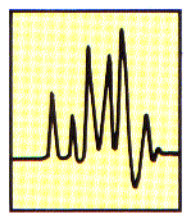
TROUBLESHOOTING

JOHN W. DOLAN



This month, in answer to several queries from readers, an error that crept into the March column will be discussed. In addition, some of the hazards involved in handling LC solvents and the use of lab records to help establish a preventive maintenance schedule for an LC system will be considered.

MORE ON STREAM SPLITTERS

The March "Troubleshooting" column (p. 234) contained an answer to a question on alternative detection that was only partly correct. The error is a common one and arises from a failure to define the difference between concentration-sensitive detectors and mass-sensitive detectors. The mistake is sufficiently common to warrant a review of the topic. The question concerned how a UV detector could be made less sensitive in order to allow its use in semipreparative separations. I recommended either detuning the detector by changing to a wavelength that is not optimal for the sample — a valid procedure — or using a stream splitter. Unfortunately, using a splitter will not solve the problem.

The reason that a splitter will not decrease the response in this case is that a UV detector is a *concentration-sensitive* detector. This means that it is the concentration of analyte in the detector cell that is measured (for example, 10^{-6} g/L) rather than the absolute amount (for example, 10^{-6} g). Therefore, because the *concentration* of analyte does not change when the stream is split (no dilution has taken place), the detector response for the split stream will be the same as it was for the original column effluent. The most common LC detectors — UV, refractive index, and fluorescence — are sensitive to changes in concentration, so sensitivity will not be affected by a stream splitter. Clearly, the way to reduce the response of such a detector is by diluting the analyte, but this is not always convenient.

The stream-splitter approach to reducing sensitivity would be effective if the detector were a *mass-sensitive* detector. Mass-sensitive detectors respond to the mass of analyte per unit time (for example, 10^{-6} g/sec) rather than to its concentration. Mass spectrometers are the most familiar mass-sensitive detectors used in liquid chromatography; gas chromatographers are familiar with two other types of mass-sensitive detectors: flame-ionization and electron-capture detectors. Any change that causes a reduction in the mass of sample entering the detector per unit time — such as using a splitter or diluting the sample — will cause a reduction in detector response.

The use of a stream splitter in liquid chromatography is appropriate when recovery of the sample is desired but a destructive detector is being used (for example, an electrochemical or a reaction detector) or when it is necessary to decrease the response of mass-sensitive detectors. The reader's request for a method to reduce UV detector response in the semipreparative mode can be best accommodated by changing the detection wavelength or by using a shorter-pathlength detector cell.

SOLVENT SAFETY

Q: I am new to the HPLC laboratory, and I find that the idea of working with all kinds of solvents makes me very uncomfortable. Can you tell me what the main hazards are and where I can go to get more information when I need it?

JWD: Chemical safety in the LC lab is really no different than in other chemical laboratories — just pay attention to the labels, use good laboratory practice and good common sense, and you should have no problems. Most of the solvents commonly used as LC mobile phases are relatively nontoxic if you are exposed to them briefly. This means that breathing a little of the vapor or spilling a lit-

tle solvent on your hands will not cause any major problems — just make certain to wash your hands well and be more careful next time. There are three main solvent hazards about which you should be concerned: frequent skin contact, frequent breathing of vapors, and fire. Each of these will be discussed briefly.

As mentioned above, skin contact with most LC solvents is not a major threat; as a matter of fact, it is almost unavoidable when working with an LC system. If a spill occurs, wash the affected area thoroughly with soap and water. Sometimes you may notice that your hands will feel very dry after a spill and cleanup. This is because the solvent has extracted some of the oils from your skin; hand cream will help the dryness. If you anticipate contact with solvents during mobile phase preparation or if you find you must clean up a spill, wear solvent-resistant gloves and a laboratory apron to protect yourself. And, of course, you should always wear safety glasses in the lab to protect your eyes.

Excessive breathing of solvent vapors should be avoided: solvents such as tetrahydrofuran, for instance, will give you a headache quite quickly. Here again, knowledge of the potential hazards and a little common sense will go a long way. The reference materials noted below give *threshold limit values* (tlv), which are the maximum allowable concentrations in air for an extended period (1,2). The tlv for methanol is 200 ppm, for hexane 100 ppm, and for tetrahydrofuran 200 ppm. To put these values into perspective, consider isopropanol, which we use as rubbing alcohol at home. The tlv for isopropanol is 400 ppm. Because we apply it without any concern for the potential hazards it may create, we can use it to judge the relative hazards of breathing other solvents. Usually, laboratory ventilation is adequate for formulating mobile phases on an open benchtop, but you may prefer to perform this task in a fume hood, especially when using an odoriferous solvent such as tetrahydrofuran. *Do not*, however, put your chromatograph in the hood: the drafts will cause detector and separation instabilities.

Fire is a real, but unlikely, hazard when dealing with LC solvents. Because most chromatographs are run in the reversed-phase mode, which uses mobile phases consisting of an organic solvent diluted with water, the mixture is made much less flammable. Nonetheless, you must be sure to keep ignition sources away from solvents because most of them will burn if ignited. This means that cigarette smoking, soldering irons, and open flames should not be allowed in the vicinity of the chromatograph. Cases also have been reported in which static electricity has started fires with tetrahydrofuran, so if static is a problem in your lab, take appropriate precautions.

