

# Emerging Technologies in the Mass Spectrometry Arsenal

**This article provides an overview of several recently developed technologies that will improve the utility and performance of mass spectrometers in a number of applications. The discussion includes photoionization, several ion mobility techniques, atmospheric pressure chemical ionization, and atmospheric pressure photoionization.**

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The willingness of a few audacious souls to navigate the uncharted areas of mass spectrometry (MS) practice can benefit the less daring among us, easing our efforts and enhancing our results. So I want to share with you some newly emergent product designs that came to light at last year's American Society for Mass Spectrometry (ASMS) liquid chromatography (LC)–MS workshop and in work presented at the Conference on Small Molecule Science (CoS-MoS) and elsewhere, all of which stand out as leading candidates for a future in MS practice.

While much of what looms on the horizon undoubtedly will prove useful to practitioners, there is nothing to match the magnitude of the technological change that the development and deployment of electrospray-based LC–MS wrought in the early 1990s. (To understand why we advanced so far so quickly in this area, see my brief review describing developments of commercial and practical importance [1].)

Electrospray ionization (ESI), which is useful particularly when the analyte of interest is polar (and many are, especially in pharmaceutical applications), is the ionization method used in about 80% of all LC–MS analyses. To better accommodate a mass spectrometer's operation in ESI mode, modern HPLC systems and methods have evolved to permit an effective "LC for MS" compromise. Flow rates of less than 1 mL/min make desolvation of the resulting liquid vapor load in the mass spectrometer an easier task, while improving the ionization efficiency. A quick calculation with water indicates that a 1-mL/min flow requires a very large, hard-working pump removing 1 L/min of vapor to maintain the needed vacuum. Columns with 2-mm diameters packed with 3.5- $\mu$ m (hybrid or nonhybrid) C18 particles allow a wide variety of applications, all within the confines of HPLC as we have come to know it.

For their part, the MS vendors have adapted to the advances in LC technology by enhancing their instruments' vacuum performance, software performance, liquid desolvation efficiency, and plumbing configuration. (For an example of their dawning appreciation of how to transport separated LC bands that evidence good fidelity, consider the early

