MILESTONE 8

Amicable separations

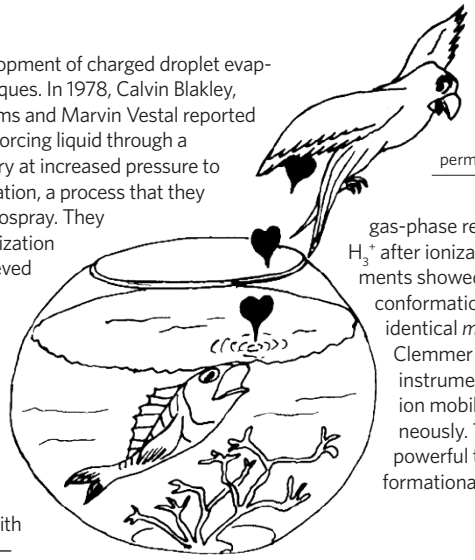
By the 1950s, mass spectrometry was a well-established technology for the analysis of volatile compounds in the petroleum, pharmaceutical and chemical industries. However, the deconvolution of spectra comprising multiple analytes was proving problematic—there was a growing desire for a rapid, online separation method.

In fact, the chromatographic techniques necessary for such separation were themselves just coming to the market. Although gas chromatography (GC) was achieving previously unimaginable separation performances, the detection methods then available gave limited chemical insight. The answer lay in coupling the powerful separation ability of chromatography with the specificity and precision of mass spectrometry.

This solution was first explored in 1955 by Roland Gohlke and Fred McLafferty of Dow Chemical Company, who hooked up a homemade gas chromatograph to a time-of-flight (TOF) instrument. This TOF instrument had been developed only recently (**Milestone 4**) and generated spectra much faster than did magnetic sector instruments (see **Milestone 1**). Soon, the team could separate mixtures of organic species and identify them—in real time.

Of course, the marriage of GC and mass spectrometry was always going to be harmonious; the gaseous exhaust of the GC was primed, ready for ionization. In contrast, pairing liquid chromatography (LC)—used for the separation of non-volatile and thermally unstable compounds—with mass spectrometry proved more difficult.

Initially, V.L. Tal'roze and G.V. Karpov tried direct liquid injection. By leaking a minute volume of LC effluent into the high-vacuum conditions of the ionization chamber, they could vaporize the sample and then ionize it through electron impact (now called electron ionization; see **Milestone 2**). Michael Baldwin and McLafferty improved this approach by switching to a chemical ionization technique (later developed and sold commercially by Hewlett-Packard). Meanwhile, others experimented with belt-drying to remove solvent before ionization (later resulting in a commercial instrument from Finnigan) or concentrating the analytes using membrane separation. A real game-changer was the development of charged droplet evaporation techniques. In 1978, Calvin Blakley, Mary McAdams and Marvin Vestal reported a method for forcing liquid through a heated capillary at increased pressure to effect nebulization, a process that they termed thermospray. They found that ionization could be achieved chemically by adding ammonium acetate to the carrier liquid. Other ionization techniques were trialed with mixed results—electrospray ionization (**Milestone 15**) was a particular triumph—and the most successful were soon incorporated by all major manufacturers into the new generation of commercial instruments. These technologies gave LC–mass spectrometry (LC-MS) a new level of usability in terms of compatible solvents and analytes. This flexibility, along with the improved speed and precision of modern LC–MS, makes it an invaluable method for the unequivocal detection of trace molecules—for example, testing for banned drugs in athletes' blood or urine.



Patrick Arpino brings the incompatibility of LC and MS to life. Reprinted from Arpino, P.J., On-line liquid chromatography/mass spectrometry? An odd couple!, *Trends Analyt. Chem.* **1**, 154–158 (1982), with permission from Elsevier.

The softness of the new ionization methods meant that even quite large molecular ions were detected intact, simplifying interpretation of the data considerably and, importantly, widening the scope for potential biological applications. Indeed, when coupled with capillary zone electrophoresis, another liquid-based separation method whereby charged species move under an applied potential, these ionization techniques proved to be useful for the identification of peptides and proteins.

In a similar way to GC, ion-mobility separation (IMS) lent itself well to a partnership with mass spectrometry, because both handle ions in the gaseous phase. Pairing IMS with a magnetic sector or a TOF instrument allowed the analysis of gas-phase reactions, such as the formation of H_3^+ after ionization of hydrogen. Later, experiments showed that IMS could separate different conformations of intact proteins that have identical *m/z* values, and, in 1998, David Clemmer and colleagues developed an instrument that could record mass-resolved ion mobilities for all analyte ions simultaneously. This approach has since become a powerful tool in the characterization of conformational dynamics of large biomolecules.

Thomas Faust, Associate Editor,
Nature Communications

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