

The Origins of Ion Spray Liquid Chromatography–Tandem Mass Spectrometry

Jack D. Henion^{1*}

Featured Article: Bruins AP, Covey TR, Henion JD. Ion spray interface for combined liquid chromatography/atmospheric pressure ionization mass spectrometry. *Anal Chem* 1987;59:2642–6.²

The 1987 paper highlighted here evolved from the ongoing desire to couple HPLC to mass spectrometry. HPLC-ultraviolet techniques were readily accepted and widely used, but the facile and practical coupling of this very capable separation science technique to electrospray ionization mass spectrometry had not yet been demonstrated.

During the late 1970s only a handful of us were experimenting with coupling HPLC to mass spectrometry to provide LC-MS capabilities. In 1979 Hewlett-Packard developed an early approach called direct liquid introduction (DLI)³ to provide one of first commercialized LC-MS interfaces. At the same time Finnigan, Inc. commercialized its “moving belt” LC-MS interface, which could produce electron ionization (EI) mass spectra via LC-MS. Neither of these commercial LC-MS techniques was easy to use or generally accepted as a useful analytical tool.

During the early 1980s a few research groups continued to explore alternative approaches to create gas-phase ions from intractable compounds for mass spectrometry (MS) detection via online LC-MS sample introduction. Marvin Vestal developed and commercialized thermospray LC-MS (1). In 1984 John Fenn published his first results describing electrospray ionization mass spectrometry (2), for which he later shared the Nobel prize. These 2 approaches contrasted with each other because thermospray LC-MS could handle standard HPLC flow rates of 1 mL/min, but electrospray was confined to flow rates of <10 μ L/min and mobile phases containing low aqueous content (3). Water is often used at high percentages in reversed-phase HPLC mobile phases, so “pure” elec-

troscopy did not look promising for real-world applications. Most of the published reports by Fenn et al. employed infusion-MS rather than LC-MS.

I attended the Asilomar conference of the American Society for Mass Spectrometry in the early 1980s. At this conference Bruce Thomson of Sciex presented some amazing results using a newly developed “ion evaporation” interface (4) coupled to Sciex’s atmospheric pressure ionization (API) mass spectrometer. This novel sprayer device operated at ambient temperature, employing high voltage applied to an induction electrode coupled with nitrogen gas for pneumatic nebulization to produce an aerosol of charged droplets. This device could handle 1 mL/min aqueous liquid flows and produced gas-phase ions of polar, intractable compounds for MS detection.

I returned to my Cornell University research laboratory where Andries Bruins of the University of Groningen, the Netherlands, had joined my research group as a visiting scientist. We commenced experiments employing a high linear velocity of coaxial nitrogen nebulizing gas as part of a modified electrospray ionization sprayer probe. Bruins experimented with this LC-MS interface, which we later named “Ion Spray,” and demonstrated significant improvements in analytical sensitivity using aqueous methanol or acetonitrile flows ranging from 0.05–0.1 mL/min. These were 10–20 times higher flow rates than with “pure” electrospray.

“Micro HPLC” was becoming available during this time, so we integrated routine online micro-LC–tandem MS (MS/MS) analysis of real-world biological and environmental extracts containing a wide variety of pharmaceutical and environmental compounds with our Ion Spray LC-MS interface. Initially we focused on very polar, medium molecular weight sulfonated azo dyes as part of our new Environmental Protective Agency Cooperative Agreement sponsored by William Budde of the EPA EMSL laboratory in Cincinnati, Ohio. Coincidentally, these very acidic sulfonic acid compounds had multiple sulfonic acid groups that were best detected in the negative ion mode. It was later learned that nitrogen nebulizing gas was particularly beneficial for facilitating the electrospray detection of the resulting multiply negatively charged sulfonic acid compounds.

Our 1987 publication on Ion Spray LC-MS has been frequently cited because it provided a meaningful

¹ Advion BioSciences, Ithaca, NY.

* Address correspondence to the author at: Advion BioSciences, Inc., 19 Brown Road, Ithaca, NY 14850. E-mail henionj@advion.com.

² This article had been cited more than 698 times since publication. Received March 4, 2009; accepted March 16, 2009.

Previously published online at DOI: 10.1373/clinchem.2009.126193

³ Nonstandard abbreviations: DLI, direct liquid introduction; EI, electron ionization; MS, mass spectrometry; API, atmospheric pressure ionization; MS/MS, tandem MS.

and practical breakthrough allowing many laboratories to begin employing LC-MS/MS techniques. Sciex was the first to commercialize this technique in the late 1980s, when my very capable Cornell graduate student, Tom Covey, joined them and helped implement our Ion Spray technology into their novel API III tandem triple quadrupole mass spectrometer. The latter instrument became a legend because so many were sold and adopted in the pharmaceutical industry. It is particularly rewarding to know that more recently our pneumatically assisted electrospray (Ion Spray) technology has been implemented in clinical diagnostic laboratories for newborn and metabolic screening (5) and a host of other clinically important applications.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: J. Henion, Advion BioSciences.

Consultant or Advisory Role: None declared.

Stock Ownership: J. Henion, Advion BioSciences.

Honoraria: J. Henion, Eastern Analytical Symposium.

Research Funding: J. Henion, Advion BioSciences.

Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Vestal ML. Thermospray liquid chromatographic interface for magnetic mass spectrometers. *Anal Chem* 1984;56:2590–2.
2. Yamashita M, Fenn JB. Electrospray ion source: another variation on the free-jet theme. *J Phys Chem* 1984;92:4451–9.
3. Whitehouse CM, Dreyer RN, Yamashita M, Fenn JB. Electrospray interface for liquid chromatographs and mass spectrometers. *Anal Chem* 1985;57:675–9.
4. Iribarne JV, Thomson BA. Atmospheric pressure ion evaporation-mass spectrometry. *Int J Mass Spectrom Ion Phys* 1976;64:2287–94.
5. Chace DH. Mass spectrometry in newborn and metabolic screening: historical perspective and future directions. *J Mass Spectrom* 2009;44:163–70.