There are several other sources of solvent-safety information. The first and most convenient source is the solvent bottle itself. Every solvent bottle label contains a summary of the chemical's hazards and recommendations for treatment when exposure occurs. Two other excellent sources are American Burdick & Jackson's solvent guide (1) and J.T. Baker's HPLC solvent reference manual (2). The American Burdick & Jackson guide lists the physical and chemical properties of each solvent as well as relevant safety data. Tables of miscibility, UV cutoff, and other useful information have made this guide a widely used resource. The J.T. Baker guide was published this year and contains much of the same information as the American Burdick & Jackson guide, but it also contains a complete set of the well-known Baker Saf-T Data Sheets, which detail hazards of and emergency treatment for each LC solvent. I recommend that every lab have a copy of both of these manuals for reference.

PREVENTIVE MAINTENANCE

Q: HPLC is used to perform the majority of my lab's analyses. I would like to set up a preventive maintenance schedule so that equipment failure becomes less of a problem. We have kept regular records on equipment usage and repair for the past year, but I'm not sure of the ways we can use these to our best advantage when setting up a maintenance schedule. What things should be included in this schedule?

JWD: As a result of the equipment records that you've kept, you already have placed a good deal of the work in establishing a preventive maintenance (PM) schedule behind you. First, you should look over these records to identify items that have failed several times during the year. The real key to designing a PM schedule that is a help rather than a hindrance is to keep the list as short as possible. To give you a sense of what you should look for, I'll briefly highlight potential problem areas of the system, from the reservoir to the detector.

There are two types of problems to look for in the pump and reservoir: contamination and

(Continued on page 507)

wear. After extended use, the frit or sinker on the column-inlet line will get blocked with dust or other contaminants. Check to see how often the frit has been replaced; in general, one to three months is considered a reasonable lifetime, and it is recommended that you replace this inexpensive part well ahead of the expected failure date. Check valves also become contaminated, but I've never seen any time-related pattern to their failure. Unnecessary changing or cleaning of check valves may cause, rather than solve, maintenance problems, and my recommendation is to leave check valves alone until they show signs of failure. Use of a frit on the solvent-inlet line will help prevent check-valve failure. Pump-seal failure is wear-related, and seal lifetime will vary with the type of mobile phase you use. Pump seals generally will last for three to six months, depending on the number of hours per day that the pump is used. Many labs find that seals should be replaced every three months because failure is common during the fourth month of use. Buffered mobile phases usually decrease seal lifetime because abrasive crystals of buffer often form on the piston and increase wear.

If a precolumn is used to precondition the mobile phase, it should be replaced regularly. The replacement period may vary from a few days to a month, depending on the pH of the mobile phase. When you begin using a precolumn, check the packing daily to see how much has dissolved by removing the inlet fitting and frit. When the packing reaches a predetermined limit (for example, 25% of it is gone) or when the pressure rises (from fine material blocking the outlet frit), replace or repack the precolumn. After you have determined the length of time that the precolumn will last, you will be able to add a replacement date for it to your PM schedule.

The lifetimes of in-line filters, guard columns, and analytical columns will vary with the type of sample analyzed. The main purpose of routine replacement of filters and guard columns is to protect the expensive analytical column. Both of these items are relatively inexpensive and should be replaced regularly, *before* they become less than effective in protecting the analytical column.

The analytical column should be tested regularly — perhaps once a week — either with the manufacturer's test sample, or, more conveniently, with a standard of the sample to be analyzed. When its performance deteriorates to a predetermined level, the column should be replaced. Use of regeneration procedures, frit replacement, back flushing, and other methods of extending column life may or may not be cost-effective, depending on the needs of a particular laboratory.

There are three common modes of detector failure. Lamp replacement is commonly placed on PM schedules. Consult your logbook to determine how long your lamps have been lasting. Some labs replace deuterium lamps every six months to avoid the time lost in troubleshooting detector problems when the lamps get old. Detector-cell cleaning can be placed on a PM schedule, but many workers place the cell in the same category as pump check valves: "If it's working, don't mess with it." This is because it is possible to rupture the seal, block a tube, or do other damage to the detector cell in the cleaning process. Electronic failures that occur in the detector, just as electronic failures that arise elsewhere in the LC system, are impossible to predict, so these should not be included in your PM schedule.

Finally, keep an adequate supply of spare parts on hand. Before a PM session approaches, be sure you have all of the necessary parts available so there will be minimal system downtime. Your list of spares should probably include an analytical column, a guard column, pump seals, check valves, frits, and a variety of fittings. Deuterium lamps have a shelf life of about six months, so, if you want a backup, don't keep two unless you really anticipate the need. Because most electronic failures are unpredictable, it is more economical to order parts as you need them and have them sent by an overnight courier delivery service than it is to keep spares on hand (unless your lab has a large number of instruments with interchangeable parts).

You won't be able to design a PM schedule that will catch all equipment failures. Be alert and aware of the normal operation of your chromatograph so that you can anticipate problems. This way you will have some flexibility when it comes time to repair the instrument, even if the failure was unanticipated. For example, when you see the pressure begin to rise (one indication of a worn-out guard column), check to be sure that you have a backup guard column in stock. If necessary, one can be ordered so that it will arrive before the problem results in too much system downtime.

REFERENCES

(1) *Burdick & Jackson Solvent Guide* (American Burdick & Jackson Laboratories, Inc., Muskegon, Michigan, 1982).

(2) *HPLC Solvent Reference Manual* (J.T. Baker Chemical Co., Phillipsburg, New Jersey, 1985).

John W. Dolan is president of LC Resources Inc., San Jose, California, and is a consulting editor for LC Magazine.