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Interview - Prof. Dr. Allan Stensballe

Overcoming Robustness Challenges in NanoLC-MS

Prof. Dr. Allan Stensballe

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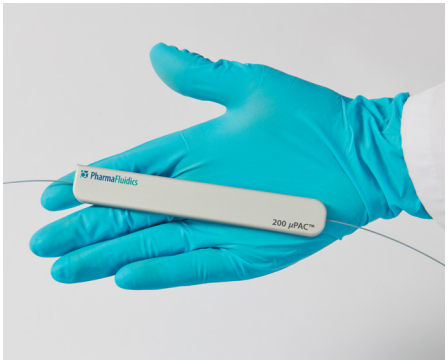
Interview with Prof. Dr. Allan Stensballe, Aalborg University, Department of Health Science and Technology, The Faculty of Medicine, Translational Biomarkers for Pain & Precision Medicine

Within the Aalborg University, Prof. Stensballe is responsible for the research facility for Translational Biomarkers for Pain & Precision Medicine. He has an extensive experience in the utilization of proteomic techniques and mass spectrometry in particular. Receiving his PhD in the early 2000s at the University of Southern Denmark, Odense, he started working at MDS Proteomics, followed by a short period at Proxeon, both located in Odense as well. This was followed by a post-doctoral fellowship at the Ludwig Institute for Cancer Research, before returning to Denmark, and accepting a position of Assistant Professor at the University of Aalborg.

In this exclusive interview, Prof. Stensballe describes the advances his team were able to make, utilizing the micro Pillar Array Column or µPAC™ technology from PharmaFluidics to push forward the stability and robustness of their NanoLC-MS workflow. This not only resulted in increased reproducibility of larger scale clinical cohort studies, but also in reduction of the time lost due to column cleaning procedures and column exchanges.

What type of biomarkers does your research focus on?



Within our research facility, we develop and investigate biomarkers, for pre-clinical and clinical studies in both human as well as model animal studies. The focus is on biomarkers from autoimmune, inflammatory and brain diseases as well as pain related biomarkers, with samples mainly originating from biofluids or tissues. Next to this biomarker research, we also work on bacterial samples for full proteome coverage and bio typing. A third, but not less important, task for the facilities is to provide other research groups with support and access to high-end LC-MS. Finally, our research extends in nanoproteomics and single-cell based investgations.
To accomplish these tasks, the research facility has a range of LC-MS systems available. The typical front-end separation systems are Thermo Scientific™ UltiMate™ 3000 RSLCnano equipped with Proflow™ technology, coupled to either Thermo Scientific Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ or Bruker timsTOF™ PRO Mass Spectrometers.

What challenges do you see for your workflows and where would the µPAC™ column fit in your biomarker research?

Our biggest challenge is to finetune all aspects of our workflows. We have employed a Clinical Proteomics approach to perform our biomarker research. This requires not only the newest generation of high scan speed instrumentation, which we have available, but also places high demands on column technology. Not just the chromatographic resolution obtained needs to be outstanding, but also column robustness and reproducibility are of highest importance. In our clinical

cohort studies, we are looking at 20-200 patients, which might result in up to 400 samples. This could require up to 1200 sample injections, that must be analysed in a high throughput, highly reproducible fashion for label free quantification. For deep proteome coverage we combine the µPAC™ chromatography with diaPASEF data acquisition technology.

"[...] the µPAC™ columns are a perfect fit for clinical cohort studies. [...] we are able to significantly reduce the time between injections directly adding to sample throughput."

To meet these requirements, the µPAC™ columns are a perfect fit. The separations performed are highly reproducible, with up to 80% of our peptides from plasma samples eluting within a gradient window of 12-56 minutes for a 60 minute total analysis time. In combination with a really remarkable low column carry-over at maximal loading capacity, we are able to significantly reduce the time required to wash and equilibrate the column between injections, directly adding to our sample throughput. In addition, we do have the experience that the free-standing pillar structure in the µPAC™ is less prone to clogging, especially compared to sub-2µm particle columns. In case of doubt, we simply backflush it, re-install and continue running it. We can also perform more injections on a column, thus reducing the number of column exchanges within the LC-MS study.

Where can you envision future use of the µPAC™ columns in your facilities?

One of the directions we would like to go is a multi-Omics approach, bringing together proteomics, metabolomics and lipidomics. Here the robustness of the µPAC™ columns would be of high importance. But also the wider flow flexibility of the µPAC™ is important, allowing to move up from the critical NanoLC flow range. This would likely increase the overall system robustness, and again help to shorten runtimes, perhaps as much as 10 minute gradient with more than 5 minute measuring windows, pushing throughput even further. So the µPAC™ ideally supplement our portfolio of nano-to cap-flow options for state-of-the-art Omics studies.