TROUBLESHOOTING

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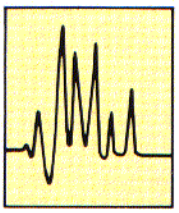

"Troubleshooting" is now adopting a question-and-answer format in order to address a greater number of problems associated with LC equipment than is possible through addressing a single topic each month. Readers are invited to submit their questions or troubleshooting tips: c/o The Editor, *LC Magazine*, P.O. Box 50, Springfield, OR 97477. Included in this month's article are two very common problems: bubbles in the system and temperature fluctuations. As the summer months approach, you should be especially conscientious about maintaining a stable lab temperature so that retention-time shifts do not invalidate your assay.

COLUMN SWITCHING

Q: I am analyzing for a trace organic in waste water on a C8 column and find that I can inject a 5-ml water sample and get a large enough peak to detect, but it creates two problems. First, there is some early eluting "garbage" that strongly absorbs in the UV, and sometimes the chromatogram doesn't return to baseline before my sample compound elutes at 3 min. Second, the injection of such a large volume of water makes the baseline quite unstable for several minutes. Is there some way to get around these problems without resorting to additional off-line sample pretreatment?

JWD: Your assay seems ideally suited for a column-switching application. In your case, this could be done manually or in an automated mode. One possible configuration is shown in Figure 1. The technique involves replacing the sample loop on the injection valve with a guard column, which is then referred to as a *loop column*. (Pack the loop column with guard-column packing, such as 30-μm pellicular C18 packing.) Now inject the 5-ml sample onto the loop column in the *load* position. The sample compound should stick to the column just as it does in the analytical column, but in this case you can wash the column with pure water to flush out as much of the unretained UV-absorbing material as possible. Next, turn the valve to the *inject* position and flush the sample into the analytical column with mobile phase. This procedure, I hope, will minimize both of your problems. First, you have flushed the interfering peaks to waste. and second, you have greatly reduced the amount of water that is injected onto the analytical column. A paper by A. Nazareth et al. and references cited therein will give you some specific ideas about how to develop a loop column cleanup method (1).

BUBBLES IN THE DETECTOR CELL

Q: I can't seem to keep air bubbles out of my UV detector cell. I vacuum-degas the mobile phase (methanol/phosphate buffer) each day before I start the LC system. The bubble problem is intermittent: it shows up as spikes in about one chromatogram out of five. If I briefly increase the flow rate, the bubble gets forced out and the system runs fine for another few injections. What can be done to prevent this from happening?

JWD: There are four areas that can contribute to air bubbles in the detector cell. One or more of these could be the source of the problem you are experiencing. The most common problem is too much dissolved gas in the mobile phase. Degassed mobile phases are much less likely to cause bubble problems anywhere in the LC system, and some systems will not operate without degassed mobile phase. Vacuum degassing is usually sufficient, but you may want to try helium sparging just to make sure. Make a helium sparger by immersing in the mobile-phase reservoir an 1/8-in. o.d. Teflon line with a solvent inlet frit on the end. Connect the other end to the regulator on a helium tank and adjust the valve so that a gently bubbling stream of helium is passing through the mobile phase. After about five minutes, the flow can be reduced to just a few bubbles to maintain the degassed mobile phase.

A second source of air is a leak in a connecting fitting. Sometimes flow conditions are such that air is drawn into the system through a loose fitting, thus creating an air leak rather than a liquid leak. To check for air leaks, carefully tighten all fittings. Fittings that connect plastic tubing are particularly prone to this problem because the seal achieved with plastic tubing is often not as good as that created by compression fittings attached to stainless-steel tubing. If you are using a well-degassed mobile phase, an air leak on the low-pressure side of the pump might not be bad enough to cause air bubbles in the pump, but it could create a problem in the detector. The air leak could exist anywhere before the detector.

A third cause of detector bubbles is insufficient back pressure on the detector cell. Dissolved air may not cause a problem in the pump or column; but when the pressure suddenly drops at the end of the column, the vapor pressure of the dissolved air is high enough to cause bubbles to form in the column effluent. Once again, degassing the mo-

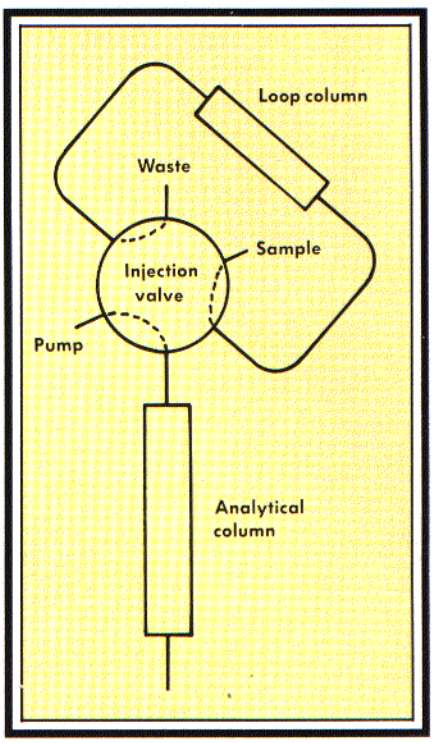

FIGURE 1: Column switching using a loop column.

bile phase helps minimize this problem. A restrictor after the detector cell may also help. Such a restrictor maintains a sufficiently high pressure to keep air in solution until after the detector. Be very careful when experimenting with restrictors after the detector because many detector cells are not built to withstand more than about 100 psi of back pressure. Some detector cells, such as electrochemical cells, may leak with any additional back pressure. Check the detector specifications in the operator's manual or check with the manufacturer if you have any questions about the pressure resistance of the cell. You can use a commercial back-pressure regulator, such as the one manufactured by Rheodyne (Cotati, California), or you can make one yourself. A few feet of 0.010-in. i.d. tubing will usually provide enough back pressure to prevent bubbles from forming in the detector. A piece of narrow-bore Teflon tubing can double as a waste line and a restrictor. You also can carefully crimp a Teflon waste line with a pair of pliers to increase back pressure.

