LC Troubleshooting

Attacking Carryover Problems

A systematic approach is the best way to solve carryover problems.

John W. Dolan
LC Troubleshooting Editor

Carryover is recognized as the presence of a small peak that appears when a blank is injected after a sample that produces a large peak. Carryover can be one of the most frustrating problems in liquid chromatography (LC) practice. Some methods seem to be immune, whereas other methods fight a constant battle with carryover. This month's "LC Troubleshooting" column focuses on a systematic approach to solving carryover problems. Readers can use the accompanying sidebar, "Steps to Correct Carryover Problems," as a quick reference guide while reading this column and when solving future problems.

Classify the Problem

The first step in solving a problem is to determine what kind of carryover you're experiencing. You can make this determination by injecting a high-level sample followed by two or more blank injections of sample matrix without the analyte (step 1). What I call *classic carryover* can be recognized by a regular reduction of peak size as blanks are injected consecutively. For example, the first blank might have a peak that is 1% of the size of the original, and then the next drops by another factor of 100. Although the washout doesn't always result in exactly the same percentage reduction with each consecutive blank, the peak size will drop significantly with each additional injection, and it should be insignificant by the third or fourth injection.

The source of classic carryover often is a small cavity in a system that acts as a reservoir of sample that becomes diluted with successive injections. Classic carryover is especially problematic when large and small analyte values are likely to be found in the same sample set such as when an LC method is used to determine the pharmacokinetic behavior of a drug in plasma. For applications such as content uniformity or product assay, classic carryover may be of little importance because all samples have approximately the same analyte concentration, so a trace of sample remaining from the previous injection will go unnoticed.

The other common type of carryover occurs when a small peak appears at a nearly constant size in all blanks, for example at 0.5% of a high-level sample. This constant carryover probably is a result of contamination and not true carryover, but the general approach to correcting the problem is the same as for classic carryover, so I will include it in the current discussion.

Constant Carryover

If the consecutive injection of blanks results in a small peak of similar size in each run, the problem is classified as constant carryover. Although most of the troubleshooting process is the same as for classic carryover, you need to conduct a couple more experiments first.

Because contamination is the most likely cause of constant carryover, you first should eliminate the most likely cause of contamination — the sample blank (step 2). First, replace the blank with a nominally identical

Steps to Correct Carryover Problems

1. Classify carryover
2. Replace blank
3. Change injection size
4. Check fitting assembly
5. Check wash solvent
   a. Use fresh wash solvent
   b. Increase wash volume
   c. Use more organic solvent in wash
   d. Adjust wash pH
6. Check washing mechanism
7. Change injection solvent
8. Check another sample
9. Change hardware
   a. Change needle seal
   b. Change injection loop
   c. Rebuild or replace valve
   d. Substitute autosamplers

blank that you feel is free of contamination. If the blank is water or a simple solvent mixture, just make a fresh batch, preferably from another source of reagents, and rinse the injection vial before use to ensure its cleanliness. If the sample is the result of extraction of the analyte from a biological or environmental matrix, you may find it more difficult to obtain a blank unrelated to the suspect sample. If all else fails, replace the blank with another solution that is compatible with the chromatographic system, such as a few milliliters of mobile phase. If the carryover peak disappears with a fresh blank, the solution to your problem may be as simple as preparing fresh blanks to use with the analysis. On the other hand, you may need to spend considerable effort tracking down the source of contamination, which may be a reagent or glassware.

As a cautionary measure, if your laboratory is involved in both trace and preparative chromatography, you should segregate the glassware. I have seen contamination problems arise when traces of analyte remain on the glassware after use of that glassware for synthesis or preparative chromatography. Washing highly contaminated glassware in the same wash cycle as analytical glassware may produce cross-contamination.

If replacing the blank did not solve the problem, try adjusting the injection volume by a factor of two or more (step 3). If the carryover peak increases or decreases in proportion to the injection volume change, it is highly probable that the blank is contaminated, so go back to step 2 and spend more time trying to identify the source. If the peak remains at constant size, continue with the steps described below.

Fittings Problems

If steps 2 or 3 did not identify the source of the constant carryover problem or if the problem is classic carryover, move to step 4. You should check all the compression fittings and tube connections that the sample contacts for proper assembly. This procedure generally is limited to the fittings on the injection valve and all fittings downstream to the detector. Stainless steel fittings rarely are a problem, but the popular polyetheretherketone (PEEK) fittings and tubing can slip under high pressures; for example, greater than approximately 4000 psi or 275 bar.

