LC Troubleshooting

Amine Adsorption — A Case Study

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Secondary retention effects can create puzzling recovery plots, especially for the first samples of the day.

The following case study began with a troubleshooting inquiry I received from a reader last summer (1). Following several exchanges of information, the reader finally solved the problem and subsequently presented the results at an international scientific meeting (2). The solution provided satisfactory results, but, as is often the case with solutions to troubleshooting problems in liquid chromatography (LC), the limited experimental evidence available prevented us from verifying all of the assumptions on which the solution was based. The result is that the problem was solved, but a certain amount of personal dissatisfaction remained because all the loose ends were not neatly tied up.

BACKGROUND

Propellants, such as gunpowder, must be stabilized to ensure safe storage and shipment. One common stabilizer is diphenylamine (DPA). LC, the accepted method for analyzing the amount of stabilizer in propellants, is used to determine the amount of residual stabilizer and its various nitro derivatives and thus to measure the level of decomposition of nitrocellulose in the propellant.

The conditions for analysis typically involve a C18 column with an isocratic acetonitrile–water mobile phase and UV detection at 220 nm. Depending on the column brand, the mobile phase is in the range of 40:60 to 60:40 acetonitrile–water. An internal standard of 4-nitroaniline is used. Analysis using normal-phase LC is also possible, but less convenient (and in the present study, DPA disappeared completely), so reversed-phase techniques are preferred.

PROBLEM ONSET

The column had been in use for about a year when the problem first surfaced. The apparent response for DPA dropped off for the first few injections of the day, then stabilized after about five runs (curve A in Figure 1). The column was flushed with methanol and the series of runs was repeated, resulting in curve B in Figure 1. Whenever I see a change in response over time, one of the first problems that I suspect is column loading. Column loading problems occur when some of the sample adsorbs onto the column, saturating active sites. Generally, this phenomenon is pronounced for the first few injections, and then the curve levels off as the active sites become saturated. However, the curves in Figure 1 show the opposite effect — I would have expected lower recovery for the first few injections and increasing recovery as the column stabilized.

One possible explanation for the behavior of curve A in Figure 1 is that the saturation process was related to the internal standard, not the DPA. If the internal standard were underrecovered for the first few injections because it was getting adsorbed onto the column, the result would be an apparent overrecovery of the analyte. To confirm this diagnosis, we needed to examine a few chromatograms and a calibration curve for the internal standard. Figure 2 shows the chromatograms for the first and fifth injections of curve A in Figure 1. It is apparent that the DPA peak (peak 4) drops off, whereas the internal standard (peak 1) remains constant. (Peaks 2, 3, and 5 in Figure 2 are other amine sample components.) Many data systems normalize the peak height to the tallest peak, however, so we needed to make sure the internal standard was really constant. The tabulated results confirmed the first impressions given by the peak shapes in Figure 2. The area of the internal standard was 163,400 counts in Figure 2a and 164,300 counts in Figure 2b, whereas the area of the DPA peak dropped from 124,900 counts to 77,580. These data indicate that the problem was associated with DPA, not the internal standard. Further confirmation was gained from examining the standard curve for the internal standard. It was lin-

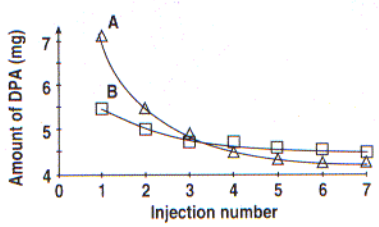

FIGURE 1: Amount of DPA detected vs. number of injections. Curves: A = original column before regeneration, B = original column after methanol wash. (Adapted with permission from reference 2.)

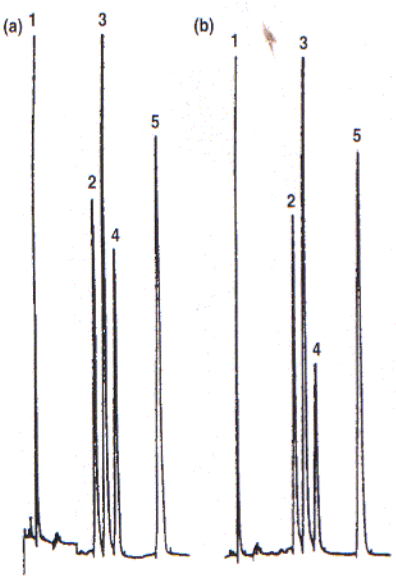

FIGURE 2: Chromatograms used to generate data shown in curve A of Figure 1, including those resulting from (a) the first injection and (b) the fifth injection. Peaks: 1 = 4-nitroaniline (internal standard), 2 = 4NDPA, 3 = NNODPA, 4 = DPA, 5 = 2NDPA. (Reprinted with permission from reference 2.)

