



MS — THE PRACTICAL ART

Q&A

Like musical instruments, mass spectrometers abide the same constancy of physics but through complex idiosyncrasies; neither pleases everyone all the time.

MS — The Practical Art, this column's running rubric, reflects a knotty truth about using mass spectrometry (MS) in general analytical practice. Despite the well-considered physics and engineering involved in mass spectrometer technology — that is, in creating ions and passing them through a series of lenses — it nonetheless seems that the quality of one's results is as much the product of art as it is of science.

Years ago, an acquaintance of mine was pursuing graduate studies involving the physics of the vibrating string. I do not know what came of his efforts, but I do remember observing with interest that the mathematical precision of the science used to predict the string's behavior and measure outcomes often went unappreciated by the end-users — the musicians.

The term “intonation,” when applied to a musical instrument, denotes how well the instrument plays in tune with itself. For example, as long as you immobilize a string at a specific point along its length, it should theoretically produce the same note each time. Yet on a finely crafted guitar, striking a harmonic at the 12th fret by dampening its vibration with your finger versus fretting the string (binding it behind the fret) to form the note can produce inconsistent results. In some positions along the length of the strings, one chord can sound perfect and another out of tune. These phenomena arise from the guitar's system of intonation, what musicians call its “temperament.”

An acoustic guitar's frets maintain a fixed tuning. The guitar's 12th fret is used to gauge whether the instrument's “equal temperament” (a system that divides the chromatic scale into 12 equal half-steps) produces the desired intona-

tion. So for any guitar, what frustrates some refined ears is that the intervals between notes are not always perfect, or “pure,” making it hard to accept idiosyncrasies in an instrument designed for precision. So, each guitar maker tries to create its version of optimal performance knowing well the physics that underlie the vibrating string, but knowing also that their instruments' particular brand of idiosyncrasy will play well in the hands of some musicians but not all.

Like musical instruments, mass spectrometers operate with the same constancy of physics. Source and ion optics interface an atmospheric ionization inlet with a mass spectrometer, delivering sensitivity and economy of function. Producing ions from analytes must obey the same physical laws, such as the (still-debated) formation of electrospray ions, gas dynamics, and desolvation to produce ions in the gas phase and conserve them via the source and ion interfaces. This is true regardless of whether those interfaces are attached to an ion trap, quadrupole, or time-of-flight instrument. Yet results often differ when parallel experiments are attempted using different instrument designs. Or worse, the same experiment is attempted on different days using the same instrument. So you might ask: How can this be when electrospray ions created from the same analyte are identical?

Apart from differences between instruments themselves, artifacts occur such as sample degradation and other vagaries of the sample handling process. Even more significant in terms of guaranteeing unequal results are the idiosyncrasies of practitioners. People can differ in their approach to calibration and tuning their instruments, and to some degree an assessment of a mass spectrometer's

“tone” can be subjective. Producers of mass spectrometers design their instruments to work well within specific parameters. The constraints vary among manufacturers — otherwise all four major instrument manufacturers would offer the same design. Assessing the common points as well as the differences, real and alleged, can appear to be quite an art.

Most in-service liquid chromatography–mass spectrometry (LC–MS) courses fail to address specific operational problems, and retaining consultants can be financially prohibitive. So one source that practitioners often turn to for advice is online discussion postings. These are perhaps the best way to appreciate that the rest of the MS world shares problems similar to yours. Yet, it is often a bit vexing to find the answers to those problems are not so clear-cut. With that in mind, I want to focus on sources to which you can go to find help, on helpful hints, and some anecdotes on spectral interpretation in upcoming columns.

One well established source (since 1995) is the discussion group (SciTechniques), maintained by moderators David Bostwick (Georgia Institute of Technology) and Sarah Shealy (Georgia State University) at <http://web.chemistry.gatech.edu/~bostwick/stms/>. John Bartmess (University of Tennessee) offers a (more or less) weekly reprise of the discussions at <https://ion.chem.utk.edu/mailman/listinfo/stms>. Perusing the discussion thread can be useful and sometimes even amusing. Perhaps, best of all, it is free.

