LC Troubleshooting

Readers Respond

John W. Dolan

Two heads are better than one. Readers' contributions help to round out and clarify several past "LC Troubleshooting" articles.

One of the hazards of being the author of a regular column is that soon after publication, readers point out missing information or statements that may be misinterpreted. Fortunately, many of you send further information and examples that help clarify and better illustrate points made in previous articles. This month I've selected some of these responses as part of the "LC Troubleshooting" column. Because some of the information has been combined from several sources and other information has been restructured to illustrate a more general point, I've placed the credits at the end to prevent inaccurate attribution.

PITFALLS OF PUBLISHED METHODS

In June, this column pointed out some of the unexpected problems you might encounter while attempting to use a published method (1). The major problems that can occur are often related to the chemistry of the sample pretreatment or chromatography, incomplete description of the instrumentation, exclusion of vital information, and the quality of the work that went into method development. The column concluded with a caution that although many published methods are thorough and reproducible, it may be difficult to tell the good methods from the weak ones without putting a lot of work into revalidation.

One reader shared his experiences of transferring methods between laboratories within the same company. He pointed out that if it is possible to communicate with the method's authors, some of the missing information can be easily recovered. In addition, cases in which seemingly poor choices were made in the method procedure may turn out to be logical choices after a more detailed explanation is given.

He illustrated the point with an example of a method for separating a target compound from processed egg yolk using a solid-phase extraction (SPE) cartridge. The method used a phosphate buffer that was adjusted to pH 5.5, which is outside the buffering range for phosphate. He decided to fix the method before putting it into service. He tried both acetate and citrate buffers but found that the recovery was poor and the necessary enzyme activity was reduced. He then made a phone call to the author of the method and found that he had just repeated the same experiments the author had originally performed. The author explained that pH 5.5 was necessary to maintain enzyme activity and that only with phosphate present was the sample consistency suitable for efficient extraction and thus satisfactory recovery. Rather than spend many days redeveloping the method, this reader used a simple phone call to solve the problems he was having.

I agree wholeheartedly that if you find a method that almost works, it may be worthwhile discussing the problem with the original developers. This is usually easy if the method was developed within your company. As the previous example illustrates, you may find that a few simple changes will make the method work well. For example, a phone call might allow you to find the dwell volume of the original instrument so that you could adjust a gradient method to work on your liquid chromatograph. Or you might find that the authors discarded the method after trying to use it for routine analysis and have replaced it with a much better one, so you need not waste time with the original.

More likely, however, you will find that the original authors of a method cannot be located, or if they can be located, they work in another area or no longer use the method. I'll say it again: If the method really is a "good" method, it will contain a discussion of the logic behind the choices that were made and will provide the reader with all the information needed to satisfactorily reproduce it. With the egg yolk method above, the author could have added a few extra sentences to explain how the conditions were selected.

When publishing methods, it is also advisable to note the *unimportant* variables. I talk to many chemists using methods for routine analysis who feel like their hands are tied because they have to adhere to method conditions dictated by others. The development laboratory, for example, may design a method with specific requirements for pH, buffer concentration, mobile-phase organic concentration, and sample preparation conditions. No matter how stringent the conditions are, however, there is always some room for variation in these parameters. Listing these limits will give the final user a little flexibility in tweaking the method when slight changes in the column chemistry or other factors require it. For example, if you know that the pH needs to be 3.50 $\pm$ 0.02, yet the percentage of acetonitrile needs to be only $\pm$5%, you'll know you have to be very careful to maintain the pH but can vary the organic somewhat to adjust retention.

WHICH COLUMN IS BEST?

After publication of the article discussing problems caused by amine adsorption (2), I received several letters criticizing my choice of illustrations (see Letters to the Editor, page 632). In that article, I included a table that showed a ranking of several commercial columns in terms of their suitability for the analysis of basic compounds. Several readers made me aware of my poor choice of columns and the fact that the data were out of date (they were taken from a book published in 1988). I

could be defensive and point out that all the complaints came from representatives of companies whose columns were not included in the list. However, some good points were made.