You can check stainless steel fittings by slightly tightening them with a wrench. The best approach for PEEK fittings and tubing is to shut off the pump, loosen each fitting, push the tubing firmly to the bottom of the tube port, and retighten the fitting. Don't try to tighten PEEK fittings with pressure in the system, because the tubing can slip in the fitting when the nut is rotated. Even if you didn't notice obvious problems, it is a good idea to repeat the carryover test (high sample followed by two blanks) to see if the problem has been resolved.

Wash Solvent Problems

The next several steps are related to the injector wash solvent. You may want to follow these steps in order or if one seems more appropriate for your sample, go directly to it. First, replace the wash solvent with a fresh batch (step 5a). When troubleshooting carryover problems, I like to err on the side of caution, so I generally replace the wash solvent with a freshly prepared batch and replace the reservoir with a clean one. The autosamplers my co-workers and I use in our laboratory work best if the wash solvent is degassed, so we helium-sparge the solvents before putting them on the autosampler. Degassing, however, is a potential source of contamination, so you may want to try using a fresh solvent with and without degassing to see if you notice any difference. After the wash solvent is replaced, cycle the autosampler through the purge or wash cycle several times to ensure that all traces of the previous solvent have been removed.

If changing the wash solvent solves the problem only temporarily, perhaps you are using an insufficient wash volume between samples. Increase the wash volume (step 5b) or the number of wash cycles to see if either of these fixes will correct the problem.

If the problem persists, the solvent properties of the wash solvent might be insufficient to remove all of the analyte from the previous injection. To my knowledge, none of the currently available autosamplers inject wash solvent, so as long as the wash solvent is miscible with the mobile phase, it doesn't need to be of similar solvent strength. My next step usually is to increase the strength of the wash solvent (step 5c). Often I'll use a wash solvent that is roughly equivalent in strength to the mobile phase, so the first step when using a stronger solvent would be to use more organic solvent or even 100% of the strong solvent (usually acetonitrile or methanol). Isopropanol makes an excellent wash solvent for many applications and proves to be more effective for removing contaminants than methanol or acetonitrile.

Most mobile phases contain a buffer, acid, or base to control the pH. For wash solvents, I generally like to avoid using buffers or salts that leave a residue when evaporated. In my experience, these additives leave white crusty deposits around the needle wash port and injection port. Thus, phosphate buffer is banned for use in wash solvents in my laboratory. However, creating acidic or basic conditions is all that is necessary for use as a wash solvent, so you can use a volatile acid or base. Workers in my laboratory often use formic, acetic, trifluoroacetic, or heptafluorobutyric acid or ammonium hydroxide to create acidic or basic wash solvent conditions. These additives are freely soluble in all concentrations of water, acetonitrile, and methanol. Typically a 0.1–1% solution by volume is sufficient. Try adding acid or base to the wash solvent (step 5d) to see if the carryover is improved. If you plan to use a basic wash solvent routinely, be sure that system components such as the injection valve seals are compatible.

Washing Mechanism

If your efforts at correcting the problem by changing the wash solvent (step 5) were unsuccessful, it is time you look into the mechanical aspects of the autosampler wash (step 6). Each autosampler design uses a slightly different wash mechanism, so you will find it helpful to consult your operator's manual for specific help. Many autosamplers use an overflow cup assembly to wash the outside of the autosampler needle. For example, the autosampler will insert the needle into a small wash station, which may look like a microvial. The autosampler pushes wash solvent through the needle, and the solvent flows up the sides of the needle from the needle tip and overflows to waste.

This process usually is effective at rinsing any contamination from the outside of the needle. In some cases, however, the wash station can become a source of contamination. If nonsoluble sample components collect in the cup or on the outside of the needle, each cycle through the wash station contaminates the needle. Some autosamplers use a septum or other wiping mechanism to physically wipe the outside of the needle as it enters and leaves the wash station. This septum can become worn out, damaged, or contaminated. If the wash station drain line is positioned improperly, contaminated wash solvent can

siphon into the wash station rather than to waste when the needle is removed. Disassemble the wash station, clean it, and check for any of the problems noted above or additional mechanical problems that are specific to your brand and model of autosampler.