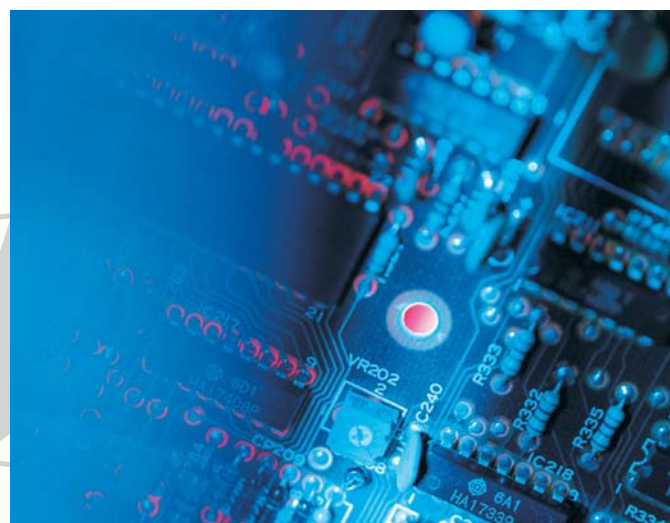


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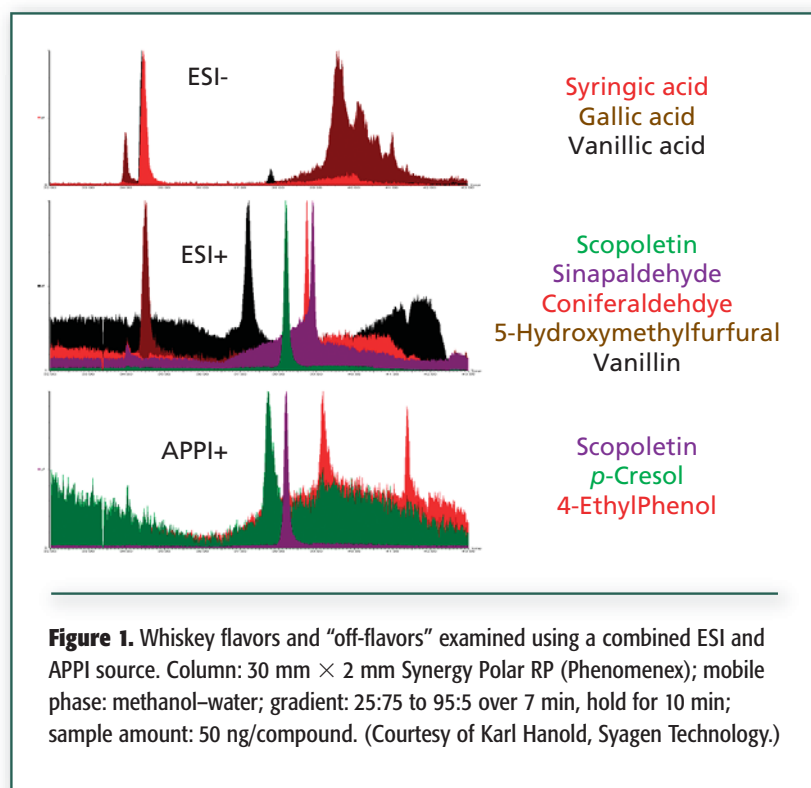
1990s-vintage Finnigan TSP interfaces and their inordinate lengths of tubing and plethora of unions.)

## Addressing Nonpolar Analytes

Yet, despite the considerable enhancements of the past decade, we still lack effective, convenient ionization techniques for the remaining 20% of nonpolar and neutral analytes of interest. In addition, we need utilitarian improvements in the way we acquire and handle data — more and better quality information for the same effort. The following are some attributive, resurgent technologies that might at last satisfy those needs.

**Photoionization.** Used for gas chromatography (GC) applications since the 1970s, photoionization for LC–MS can be traced to Chen's work on LC applications during the early 1980s (2). MDS/Sciex (Ontario, Canada) recently commercialized photoionization through the work of Bruins and Robb (3). Agilent (Wilmington, DE), Thermo Finnigan (Waltham, MA), and Waters (Milford, MA) did likewise through work by Syage and Hanold (4).

**High field asymmetric mobility.** Commercialization of high field asymmetric ion mobility by Guevremont (Ionalytics, Ontario, Canada) and Miller (Sionex, Waltham, MA) (5, 6)



commercialized variants of high field asymmetric ion mobility spectrometry (FAIMS) used in conjunction with MS (Figure 2). Varian (Palo Alto, CA) recently commercialized the Sionex uDMX sensor for GC use, and Sionex is investigating the sensor’s potential for LC application. Ionalytics, meanwhile, already has established primacy with most major manufacturers regarding various aspects and applications of the technology for LC use.

#### Multiple ionization mode designs.

These attract continuing interest. Some long-held preconceptions about the way atmospheric pressure chemical ionization (APCI) works are being challenged

as follows: APCI does not in fact require higher flow rates than ESI does. Nor does it require a separate probe. Indeed, one can achieve the requisite sensitivity by artfully implementing desolvation strategies.

Agilent recently indicated its plans to introduce a multimode ionization MS source that uses infrared lamps to achieve the dry vapor necessary for successful APCI operation. Waters’ ESCi multimode ionization design has been well received in the past two years and now applies to the manufacturer’s entire MS product line.

