A Q&A

Silicon Wafer Technology Makes LC Analysis for Proteomics More powerful and Effective, A User's Experience

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As the field of proteomics grows more complex, traditional separation techniques like nano-liquid chromatography (LC) have a harder time extracting critical information efficiently. PharmaFluidics' µPAC™, a breakthrough in column design, helps bring LC analysis up to speed. *LCGC* recently sat down with Geert Van Raemdonck, global field support expert at PharmaFluidics, to discuss this topic. After using the µPAC™ at the University of Antwerp, Van Raemdonck was convinced about the benefits of micro-pillar array column technology. His enthusiasm resulted in him joining the team of PharmaFluidics.

LCGC discussed with Van Raemdonck the unique properties of the µPAC™ columns and got a user's-eye view of what it's like to work with them in proteomics applications. Van Raemdonck also shared his ideas about how future products could meet even more advanced analytical needs.

LCGC: What are the biggest differences between the µPAC™ columns and conventional nano-LC columns?

Van Raemdonck: The biggest difference of the column is the way the backbone of the stationary phase is manufactured. µPAC™ incorporates a chip made of a silicon wafer, and freestanding pillars are etched out of the wafer, which results in a perfectly ordered structure. This leads to a high separation performance because there is almost no peak dispersion. Sharp peaks also lead to higher sensitivity, so that small amounts of molecules can be detected more easily.

In addition, back-pressure is significantly lower as compared to conventional columns, which allows you to operate your column at a broader flow range, going from about 300 nL up to 1 µL per minute.

And last, since the µPAC™ column is etched out of a silicon wafer, there is no batch-to-batch variation, as every column that is produced is exactly the same.

LCGC: For which applications are these µPAC™ columns best suited?

Van Raemdonck: Special applications seen in the field of proteomics are analyses of samples with low amounts of peptides—for instance, the analysis of biopsies or protein extracts from a tissue. But, there's also the opportunity for the analysis of complex samples that require longer gradients, as for biomarker discovery. The technology is also suitable for chemically labeled samples like iTRAQ or TMT, where the gradients applied for separation often last four hours and longer.

Additional applications are in pharmaceutical fields, like in the detection of small subtle differences in biopharmaceuticals, and biosimilars, like for antibody production and quality

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control. In metabolomics, it might also be helpful to use µPAC™ columns because of the low concentrations of molecules, and also in lipidomics experiments, where a high resolution is required.

LCGC: Are there any drawbacks to these types of columns compared with classical columns?

Van Raemdonck: In my opinion, there are no specific drawbacks related to the µPAC™ columns. However, I think it's important to keep a few things in mind.

First of all, the back-pressure of the µPAC™ should always stay below 350 bar, or 5,000 PSI, to prevent any damage to the freestanding pillars that form the separation bed of the column. This maximum back-pressure is lower than for classical nano-LC columns, but it also lowers the shear force of the LC instrumentation.

Secondly, it's important to ground the column in case of applying a high voltage to the spray emitter of the mass spectrometer. This prevents any charging effects since the column is made of a semi-conductive silicon chip.

Finally, the price of a µPAC™ column is higher than most commercially available columns. However, since the lifetime of a µPAC™ column outperforms any conventional nano-LC column, the price per injection will be at least equal or even lower.

LCGC: What product improvements would you like to see in the future from a user's point of view?

Van Raemdonck: I think an introduction of trapping columns would be very useful since this would reduce the loading time and also offer higher flexibility in the sample volumes that can be injected into the column.

Further, it would be interesting to have the addition of some products to our current portfolio—like, for instance, those with bigger pore sizes in combination with other coatings, like reverse-phase C8 or C4, which could be applied in peptidomics and top-down applications.

And finally, if it would be possible to integrate the grounding mechanism, that would also be very handy.

LCGC: Did you encounter any issues during the installation and first use of the column?

Van Raemdonck: I used the µPAC™ for the first time when I was working at the University of Antwerp. The installation was done perfectly by the team of PharmaFluidics. One thing that you have to keep in mind is that you have to adapt your methods to the internal volume of the 200-cm column. This is about 9 µL, so it's important that at the end of your gradient, you provide enough equilibration time to equilibrate your column before you inject your next sample. If you desire, you can also increase your flow rate (due to the lower back-pressure) in order to reduce this equilibration time.

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LCGC: Why is grounding the column so important?

Van Raemdonck: The column is made out of a single silicon wafer that's semi-conductive. So, when the column is not grounded and when a high voltage is applied to the spray emitter that is used to transfer your molecules to your mass spectrometer, the current can actually reach the column and this will influence the retention of the column.

So, this will result in some charging effects that will retain your tryptic peptides, which will result in significantly broader peaks. And this discrepancy can be really big—you'll see it immediately when a column is not grounded; you'll be having very broad peaks. Unfortunately, adequate grounding is often a step that's forgotten.

LCGC: For which proteomics applications do you see the biggest potential for the µPAC™ technology?

Van Raemdonck: On the one hand, there are the small sample sizes that I already mentioned—like the protein or peptide extractions from tissues and biopsies. Furthermore, there are also the top-down proteomics applications with the C4 or C8 coating. But, there are also opportunities for protein–protein interactions and host-cell protein identifications since there will be very tiny amounts of compounds detected.

There is also a benefit to using the µPAC™ technology for targeted applications like scheduled parallel reaction monitoring, since the elution profiles can be set really narrow. So, you can include much more of the compounds that you want to validate in a single run. And further, it could also be very interesting to use the very stable retention times of the µPAC™ columns for data-independent analysis.