I edited some samples of the exchanges, hoping to improve their coherence and also to ensure against unintended emphasis on either products or individuals. The exchanges lie buried as discussion threads, and digging them out can prove daunting. But it is simple enough to subscribe to the mailings, and have them show up periodically in your inbox. The helpful hints range from the obvious to what appear the beginnings of an advanced-degree thesis.

Modifier, Linearity, and Response Problems

This plea for help is by a chromatographer with a modifier problem:

I have several positively charged

(amine) compounds, which, for chromatographic reasons, must be analyzed using a basic (triethylamine) buffer mobile phase. Because the mobile phase is only 0.1% triethylamine and about 50% acetonitrile, I thought I could simply acidify the effluent with a postcolumn infusion of formic acid (10:1 effluent–formic acid) via a tee. Unfortunately, I cannot see anything, even at high sample concentrations. Where am I going wrong?

Here are some helpful reader responses:

Have you ever gotten an LC–MS signal from your substances? If you have, was triethylamine then included in the mobile phase?

(Comment: Triethylamine is highly ionic and can compete unfavorably with your analyte, especially if its concentration is much higher than the analyte's. You might find some additives like triethylamine work better in negative rather than positive mode. Triethylamine can also present a persistent [m/z 102] background ion. Surprisingly, many do not first try to omit modifiers from their chromatography when transferring to the gas-phase environment of LC–MS from the condensed phase world of UV-only. Sometimes uncharted changes in equilibrium can and do take place in the transition. Sometimes it can be impractical — for instance, when it would require showing equivalence with an existing method based upon an accepted new drug application — but it can nevertheless provide a simplified comparison.)

Are you sure you are introducing the intended percentage of formic acid?

Do the interface and postcolumn device work?

(Comment: Anytime a novel — that is, a less-than-satisfactory — response is encountered, operators must verify that the system is working correctly. An easy way to do this is to include a small amount of “known good” analyte to the postcolumn infusion. At the very least, every practitioner should have a sample that gives a well-documented response at hand.)

This spectrometrists has a linearity problem:

I have problems with the linearity of some compounds while using LC–MS in negative ion mode. The calibration curves are very nonlinear. The concentration is in the same range as that of other

linear compounds. The compound is an acid, like the other compounds, but others do not exhibit this problem. I have tried several eluent compositions, but without success.

(Comment: The distracted analyst comes to various seemingly reasonable solutions, such as considering what eluent additives he or she could try that would improve the method. Is acetic acid better than formic acid? Is methanol or acetonitrile better? Yet the solution could be much easier.)

Readers forwarded these ideas:

This could be caused by adsorption (compound-dependent or analyte-dependent). Changing from stainless steel to PEEK might improve performance.

I had similar problems when I worked with a [triple quadrupole]. We could not find the problem [replacing solvent type–acid, and so forth] and an engineer suggested we lower the temperature of the source. We were running at 180 °C and lowered it to 150 °C. That solved the nonlinearity problem. Sometimes solutions are very simple.

(Comment: Amen. And sometimes they are not. Indeed, not all linearity problems can be resolved in the discussion group format.)

This chromatographer missed both linearity and response:

I am hoping that someone can help me. I am developing a method to analyze alkylphenol ethoxylates by electrospray LC–MS, and I ran into a problem. The analytes in my method give exclusively $(M+Na)^+$ ions, so I am monitoring those. During a sequence of three injections the chromatogram baseline remains stable for a period of time. Then it drops for a few minutes only to rise again. Any peaks during the baseline drop are much smaller than where the baseline does not drop. This plays havoc with my quantitation, especially when the internal standards are eluted during this time.

The baseline suppression does not happen at the same time during each of the LC runs. It migrates all over the place, showing no discernable pattern. Because the depression of the peak height is much greater than the magnitude of the baseline suppression, I am assuming that what is happening is a disruption in the ionization process, leading to low and irregular ion formation and

sampling efficiency. The first thing I thought was that this was perhaps a competition effect from coeluted interferences. So, I put 50 μM of sodium acetate in the mobile phase. All this did was junk up the spectrum with a bunch of clusters and reduce the overall sensitivity (10 μM seemed to be okay, though). In any case, the baseline problem persisted. My next thought was that it might be a problem with really nonpolar stuff sticking to the column and carrying over into the next run (my runs are short, about 15 min) because I was running isocratic. Nope, I inserted a 15-min flushing–equilibration step, and this still did not get rid of it.

