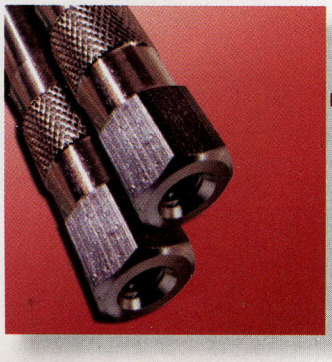


LC TROUBLESHOOTING

300 and Counting

This is John Dolan's 300th "LC Troubleshooting" column. Here, he takes a look through the archives.

John W. Dolan
LC Troubleshooting Editor

This month, I'm going to take the liberty of a little reminiscing and review. It is hard to believe, but this is my 300th consecutive "LC Troubleshooting" column to appear here. I don't have the longest tenure, though — Ron Majors started writing his "Column Watch" columns with the second issue of *LC Magazine* in April of 1983, so he has seniority. Dennis Runser started writing the "LC Troubleshooting" column in the very first issue, but tired of it after three installments. I was recruited, along with Vern Berry, to pick up the baton and continue. Because the magazine's title then was *LC Magazine*, we just titled the column "Troubleshooting." Vern wrote with me for 11 columns, then moved on to writing meeting reports and I continued as the editor of this column. The magazine title changed to *LCGC* in April 1986, but the column didn't change titles to "LC Troubleshooting" until September 1987. From its onset through 2004, *LCGC* comprised 11 technical issues and one Buyers' Guide (in August) each year; commencing in 2005, there were 12 issues a year with technical content, plus the Buyers' Guide.

I'd like to thank the staff of *LCGC* for their long years of support. I've survived several editors and numerous staff changes over the years. The original magazine was the brainchild of Ed Aster, of Aster Publishing in Eugene, Oregon. The only remaining staff member from the Aster days in Eugene is technical editor, Steve Brown, who started in 1989. Steve is the guy who has caught innumerable mistakes in equations or other subtleties that tend to slip through the cracks when you try to proof your own writing. His associate, Lisa McAdam, was responsible for mentoring me for years, correcting my style, grammar, and punctuation to adhere to the Aster style book and good writing practices. This pattern of striving for technical excellence is something that has remained over the years and continues with the current editor, David Walsh.

The content of my columns, however, comes from you, the readers. The only time in all these years that I've been asked by *LCGC* to write (or not write) on a particular topic was for the 25th anniversary issue, which was appropriate. The remainder of the topics have come from my interactions with liquid chromatography (LC) users, through mail or e-mail, in classes I teach, in laboratories where I have worked, or in my consulting practice. The problem each month is not trying to think up a topic, but choosing from the many possible ones available.

As I prepared this article, I spent some time going through my "LC Troubleshooting" archives, looking for patterns and common threads over the years. Let me share a few of these. As mentioned earlier, *LCGC* readers are a significant source of the topics — I counted 50 columns that focused directly on problems that had been submitted by readers. Some of those problems and their solutions ended up as at least 21 case studies. The case-study format is an excellent way to take a specific problem and in the presentation, show how general

principles apply to the specific case as well as other applications.

I don't use coauthors or contributing authors as much as Ron Majors does, so I was surprised to count 50 columns that fell into this category. A large portion of these coauthors were staff members of the laboratory I used to manage. We had a running joke that if you really messed up, you could get an article in *LCGC*. That's not quite true; it's more about encountering a problem and solution from which others will benefit. After all, we all make mistakes. The coauthors of these problem-oriented columns were willing to share their knowledge so that others might be able to avoid future problems. I'm the first to admit that my expertise has definite bounds, so I've been happy to have contributions by experts in their own fields. Among these are Bill Tindall on pH (1), Ken and Carol Collins on stainless steel surfaces (2), and Craig Young on the evaporative light scattering detector (3). And readers often have clever ways to look at a problem that will benefit others; the latest of these is Jennifer Birchett's contribution on how to get more out of the data you collect (4).

By the way, if you are interested in viewing the back issues of "LC Troubleshooting," all of the issues of *LCGC* back to January 2002 (volume 20, number 1) are available on the *LCGC* website (www.chromatographyonline.com) in the Archives section.

