# LC Troubleshooting

## Fixing Problems on the Cheap

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*What happens when you can't correct a problem properly?*

The saying "time is money" holds true, but we've all been in situations in which time was the only commodity we possessed in abundance. Usually, I make recommendations in this column about how to fix a problem properly and avoid cutting corners when making a repair. This advice is sound; however, shortcuts and quick-and-dirty fixes sometimes can help you through a crisis.

I remember the advice of John Muir, whose book, *How to Keep Your Volkswagen Alive* (1), helped me get my car through several years of graduate school. He said that if you performed a quick fix instead of a thorough one, you needed to make — and keep — a promise to correct the problem properly as soon as possible — otherwise your vehicle would remember and get even by breaking down at some inopportune moment. Some days I think liquid chromatographic (LC) systems play the same game. So the tips in this month's "LC Troubleshooting" are primarily meant to get you through a crisis when the right parts are not available or something else prevents you from making the proper repair.

### VOID FILLING

Generally, it isn't fruitful to repair a column void. Voids occur because the column packing material has either dissolved or become crushed, so although filling the void may restore most of the column efficiency, it is unlikely that any changes in selectivity will be corrected. Void filling can be justified in emergencies or when you are using expensive specialty columns.

To fill a void, remove the column inlet fitting and frit and fill the void with a slurry of packing material that you have recovered from an old column. If the void is 1–2 mm deep, a single filling may be sufficient. For deeper voids, you will probably have to flush the column at a pressure of 2000–3000 psi to compress the material after each addition and then remove the frit and add more packing as necessary. After you have successfully filled the void, reverse the column and use it in the reverse direction. Vendrell and Aviles (2) reported that after void filling, column reversal provides better column performance and peak symmetry than running the columns in the normal direction.

### SOLVENT SUBSTITUTES

HPLC-grade solvents should be used whenever possible. Their added purity will reduce the number of chromatographic problems you are likely to encounter. If you run out of solvent or if your in-house water purification system fails, you can use less-pure solvents and still obtain satisfactory results. ACS-grade solvents will provide good results, especially with isocratic analyses. Distilled water purchased at your local supermarket will also yield satisfactory results. Gradient runs are more sensitive to solvent impurities than are isocratic ones, so these solvent substitutes are likely to produce noisier baselines and more spurious background peaks with gradient analyses than with isocratic runs. Why not use lower grade, less-expensive solvents routinely? The impurities from these solvents tend to build up on the column, increasing the background noise and possibly causing some changes in column selectivity. It is always best to use the highest quality reagents available.

If you find yourself beginning to run low on solvents for isocratic analyses and you are unsure that they will last until you get a new shipment, you can recycle the mobile phase. Just route the waste line from the detector back into the mobile-phase reservoir. You should add a stir bar to the reservoir and stir the mobile phase so that it is uniformly mixed. Even with dirty samples, the solvent must pass through the column several times before you will notice appreciable baseline problems with recycled solvent. Of course, this technique will not work with gradient or on-line mixing applications.

### FRIT CLEANING

Often, you can clean column or mobile-phase reservoir frits by sonicating them in acid. [See last November's "LC Troubleshooting" for instructions on changing frits (3).] First rinse the frit with water to remove any solvents, then place it in a beaker of diluted nitric acid (dilute one part acid with five parts water), and sonicate for 15 min. (Take the standard precautions for working with acids.) Then rinse and sonicate the frit in distilled water. Use fresh-water rinses until the pH of the rinse water remains constant. Generally, frit cleaning is not cost-effective. Frits are inexpensive, and you probably will spend more for the labor to clean one than it would cost to buy a new one. Furthermore, frit cleaning is not always successful, so don't try to recover used frits unless you are unable to replace them with new ones.

### TEMPERATURE CONTROL

For stable retention times, you should maintain the column at a constant temperature. The best method for maintaining temperature is using a column heater designed for this purpose. A homemade column heater can be constructed from a piece of glass or plastic tubing, a couple of rubber stoppers, and a circulating water bath. Choose tubing that is large enough to slip over the column. Bore a hole in each stopper for a connection to the water bath and a small hole or slit for the column inlet and outlet tubing. Water jackets can be rather messy, but if the water bath has good temperature control, you can obtain excellent results.

