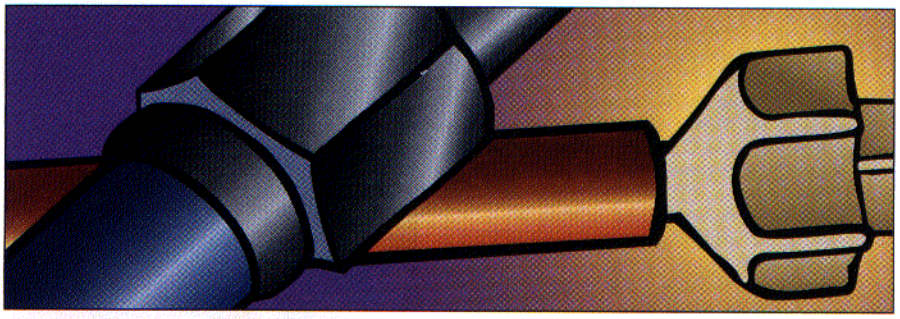
LC Troubleshooting



LC Troubleshooting On-Line with *Chromatography Forum*

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Now you can find more troubleshooting help at http://www.lcgcmag.com.

In this month's "LC Troubleshooting" column, I'll talk about an exciting new way to find and share troubleshooting advice for your liquid chromatography (LC) separations and hardware: *Chromatography Forum*. Our new Internet discussion group will allow you to post questions about problems you have and receive advice from other participants. You also can read the questions and comments submitted by others and add your own comments. By combining the expertise of the entire readership of *LC•GC*, everyone should benefit from this new forum.

You might ask why you should participate. Perhaps some background is in order. When I started writing the "LC Troubleshooting" column in 1983, 16 years and approximately 165 installments ago, only 25,000 subscribers received *LC Magazine* (*LC•GC*'s former name); today more than 86,000 readers receive *LC•GC* and its sister publications *LC•GC International* and *LC•GC Asia Pacific*. In the beginning, my co-author, the late Vern Berry, and I received perhaps one or two letters a month with troubleshooting questions. Today, I rarely receive letters; instead the questions come primarily via e-mail. And, as you might expect, I receive many more questions. So what was an interesting sideline activity a few years ago — fielding your questions has become a much larger task — making it harder for me to provide an immediate response. I hope this new forum will provide you with immediate answers from the broad expertise of *LC•GC*'s readership.

With the advent of *Chromatography Forum*, you'll find a number of features that are impractical in the written version of "LC Troubleshooting," which you will continue to read each month in *LC•GC*. First, you'll receive immediate feedback. For one of your questions to appear in print in this column, it takes a minimum of two months (and often six months or more). Although I try to answer your questions promptly via e-mail, no one else receives the benefit of your experience. In *Chromatography Forum* any reader can see the question and provide an answer. Second, you'll benefit from a broad base of expertise. I have a wide range of experience, but, for example, I've never had hands-on experience with an evaporative light-scattering detector — hundreds of readers could offer better advice about that detector. Third, in the "LC Troubleshooting" column, I usually modify your questions slightly to make them apply to a wider audience, and I rarely mention equipment brand names. Specific questions about specific equipment and columns are fair game in *Chromatography Forum*.

HOW DOES IT WORK?

How can you take advantage of *Chromatography Forum*? First, you have to go there. Access is via either *LC•GC*'s or LC Resources Inc.'s (co-sponsor of *Chromatography Forum*) World Wide Web (WWW) pages. Just go to http://www.lcgcmag.com or http://www.LCResources.com and select the *Chromatography Forum* option (see Figure 1).

Inside the forum you'll have several options. You can submit a question by selecting the *Post Message* option and following the instructions. It's as simple as typing the question. The confidentiality of proprietary information is of utmost concern to most of the readership, so be sure to suitably launder your question so that it does not reveal information that you would not like to share with a competitor. For the most effective responses to your questions, include a brief description of your LC system, including brand names and model numbers. A chromatogram is worth a thousand words, so submission of chromatograms is encouraged. More on this topic later.

A second option after entering the forum is simply to scroll down and read the questions and answers submitted by other participants. You can view submitted questions and read the accompanying answers. You may find a dialog back and forth between two users, or suggestions and comments submitted by several users. I'm looking forward to reading this section regularly to pick up new ideas and learn about applications with which I have little experience.

A third way to participate in the forum is to supply answers to questions or additional comments to existing answers. This area is where you can share your expertise. We each have our specialty, and by sharing with others, we'll all become better chromatographers. To add to a current discussion or answer a question, select the *Post Follow-Up* option and type away at the end of a question or comment. Just remember the rules of common courtesy and don't say anything you'll regret later.

As always, we welcome your feedback to "LC Troubleshooting," which will continue as usual each month in *LC•GC*. Sending me e-mail at the address provided at the end of this column is an easy way to make suggestions or attempt to clarify topics covered in this column.