A final cause of air-bubble problems in the detector is a dirty detector cell. Microscopic particles of dirt or column packing that get lodged in the detector cell may not cause any problems as particulates, but can act as nucleation sites for bubble formation. Dissolved air that otherwise would flow smoothly through the system may collect at these points and gradually grow into a bubble. The solution to this problem, of course, is to clean the detector cell. Flush the mobile phase from the cell with water, and then carefully draw 50% nitric acid through it and let stand for a few minutes — or follow the manufacturer's recommended cell cleaning procedures. Rinse thoroughly with water and reinstall the cell.

You can generally eliminate detector bubble problems if you use degassed mobile phases, occasionally clean the detector cell, and maintain a slight back pressure on the cell.

If you have bubbles in the detector and have trouble getting them out, here are a few tips:

- degas the mobile phase (this will often allow the bubbles to redissolve)
- increase the flow rate and/or the back pressure across the cell (the pressure increase forces air back into solution)
- change to a low-surface-tension mobile phase (pure methanol will often flush bubbles out quickly)
- when connecting a new column, flush several column volumes of mobile phase through it to remove any residual air before connecting it to the detector.

RETENTION-TIME DRIFT

Q: I have a problem with retention-time drift in my LC system. When I start up the system at 8 AM, the retention times slowly decrease until they stabilize at about 9:30 or 10:00 AM. In the afternoon, I often see the reverse drift at about 4:00 or 4:30 PM. I don't think the problem is in the mobile phase because it occurs even if I leave the mobile phase flowing slowly overnight.

JWD: I suspect you have a lab temperature problem. You might check the building heating cycle to confirm this. When the heat is turned on in the morning the lab temperature rises, and in the late afternoon it falls when the thermostat is turned down for the night. Increasing the column temperature will give lower retention times. Some labs experience the reverse problem in the summer when the lab is cooled by air conditioning during the day but heats up at night when it is turned down or off. I would suggest thermostating the column to solve this problem. If your liquid chromatograph does not have temperature-control capability, an auxiliary column heater can be purchased from most LC supply vendors. Set the column temperature slightly above room temperature (for example, 30 °C) for the best stability. Be sure to insulate the connecting tubing as well by wrapping it with foam strips or by enclosing it in a piece of Tygon tubing.

COLUMN-TO-COLUMN REPRODUCIBILITY

Q: We run an assay for a basic and a neutral compound on a C18 column but have a problem achieving the same separation when the column has to be replaced. Sometimes the peaks are so close together with a new column that we can't use it at all. What can we do to avoid redeveloping the assay conditions each time we change columns?

JWD: The problem you have is not uncommon when two different types of compounds are assayed in one procedure. In your case, the neutral compound may be retained by hydrophobic retention and the basic one by a silanophilic interaction. This means that any change in surface coverage of the bonded phase will change the selectivity, and, thus, peak spacing will change. There are three ways to minimize this problem. First, try to make the assay conditions less dependent on the silanol interactions. This is done by adding a silanol-suppressing agent such as triethylamine to the mobile phase. Many chromatographers add triethylamine at the 5 m*M* level to any mobile phase whenever basic compounds are to be analyzed. In this way, the mobile phase will help to minimize differences in bonded-phase coverage from one column to the next.

A second way to minimize the problem is to purchase columns that are as similar as possible. This means that not only the manufacturer and the phase type should be the same, but the batch number should be the same as well. Many manufacturers will send you columns from the same batch if you request them in advance. Purchasing a year's supply of columns in a single order can be cost-effective if it means that no time is wasted redeveloping the assay each time a column is changed.

Finally, you may need to try another LC method to eliminate your problem. In your case, ion-pair chromatography might give much better column-to-column reproducibility for your assay than a reversed-phase method.

CHANGING PEAK HEIGHT

Q: When I start the chromatograph in the morning, one band in four of my standard gives a peak that is much too small. It is a little larger in the second injection, and by the fourth or fifth injection it is the proper height and is fine for the rest of the day. What could be causing this? Is there any way to get a faster start? (Four or five 15-minute assays waste a lot of time.)

JWD: The phenomenon of having to "load" the column with a compound before the assay is stable is fairly common. It appears that there is a specific interaction or an active site that needs to be saturated before you get as much compound out of the column as you put into it. Often this kind of problem is more trouble than it is worth to eliminate, so you should try to minimize the trouble it causes. Two approaches can be taken. First, you could combine your first five injections into one by injecting a "slug" of the problem compound. In this case, inject the compound at ten times the normal level and see if you can load the column all at once. An alternative to this approach is to put a trace amount of the compound in the mobile phase. This may or may not be satisfactory, depending upon the purpose of the assay and the chemical characteristics of the compound.

REFERENCE

(1) A. Nazareth, L. Jaramillo, B.L. Karger, R.W. Giese, and L.R. Snyder, *J. Chromatogr., Biomed. Appl.* **309**, 357–368 (1984).

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