Fifty Years of HPLC

By František Švec, Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

My first encounter with chromatography dates back more than 30 years. My first projects focused on the application of polymers in separation science by developing a classic format of stationary phase – beads. But this format was soon replaced by a new approach: monoliths, of which we knew very little at the time.

The monolith boom came in the 1990s, and has continued growing since. My current position, supported by the STARSS (Specialized Team for Advanced Research in Separation Science) project, is exceptionally well placed for applying subtleties of separation science, and particularly chromatography.

This project means not only conducting excellent research using high-end instrumentation, but also teaching our students and postdocs. They are the next generation to whom we will hand over the baton, expecting them to love chromatography just as we do, and to discover creative solutions to society's significant challenges, including environmental issues, food safety, affordable health care, and the development of drugs against new targets.

HPLC has developed significantly since its advent more than 50 years ago. Focusing just on the column technologies:

- 1966: Horvath and Lipsky used stationary phases with a particle size of around 50 µm packed in up to 275 cm long columns. The particle size has decreased ever since.
- 1990s: New columns format – monoliths – invented. Monoliths allowed HPLC separation at unprecedented speed – separating molecules as large as proteins in mere seconds.
- Today: Sub-2 µm particles are a current industry standard. Horvath's idea of pellicular beads has transformed to give today's core-shell particles. These developments have led to a significant increase in column efficiency, meaning excellent separations could be achieved with much shorter columns.
- Tomorrow: What can we expect to see in the future?
  - Even smaller particles? This would require chromatographic hardware capable of tolerating higher pressures and dealing with associated challenges.
  - Narrower columns? Perhaps, since narrower columns require smaller flow rates to achieve desired velocity, thus reducing consumption of the mobile phase and attempting "green" chromatography.
  - Micromachined columns? Using processes typical of microelectronics, the preparation of columns containing arrays of pillars in a channel are emerging.
  - 3D printing? I anticipate that columns will be produced this way. Early birds are already emerging on this front; printed monolithic columns will be designed in silico. However, high-resolution and rapid 3D printers are as of yet are unavailable.

Alongside column breakthroughs, detection methods have also taken impressive strides. The introduction of electrospray ionization to support MS by Fenn in the 1980s is a great example, and an essential breakthrough for the field of proteomics. Despite the availability of several approaches in the detection area, MS has become the most frequently used characterization technique for multistep analytical procedures. Significant improvements in MS instrumentation have been achieved in recent years, meaning spectrometers are both more accurate and more sensitive.

Yet, numerous sample preparation steps precede the chromatographic analysis. This area will remain a major target of studies for the foreseeable future, as the diversity of samples to be analyzed is close to infinite. Their preparation will require tailor-made, integrated, and high-throughput methods, alongside a decrease in both sample and instrument sizes, as well as automation, robotics, and multiplexing.

In short, there is much that must be achieved. Thus, the problems we face will not only keep us busy, but provide ongoing challenges for the next generations of analytical scientists and engineers.