MINDING THE STORE

Although most of the discussions will rely upon the input from you and other readers, some administration is necessary. As joint sponsors of *Chromatography Forum*, *LC•GC* and LC Resources are responsible for the administrative details. *LC•GC* is providing the space and general arena for promoting the forum. LC Resources will provide the technical expertise. Our software group will make sure the bits and bytes work properly. Other

LC Resources technical staff members and I will monitor the technical content. Although we'll be providing some responses to questions, don't depend entirely on us or this venture will fizzle.

WHAT ABOUT CHROMATOGRAMS?

Because a chromatogram can provide so much information, we encourage you to submit a chromatogram or two along with your questions. The best way to submit a chromatogram is as an attachment. Because so many data systems are available, it will not be very effective to post the chromatogram in the native format of the data system. For example, our laboratory uses a particular brand of data system, but if I post a file 990513jd.03R, only readers with the same software will be able to view the chromatogram. Instead, you should save the file in the Analytical Instruments Association (AIA) or Analytical Data Interchange (ANDI) format, sometimes called CDF files for the default file extension for this format. This file format is a standard interchange format that allows a file to be saved from one data system and read by one of a different brand. The format option is available through the File Export menu or through conversion utilities in most data systems. To view the AIA file, you can read it on your data system via File Import or use the universal AIA viewer, Chrom Merge (LC Resources), which can be downloaded from the *Chromatography Forum* WWW page.

If you don't have access to AIA files, you can post your chromatogram in one of the standard graphics formats (TIF, BMP, or JPG) that can be read by most graphics programs. For maximum flexibility, however, we encourage the use of the AIA format.

RULES OF THE ROAD

Courtesy should be the byword of *Chromatography Forum.* Traditionally I have minimized references to specific equipment and columns in the "LC Troubleshooting" discussions, mainly because I've tried to make the material apply to many situations rather than to problems with specific vendors' instruments. This situation will not be the case for the *Chromatography Forum.* People can submit inquiries asking who has experience with Brand X check valves used with Brand Y pump, or what is the best 3-μm d_p column to use when trying to convert a method from a Brand Z 10-μm d_p C18 column. If you have a problem with a specific detector, share it as an objective question so others can help you, and not as a passionate insult about the performance of the manufacturer's employees on standardized intelligence tests. Remember, the reason *LC•GC* is free and of such high quality is because it is supported by the advertising of vendors that supply us with equipment, columns, and supplies. Without these products, we'd all be out of business.

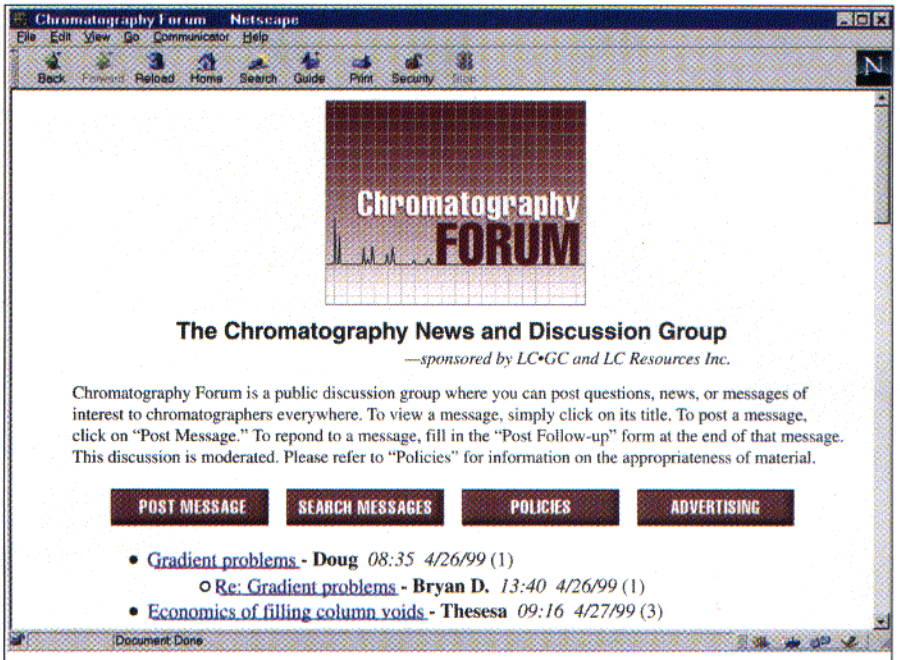

FIGURE 1: The *Chromatography Forum* home page. Readers can access the site via *LC·GC*'s WWW page (http://www.lcgcmag.com) or LC Resources' WWW page (http://www.LCResources.com).

SO HERE WE GO

Here are the first questions and answers you'll see on entering *Chromatography Forum*; feel free to add your suggestions and feedback on-line.

SOLVENT FILTRATION

Q: I hear mixed messages about filtering solvents. Some of my colleagues filter all solvents used in their LC systems, whereas others seem to filter the mobile phase only part of the time. Shouldn't you filter everything?