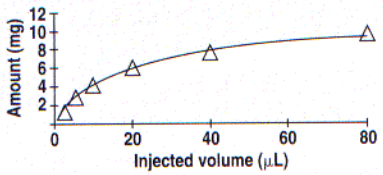

FIGURE 3: Apparent amount of DPA in various sample volumes. (Adapted with permission from reference 2.)

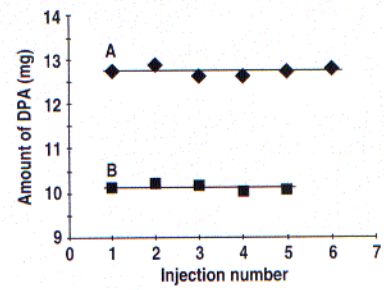

FIGURE 4: Amount of DPA detected vs. number of injections with and without TEA in the mobile phase. Curves: A = original column with 2.5 mM TEA added to mobile phase, B = second brand of column with no TEA in mobile phase. (Adapted with permission from reference 2.)

ear over the region of interest and passed through zero, so little evidence indicated a problem with the internal standard.

So far, the problem was elusive. The initial chromatographic behavior seemed to suggest some kind of column loading problem, but the first few experiments contradicted that conclusion.

LOADING STUDIES

Another experiment was performed to determine whether the recovery of DPA was related to the mass of injected sample. Figure 3 shows a plot of the amount of DPA detected vs. the volume of standard solution injected. Because the sample contained the internal standard, the amount of DPA detected should have been independent of the injected volume. In fact, the lower-mass injections showed significant loss in the amount of DPA detected. This is the result you would expect to see when a portion of the analyte is adsorbed onto the column. If a fixed mass (determined by the number of active sites and the chemistry of the system) is adsorbed, the apparent loss of sample will be much more pronounced for low-mass samples than for high-mass ones.

A TRIAL FIX

At this point, we had some evidence that an adsorption problem was occurring. Another way to check this conclusion is to use a mobile-phase additive that is strongly adsorbed by active sites on the column. Triethylamine (TEA) is often the compound of choice for saturating active silanol sites responsible for adsorption of basic compounds. TEA in the mobile phase is small enough to easily penetrate to the silica surface and therefore equilibrates rapidly with the column. I generally recommend adding ~25 mM TEA to the mobile phase in cases such as this. The reader was hesitant to use more than 2.5 mM TEA because the pH of the mobile phase exceeded 8 when more was added. (In general, column life is shortened when the mobile-phase pH is greater than ~7.5, but the pH could have been controlled with a buffer.) The experiments that produced curve A in Figure 1 were repeated with the modified mobile phase, and the results are shown in Figure 4 (curve A). The drop-off in response with the first few injections no longer occurred, confirming the assumption that a loading phenomenon was responsible for the irregular behavior of DPA in Figures 1 and 3.

COMPARING COLUMNS

During the search for a solution to the problem, other column brands were tried. The best brand tested showed little change in response with the number of injections, as curve B in Figure 4 shows. Interestingly, the original column brand with added TEA yielded ~20% better DPA recovery than did the second brand with no TEA added. Adding TEA to the mobile phase of the new column yielded results similar to those obtained when TEA was added to the original column.

A number of workers have classified commercial LC columns according to the quality of separation they provide for basic compounds (which correlates with reduced peak tailing). One such classification is shown in the accompanying box (3). Columns listed toward the top of the list are better suited to separations involving basic samples, whereas acidic samples are separated better by columns lower in the list. Obviously, this classification is not exhaustive, and differences between adjacent listings may be insignificant. For the present study, the better column was four places higher in the listing than the original one.

Commercial Columns Ranked According to Suitability for Basic Compounds*

Zorbax Rx (Rockland Technologies)	Most suitable
Vydac (The Separations Group)	
Rsil (Alltech Associates)	
Nucleosil (Macherey-Nagel)	
Polygosil (Macherey-Nagel)	
Novapak (Waters Division of Millipore)	
μ-Bondapak (Waters Division of Millipore)	
Supelcosil DB (Supelco)	
Spherisorb 2 (Phase Separations)	
LiChrosorb (E. Merck)	
Chrompak	
Hypersil (Shandon Scientific)	
Perkin-Elmer	
Supelcosil (Supelco)	
Zorbax (Rockland Technologies)	
MicroPak (Varian)	Least suitable

*Those least suitable for basic compounds are most suitable for acidic compounds.

LESSONS LEARNED

We can learn several things from this case study. Perhaps most obvious is that we can arrive at a satisfactory solution without having a complete understanding of the problem. I still cannot give a satisfactory explanation of curve A in Figure 1 (perhaps a reader can provide a clear answer), yet we found a solution that satisfies the requirements of the method.

