Troubleshooting

# The Future of LC Troubleshooting — 10 Years Later

**John Dolan revisits 10-year-old predictions and makes some new ones for the coming decade.**

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In March of 1992, *LCGC* was entering its 10th year of existence and high performance liquid chromatography was early in its third decade of popular use (1). I wrote an "LC Troubleshooting" column titled, "LC Problems — Past, Present, and Future." It was interesting to reflect on the changes that had occurred as liquid chromatography (LC) developed into a mature analytical technique. I also made some predictions about developments that might occur in the field of LC in the next 10 years. This month, I'd like to look back at some of the changes that have taken place in the past 10 years, see how my predictions scored, and make another set of predictions for the next decade.

## Yesterday and Today

In March 1992, I cited five areas in which advances had been made that reduced the amount of time spent troubleshooting LC problems. Let's first look at these areas and see if further progress has been achieved.

**Pump seals:** Manufacturers made great advances in pump seal technology, which significantly extended the useful lifetime of seals, during the 1980s. I don't think such dramatic advances have occurred in the past 10 years, but today I classify pump seal problems as minor. This change primarily is the result of a better understanding of pump seal care. Users have learned the importance of not leaving buffers and salts standing in unused LC systems because salt crystals can form. Restarting systems containing old buffers can cause abrasion of the pump seals and shorten their useful life. Today, most users either flush the buffer at the end of a run sequence or reduce the flow rate to 0.1 mL/min so the pump never stops. With reasonable care, pump seals need to be changed only once or twice a year, which moves seal replacement into the routine maintenance category.

**Air bubbles:** In the past, air bubbles were a major source of irritation for LC system operators. Again, improved knowledge that mobile-phase degassing can eliminate most air bubble issues has reduced the magnitude of bubble problems. Built-in degassing systems, in which the mobile phase was helium-sparged and then pressurized, were popular with several manufacturers and some aftermarket suppliers. These systems reduced or eliminated air bubble problems, but they were complex, leak-prone, and cumbersome. During the past 10 years, in-line vacuum degassers have become more popular and are available as standard equipment on many of today's systems. In these devices, degassing is accomplished by pumping the mobile phase through thin-walled tubing that passes through a vacuum chamber. Air bubbles pass through the tubing to waste, and the liquid continues to the pump, much as a Gore-Tex rain jacket sheds the rain, yet lets the air pass through. So bubble problems are quite manageable today and are not a primary irritant to most chromatographers.

**Detector lamps:** Manufacturers have made a progressive improvement in the performance of deuterium lamps used in UV detectors. Ten years ago, a detector lamp could be expected to last approximately 1000 h, which was a great improvement from the early days of UV detectors. Today, it is common to look at the usage meter when a lamp is replaced in my laboratory and discover that it has been in service for more than 2000 h. Detector lamps no longer are considered a focal point for troubleshooting efforts.

**Column failure:** Ten years ago, nearly everyone was using 5-μm $d_p$ spherical packing materials in their columns. Batch-to-batch and column-to-column reproducibility was acceptable. Retention reproducibility and peak tailing problems were fairly well understood. Although tailing still was a major issue, using mobile-phase additives such as triethylamine helped reduce tailing to acceptable levels in most cases.

I think that column technology is the area of LC in which the greatest advances have occurred in the past decade. Perhaps the most significant change has been the introduction of Type B silica. Previously, the silica particles used in columns were contaminated with metals and had a fairly high population of acidic silanol groups on their surfaces. This caused strong band tailing, especially for basic compounds. Type B silica is synthesized to eliminate metals as much as possible and treated to generate a homogeneous surface of less-acidic silanol groups. The result is a silica particle that exhibits much less propensity for peak tailing with bases, so triethylamine and other additives seldom are needed. Further advances in reducing peak tailing have resulted from the development of sterically protected packings, the substitution of carbon for silicon in the silica polymer, and high-density bonding techniques.

One attempt to improve column stability was the development of the polar embedded phase — sometimes called an amide or carbamate phase — in which a positively charged functional group was bonded near the base of a bonded phase. It isn't clear to me that this phase increased bonded-phase stability, but it has yielded two very useful characteristics. First, the polar embedded phases have chemical interaction characteristics that are different enough from traditional C8 or C18 phases that they can be used to gain column selectivity in difficult separations. A second benefit is that these phases do not collapse in 100% water mobile phase, whereas most C8 or C18 columns are limited to mobile phases with at least 2–5% organic solvent. This characteristic can be quite useful with polar analytes.

