Trends and Perspectives on Future Developments in Liquid Chromatography

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Since the early 1970s, there has been a tremendous surge in the development and refinement of high performance liquid chromatography (HPLC) instrumentation as well as in HPLC applications. The molecules can be very small or their molar mass may range from thousands to hundreds of thousands. In a short time, vast amounts of highly reproducible data can be generated. The use of HPLC has not just been confined to analytical chemistry or biological sciences. HPLC is frequently used as a powerful separation technique in all branches of science or industry that require the separation of components in a complex mixture.

The characterization of different molecules requires a variety of chromatographic approaches, including reversed-phase, ion exchange, and size exclusion. Reversed-phase has been responsible for almost all applications in chromatographic separations because a majority of analytes can be separated by this technique. Hydrophilic interaction liquid chromatography (HILIC) has emerged as an alternative for the separation of polar analytes and hydrophilic compounds that are problematic in other separation modes. It has become more routine because of the reliable and stable results it produces. Innovations in stationary phases have focused on the development of more stable supports, new bonded phases, and novel materials. These innovative materials must be capable of improving specific selectivity and providing faster analysis times. There is still potential for further improvements and new materials.

The availability of reliable and sensitive detectors underscores the success of HPLC as a multipurpose analytical technique for miscellaneous applications. Three categories of HPLC detectors have featured in recent years: ultraviolet (UV) detectors for molecules with chromophores, evaporative light scattering detectors (ELSD) or charged aerosol detectors (CAD) for non-chromophoric compounds, and mass spectrometers (MS) for general use. There is continued growth in the use of liquid chromatography with tandem mass spectrometry (LC-MS/MS). This use is because the improvement in MS technology — both hardware and software — allows highly sensitive and selective methods to be developed for diverse applications, including clinical research, food safety, biopharmaceutical research and development, environmental protection, and “omics”-related research. Several areas are growing fast with the use of LC-MS because MS can now provide a level of sensitivity and selectivity that opens doors to replace other technologies, for example, costly immunoassays in clinical laboratories. However, this development requires very easy-to-use, robust instruments and the production of reliable, high-quality data, regardless of the user’s experience.

Advances in column technology (smaller particles, superficially porous particles) and instrumentation

(pressure capabilities, detector cell size) have provided the means to increase peak capacity. Hyphenating comprehensive two-dimensional liquid chromatography (LC×LC) with high-resolution MS is also an effective way to significantly increase resolving power and peak capacity in LC. In the near future, if more tools are developed to make this powerful technology simpler, LC×LC will also be used for routine analysis. Software developments to improve “feature recognition” should be investigated further.

Although much effort has been made in simplifying data processing, it remains far from fully automated and consumes marked amounts of time and analytical expertise. The implementation of artificial intelligence to assist data processing, self-diagnosis, and self-maintenance — in order to reduce human intervention in daily laboratory routine — should be developed to improve workflow solutions.

In the future, LC will continue to be the separation technique of choice, and nano-LC and capillary LC are the way forward for faster LC and greener technology. The demand for increased sensitivity, throughput, and robustness has made capillary LC a potential technique because of its ability to provide increased MS sensitivity compared to typical analytical flow LC-MS. An additional advantage includes lower solvent consumption and sample size. However, improvements in micro-LC instruments are required, for example, decreasing dead volumes to make them more efficient.