Solvent, Chemistry, and Other Myths in LC-MS

Sometimes we can be surprised to find we have overlooked details in areas where we are otherwise so well versed. Then, forced to have another look, find we have opened a wealth of understanding. The art and science of chromatography often seems to offer opportunity for passionate debate. Over the past seven years, since CoSMoS conferences began, the chromatography discussion sessions have been the most highly rated, often inciting the most vigorous debate. A now-famous quote from former U.S. Secretary of Defense Donald Rumsfeld might apply well: "There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things we know that we now know we don't know. But there are also unknown unknowns. These are things we do not know we don't know." (This quote was taken from a press briefing February 12, 2002, on the increasingly unstable situation in Afghanistan.)

A recent discussion at CoSMoS 2009 (Boston, Massachusetts, see www.CosMoScience.org) proves Rumsfeld's observations aptly describe the state of much of the underlying, and often misguided, assumptions we bring to bear when applying our understanding of chromatography to liquid chromatography–mass spectrometry (LC–MS) practice. The inherent value of the charter principle of CoSMoS since its founding in 2003 is crafting open discussion workshop sessions to encourage a free exchange of ideas, leading to enhanced practical knowledge.

One might, for example, approach LC–MS with a wealth of condensed phase knowledge based upon years of chromatography practice and accept the prospect that protonation (and therefore sensitivity) is enhanced at least in positive-mode electrospray by ensuring the analyte is more basic than the solvent. While at odds with accepted practice previous to widespread MS use, adding a volatile modifier such as acetic acid in place of the usual, less-volatile mobile phase components found in LC practice becomes commonplace. In many ways, our solvents of choice have followed similar paths.

Marketing literature — for example, a trade column written by a scientist employed by industry — while typically designed to be useful, can nonetheless be biased, seeming to further confound understanding. The same can be true for conference presentations. A speaker who brings a lifetime of interest, effort, and achievement in a given area will necessarily color the presentation and typically place too much burden of preparation and prior knowledge on listeners much less familiar with the topic. The members of the CoSMoS planning board believe providing a platform for ideas is, of itself, not enough and that ideas need "rounding out" or not debunking outright.

For example, the commercial success of a new technology such as ultra-high-pressure liquid chromatography...
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acetonitrile. Prices are falling but have not reached preshortage levels and I doubt they will."

"The demand for acetonitrile is slow at this time. That seems partly a seasonal effect, partly some inventory or production planning effects and partly due to the fact that users have gone to other solvents. At this time, we are not sure the magnitude of each factor. However, we do expect demand to improve into 2010."

**Aftermath of the "Crisis"**

Though perhaps a true "crisis" did not materialize, the shortage prompts us to reflect on critical aspects of our work. The success over many years of reversed-phase LC has increased the utility of acetonitrile. From Goetzinger and Farrell's notes:

- Analytical separations use relatively small amounts of acetonitrile.
- A 5–10 min LC analysis consumes 5–20 mL which even at $100/L is less than $2 per sample.
- Preparative separations using more than 200 mL per sample is an issue.

Goetzinger and Farrell also speculate that a normal-phase technique like supercritical fluid chromatography (SFC), in which the preferred solvent is methanol, will attract a lot of attention in the future despite the nontrivial process of transferring from one separation mechanism (reversed-phase LC) to SFC. In the prevailing opinion, it seems SFC today might be where high performance liquid chromatography (HPLC) was in the 1980s. So SFC stands as an impressive prospect.

**Smaller Internal Diameter Columns**

Another commonly raised issue is the various benefits — and tradeoffs — of using smaller internal diameter columns. Solvent consumption is proportional to the square of the column internal diameter. Peak volume also decreases to the same extent, which is clearly a problem for concentration-dependent detectors, but in view of electrospray's dependence upon electrohydrodynamics, not so much of a problem for MS detection.

Reducing the flow rate (overall or by postcolumn splitting) has long been a mainstay practice in LC–MS to improve sensitivity. However, any impediment to a well-formed separation (changes in tubing internal diameter, increased tubing length, or junctions in the flow path) will result in dramatic loss of fidelity and sensitivity, even more evident at reduced scale. Mass spectrometers, unlike UV detectors, are mass-sensitive so less solvent requiring vaporization results in a more uniform population of smaller droplets that desolvate to the "magic" 10-μm radius faster, liberating ions more uniformly. If peak width is maintained equivalent in the MS trace as seen in UV, one can see an apparent gain in sensitivity. A reduction of either mass or concentration at peak maximum will, however, result in less than optimum results.

