PharmaFluidics
The micro-Chip Chromatography company

Interview - Dr. Duncan Smith

Robustness and repeatability - The promise of µPAC™ in low flow LC/MS

Dr. Duncan Smith

CANCER RESEARCH UK | MANCHESTER INSTITUTE

Interview with Dr. Duncan Smith, Cancer Research UK (CRUK) Manchester Institute

Within Cancer Research UK Manchester Institute, Dr. Smith is Head of Mass Spectrometry, responsible for innovative biological mass spectrometry workflows to cancer research groups. Dr. Smith has extensive experience with mass spectrometry and liquid chromatography, and nanoLC in particular. The mass spectrometry facility offers routine services and advanced method development with multiple mass spectrometry instruments from different manufacturers.

In this interview, Dr. Smith explains how the micro Pillar Array Column technology or µPAC™ from PharmaFluidics has helped to increase both the nanoLC/MS robustness and throughput. Especially the repeatability and durability of the µPAC™, as well as the flow rate flexibility, helped reduce the number of column exchanges due to lost performance or application switching, resulting in easier to use workflows and increased productivity.

How did you learn about PharmaFluidics and µPAC™?

I first learned about PharmaFluidics and the micro Pillar Array Columns during discussions with some of your first users. I have been using packed-bed nanoLC columns for quite some time now, and the idea of low pressure extra-long columns without carry-over was very tempting.

After I had the opportunity to discuss the technology, the possibilities and benefits with PharmaFluidics, I decided to try it for myself. After positive initial testing against my typical 25 cm and 50 cm nanocolumns, I have placed a single 50 cm long µPAC™ in my nanoLC/MS set-up, and it has been running for over five months without exchanging it, running both short or long gradients.

As a core facility, what types of samples would you typically run on your LC/MS?

Running a core facility, a wide range of samples are coming through for LC/MS analysis. We support about 25 groups, and their samples range from protein SDS-gel bands, single proteins, low and medium complexity protein mixtures (up to 100 proteins), all the way up to complex human proteomes and isoforms, which can be run in labelled and unlabelled methods.

Until half a year ago, this variety of sample complexity would require us to change from 25 cm to 50 cm length columns, as sample complexity goes up. The 25 cm long nanocolumn would allow us to run less complex samples, with a reasonable throughput. The 50 cm long nanocolumn would be used for the samples with more complexity, running with longer gradients. Of course, this would require to shutdown and restart the instruments, to be able to exchange the column to maintain optimal performance and throughput.

What is the main advantage of the µPAC™ column for your lab?

In my opinion, that would be two. First, the wider application flexibility, followed by the actual robustness of the µPAC™.

That low backpressure over the nanocolumn, less than 50 bars at 300 nL/min, does provide some true flexibility. For us, that means that lower complexity samples can easily be run at higher flow rates, like 600-1000 nL/min, but still have that separation performance and resolution from a 50 cm long column. When more complex samples come in, all we need to do is change the gradient conditions in the methods. Simply reduce the flow rate down to 300 nL/min, create a more shallow gradient over a longer runtime. This allows us to perform 48 samples/day for the less complex samples, using 30 min runtimes, and using the exact same set-up to analyse 12 high complex samples/day using up to 120 min runtimes. All this is achieved without user interaction on the setup.

"Exchanging columns is a necessary evil [...] However, the µPAC™ has been running for over five months continuously, taking several thousand of injections [...]."

Robustness is also a key issue. Exchanging columns is a necessary evil, but it does take time to get it done. However, the µPAC™ has been running for over five months continuously, taking several thousand of injections, samples, blanks and QC samples. One of the things we found, is the much reduced column carry-over of the µPAC™. This has helped us reduce the number of blanks or QC samples we run, directly impacting our throughput. Of course, the fact that the µPAC™ takes that many injections without losing any of its performance is incredible and simply means fewer column exchanges and more instrument uptime.

Where do you see extra possibilities to employ µPAC™ in your lab?

One of the applications I have in mind is to run capillaryLC coupled to mass spectrometry. Especially for the less complex samples, flow rates of 4-8 µl/min would be ideal to further increase sample throughput. We already have the LC available, as well as a mass spectrometer with capillary flow interface.

Another direction I can see is more dedicated to phosphoproteomics.

The µPAC™ acts in a unique manner compared to any RPC18 column I've used before. We detect a far greater number of multiple phosphorylated peptides on µPAC™ columns than conventional silica based chemistry. We hypothesise the µPAC™ columns have a significant improvement in multiple phosphorylated peptide detection simply because they have fewer active sites where these peptides are largely lost on conventional chemistries. This could help in the recovery of multiple phosphorylated peptides, allowing more accurate identification and quantitation of phosphorylation sites, especially when you consider many kinase target sites are clustered in close proximity.