#### Ultrahigh-pressure chromatography.

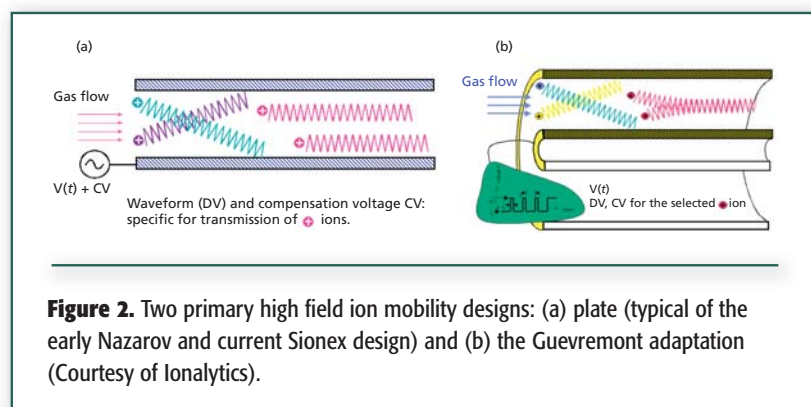
Derived from the work of Professor Jim

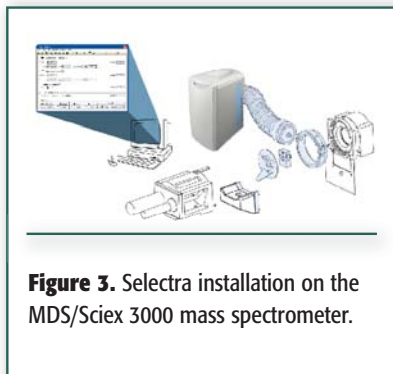
Jorgenson (University of North Carolina, Chapel Hill), ultrahigh-pressure chromatography was introduced this year by Waters. Though not a technology dedicated to LC–MS practice, ultrahigh-pressure chromatography has gained significant standing in the industry and forced a rethinking of the approaches to method development.

#### Photoionization

Several sources — publications, presentations, and sales figures — suggest that practitioners are steadily adopting the atmospheric pressure photoionization (APPI) technology. Though still in its commercial infancy, the technology attracts several avenues of research. Among them is a quest to improve sensitivity (a long-time nemesis preventing wider adoption of the technology) and another to couple the APPI with ESI (rather than allowing APPI technology to remain bound to the APCI probe and its “complementary” ionization to form the aerosol.) In August, Karl Hanold of Syagen (Tustin, CA) presented a paper at CoSMoS setting forth early work toward developing a combined ESI–APPI source (Figure 1). The model achieves APPI sensitivity equal to existing designs using practical flow rates (currently < 300  $\mu\text{L}/\text{min}$ ). A debate has emerged regarding the need to use a dopant, however. An impurity (such as toluene or benzene) added in small amounts to a pure substance to change the latter’s properties, dopant is still considered compound-dependent. Nevertheless, it seems Syagen’s model, which differs from Sciex’s in the design of its lamp (though both use krypton gas discharge), might reduce dependence on dopants.

Dopants, which can be quite contrary because they interfere with chromatographic performance, are indeed noteworthy. In their simplest characterization, a spray containing elements that possess ionization potentials (IE) both above and below the output of the gas-discharge krypton lamp (about 10 eV) responds accordingly. That is, solvents and gases present in the source — such as  $\text{O}_2$  (IE = 12.07 eV),  $\text{N}_2$  (IE = 15.58 eV), and  $\text{H}_2\text{O}$  (IE = 12.62 eV) — have IE values greater than 10 eV and therefore fail to ionize. This is a dominant advan-





**Figure 3.** Selectra installation on the MDS/Sciex 3000 mass spectrometer.

tage because common atmospheric gases and ubiquitous HPLC solvents are of no interest in our analyses, and we would choose to avoid dealing with them as “noise.” In fact, the spectra produced by APPI are likely to be more noise-free than those produced by APCI, which creates ions in the plasma to transfer charge (if we were able to achieve a theoretical equivalent measurement). In cases where analytes do exhibit ionization potentials in the region above 10 eV, they are candidates for dopant mediation. Using dopant often increases the signal detected for the analyte of interest. However, the dopant’s recombinant energies also cause a concomitant increase in numerous background ions, yielding increased noise without an accompanying increase in signal-to-noise ratios or detection limits. For a fundamental discussion of how common dopants work, I recommend reference 12. This material discusses experiments performed with acetonitrile (IE = 12.2 eV) in its deuterated form and thus attempts to explain the role this common solvent plays in ionization. It also offers a review of the common dopants toluene and benzene.

