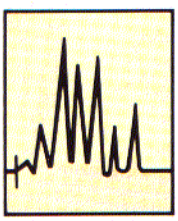
LC TROUBLESHOOTING

Mobile Phase Problems

JOHN W. DOLAN



This month, two mobile phase problems are discussed, one in a contribution from a group of readers and the other in a question submitted by another reader. In addition, two people have come up with possible solutions to a problem with peak-area ratios that was discussed in an earlier "LC Troubleshooting" column.

ACIDITY IN CHLORINATED SOLVENTS

Note: A quote from the contributors' cover letter is a good introduction to this contribution: "I believe that too many of us take solvent purity for granted just because the label tells us that it is good. What may be good for one purpose may not be good for another, and in some cases a less expensive grade may be better for a given application." If you have found specific solvent-related problems, please let me know. Write to *LC•GC*, P.O. Box 10460, Eugene, OR 97440. (The following contribution was submitted by W.A. Nichols, A.C. Mayer, and M. Alexander, Central Analytical Department, Olin Research Center, Cheshire, Connecticut.)

A minor impurity in a solvent that is used to elute compounds from an LC system can create perplexing problems if the chemist assumes that the mobile phase is "clean." That is also true of extraction methods in which the solvent is used in relatively large quantities and then is concentrated in order to extract trace components from a sample matrix.

Potential problems with "pure" solvents were discussed in an earlier installment of "LC Troubleshooting" (1). In that case, the manufacturer added a small amount of ethanol to chloroform as an inhibitor, but that modified the polarity of the solvent enough to make it an effective eluent for a particular separation. When the user switched to another grade of chloroform, in which a hydrocarbon was used as an inhibitor, the mobile phase would no longer work. It was found that a polar component (ethanol) had to be added to produce a mobile phase that could be used.

We recently had an experience with methylene chloride in which certain batches rapidly destroyed LC columns for a particular application. The original observation that prompted this study was made during the application of an established LC method using a silica column and methylene chloride modified with 2-propanol as the eluent. Successive injections of a standard material (a metal complex) gave smaller peaks over a fairly short amount of time. Different lots of methylene chloride from various manufacturers were tried; the best lots for this application were those from Burdick & Jackson (Muskegon, Michigan).

TABLE I: pH AND CHLORIDE CONTENT OF SELECTED SOLVENTS

Brand*	Grade	Lot Number	ppb Chloride	pH
Fisher	pesticide	860785	781	6.32
J.T. Baker	HPLC	545122	303	6.91
Burdick & Jackson	distilled in glass	AO 303	5	7.22
Burdick & Jackson	distilled in glass	AJ 189	6	7.19

*solvents listed in order of increasing analyte stability

The following conditions were used: A 5060 liquid chromatograph (Varian Instruments Division, Walnut Creek, California) was fitted with a UV detector (also from Varian) operated at 235 nm. A 25 cm × 4.6 mm Zorbax Sil column with 5-μm particles (Du Pont Co., Wilmington, Delaware) was used. The mobile phase was methylene chloride with 0.25 vol % 2-propanol. A 20-μL sample of a 75-ppm solution of a proprietary metal complex was injected.

The only apparent difference between experiments was the grade of the solvent used, although all of the solvents allegedly were stabilized to prevent the formation of free HCl. We extracted several different lots of methylene chloride from various vendors with water and, using ion chromatography, examined them for extractable chloride. We also determined the pH of the extracts and found that there was a correlation between the amount of acidity and the stability of the chromatographic system toward the compounds being injected.

The results, reported in Table I, showed significant differences in the levels of free HCl. In an effort to explain these results and verify the inhibitor content that was specified on the product labels, we examined the solvents by high resolution gas chromatography using a Finnigan Mat (San Jose, California) ion trap detector.

The methylene chloride samples examined were two lots of Burdick & Jackson distilled-in-glass grade, Fisher (Pittsburgh, Pennsylvania) pesticide grade, and J.T. Baker (Phillipsburg, New Jersey) HPLC grade. The Burdick & Jackson label stated that *cyclohexene* was used to prevent formation of free HCl, whereas the Fisher and J.T. Baker labels indicated that *cyclohexane* was the stabilizer. The ion trap experiments confirmed the presence of the respective inhibitors and verified that "cyclohexane" was not a typographical error. As indicated by the data in Table I, only cyclohexene appears to be effective. Thus, if an application for methylene chloride arises in which the free HCl must be low, only products stabilized with cyclohexene should be considered.

SOMETHING'S GROWING

Q: I am separating ions (F^-, CO_3^{2-}, Br^-, Cl^-, etc.) on a 3-μm C18 column using a mobile phase of tetrabutylammonium hydroxide (0.4%, w/w) adjusted to pH 8.0 with potassium hydrogen phthalate. Detection is at 280 nm. I've changed the mobile phase daily because I've suspected bacterial growth. Still, the column becomes blocked in about two weeks. Is there something that I can add to the mobile phase to stop the column from deteriorating?