Although I did not predict the use of smaller particles, 3.0- and 3.5-µm $d_p$ media have become popular. These small particles allow chromatographers to obtain roughly the same separation on a 75–100 mm column that they could with a 150-mm column packed with 5-µm particles. This change can reduce separation times by half. Particles smaller than 3.0 µm have been commercialized, but their application is limited because of high column back pressure.

Other changes in column-use patterns relate to the increasing popularity of mass spectrometers as LC detectors. In general, because mass spectrometry (MS) systems provide specificity, analysts require less chromatographic separation with LC–MS than with LC–ultraviolet (UV) applications. Thus, they can obtain sufficient separation with LC–MS using a 50-mm-long column packed with 3.5-µm particles, whereas a 150-mm column might be necessary for LC–UV. Although LC–MS units can accept 1.0–1.5 mL/min solvent flow rates, they work better at lower flow rates. This development has resulted in the increased use of 2-mm i.d. columns, which require only 0.2 mL/min flow rates to generate the same linear velocity as a conventional 4.6-mm i.d. column operated at 1 mL/min. Faster separations translate to higher sample throughput for most LC–MS separations when compared with conventional LC–UV separations. However, the autosampler cycle time can become a bottleneck with run times less than 5 min, so autosampler performance leaves room for future improvements.

> ***I predict some of chromatography's bread-and-butter columns in 2012 will be something today's chromatographers haven't thought of yet.***

**Fittings:** The last area that I discussed in the March 1992 column was fittings and tubing. Polyetheretherketone (PEEK) fittings and tubing were becoming popular, and finger-tightened fittings had made major inroads in the traditional stainless steel fitting market. Today, nearly every chromatographer I know depends upon finger-tightened fittings and PEEK tubing for LC system connections. Stainless steel still is used for the more permanent and high-pressure connections, but the convenience and reliability of PEEK has made this material the primary tool for connections that need to be changed regularly, such as columns.

## How Accurate Were the Predictions?

I ended the 1992 column with a few predictions about what might develop by the year 2002. Let's see if any of them came true.

**Replacement components:** We had seen the introduction of cartridge check valves and cartridge columns by the early 1990s, and I predicted this trend of modular replacement components would grow. At least one manufacturer produced quick-disconnect pump heads, and I expected this instrument to become standard. I predicted that other parts would be easily removed and replaced, with the problem components returned to the manufacturers for refurbishment.

*The verdict?* Wrong! In general, LC system design has not changed in the direction of modular replacements. Some manufacturers have designed their systems so that entire subassemblies are replaced rather than servicing a component of that assembly; for example, the proportioning valves of low-pressure mixing systems. This capability reduces service time if users have a service contract on the system but often results in a significant increase in cost for user-serviced systems. In general, I don't see that it is any easier to replace the injection valve on an autosampler or deuterium lamp on a UV detector than it was 10 years ago. Even cartridge columns and check valves don't seem to be used as much as they were in the past.

**Built-in diagnostics:** In the early 1990s, some companies sold LC systems that had some internal diagnostic capabilities. I predicted that more manufacturers would provide these functions and that users would be able to tell the volume of solvent pumped through the system, when pump seals or check valves were ready to be replaced, and when maintenance was due. Columns would be bar coded to facilitate recording a history of each column and help determine when a column was due for replacement.

*The verdict?* Significant improvement has occurred in this area. Some kind of electronic monitoring is a standard feature in most systems now. The most common features are piston-stroke counters and lamp-life meters. Some systems will record the pressure for each injection. At least one manufacturer produces a system to bar code columns, but it hasn't caught on as a widely used tool. Although many systems now have various use recorders as standard features, users often must dig into the diagnostic portion of the system to access these data — it appears to me that these features often are designed more for the manufacturer's service technicians than for end users.

**Remote servicing:** I predicted that every LC system would have a built-in modem so that service technicians at factories could diagnose LC problems remotely. I thought this capability would reduce downtime and on-site service calls and, thus, improve analytical laboratories' efficiency.

*The verdict?* Wrong! Although some LC systems can perform some kind of remote diagnostics, I don't see this feature in regular use. I'm not sure why. We have quickly adapted to the Internet in the past 10 years, but servicing LC systems seems to be a hands-on activity, either for users or service technicians. In my laboratory, the LC–MS units have remote communications, but my colleagues and I have used this function only a few times. I still think that remote diagnostics and servicing has plenty of room for improvement.

**Push-button experts:** I predicted that expert-system software would be common for diagnosing instrument problems and guiding method development. This software could incorporate user preferences and instrument performance trends to help chromatographers perform at a higher level.

