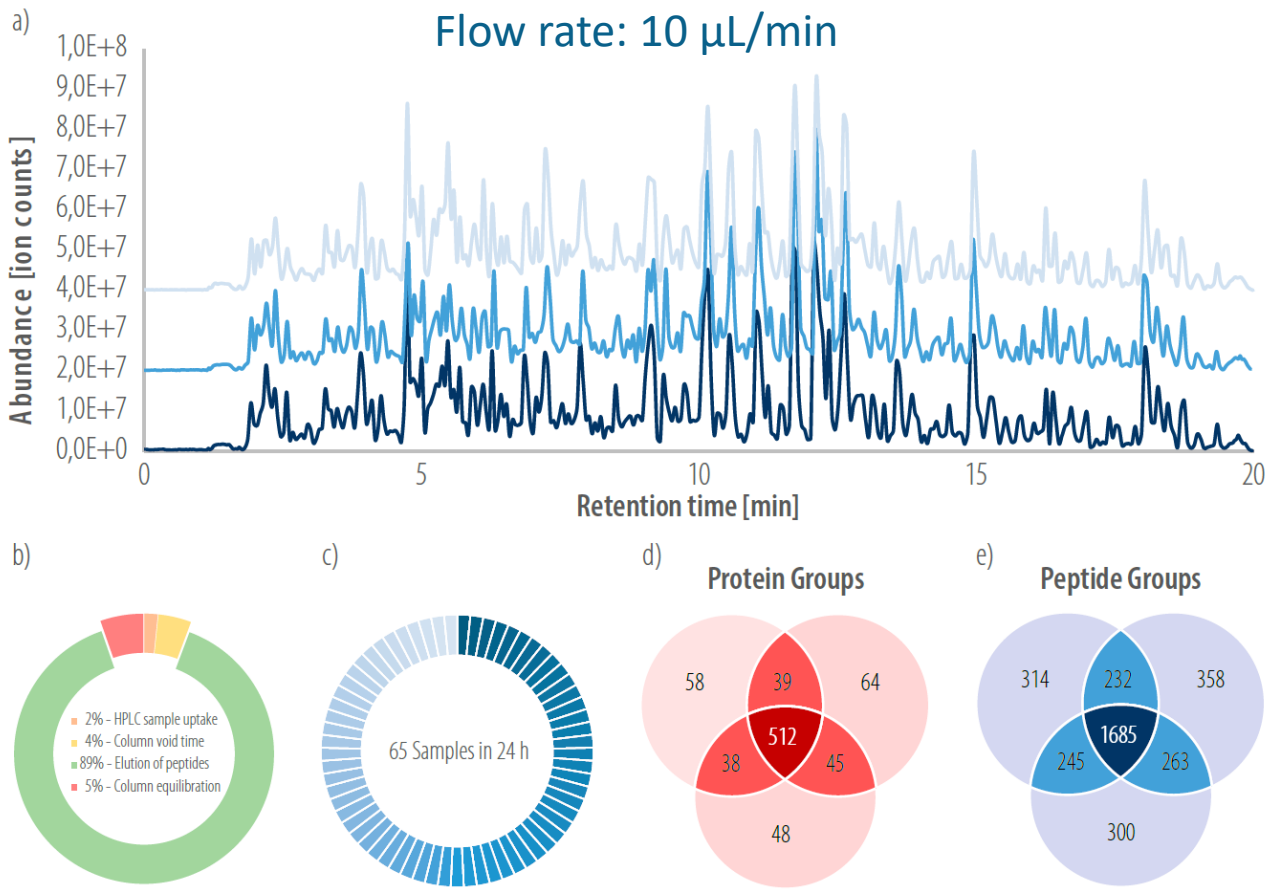
This article is a preprint and has not been certified by peer review [what does this mean?].