### **Ion Mobility, Differential Mobility, and FAIMS**

Of the current trends cited in the analytical press, ion mobility is one of the more interesting. Although we can improve capacity with smaller particles and higher pressures, and we can improve the accuracy of our results with ion cyclotron resonance (ICR) MS, we have nonetheless reached the technological limit of our ability to improve selectivity. Ion mobility is a technology that Nazarov and others (7) brought to the U.S. from the for-

mer Soviet Union. Since the September 11, 2001 terrorist attacks, ion mobility is the technique applied most frequently in the explosives scanners that airports use to screen passengers before they board aircraft. Indeed, one published estimate puts the annual total for such screenings at 100 million analyses (8). Recent work by Sionex (9) indicates the broader possibilities in the field and their relationship to each other — whether referred to as

ion mobility, differential mobility spectrometry, or FAIMS.

The Selectra device (Ionalytics) is perhaps the best-known laboratory application of FAIMS technology. Selectra units are external devices positioned between the mass spectrometer source and inlet (Figure 3). Essentially, an ion’s selection occurs as it passes through a high field voltage. A compensating voltage is applied, which characteristically affects

**Table I. QC samples (25 ng/mL drug) containing 0 and 2500 ng/mL metabolite, respectively; from a run performed with the Waters Micromass Technologies Quattro Micro system (Courtesy of Mohammed Jemal, Bristol-Myers Squibb, NJ).**

|               | LC-FAIMS-MS-MS |               | LC-MS      |               |
|---------------|----------------|---------------|------------|---------------|
|               | Metabolite     | No Metabolite | Metabolite | No Metabolite |
| Nominal       | 25             | 25            | 25         | 25            |
| Replicate 1   | 25.3           | 23.7          | 24.7       | 48.1          |
| Replicate 2   | 22.5           | 23.4          | 23.9       | 47.5          |
| Replicate 3   | 24.8           | 22.5          | 25.4       | 46.3          |
| Replicate 4   | 25.7           | 25.0          | 24.8       | 48.1          |
| Replicate 5   | 26.7           | 24.1          | 26.2       | 47.2          |
| Mean          | 25.0           | 23.7          | 25.0       | 47.4          |
| RSD           | 6.2%           | 3.9%          | 3.4%       | 1.6%          |
| % Theoretical | 100            | 95.0          | 100        | 190           |

the ion's mobility based on conformation rather than simply on mass-to-charge ratio as in MS.

Practitioners agree that the ability to eliminate molecular and isobaric mass interference problems in quantitative exercises is invaluable. Mohammed Jemal (Bristol-Myers Squibb, NJ) points out that through conversion, metabolites can produce the same ion in the mass spectrometer source as their parent drug does. Yet for an analyte's positive identification, the pharmaceutical industry nevertheless relies on the selected reaction monitoring transition from the parent compound to a related "product ion," which occurs after the parent collides with a gas in the radio frequency-only section of the triple quadrupole. But this method is not reliable if the products and transitions for drug and metabolite are identical.

Here are some typical problem pairings:

- Carboxylic acid drug and acylglucuronide metabolite
- Alcohol drug and O-glucuronide metabolite
- Amine drug and N-glucuronide metabolite
- Amine drug and carbamoyl glucuronide metabolite
- Amine drug and N-oxide metabolite
- Hydroxy acid drug and lactone metabolite
- Lactone drug and hydroxy acid metabolite
- Thiol drug and disulfide metabolite
- Prodrug and its drug

Obviously, the potential problems this list reveals are extensive.