The next best thing to temperature control is temperature stabilization. Often, you can stabilize the column temperature by insulating the column so that it is less influenced by changes in laboratory temperature. My favorite way to insulate the column is to use a piece of foam water-pipe insulation. Just buy a piece of pipe wrap at your local hardware store, cut it to the desired length, and snap it around the column. I have seen analysts use a column box as an insulator in an emergency situation. Cut a slit in each end of the box for the connecting tubing, insert the column in the box, and close the lid. You can insulate transfer tubing from the injector to the column or from the column to detector with a piece of clear plastic tubing that has been split lengthwise and slipped over the line.

The LC system should be protected from unnecessary drafts. In a laboratory with otherwise acceptable temperature stability, a heating

or air conditioning vent blowing directly on an LC instrument can cause unnecessary temperature excursions. You may be able to block a vent or redirect the airflow by constructing a deflector from cardboard and duct tape.

## COLUMN FLUSHING

You should flush your columns regularly using the strong solvent from your mobile phase. Most workers like to flush 10 or more column volumes (about 25 mL for a 25 cm × 4.6 mm column) of strong solvent through the column at the end of each day to remove strongly retained materials. Occasional flushing with a stronger solvent such as methylene chloride can remove even more strongly retained materials.

Sometimes you can extend the life of a column by flushing it with a more-aggressive wash solution to remove contaminants. For example, if you suspect column contamination by metal ions, a chelator such as EDTA might add an extra dimension of column cleanup. A 4 M guanidine solution can flush out proteins.

If you are analyzing samples with ionizable components, changing the pH of the mobile phase can help remove strongly retained materials. Use your knowledge of the sample chemistry to help you decide which wash solution to use. Remember, if the column is already useless, you aren't going to hurt anything by flushing it with an experimental wash solution — as long as it does no damage the rest of the system.

You can accelerate flushing by reversing the column first — the strongly retained materials have to move only a few millimeters rather than several centimeters to leave the column. Always disconnect the outlet end when flushing the column with any solvent other than standard mobile-phase components to avoid washing contaminants into the detector flow cell.

Some workers have successfully extended their columns' lives by removing small amounts of contaminated packing material from the heads of columns and replacing it with clean packing. This procedure likely will cause other problems because of the disturbance of the packing material and may not be effective at removing contaminants, but it is sometimes worth a try as a last resort.

## TUBING SUBSTITUTIONS

I always advise chromatographers to minimize the volume of the tubing that connects the column to the injector and detector; often this means using 0.007- or 0.010-in. i.d. tubing with a total length of ≤25 cm to plumb the column. These guidelines minimize the band broadening caused by extracolumn effects. Sometimes longer runs of tubing are necessary, and larger diameter tubing is all that is available.

If you are using a standard 25 cm × 4.6 mm column packed with 5-μm $d_p$ particles, it is surprising how much tubing can be used without compromising most separations. If you do substitute larger diameter tubing for an emergency application, be sure to label it prominently with colored tape. Although the tubing may not cause problems in the present application, it could be a problem source if a smaller column were installed — and it is nice to be forewarned in these situations.

## WHAT IS THE IMPACT?

Whenever you decide to take one of the shortcuts mentioned above or to change the LC system in some other manner that could compromise its optimum performance, you must evaluate the impact on your analytical results. For many LC separations, the method is set up to provide excess resolution. If this is the case, you can make changes to the system that will significantly degrade the performance and still obtain satisfactory results. For other methods, even the slightest change will make the results useless.

As with John Muir's recommendations above, fix the system the correct way if it is possible. Use a quick-and-dirty fix only when you can't make the proper repair and must keep the system running. And remember to fix the problem in the correct manner as soon as possible — LC systems seem to remember when we don't treat them right.

**Fix the problem as soon as possible — LC systems remember when we don't treat them right.**

## REFERENCES


(1) John Muir, *How to Keep Your Volkswagen Alive* (John Muir Publications, Santa Fe, New Mexico, 1969).

(2) J. Vendrell and F.X. Aviles, *J. Chromatogr.* **356,** 420–422 (1986).

(3) J.W. Dolan, *LC•GC* **11**(11), 790–792 (1993).



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