PSM, peptide and protein identifications obtained for a dilution series of HeLa tryptic digest (5 to 0.25 ng injected)

Increasing throughput with µPAC™ capLC

Gradient time: 17.5 min
Flow rate: 10 µL/min



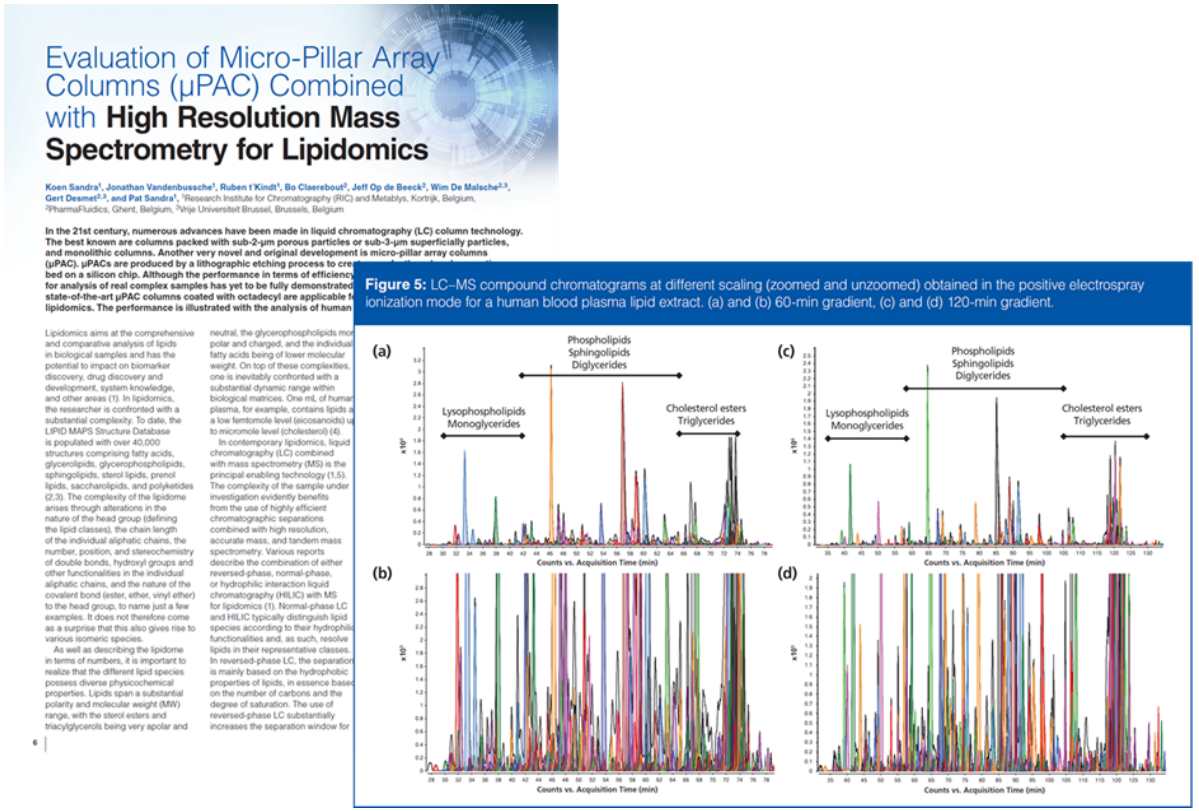
Small sample volumes in metabolomics/lipidomics

- Separations using 2.1 mm ID columns
- Small model organisms provide smaller sample volumes ≥ 1 µL
- Requiring more sensitivity in LC/MS

Zebrafish

E coli

C elegans



Evaluation of Micro-Pillar Array Columns (µPAC) Combined with **High Resolution Mass Spectrometry for Lipidomics**

Koen Sandra[1], Jonathan Vandenbussche[1], Ruben t'Kindt[1], Bo Claerebout[2], Jeff Op de Beeck[2], Wim De Malsche[2,3], Gert Desmet[2,3], and Pat Sandra[1]. [1]Research Institute for Chromatography (RIC) and Metablys, Kortrijk, Belgium, [2]PharmaFluidics, Ghent, Belgium, [3]Vrije Universiteit Brussel, Brussels, Belgium

In the 21st century, numerous advances have been made in liquid chromatography (LC) column technology. The best known are columns packed with sub-2-µm porous particles or sub-3-µm superficially particles, and monolithic columns. Another very novel and original development is micro-pillar array columns (µPAC). µPACs are produced by a lithographic etching process to cre... bed on a silicon chip. Although the performance in terms of efficiency... for analysis of real complex samples has yet to be fully demonstrated... state-of-the-art µPAC columns coated with octadecyl are applicable f... lipidomics. The performance is illustrated with the analysis of human

Lipidomics aims at the comprehensive and comparative analysis of lipids in biological samples and has the potential to impact on biomarker discovery, drug discovery and development, system knowledge, and other areas (1). In lipidomics, the researcher is confronted with a substantial complexity. To date, the LIPID MAPS Structure Database is populated with over 40,000 structures comprising fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (2,3). The complexity of the lipidome arises through alterations in the nature of the head group (defining the lipid classes), the chain length of the individual aliphatic chains, the number, position, and stereochemistry of double bonds, hydroxyl groups and other functionalities in the individual aliphatic chains, and the nature of the covalent bond (ester, ether, vinyl ether) to the head group, to name just a few examples. It does not therefore come as a surprise that this also gives rise to various isomeric species.