The study's dramatic conclusion indicates ion mobility can eliminate one of the traditional means of discriminating analytes of interest, the chromatography itself.

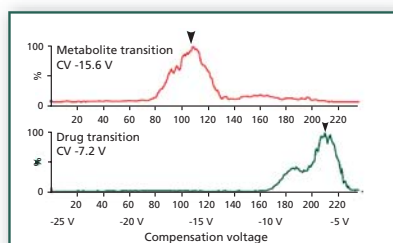
### Ultrahigh-Pressure LC

The recent commercialization of work from Professor Jorgenson's Chapel Hill labs, often referred to generically as ultrahigh-pressure liquid chromatography, brings a potential to increase information derived from typical LC-MS analyses. Commercialized by Waters Corporation as UPLC, the increased peak capacity over HPLC enables definition of chemical entities that might have been obscured by coelution under the broader HPLC peaks. Concentrating peaks into bands of (typically) 2-s width raises the potential for increased sensitivity by favoring the mass spectrometer's response to improvements in S/N.

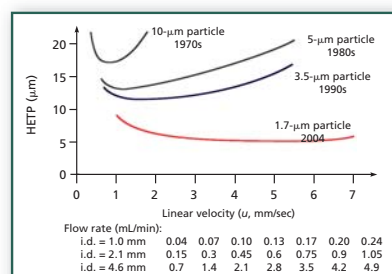
The comfort zone we established for ourselves in separations practice changes — familiar parameters such as flow rates, particle sizes, even our appreciation of van Deemter curves — as we increase operating pressure from ;2000 psi to as high as 20,000 psi. Reducing particle diameter to less than 2  $\mu\text{m}$ , and thus approaching the theoretical limit described in 1969 by John Knox (the "Knox equation") (10), forced chromatographers to grapple with the attendant problems of increased mechanical stress and exaggerated thermal effects.

The improvements in MS performance come as a somewhat counterintuitive consequence of theory. Viewed as changes in efficiency due to linear velocity depicted as a van Deemter plot, columns packed with 1.7- $\mu\text{m}$  diameter particles perform better regardless of flow rate. I say this finding is counterintuitive because it contravenes what we have become accustomed to in HPLC practice. While all columns show diminished performance at extremely low linear velocities, those with smaller diameter particles perform better and show less performance deterioration due to increases in linear velocity (Figure 5).

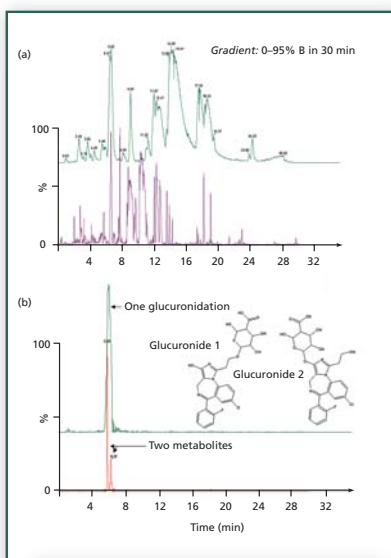
The ultimate advantage of ultrahigh-pressure LC over HPLC, particularly for mass spectrometrists, lies in the newer technology's increased ability to characterize complex mixtures by separating to a greater degree chemically distinct species in the temporal domain. This, of course, leads to increased MS sensitivity, quantitative response, and spectral purity, as shown in Figure 6.



**Figure 4.** FAIMS compensation voltage scan for a drug and its metabolite as shown by the Waters Micromass Technologies Quattro Micro MS system (Courtesy of Mohammed Jemal, Bristol-Myers Squibb, NJ).