The problem seems to occur most often with samples. But when it happens with standards, it really ruins quantitation. It does not happen in negative mode; I get a nice baseline. Does anyone have an idea about what is going on here? Surely I am not the only person who has ever seen this problem.

(Comment: In this case the submitter's reasoning was absolutely on target. The problem is widely recognized as the bane of quantitative MS in complex mixtures, and numerous publications are available for guidance. If I were to recommend one, it would be "Ion Suppression: A Major Concern in Mass Spectrometry," a tutorial written by Lori Lee Jessome and Dietrich Volmer, which appears in an upcoming issue of LCGC. It serves as not only a very comprehensive overview of what painstakingly has been collected in recent practice, but as a collection of references to pursue as needed.)

Mass Spectral Interpretation Problems

We will examine this topic in detail soon, in another column. For the moment, though, consider that atmospheric ionization work often can create adducts that range from the common to the exotic, making spectral interpretation and quantitation difficult.

A case of mystery ions — common sodium in a not-so-common mode:

Could anybody give me some clues about the mechanism of sodium adduct formation under atmospheric pressure chemical ionization (APCI) so that I can control it? The Na^+ adduct is commonly formed in solution in electrospray

ionization (ESI). Is it possible the Na^+ adduct can form in the gas phase by APCI?

(Comment: The issue remained unresolved after a long but interesting and informative exchange. In the ensuing exchange, which I summarize here, the consensus was that sodium adducts form in solution requiring a considerably higher voltage rather than forming in the gas phase.)

Perhaps because of APCI's higher probe temperatures, certain analytes can be desorbed into the gas phase, resulting in a mixed electrospray and APCI spectrum. [A mixed spectrum is not uncommon and is in fact being exploited in various mixed or multimode ionization source designs]. If it is of interest to determine which mechanism predominates, a high-speed switching design like the ESCi multimode ionization source (which comes standard on all Premier products from Waters Corporation, Milford, Massachusetts) can be employed to characterize the response between the two mechanisms with good, reproducible fidelity. The Turbo V design (MDS Sciex, South San Francisco, California) offers similar common source abilities. If your purpose is simply to ionize the majority of molecules in a solution, a simultaneous design such as the Agilent (Santa Clara, California) multimode will suffice. All of these devices are of course limited by each vendor for use with their particular instrument line.

You can minimize or avoid sodiation by using plasticware instead of glassware. Glass surfaces produce infinite amounts of sodium, which seems to affect some compounds readily. Also, you might see in the sample history that sodium buffers were used in the prep.

Because the concentration of sodium in solution is not fixed, using sodium-adducts for quantitation can present problems. One reader suggests adding 1 mM sodium acetate to the eluent and then running in ESI mode rather than APCI mode. Even though APCI is preferred, studies indicate it is less susceptible to suppression. This suggestion is not so extreme these days: Z-spray and similar designs minimize contamination and clogging.

You might find that expending a bit of effort to hold contamination to a mini-

um improves downstream interpretation and response. Two major sources of contamination in reagent prep are the containers used for preparing solvents and the transfer devices. Detergents and surfactants can increase propagation of ESI ions and therefore sensitivity. Unfortunately, the increased sensitivity is for the surfactant contaminants, not the analytes. So, as an old hand at electrochemistry once told me, do not use anything in your preparative practice that you would not want to see in your spectral background. Segregate LC-MS containers and measuring devices for LC-MS use only. Do not use detergents, and rinse containers repeatedly before setting them aside to dry. Good practice also dictates preventing acids and plastics from coming together.

Readers' remarks, though they sometimes fail to offer technical help, always remind us that we do not face these problems alone — cold comfort, perhaps. So if your problem seems instrument-specific, you likely will find someone who can sympathize. Just remember that LC-MS practice can be quite a complex orchestration of sample preparation, instrument condition, and suitability for the intended use, not to mention your own level of understanding — all of which are evolving at different rates. Clearly, if there is one benefit to point out from among LC-MS courses, reference literature, and other self-help sources, it is the decision you make before purchasing your instrument. Weigh the accessibility and history of your service and sales contacts well in the equation. Unless you are blessed with a colleague who is an experienced operator — which aside from major pharma sites can be rare — these folks are your best allies.

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