*The verdict?* Marginal progress. Several groups, including my company, have attempted to design expert systems for LC, but to date, none of these efforts has generated a successful product. I attribute this failure to the huge effort required to encode enough specific information to be of practical use. With the widespread use of CD-ROM drives and disks in computer systems, some manufacturers offer video-assisted instruction for common system maintenance techniques such as changing pump seals or check valves. One unexpected benefit of the widespread use of the Internet has been the availability of expert advice anywhere at any time. User groups and technical forums, such as "Chromatography Forum," allow users to post problems and get advice from experts around the world. So, although expert systems have faltered, the availability of expert advice for chromatographers is greater than ever.

## And What Does the Future Hold?

If I were generous (and, well, I'm the scorekeeper, so I'll be very generous), I'd give myself an approximately 25% success rate on my predictions from 1992. Let's see if I can do better for the next 10 years. Here are some prognostications, which are driven by the need for faster separations and more universal detection.

**Detectors:** During the past 10 years, LC–MS and LC–MS–MS have become standard tools in analytical laboratories, especially in the pharmaceutical industry. I predict that the MS detector will become as common in 10 years as the diode-array detector is today. Two things must happen. First, the price must fall significantly. An LC–MS–MS unit costs $250,000–$350,000, which is too much to justify for a routine detector for many LC applications. If some clever manufacturing, design improvements, and increased production volume could reduce the cost by an order of magnitude, we could see an LC–MS system in every laboratory. Second, although today's LC–MS units are much simpler to operate than those of the past, they'll need additional improvements so that little MS skill is needed for operation. Automatic setup and tuning would go a long way toward making these instruments easier to operate.

**Autosamplers:** Most of today's autosamplers were designed to support separations on 150–250 mm columns that need 10–20 min per run. In these designs, a preinjection cycle time of 1 min or more has little effect upon the overall throughput. However, laboratories want run times of less than 5 min, so an autosampler must not contribute significantly to the overall run time. For example, my laboratory just replaced four conventional autosamplers with new ones that have 15-s cycle times so that run times on the LC–MS–MS systems are not compromised.

Carryover is another area that needs additional attention. The injection valves that form the basis of most of today's autosamplers were designed in the 1970s. Although these valves are reliable, many have significant carryover problems. Carryover can be considered in two aspects. First, consider carryover as a percentage of the previous injection. This amount generally is no problem as long as the carryover is less than approximately 0.1%, which many of today's units provide. Second, a potentially more serious problem with trace analysis is absolute carryover. Absolute carryover refers to some amount of carryover resulting from adsorption or other processes that is not necessarily proportional to the size of the previous injection. I have noticed this problem to be of special interest with trace analysis by LC–MS–MS. For example, when the injected sample contains less than 10 pg/mL of analyte, it doesn't take much absolute carryover from a more concentrated sample to yield an unacceptable percentage carryover relative to the lower concentrations. Improvements in autosamplers to reduce carryover would be welcomed.

**Columns:** I'll repeat one of my predictions — maybe it really is a wish — for bar coding or some other convenient and automatic way to track column use. In the past, chromatographers have been relatively spoiled by having columns that generate more theoretical plates than needed for separations. Thus, they could use columns that were significantly degraded and still obtain good results. Looking into the future, I see more demand for high-throughput separations, which require maximum column performance in a minimum amount of time. As such, users will need a simple method to monitor the health and history of a column so that the columns can be replaced in a timely manner. The paper records that most analysts use today just won't do the job adequately.

I'm also looking forward to some new, as yet undefined advances in column technology. Perhaps it will be something like the silica rod columns that were introduced in the past couple of years, a new phase such as the embedded polar phase, or a leap forward in silica chemistry such as the Type B packing material. I'm not sure what the advance will be, but I predict some of chromatography's bread-and-butter columns in 2012 will be something today's chromatographers haven't thought of yet.

**Remote diagnostics:** I'll vote again for improved remote diagnostics. I see more dependence upon computers and computer networks in all aspects of laboratory operation. It is only natural that LC systems should make better use of this technology for diagnostic purposes. I believe that remote access and built-in diagnostics with automatic messages will be common. In isolated cases, I've seen LC–MS systems that diagnose each chromatogram, notify users by telephone when a fault is discovered, and relay information about the nature of the fault. It is reasonable to expect this kind of instrument monitoring to become routine.

## Call Me in 2012

It's fun to look at predictions from the past and evaluate their accuracy. It is interesting that what I consider two of the biggest advances in LC of the past decade — Type B silica and embedded polar phases — and the widespread use of, or shall I say dependence upon, the Internet — were not among my predictions in 1992. In the same manner, I expect we'll look back at 2002 from our 2012 vantage point and make the same observation — the unexpected was the most significant.

## Reference

(1) J.W. Dolan, *LCGC* **10**(3), 202–210 (1992).

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