JWD: Two problems could cause column blockage: microbial growth in the mobile phase or chemical degradation of the column. I think that you were right to suspect microbial growth — your mobile phase is an ideal home for "bugs," offering both a carbon and a nitrogen source. Your first step, changing the mobile phase daily, is a start, but is obviously not enough. Be sure that you flush the system completely at the end of each day so that no buffer residues remain in the reservoir, pump, tubing, or column. You may inadvertently be leaving enough buffer in the system to allow for microbial growth when the instrument is not in use; the bugs might grow at night and collect on the column frit in the

morning. Flushing the system will also remove buffer salts, thus prolonging pump-seal life. If you flush the system with an aqueous organic mobile phase (for example, 50% methanol–water), you will not only kill most of the bugs, but you will also remove any organic residues that may have built up on your column during the day. If you flush with an organic mobile phase, be sure to flush out the buffer with water first so that you don't precipitate buffer in the system when organic is added.

If you work from a stock solution of buffer, be sure that it is prepared with sterilized water, made at a high enough concentration to prevent microbial growth, and stored in a refrigerator until use. After you mix up a batch of diluted mobile phase, filter it through a 0.2-μm (bacterial) membrane filter before use. Use a sterile reservoir, and keep it covered during use. These steps should reduce your problems by minimizing contamination and shortening the time that the environment is favorable to growth. Some workers have found that adding sodium azide ($\sim$0.1% by volume) or methanol (1–2% by volume) will retard microbial growth sufficiently to eliminate problems as long as reasonable care is taken when the mobile phase is prepared. (First, check to see if either of these additives causes chromatographic problems for your particular assay.) Using a guard column and/or in-line filter may also extend column life.

The pH of your mobile phase could be another source of problems because silica-based columns are not very stable above pH $\sim$7.5. If your column is deteriorating because of this, you may observe changes in retention over time. Some workers have found that column life is extended if a precolumn (saturator column) is used upstream from the injection valve. The precolumn, often an old analytical column, somehow conditions the mobile phase so that the analytical column is not degraded as quickly. A better choice would be to switch to a polymer-based reversed-phase column or an appropriate polymer-based ion-exchange column. For mobile-phase selection with these common ions, a good place to start is with the applications notes from one of the column manufacturers.

PEAK-RATIO VARIABILITY

In an earlier "LC Troubleshooting" column (2), a reader reported a problem with inter-laboratory peak-height–ratio reproducibility, even though the same method and same model of detector were used in each case. Several suggestions were given on how to isolate the problem further, but the problem was left open for reader response.

One reader (3) suggested that the problem may have nothing to do with the LC system or the method, but might be an integrator problem. If different models of integrators or computers were used, differences in the integration algorithms could be the source of the problem. If the same model of integrator or computer is used in each lab, the settings for integration parameters could cause the described problem. For example, if the peak detection is set to be less sensitive (by selecting a higher noise-rejection or threshold setting), the areas of all peaks in the chromatogram will be reduced. The area of the smaller peaks will be affected the most, however, because a constant value will be subtracted from the peak height. Thus, the peak-area ratio will vary, especially if one peak is large relative to the other. This hypothesis could be checked by assaying the same mixture of standard compounds at all three lab locations and then comparing the integration results for peaks of various sizes.

Another reader (4) suggested that inter-laboratory flow rate variations could account for changes in peak-area ratio variability, especially if columns with different dead volumes were used. This might not seem valid at first, if you think about the effect of flow rate on a separation. After all, we know that peak area is not affected by flow rate — when flow rate is changed, peak heights in the chromatogram change, but peak areas stay constant. For example, increasing the flow rate causes compounds to elute earlier. Earlier elution results in narrower peaks, and because area is conserved, the peaks are taller. Under normal circumstances, flow-rate variations would have no effect on the peak-area ratio, but they could if the integration parameters were different, as suggested above.

REFERENCES

(1) J.W. Dolan, *LC•GC* **4**, 894–897 (1986).
(2) J.W. Dolan, *LC•GC* **5**, 554–556 (1987).
(3) J.B. Li, personal communication.
(4) G.I. Rudd, personal communication.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc., of Lafayette, California, and is a consulting editor for LC•GC.

Bulletins

HPLC '88: Call for papers. The 12th International Symposium on Column Liquid Chromatography will be held in Washington, D.C., June 19–24, 1988. Theory, instrumentation, applications, and new developments in the field will be presented. Among the topics to be covered in lecture sessions or discussion groups are biotechnology, detection, micro-LC, and methods development. Abstracts of approximately 150 words for posters and papers will be accepted until October 30, 1987, by the Symposium Manager, Barr Enterprises, P.O. Box 279, Walkersville, MD 21793.

Capillary meeting: Call for papers. Abstracts will be accepted until December 1, 1987, for the Ninth International Symposium on Capillary Chromatography, to be held May 16–19, 1988, in Monterey, California. The meeting will address micro separation techniques, including capillary GC, capillary LC, and capillary SFC. Send 300-word abstracts to P. Sandra, PhD, R.I.C., P.O. Box 91, B-8610 Wevelgem, Belgium.