As well as describing the lipidome in terms of numbers, it is important to realize that the different lipid species possess diverse physicochemical properties. Lipids span a substantial polarity and molecular weight (MW) range, with the sterol esters and triacylglycerols being very apolar and neutral, the glycerophospholipids mo... polar and charged, and the individual fatty acids being of lower molecular weight. On top of these complexities, one is inevitably confronted with a substantial dynamic range within biological matrices. One mL of human plasma, for example, contains lipids a... a low femtomole level (eicosanoids) u... to micromole level (cholesterol) (4).

In contemporary lipidomics, liquid chromatography (LC) combined with mass spectrometry (MS) is the principal enabling technology (1,5). The complexity of the sample under investigation evidently benefits from the use of highly efficient chromatographic separations combined with high resolution, accurate mass, and tandem mass spectrometry. Various reports describe the combination of either reversed-phase, normal-phase, or hydrophilic interaction liquid chromatography (HILIC) with MS for lipidomics (1). Normal-phase LC and HILIC typically distinguish lipid species according to their hydrophilic functionalities and, as such, resolve lipids in their representative classes. In reversed-phase LC, the separation is mainly based on the hydrophobic properties of lipids, in essence based on the number of carbons and the degree of saturation. The use of reversed-phase LC substantially increases the separation window for

6

Figure 5: LC–MS compound chromatograms at different scaling (zoomed and unzoomed) obtained in the positive electrospray ionization mode for a human blood plasma lipid extract. (a) and (b) 60-min gradient, (c) and (d) 120-min gradient.

User comments on robustness

Als antwoord op @UCDProteomics en @Thomas_Beer

Have you seen PharmaFluidics now have a capillary flow column? I've been blown away by the micropillar chemistry in nano and it's run (in direct injection) for 6 months straight-absolutely no loss in performance. Expensive but only bought one 👌

Tweet vertalen

11:05 p.m. · 9 mrt. 2020 · Twitter for iPhone

1 Retweeten **1** Vind-ik-leuk

Brett Phinney @UCDProteomics · 9 mrt.
Als antwoord op @duncoafc en @Thomas_Beer
What flow rate were your running at ? I tried one and I could not get it to work well. They have that really stupid half meter outlet tubing on the end . I'm think of trying it again and using a higher flow (1.5 ul/min) and a easyspray cap emitter

Dunc Smith /// @duncoafc · 9 mrt.
280 nl/min routinely but also brilliant for ballistic gradients at 600nl/min for injection to injection in 30 mins with 20 min separation for higher throughout of simpler samples. Stunning and I think micro flow could be its real sweet spot

Brett Phinney @UCDProteomics · 9 mrt.
Thanks ! What length are you using? It's so expensive I'd be frightened to do direct injection on it . I'm planning to demo it again soon. Maybe I'll have better luck

Dunc Smith /// @duncoafc · 9 mrt.
50cm.....got a 2M in a drawer but not had the time to play yet. It hasn't missed a beat-really impressive and running at lower pressure is so much more chilled 👌

Back in 2017, Goran Mitulović was one of the first to get his hands on the brand-new 200 cm µPAC™. As the head of the Proteomics Core Facility of the Medical University of Vienna, the potential of the new separation technology to improve his analytical services intrigued him. Recently, he has co-authored a paper detailing his first experiences with the column, optimizing the analytical workflow and benchmarking its performance. These initial results proved so good that µPAC™ has since been introduced as the state-of-the-art for all proteomics analyses at the Core Facility. We spoke to Dr. Mitulović about his work and his recent publication.

Dr. Goran Mitulović

The art of tinkering

Interview_Goran-Mitulović_Towards-clinical-proteomics-applications.pdf

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22 • 1 Comment

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Goran Mitulović • 1st
Actively pursuing new challenge! Experienced analytical scientist (chromat... 6d...

Well, I am happy to say that a single 200-cm column has handled already more than 2000 injections and is still running without performance loss. Thanks #Pharmafluidics.

PharmaFluidics

Join us in the meet & greet