Topics for Conversation

I'm often asked about whether or not I rerun columns from the past. The answer is that I've never rerun a column, per se. However, there are common topics that are repeated over the years. Some of these are specific problems that readers submit and others are general problems, such as check valves or column maintenance, that new readers will benefit from reading. My regular readers know that one of my soap-box topics is preventive maintenance. I believe that most LC problems can be avoided or controlled by a good preventive maintenance program. Part of this is a solid understanding of how the different parts of the instrument work so that you will know what is likely to fail. I counted 29 columns that dealt specifically with how different equipment modules work. There always will be problems with the separation, at least as long as we persist in injecting samples into the column. There were at least 28 columns dealing with specific problems with the chromatogram, such as split peaks, tailing peaks, and retention drift.

I'm often asked about whether or not I rerun columns from the past. The answer is that I've never rerun a column, per se.

Sometimes a topic is just too large to give it ample discussion in a single column. I like to keep the columns short and sweet. I don't know about you, but when I look at the length of an article, if it looks like I can get through it in a short session, I'll read it right then, otherwise it gets set aside and perhaps forgotten. So sometimes a topic gets split up into more than one column. My archives showed 16 such series of columns, for example, the most of contributed columns mentioned previously (1–3) are multi-installment in nature. The most popular series (measured by reader feedback) in the last few years are the five-part series on calibration curves (5) and the seven-part series on method development (6).

A quote from I.M. Kolthoff, considered the Father of Analytical Chemistry, is one of my favorites: "Theory guides, but experiment decides." This is definitely true with LC, but too often, we seem to be scared away by equations and prefer the "blind dog in the meat house" approach of random experimentation until we get the result

we want. In nearly 30 columns, I have explored the theory–practice interface for LC. I strongly feel that application of some of the basic relationships of LC, such as retention factor, plate number, the fundamental resolution equation, and propagation of errors, can be used to develop more dependable methods and to help us correct problems when we encounter them. This is certainly true with the method development series (6) mentioned earlier and its predecessor six-part series on method development called "Starting Out Right" (7).

Some Favorites

I find that simple rules of thumb, mnemonic devices, and estimations help me to remember what is important and encourage me to make simple checks to keep the LC system operating properly. Some of these topics are the subject of columns that stood out as I reviewed my database.

My five troubleshooting rules of thumb (8) have been a standard part of the LC troubleshooting classes that Tom Jupille and I teach. These are:

1. The Rule of One (change just one thing at a time)
2. The Rule of Two (make sure a problem is repeatable)
3. Put It Back (if a part is substituted and it doesn't fix the problem, put it back)
4. Throw It Away (don't spend more time trying to salvage a part than it is worth)
5. Write It Down (if it isn't documented, it didn't happen)

One of my favorite posters is titled "The Seven Last Words of [X] " (add the name of your favorite organization): "We never did it that way before." Sometimes I think we should flip this around for the chromatography lab: "We've always done it that way before." Often we take our laboratory training from someone who learned a good deal from the school of hard knocks, but has not changed with the times. This results in continuation of practices that are no longer appropriate. One series of columns titled "Useful or Useless?" (9) examined some of these topics. Useful: guard columns, in-line filters, tubing selection; useless: saturator columns, frit cleaning, void-filling, ramping flow rate. We need to carefully examine if all our laboratory practices really make sense.

When David Letterman made top 10 lists popular on his Late Show, I decided to join ranks with my "LC Columns — The Top 10 List" (10).

1. Dedicate columns (columns have fewer problems if you don't use them for multiple methods).
2. Use in-line filters (catch any particles on an inexpensive filter rather than an expensive column).
3. Flush columns regularly (before contaminants cause problems).
4. Use guard columns (keep strongly retained material off the analytical column).
5. Control temperature (both retention and selectivity can change if the column temperature changes).
6. Avoid the void (minimize extra-column volume for best performance).
7. Retire early (the column is a consumable item — replace it before it ruins your results).
8. Ensure the supply (use columns that you are confident will have the same characteristics the next time you buy one).
9. Minimize garbage (sample cleanup, and filtering or centrifugation to remove particles are two steps that can greatly increase column lifetimes).
10. Pure is good (use HPLC-grade solvents and reagents so that you don't inadvertently contaminate the column).

