LC TROUBLESHOOTING

More on Solvents

JOHN W. DOLAN

This month's column is based partly on information contributed by a solvent manufacturer in response to an earlier "LC Troubleshooting" article. We are reminded that minor ingredients in solvents — like the minor ingredients in packaged foods — can be important. In addition, a reader's question about retention drift with unattended runs is addressed.

TABLE I: CHLORIDE CONTENT OF SELECTED SOLVENTS*

Brand*	Grade	Lot	Date of Manufacture	Chloride (ppb)
Fisher	pesticide	860785	winter 1986	781
J.T. Baker	HPLC	545122	fall 1985	303
Burdick & Jackson	distilled in glass	AO303	spring 1986	5
Burdick & Jackson	distilled in glass	AJ189	winter 1983	6

*solvents listed in order of increasing analyte stability

SOLVENT ADDITIVES

Most users assume that the solvents used for LC mobile phases are "pure," but as was discussed in previous installments of "LC Troubleshooting" (1,2), stabilizers are often added to HPLC-grade solvents. For most applications, these stabilizers are innocuous, but problems can arise in certain cases. In one case study reported earlier (2), the users observed column degradation because of residual HCl in the methylene chloride component of the mobile phase. When the methylene chloride was stabilized with cyclohexene, low levels of HCl were observed, whereas higher levels were found when cyclohexane was used as a stabilizer. Table I shows the chloride content of the solvents tested. The authors concluded that "if an application for methylene chloride arises in which the free HCl must be low, only products stabilized with cyclohexene should be considered."

A manufacturer of solvents responded to that particular case study with further information on solvent stability (3). Although cyclohexene is a more effective stabilizer than cyclohexane for methylene chloride, there are potential problems associated with cyclohexene as well. The most serious of these problems arises when cyclohexene is used for certain gas chromatography (GC) assays. When chlorinated water samples are extracted with methylene chloride that is stabilized with cyclohexene, dichlorocyclohexane is produced as an artifact. It is easily detected with modern GC equipment and may complicate the interpretation of environmental test data. The problem can be overcome by using amylene-stabilized methylene chloride. As was seen earlier with chloroform (1), however, amylene is not without its own potential problems.

So, what is the bottom line on solvent stabilizers? Some solvents (for example, tetrahydrofuran) can be ordered with or without additives. Other HPLC-grade solvents may not be available without stabilizers. Two practices can help to alleviate solvent additive problems. First, consider the chemistry involved when you select a solvent for sample or mobile phase preparation. If chemical interactions might cause problems, switch to a solvent that has another stabilizer (if possible). Alternatively, test for the consequences of the interactions by spiking samples with high levels of stabilizer to determine the outcome. Second, be explicit about the solvents to be used in a given method. Once a method has been validated, include stabilizer information when designating the solvents. Using solvent with the same part number from one manufacturer should be sufficient, but if it is necessary to switch to another manufacturer, be sure to specify the stabilizer as well as the solvent.

The age of the solvents should also be considered when solvent-related problems arise. It was pointed out (3) that solvents should be used within one year of purchase or discarded. This practice decreases the potential for problems caused by product instability. The original work reported in Table I was performed in the fall of 1986. At that time, one lot of solvent (AJ189) was three and a half years old, and another lot (545122) was at the end of its useful life. (In this case, these solvents apparently had not degraded, but in practice they should have been discarded.) The other two lots were still fresh. To help reduce problems, rotate your solvent stock and discard solvents after you have had them for a year. If you are not sure of a solvent's age, call the manufacturer to get the lot number decoded.

RETENTION-TIME DRIFT

Q: Our laboratory relies heavily on unattended overnight runs to process our large sample load. Retention-time drift often is a big problem because once a peak drifts out of the retention "window," the data system no longer considers it present in the sample. As a result, we have to manually recalculate many more runs than should be necessary. Do you have any suggestions on how we can solve this problem?

JWD: Retention-time drift in liquid chromatography (LC) can be caused by a number of system changes. As can be seen in Table II, the importance of some variables may change when the LC mode is changed. There are four possible causes of your problem — temperature fluctuations, variations in mobile phase composition, column aging, and flow-rate changes. These problems are listed and considered in the order that they are likely to occur in your case.