Second, more than one usable solution is possible. In the present study, 2.5 mM TEA was added to the mobile phase to give satisfactory results. I would have used a greater concentration of TEA and adjusted the pH by using a buffered mobile phase. A second brand of column produced better results than the first brand; the reader could have switched to the new column and perhaps avoided the use of TEA altogether, but for other reasons he continued to use the first column.

Finally, it is important to be aware that column-loading phenomena are quite common. Often it takes several injections of samples or standards at the beginning of a set of runs for the signal to stabilize. Sometimes additives, such as TEA, can be used to overcome this problem, but many times it is more effective to make several injections to "prime" the system. Some workers successfully prime their systems by making one or two injections containing perhaps 10 times the normal sample mass to

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Select one with readout precision of 0.01 s. Some gas chromatographs include a stopwatch function on the display that includes flow, split-ratio, and linear velocity calculations.

Syringe: Keep two syringes on hand, one 10-µL syringe for injecting methane or butane to measure t_M and for ascertaining that the flame is lit and carrier gas is flowing, and the other for making test-mixture injections.

Syringe-cleaning wires: Syringe-cleaning wires may be used in an emergency to clear septum particles or other debris from syringe needles.

Test mixtures: Column and detector test mixtures are useful for verifying column performance and detector sensitivity. Keep a fresh vial of each type on hand. Column test mixtures are available for polar and nonpolar columns, and test mixtures are available for each detector type. Some manufacturers provide a detector test mix that combines components for testing several different detectors. Once opened, test mixtures can be kept for a while in septum-sealed vials. Their life is limited by gradual evaporation. If you keep test mixtures in vials, remove the vial cap rather than puncturing the septum when withdrawing liquid for injection.

Tubing, deactivated fused-silica: When necessary, 5–10 m of deactivated 0.53-mm i.d. fused-silica tubing can be used with a press-fit connector as a retention gap. Shorter pieces can serve as a column-to-detector adapter when you don't want to put the coated column end into a detector to avoid column bleed or polyimide breakdown at the hot internal detector temperatures.

Tweezers: A pair of tweezers can hold small nuts or ferrules, eliminating the risk of contaminating the parts with skin oils and preventing you from being burned by hot items. Some tweezers have a convenient locking feature that frees one hand for other tasks.

Typewriter correction fluid: Use typewriter correction fluid to mark the position on a column that corresponds to the correct column penetration depth into an inlet or detector. Measure the depth after inserting the column into the nut and ferrule and making a fresh cut on the column end.

Vial crimper: Vial crimpers attach aluminum crimp-top seals to autosampler vials. Several crimp-top sizes are commonly used for GC: 8 mm for 0.8-mL vials, 11 mm for 1.5-mL and 2.0-mL vials, and 20 mm for 5-mL and 20-mL vials. Hand crimpers are the least expensive but have no interchangeable jaws to accommodate the various sizes. Bench-top crimpers are less mobile, but the jaws can be interchanged quickly.

Vial decapper: Vial decappers perform the opposite function of crimpers and remove crimp-top seals from vials. Decappers come in the same sizes as the crimpers. They resemble a pair of pliers. Some caution is required to avoid breaking the neck of the vial. Once caps are removed, the contents may be properly disposed of and the vial cleaned and reused.

Wire brushes: Wire brushes can dislodge particles and debris from detector parts and some sealing surfaces. Be careful not to score polished metal surfaces or damage ceramics. It is better to replace an extremely dirty flame jet or collector than to forcibly clean it off.

Wrenches, open-ended: I have an assortment of open-ended wrenches in inch sizes, plus a metric set. I keep two or three of each of the following sizes: ¼, ⅜, 7/16, ½, and 9/16 in. These are the most common sizes, and I often use two at once to prevent counter-rotation while tightening or loosening fittings.

Wrenches, adjustable: I have one large 1-ft. adjustable wrench that looks like it belongs in an automotive garage. I use it for attaching or removing pressure regulators on gas tanks. I also have a smaller 6-in. adjustable wrench that I use occasionally if I don't have the exact open-ended wrench size handy.

REFERENCES

(1) J.V. Hinshaw, *LC•GC* **10**(7), 516–522 (1992).
(2) J.V. Hinshaw, *LC•GC* **9**(10), 698–703 (1991).

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speed up the loading process. Because saturated active sites may result from interactions with the sample compounds or from materials in the sample matrix, it may be more effective to inject a "real" sample (using a control sample or extra sample from earlier runs) rather than a calibration standard when loading the column.

REFERENCES

(1) T. Lindblom, personal communication (1992).
(2) T. Lindblom, paper presented at the 9th Symposium on Chemical Problems Connected with the Stability of Explosives, Margretetorp, Sweden, August 1992.
(3) L.R. Snyder, J.L. Glajch, and J.J. Kirkland, *Practical HPLC Method Development* (John Wiley & Sons, New York, 1988), p. 62.

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