I'll end with "My Favorite Shortcuts" (11), a summary of tricks and techniques that I find useful to estimate chromatographic changes. You'll have to look up most of the details in the reference.

1. Retention factor k (estimate from the column dead-time, t_0; ideally $1 < k < 20$ is desired).
2. Rule of three (k changes approximately threefold for each 10% change in organic solvent in reversed-phase).
3. Is t_0 reasonable? (estimate t_0 from the column dimensions.)
4. What about k for gradients? (the gradient retention factor k^* is calculated differently from isocratic k and

is influenced by different factors)

5. TGIF (one simple way to make gradients "equivalent" is to keep the gradient volume –the product of gradient time t_G and flow rate F constant when making changes).

6. How much can I inject? (if you inject in mobile phase as the injection solvent, you usually can inject up to 15% of the volume of the first peak without problems).

7. Column efficiency (a simple estimate of the column plate number N often is a more meaningful reference value than the manufacturer's column test results).

8. Temperature and retention (isocratic retention changes approximately 2% for a 1 °C change in temperature).

9. Keeping life simple (use the KISS principle "[Keep It Simple, Stupid"]; simple methods are more reliable).

Conclusions

Now that I'm done with column 300, what can I conclude regarding "LC Troubleshooting" after nearly 27 years of writing about it? Some problems will never go away. They are part of the process. Equipment will wear out or be abused. Columns won't last forever. And, as radio talkshow host Dave Ramsey is fond of saying, "I have a Ph.D. in Stupid." I think we can all relate to that one, so operator error will never go away. On the other hand, some things have improved greatly over the years. Degassing, which was near the top of the problem list 20 years ago, is seldom a complaint with the nearly universal use of in-line degassers. Column technology has improved so that peak-tailing problems are a faint shadow of their ancestors, and with most suppliers, we can be confident of getting another column a year or two from now, just like the one we're using. And while we tend to complain about some of the regulatory scrutiny under which most laboratories live, such regulations have improved our habits of preventive maintenance, ensuring that system suitability passes, and keeping good records of our work, all of which make system operation more reliable and increase the quality of the data generated.

So keep those cards and letters coming. I'm always looking for the next topic, an interesting case study, or another coauthor.

References

(1) G.W. Tindall, *LCGC* **20**, 1028–1032, 1114–1118 (2002).

(2) K.E. Collins, C.H. Collins, and C.A. Bertran, *LCGC* **18,** 600–608, 688–692 (2000).

(3) C.S. Young and J.W. Dolan, *LCGC* **21,** 120–128; 22 (2004), 244–250 (2003).

(4) J. Birchett and J.W. Dolan, *LCGC* **28**(4), 292–301, (2010).

(5) J.W. Dolan, *LCGC* **27**, 224–230, 306–312, 392–400, 472–479, 534–540 (2009).

(6) J.W. Dolan, *LCGC* **25**, 546–552, 632–638, 704–709, 944–950, 1014–1020, 1094–1102, 1178–1182 (2007).

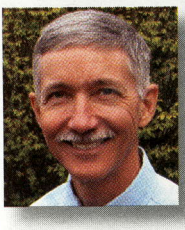
(7) J.W. Dolan, *LCGC* **17,** 1094–1097 (1999); **18,** 28–32, 118–125, 286–294, 376–382, 478–487 (2000).

(8) J.W. Dolan, *LCGC* **6,** 304–308 (1988).

(9) J.W. Dolan, *LCGC* **16,** 544–546, 635–636 (1998).

(10) J.W. Dolan, *LCGC* **21,** 262–266 (2003).

(11) J.W. Dolan, *LCGC* **22,** 1074–1080 (2004).



John W. Dolan

"LC Troubleshooting" Editor John W. Dolan is Vice-President of LC Resources, Walnut Creek, California; and a member of LCGC's editorial advisory board. Direct correspondence about this column to "LC Troubleshooting," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail John.Dolan@LCResources.com.

For an ongoing discussion of LC troubleshooting with John Dolan and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.org.