Injection Solvent

The injection solvent serves as a carrier to facilitate transfer of the sample onto the column. In general, and especially if small injection volumes (less than 20 μL) are used, the choice of injection solvent is unimportant. As long as the injection solvent is no stronger than the mobile phase and the injection volume is no larger than approximately 15% of the resulting peak volume, the injection solvent composition will have little effect on the chromatography. For this reason, chromatographers should choose their injection solvent for ease of sample preparation. For example, if the sample is highly water soluble, the injection solvent could be water, even though the mobile phase contains 50% organic solvent. With some samples, the selected injection solvent may facilitate adsorption of the sample on system components such as the autosampler loop. This practice most commonly becomes problematic when the injection solvent is 100% water or buffer. Often, adding a small amount of organic solvent such as 5% methanol or acetonitrile to the injection solvent will eliminate any sample adsorption. Check for problems related to the injection solvent by changing to another solvent or by adding more organic solvent to the injection solvent (step 7).

Sample-Specific Carryover

By now you've tried all the easy fixes to carryover problems, so it is worth the trouble to see if the problem is specific to your sample (step 8). Sometimes you can check by examining another peak in the sample. For example, if your method uses an internal standard, try injecting a large amount of internal standard and then injecting blanks without an internal standard. Perhaps you can inject another chemically related compound that will be eluted under the current method conditions. If all else fails, you can change the mobile phase or column and run another method.

If you find that the carryover problem is common to different compounds or method conditions, most likely the carryover is a physical problem associated with the autosampler hardware or system plumbing. If the carryover is specific to one compound, you should search for a chemical solution to the problem, such as changing the injection or wash solvents.

Hardware Changes

If you're still trying to solve the carryover problem, it is highly likely that the problem is related to the autosampler hardware. At this point, you should substitute parts until you locate the problem. Often the easiest first step is to try a manual injection valve or to replace the autosampler with one that has not been exposed to the compound of interest (step 9d). If either of these approaches correct the problem, it confirms that the problem is in the autosampler and further troubleshooting efforts along this line are worthwhile.

If you haven't done it already, replace the needle seal on the injection valve (step 9a). Many autosamplers use a polymeric seal to prevent sample loss during sample loop filling. These seals can become worn and require occasional adjustment or replacement. Autosampler designs in which the needle and loop are combined may use a graphite or hard polymer seal to provide high-pressure sealing. If this seal is worn or damaged by improper needle alignment, you may observe increased carryover even if the fitting doesn't leak. When the needle seal is replaced, carefully examine the tip and outside of the injection needle. If any roughness or corrosion is observed, replace the needle to avoid rapid wear of the new seal.

Sometimes the sample adsorbs on the sample loop because of the characteristics of sample and injection solvent. Replacing the loop (step 9b) with one of different composition can help solve this problem. Injection loops are available in stainless steel, PEEK, and titanium. A combination of a new loop material and a different injection solvent (step 7) should eliminate sample adsorption on the loop.

The internal components of the injection valve will become worn over time; repairing or replacing the injection valve (step 9c) will be necessary from time to time. A worn injection seal can distort the flow of fluids through the valve and may create conditions that facilitate carryover. In other cases, sample can adsorb on the polymeric seals inside the valve. Seal materials of different composition help address this problem.

Other components specific to your brand and model of autosampler can be cleaned or replaced. Once you've exhausted the repair options, the only option left is to use another autosampler (step 9d). If you take this route, be sure you are not just making a substitution that delays the recurrence of carryover problems. For example, my laboratory has two models of the same brand of autosampler; one model is much less susceptible to carryover than the other. So if we just replace a problem unit with another clean unit of the same model, the carryover problem likely will recur before long.

Conclusion

Carryover problems can be some of the most vexing quandries in LC. It is tempting to try a quick fix such as changing the wash solvent and seals and thoroughly cleaning the system. Sometimes this route will work, but it rarely identifies the cause of the problem. Thus, when the problem shows up again, you have little experience to help you solve it more quickly the next time. In my experience, carryover problems of one sample type often show up as carryover problems for another sample. By taking the time to systematically find the cause of the problem, you will be able to locate and correct the problem much more quickly in the future.

John W. Dolan

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of LCGC*'s editorial advisory board. Direct correspondence about this column to "LC Troubleshooting,"* LCGC*, 859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.*

For an ongoing discussion of LC troubleshooting with John Dolan and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.com.