A: There is no harm in filtering every mobile phase you use, but you may not be gaining much by your efforts. Most workers filter solvents using a vacuum filter and a 0.5- or 0.45-μm porosity, 47-mm diameter membrane filter. At first glance, you would think that all solvents would benefit from filtration, but it is not necessarily the case. Look at the specifications for the high performance liquid chromatography (HPLC)–grade methanol or acetonitrile component of the mobile phase. Either on the bottle or in the accompanying literature, you'll find that these solvents were filtered through a 0.22-μm filter before bottling. This porosity filter usually is considered a biological filter that removes bacterial contaminants from the solvent in addition to any particulate matter. Most of us use laboratory-generated HPLC-grade water from a multistage water purification system. Usually the last step in water preparation is passage through a 0.22-μm filter. So solvents that nominally are clean at the 0.22-μm level won't gain anything by passage through a 0.45-μm filter. This conclusion assumes, of course, that your glassware is dust-free.

What about mobile-phase additives? In my experience, the HPLC-grade additives also are filtered at the 0.22-μm level during preparation. For this reason, the standard operating procedure in my laboratory does not require filtration of any mobile phase that contains only HPLC-grade liquids. Solids are a different story.

Consider, for example, the salts used to make buffers. The specifications may indicate that 0.22-μm filtration was part of the manufacturing process, but two other potential problems exist. First, buffer salts sometimes have solubility problems, especially with acetonitrile as an organic solvent. Second, although the buffers are chemically pure, they may contain bits of foreign matter. For example, if you look inside the lid of the buffer bottle, you may notice that the liner is scratched where it rubs on the rim of the bottle. Bits of PTFE from the liner can drop into the bottle and end up in the mobile phase. Trace amounts of particulate material can foul check valves, causing pressure and flow problems. For this reason, the same standard operating procedure that allows us to skip filtration for HPLC-grade liquids requires filtration whenever solids are added to the mobile phase.

What's the best way to filter solvents? I prefer the membrane filters mentioned above with 0.45-μm porosity. PTFE membranes are difficult to wet and filter very slowly. Prewetting PTFE with a few drops of pure organic solvent (methanol or acetonitrile) may help speed filtration. I prefer one of the alternate

membrane materials designed for filtering mobile phases. Consult the filter manufacturer's literature to determine solvent compatibility before you use a filter for the first time.

I have seen advertisements for solvent filters that incorporate reversed-phase particles or carbon in the filter membrane. These filters are purported to provide additional mobile-phase cleanup by adsorbing trace organic impurities. This claim sounds like a logical improvement to mobile-phase preparation, but I have no experience with such products. Please add your experiences with these materials to *Chromatography Forum*.

PROPORTIONING PROBLEMS

Q: I'm experiencing problems with my LC pump. When using low-pressure mixing, I often observe proportioning errors, exhibited as retention times that are too short and inconsistent. When I run premixed mobile phase in the A reservoir, bubbles sometimes appear in the pump. The same solvent pumped from the B reservoir works perfectly. The system uses low-pressure mixing.

A: I suspect that the inlet-line frit in the A reservoir is partially blocked. Low-pressure proportioning works by alternately opening the proportioning valves to allow solvent A or solvent B into the mixing chamber. For example, to make a 50:50 mixture of A and B, the A valve would be open half the time and the B valve would be open the other half. The valves' cycle time is usually less than 1 s. The pump maintains a steady flow rate. If the inlet frit in the A reservoir were partially blocked, it would restrict flow into the mixer. Thus, when the A valve opened, less than the desired amount of the A solvent would enter. This low flow also would create a slight vacuum. When the B valve opened, solvent would flow freely, and a little extra solvent would enter the mixer to make up the pressure deficit created by low flow from A. Two simple tests should confirm this hypothesis. The easiest test is to remove the inlet frit on inlet line A. If the problem goes away, the frit is faulty. Alternatively, exchange the A and B solvent reservoirs and make the appropriate adjustments to the system controller to obtain the same solvent mixtures. If the retention times now are longer because of a shortage of the stronger A solvent, the A reservoir is underdelivering. In either case, replace the frit. Don't try to clean the frit — this practice is only marginally beneficial and the cost of time spent would exceed the cost of a new frit. I like to use large-porosity 10-μm frits to minimize resistance to solvent flow.

The purpose of the frit is twofold. First, it serves as a sinker to hold the inlet tubing in the bottom of the reservoir. For this reason, I prefer stainless steel over PEEK, but it is a personal choice. The second function of the frit is to provide minimal filtering of the mobile phase. The inlet-tubing frit is not a substitute for normal mobile-phase filtration but rather eliminates dust particles that might enter the reservoir. Finally, I prefer one of the frits that is designed to draw solvent from the bottom of the frit. Sooner or later, you'll perform a method where you need every last drop of solvent in the reservoir, and this type of frit will help you out of a bind when you didn't prepare enough mobile phase.

CONCLUSION

I'm excited about the advent of the on-line *Chromatography Forum*. I've found that e-mail is a highly effective way to communicate, especially with others who live several time zones away from me. Having access to 24 h/day, 7 days/week troubleshooting support seems like a natural extension to this form of communication. I hope you'll join us in making this new troubleshooting venue successful.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of LC·GC's editorial advisory board. Direct correspondence about this column to "LC Troubleshooting," LC·GC, 859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.