**Figure 5.** The van Deemter plot changes with particle size (Courtesy of Waters Corporation).



**Figure 6.** Characterizations from complex mixtures: metabolite identification of the anticonvulsant Midazolam in rat liver bile. Shown are (a) a challenging separation due to the bilirubin components in bile ion-pairing and the high concentration of bile salts, and (b) improved peak capacity and information about the glucuronide metabolites of Midazolam ( $m/z$  548.125). The analyses were performed using an ACQUITY/QTOF system (Courtesy of Robert Plumb, Waters Corporation, and Ian Wilson, Astra Zeneca, U.K.).

## Multimode Ionization

In 2003, my work at Waters (11, 13) led to the commercialization of a design that combines ESI and APCI ionization modes in a single MS source. Thus, this is admittedly an area of personal interest. But it also is an area of interest for all practitioners who have ever struggled with the sometimes unanticipated effects of heat, gas behavior, and other elements of MS source function and a chemically diverse world of samples.

The Waters design, called ESCi multimode ionization, applies the inherent geometry of the source to provide secondary desolvation, a necessary step to achieve a sufficiently dry vapor to promote APCI ionization.

Agilent recently announced plans to

introduce a similar combination source, and I was pleased to have the work presented this year at both the ASMS LC-MS workshop in Nashville, TN, which I organized, and our inaugural CoSMoS conference, held at Roger Williams University, Bristol, RI. Agilent's approach to providing the necessary secondary desolvation consists of incorporating infrared heat lamps in the source to further desolvate the aerosol after its initial formation by the single ESI probe. Taking advantage electrostatically of the difference between the preformed ESI ions and nonionized analytes in the spray, they are able to manipulate the result, directing the ESI ions into the inlet while applying further desolvation and APCI to the remaining analytes in the spray.

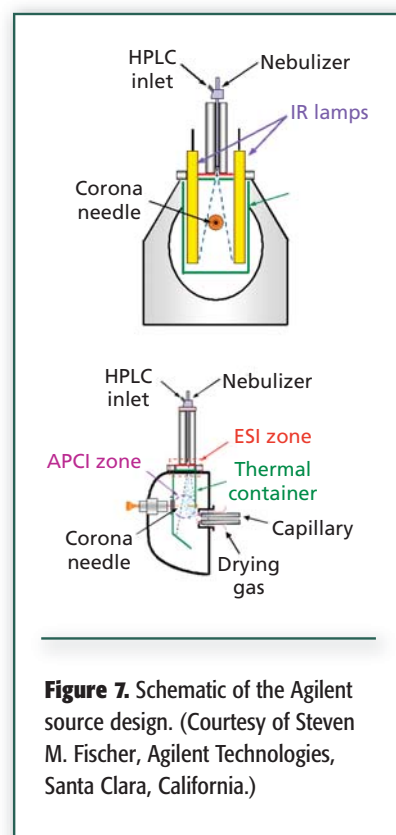
The Waters design is able to create specific boundaries between the ESI and APCI ionization mechanisms, whereas the Agilent product does not. Creating and reproducing such boundaries at millisecond intervals with good fidelity gives the added advantage of being able to use the device for research purposes as well as high-throughput sample processing. For instance, monitoring which ionization mechanism is promoted based upon solution changes. The Agilent product requires fitting an optional source enclosure in place of the standard ESI one. According to their published results, the optional source's ESI sensitivity is slightly compromised, however, falling somewhat below that of the standard source.

The Agilent design benefit is that it increases the duty cycle. In essence, the ability to ionize in both ESI and APCI modes is always present. Nevertheless, with the latest power supply and software improvements found in its Premier line of mass spectrometers, the Waters interscan delay (ISD) can be reduced to as low as 5 ms. Points of differentiation between the two designs might be less important when employed at their primary task, namely, screening thousands of samples in early drug discovery activities.

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**Figure 7.** Schematic of the Agilent source design. (Courtesy of Steven M. Fischer, Agilent Technologies, Santa Clara, California.)

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