An example of how our thinking can be changed by such disruptions in our normal practice came from a colleague in Japan a few years ago. Jun Yonekubo was developing an analytical separation to determine additives in polymers. The typical approach to LC–MS analysis of such antioxidants requires using atmospheric pressure chemical ionization (APCI) because they are not amenable to electrospray ionization (ESI). Historically, we needed to change the separation to accommodate the higher flow rate of APCI. Where a 2-mm i.d. column used for ESI might optimize at a flow rate of 0.250 mL/min, the linear velocity for optimum traditional APCI was closer to 1 mL/min. Considering the negative effects on droplet formation — the need for increased...
desolvation of higher vapor loads — and any gains expected from increased column loading on a 4.6-mm i.d. column quickly disappears. Yonekubo-san found having the freedom to reduce the column diameter and flow rate using the multimode approach of ESCi (a registered trademark for Waters Corporation multimode technology), where APCI is performed under “ESI conditions,” also condensed the separated bands at this reduced scale, which favored the MS need for higher signal-to-noise ratios. UV detection can better handle a rather diffuse, broad peak with minimal signal-to-noise ratios because the peak is characterized with contiguous wavelengths. MS response improves as signal is better differentiated from noise because the spectrum for even a well-formed separated band is composed of a wealth of discrete ions.

Speaking with some retrospective in the wake of the acetonitrile event, Goetzinger offered some observations: “Reducing particle diameter from 3 µm to 1.8 µm (with optimized equipment) allows separation time to be reduced by 40%. Assuming you need the same number of plates (that is, going from 50 mm to 30 mm length) the amount of eluent is respectively reduced.”

Bearing in mind Smith’s comments on “defining fast LC” as increasing information not just speed, Goetzinger remarks “Given the advantages of smaller particles, UHPLC is probably something we’ll all be doing in a few years since pressure drop is cheap!”

The moderators of the CoSMoS workshop expressed this caveat: Beware of performance losses when moving to smaller internal diameter columns. Practitioners have always been encouraged to place an emphasis on low-dead-volume, well-designed systems. This is certainly true when working at reduced scale. Pay attention to splitting for hpyphenated techniques as peak volume is reduced and disrupting the well-formed separation is possible.

Are There Real Alternatives?
Acetonitrile has become the popular solvent of choice due to low viscosity when mixed with water, enabling high flow rates. The high diffusivity of water–acetonitrile mobile phases provides high separation efficiency and little UV absorbance at low wavelengths for good detection limits. Alcohols, ethers, and acetone don’t perform comparably. All contribute to high viscosity in aqueous mixtures, leaving only methanol as a viable alternative.

Acetonitrile, due to its elutropic strength, has become a favorite for many applications. Even though one might think an aprotic solvent would not perform well in the electrospray process, there are numerous ways to provide protons at atmosphere as opposed to vacuum ionization processes. Methanol has so far shown little benefit when employed with monolithic columns and mixing methanol with acetonitrile is of limited value as Goetzinger points out, resulting in “issues with reproducibility, long-term stability . . . possibly the worst of both worlds.”

Replacing acetonitrile is a question of balancing chromatography performance, pressure and cost. Until the 1990s, methanol was the reversed-phase solvent of choice. When particle diameters decreased from 5 µm to 3 µm, acetonitrile popularity rose accordingly. Methanol requires about 10% higher organic content to produce the same retention factor and selectivity may change. The increased viscosity resulting from using methanol results in increased pressure drop and, typically, a reduced efficiency.

David Masters-Moore (Pfizer, La Jolla, California) makes similar arguments. Investigating alternatives in high-volume purification solvent usage, his group has recently reviewed their practice. Their study compared mixtures of solvents selected to reduce overpressure risks: ethanol, isopropanol, and n-butanol in combination with methanol. Using a simple mixture of parabens, Masters-Moore found 30% butanol and isopropanol and 60% ethanol–methanol mixtures performed most similar to acetonitrile (Figure 5). All had adequate resolution and acceptable pressure gradients. In his estimation, no plumbing, column, or temperature changes were required. The pressure gradients for each mixture were similar to methanol alone.

Others have taken a careful look at some of the underlying behaviors and found we sometimes misinterpret what we see (4). Butanol mixtures, for instance, generate more heat than other alcohol aqueous mixtures and measuring the column temperature can show a disparity between the actual temperatures measured not visible in retention changes. So we might not see much need to amend our practice.

As always, I am indebted to those who graciously share their efforts for the

![Figure 5: Gradient elution retention comparison of candidates for acetonitrile replacement mixtures. (Courtesy of David Masters-Moore, Pfizer, La Jolla, California.)](image-url)
benefit of the rest of us. David Masters-Moore is currently a Senior Research Scientist at Pfizer La Jolla. He has provided chromatography and MS expertise for multiple pharmaceutical, natural products, and agrochemical companies. Bill Farrell is Senior Principal Scientist in High Throughput Analysis and Purification, Pfizer Global Research (La Jolla, California) and a director for the Society of Small Molecule Science, which organizes the CoSMoS conference each year. Wolfgang Goetzinger is Director, Research, Molecular Structure, at Amgen (Cambridge, Massachusetts) and a board member of CoSMoS since 2006. Jerry Richard is President of Purification Technologies Inc. (Chester, Connecticut).

References
(4) W. Goetzinger, publication in process for 2010.

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