Temperature fluctuations are common in overnight LC runs. Many labs are climate-controlled only during the day. As can be seen in Table II, a 1–2% change in retention is possible for each 1 °C change in column temperature. The easiest way to overcome this problem is to make sure that the column is maintained at a constant temperature. Often, wrapping the column with insulation or placing it in a draft-free compartment is sufficient, but a column oven may be necessary. For best results, operate the oven slightly above room temperature (for example, at 35 °C). Be sure to insulate the connecting tubing between the column and detector to minimize baseline drift. When large temperature fluctuations occur, it may be necessary to heat the solvent reservoir(s) to prevent temperature swings in the column. If you heat the solvent, however, be aware of potential evaporation problems (see below). You can check for temperature problems by running a recording thermometer for several nights to determine

TABLE II: EFFECT OF CHANGE IN SEPARATION CONDITIONS ON SAMPLE RETENTION

Variable	Method	Change in Variable	Average Change in t_R
Flow rate	all methods	+1%	−1%
Temperature	all methods except SEC	+1 °C	−(1–2%)
Mobile phase composition: Organic solvent	reversed-phase*	+1 vol %	−(5–10%)
pH	reversed-phase	+0.01 unit	±(0–1%)
Strong solvent	normal-phase	+1%	−(1–2%)
Buffer, organic solvent	size-exclusion	+1%	0%

*including ion-pair LC

whether temperature fluctuations correlate with retention changes.

Variations in mobile phase composition can dramatically change retention, as is shown in Table II. There are three reasons for this. First, when premixed mobile phases are allowed to stand before use, the more volatile component(s) of the mobile phase can evaporate, thus changing the retention characteristics. Generally, evaporation is not of much concern with reversed-phase methods, unless the reservoir is heated during use. Normal-phase methods, however, can be more sensitive to changes in a minor mobile phase component. This is because minor components can deactivate the column, either intentionally or unintentionally. For example, many workers add traces of water or alcohol to control column activity with silica columns. Similarly, the uptake of atmospheric water by dry solvents, such as hexane, can cause retention times to change significantly. You can usually eliminate evaporation problems by capping the reservoir.

A second mobile phase problem could occur if improperly recycled solvent is used. Recycling is a valid and widely practiced method of conserving solvent for isocratic methods. If you choose to recycle your solvents, however, be sure that the reservoir contains a minimum of about 3 L of mobile phase and that it is constantly stirred, so that sample contaminants or other small changes in the column effluent are diluted out before their next pass through the system. Also, be sure to cap the reservoir to minimize evaporative losses. If you suspect that solvent recycling is your problem, run your samples for a few nights without recycling the mobile phase to see if the problem disappears.

A third mobile phase problem could be caused by malfunctions in the hardware when on-line mixing is used. This is unlikely in your case, because you would probably see the same problems during daytime runs.

Column aging can result in retention-time drift as the packing surface changes over time. Again, this is not likely to be your problem because you would also see it with daytime runs. If you suspect column aging, however, replace your column with a new one to determine for sure that that is the problem. If column lifetimes are unacceptably short, consider improving sample cleanup, using a guard column, and/or using a precolumn (saturator column).

Finally, changes in flow rate sometimes cause retention drift. Because flow rate changes are also related to hardware, you would probably observe them in the daytime as well. Again, it is unlikely that they are causing your problem.

In addition to controlling the variables mentioned above, you might be able to increase the retention time window that the data system uses for peak identity and, thus, allow more retention drift to occur before the peak moves outside the window. This solution may or may not be useful, depending on the complexity of the chromatogram. And finally, you could change your standardization technique. Running standards more often — for example, once every 5 samples instead of once every 10 — may allow the data system to keep up with the drift. Internal standardization may also help, especially if relative, rather than absolute, retention times are used.

REFERENCES

(1) J.W. Dolan, *LC•GC* **4,** 894–897 (1986).
(2) J.W. Dolan, *LC•GC* **5,** 874–878 (1987).
(3) J.T. Przybytek, American Burdick & Jackson, personal communication.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc., of Lafayette, California, and is a member of the Editorial Advisory Board of LC•GC.

Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future columns. Write to The Editor, *LC•GC*, P.O. Box 10460, Eugene, OR 97440.