First, it is important to realize that column technology development is a dynamic, not a static, process. Any manufacturer that plans to stay in business is constantly improving its columns. As a result, many manufacturers offer specially prepared columns to help overcome the tailing problems that are encountered when analyzing amine-containing samples. In addition, more and more columns are available for the analysis of specific sample types. These specialty columns may be available from a single manufacturer, or they may be widely available. New columns are introduced almost daily, so even if I included an up-to-date list in this article, it would be out of date before it was printed. Check with your favorite supplier to see if they have a column that will give you a better separation than the one you currently use.

Second, the direct column comparisons that I have seen are most often the result of a manufacturer's efforts to find out how its columns compare with others or to show that a particular column is better than other commercial columns. Comparative column testing is expensive and is seldom done except by self-interested manufacturers, so the data tend to be somewhat biased. Consider the problem of testing just C18 reversed-phase columns. *LC•GC*'s 1993 Marketplace Issue listed 117 suppliers of reversed-phase columns (3). If each supplier offered just two types of columns — a standard C18 and a base-deactivated C18 — it would require procuring ~250 columns to test. We should test at least two samples of each column type to give a fair test, so we're up to 500 columns. If we assume a cost of $300 per column, we're looking at $150,000 just for the columns. A cursory test of the standard toluene, benzene, and uracil test mix would take about 30 min per column if everything went smoothly — about a month in all. How long would thorough testing take? Certainly several times longer. As you can see, any comparison will necessarily be selective, incomplete, and out of date.

What can you do to be sure you are using the best column for your separation? First, resign yourself to the fact that you will never know if you have made the best choice. Select one or several suppliers in which you have confidence, look over their literature, and select a column or two to try. When you find a satisfactory column, use it. As a colleague of mine often points out, "Better is the enemy of good enough." Your goal should be a satisfactory separation of your sample, not the best possible one. Fortunately, even though large differences can exist between columns, many (or perhaps most) suppliers can sell you a column that will provide an adequate separation when the conditions are adjusted and the right mobile-phase components are used. So choose a column that looks like it will do the job and develop your method on it. You need to decide early in the process which column you will use. Once the method is developed, it is seldom fruitful to change from one column brand to another because it will require a significant investment in revalidating the method.

UNIQUE SPE APPLICATIONS

The topic of mobile-phase contamination comes up regularly. The ideal practice of using fresh HPLC-grade mobile-phase components for each run is impractical for some workers. HPLC-grade water can be purchased or prepared in the laboratory with a commercial water purification system. One reader, who has to rely on reverse osmosis and deionization for his water source, has found that he can successfully remove remaining organic contaminants by passing the water through a C18 SPE cartridge. He suggests replacing the cartridge at the first sign of spurious peaks in the chromatogram. I think it would be more effective to keep careful records of the use of the cartridge so that it could be replaced before contaminants break through. Replacement of the cartridge after every few liters of use would add an insignificant cost to the water production and ensure more consistent quality.

Solvent recycling has been covered previously in this column (4,5). A reader pointed out a commercial product (Anapharm Instruments Inc., Bound Brook, New Jersey) that uses a device similar to an SPE cartridge to provide some cleanup of recycled solvent. The device is mounted on the cap of the solvent reservoir. The mobile phase is recycled in the traditional manner by routing the waste stream back to the reservoir, but it first passes through this scavenger cartridge. As long as the capacity of the cartridge is not exceeded, it will retain hydrophobic contaminants. The effectiveness of this method depends on solvent strength — mobile phases with a large organic content would be expected to be less improved than highly aqueous mobile phases.

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WATER PURIFICATION TIP

A manufacturer of HPLC-grade water purification systems sent a reminder that 18-megohm water is not necessarily organic-free water. For low-UV detection, the water needs to be free of organic compounds, thus carbon cartridges are used to remove organics. For best results, the carbon cartridges should be mounted downstream from the ion-exchange cartridges because they will trap any organic materials released from the ion exchangers.

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