Episode 1: Why Liquid Chromatography is a Must for Mass Spectrometry?

Although LC technology plays an essential part in LC-MS analysis, it has often been overshadowed by advances in mass spectrometer performance and usability. In this six-part series, we will uncover the crucial role of nano-, capillary-, and micro-flow LC separations in proteomics and biopharma LC-MS applications.

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LCGC: While working in the field of LC-MS, where do you see the fastest technology update and development progress?
BOYCHENKO: Technology innovation continues to drive LC-MS due to the demands for higher sensitivity, better selectivity, and more precise data requirements. And these demands are coming from stricter regulations for food and environmental quality but even more from biology research such as the development of new biotherapeutics or translational research and precision medicine. MS has a faster technology update cycle, as there are a lot of options to advance for the detection capabilities, for example, by enhancing the ion transmission or combining different ion sorting or accumulation options.

LCGC: Your primary focus is separation sciences and new LC technologies. Don't you worry that advancements in MS will make LC obsolete in near future?
BOYCHENKO: All the progress in MS is impressive, but there is still no way that MS can exist without coupling with efficient techniques for separation, especially for complex samples. In fact, the trend is the exact opposite. Samples are getting more and more complex, and researchers are investing more time into exploring options for getting maximum peak capacities, more separation power in the shortest possible time, and even utilizing different alternatives for classical reversed-phase separation columns. So, they are exploring different chemistries on a routine basis as well as multi-dimensional separations.

LCGC: You mentioned that LC is required for MS. Can you give some examples of this?
BOYCHENKO: Yes, the first and main benefit is the separation of compounds in time. MS is a really powerful technique that can be used for many applications, but if you look at complex proteomics samples with thousands of individual peptides, there is no way an MS instrument can handle all of them simultaneously. Reducing the sample complexity is an important task, and it's needed on the other side to eliminate the effect of ion suppression for achieving better sensitivity.
LC separations allow us to reach a wider dynamic range of mass spectrometers, so without separations, quantification limits would be very high. Another benefit is related to making the quantification precise, as separation allows compounds to concentrate on the column and elute them in short times that lead to lower quantification limits and better precision.

LCGC: Thermo Fisher Scientific recently released a new ultra-high-performance liquid chromatography (UHPLC) system specifically designed for low-flow LC-MS applications. What were the main drivers for this development?

BOYCHENKO: One of the main drivers was the needs of the researcher—instrument users—in addition to application areas. We had a lot of interest in moving low-flow LC into the translational field or clinical research. Those movements are defining how the next instrument should look. We already have two high-performance platforms for nano-LC separations and proteomics applications, and they are used by thousands of LC-MS researchers across the globe, but the demands for better performance, increased robustness, analysis of large cohorts, and increased productivity to make this analysis a day-to-day routine are driving innovation for LC technologies. We spent a lot of time trying to understand what the exact requirements are and what is crucial to develop a next-generation platform.

LCGC: What is making this new nano-, capillary-, and micro-flow LC different or maybe even unique?

BOYCHENKO: There are several LC fields where performance improvements are not critical. There the goal is to focus on the uptime increase, reduction of cost per sample, and that the systems are compliance-ready. On the other side, we have fast-developing areas of low-flow LC and LC-MS analysis, where innovations are essential to boost the performance and make high-sensitivity analysis accessible not only for high-skilled operators but also to provide access to the community of scientists with different backgrounds in their base education. With that, we focused on the development of several unique features that made the system robust: maintenance-free low-dispersion valves; increased pressure capabilities; and specific vial bottom detection technology. To make the analysis quantitative, we introduced multi-wash options to minimize carryover. All of this is needed to bring low-flow LC-MS to the next level, so there are no issues running it 24/7. Now not only users of the same instrument but also users across different laboratories can compare the results and complete the analysis of large sample cohorts from large biobanks of biofluids, for example.

LCGC: For a long time, nano-flow LC has been the gold standard in LC-MS proteomics—where do you see the applicability of capillary- and micro-flow LC-MS?

BOYCHENKO: Nano LC-MS proteomics has allowed us to make a lot of discoveries already, and those discoveries are published in thousands of peer-reviewed papers. But the real issue is to translate those findings into useful applications that can then go into clinical research, for example. Here, the main need is to increase throughput and robustness, so those findings can be validated by large sample cohorts. Capillary- and micro-flow LC-MS can be excellent tools to analyze, for example, thousands of clinical samples and get statistically significant results.

LCGC: Do you think the field of high-sensitivity LC-MS analysis is transforming? What are the recent trends?

BOYCHENKO: Yes, the technology is transforming the field and is opening new application areas. For example, single-cell proteomics was not possible a few years ago, and now we have results where thousands of single cells are analyzed with the identification and quantification of thousands of proteins in each individual cell. LC technology is critical for the success of such applications because it allows in many ways to improve sensitivity, for example, by lowering the flow rate to increase electrospray ionization (ESI) efficiency, as well as to maintain high-separation quality. Nano LC-MS will be and continue to be a gold standard for discovery proteomics and analysis of post-translational modifications, but the translational research will rapidly adopt higher flow rates like capillary-flow and micro-flow LC.

LCGC: With such high potential for LC-MS technologies and many new application areas, where do you think we will see the fastest adoption of low-flow LC-MS technologies?

BOYCHENKO: The fastest adoption is happening with translating proteomics discoveries into clinical research. There is potential for low-flow LC-MS to become a standard for routine analysis.

LCGC: Do you think this will ultimately affect our lives?

BOYCHENKO: There are already some great examples of the applicability of LC-MS for the assessment of an individual's status. Having this extended to screening purposes or the day-to-day testing will change our lives and how we monitor the health of individuals.

LCGC: Tell me something true in LC-MS analysis that many don't agree with you on.

BOYCHENKO: There are many strong opinions and beliefs on the topic of sensitivity gains and LC-MS. At one point, I even gave a presentation on the demystifying of sensitivity-gains perception in LC-MS where ESI is used. It's commonly accepted that ESI-MS behaves as a concentration-sensitive detector, but it is important to understand that sensitivity in this respect is mainly linked to better ionization efficiency and lower flow rates rather than reduced columns' internal diameter.