LC Troubleshooting

Not Another Leak!

Drip, drip, drip — are leaks inevitable?

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Leaks seem to be a part of life when working with liquid chromatography (LC) systems. I have never worked with an LC system that didn't have an occasional leak, but most leaks can be prevented. This month's "LC Troubleshooting" will discuss LC system leaks, what they tell us, and how best to avoid them.

Leaks result from one of three problem sources — wear of system parts, loose components, or excessive pressure. Let's look at each of these areas and try to determine if some preventive maintenance procedures will minimize the occurrence of leaks.

Wear of System Parts

Any part of an LC system that moves regularly as part of its system function is subject to wear. Each of these moving components contacts another system part during its operation, and the resulting friction between the two parts will cause wear of one or both parts. The main locations of these parts are in the pump and the autosampler.

Pump seals: Pump seals perhaps are the most wear-prone part of an LC system. With each piston stroke, the piston rubs against the seal. Pump seals generally are made of an inert polymeric material, such as fluorocarbon polymer, and sometimes a filler, such as glass fiber, to improve mechanical properties. Many pump seal formulations and designs are available, but all perform the same function — they allow the pump piston to move without mobile-phase leakage. Three properties are of special importance: Flexibility is required to ensure that the seal fits closely to the piston. Hardness is needed to ensure that the seal doesn't wear too fast. Chemical resistance is necessary to ensure that the seal doesn't dissolve. Each manufacturer strives to optimize these properties for the best seal performance.

The pump seal is not completely leak-free by design. A film of mobile phase adhering to the pump piston acts to lubricate the seal and extend seal life. As long as this leak does not progress beyond the film stage to the drip stage, users generally will obtain satisfactory pump performance.

Seal lifetimes are related directly to the composition of the mobile phase, the cleanliness of the system, and the condition of the piston. Obviously, analysts don't want to use a mobile phase that is chemically incompatible with the pump seal, but this mistake can happen. Some pump seals are optimized for reversed-phase use, whereas others work best under normal-phase conditions, and still others work well for both conditions. I remember one case in which an LC system that was used in reversed-phase mode was switched to normal-phase mode while containing pump seals that were incompatible with normal-phase solvents. The plasticizers leached out of the seal material, contaminated the entire system, and caused leaks in short order. It took several days of cleaning before the system could be used again.

By design, a thin film of mobile phase adheres to the piston to provide lubrication. When the LC system is shut down, the film behind the seal evaporates. The next time the pump is started, the piston is rewetted after a few strokes, and the system works as desired. A potential problem exists when nonvolatile buffers or salts such as phosphate or sodium chloride are used. When the residual film of mobile phase evaporates, a crystalline buffer residue can be left behind. This forms an abrasive surface on the piston that can cause premature seal wear. Of course, the residue redissolves shortly after the system is restarted, but seal wear is accelerated under these conditions.

Two preventive maintenance practices can help to extend pump seal life. One is to ensure that the LC system never is shut off with buffers in the system. Before shutdown, replace the buffer component of the mobile phase with water and flush the buffer from the system. For example, if a mobile phase of 65% acetonitrile and 35% buffer was used, wash the system with 65% acetonitrile and 35% water. Generally 10 mL or more of this flush solvent is sufficient. Then use a strong solvent such as

100% acetonitrile to remove strongly retained materials from the column. It is best to avoid switching directly from buffered mobile phases to 100% strong solvent because buffers can precipitate. A second way to extend seal life is to flush behind the pump seals. Many LC systems are designed with a passage behind the pump seals that can be flushed to remove salt contaminants. (The drain for this passage is the same place from which mobile phase drips when the pump seals wear out.) This flushing procedure generally is unnecessary when buffer concentrations less than approximately 25 mM are used for reversed-phase applications; however, when using high buffer concentrations or ion-exchange mobile phases, analysts can observe significant improvements in seal life. Just flush 10 mL or so of water through the flushing channel to remove water-soluble salts. I like to follow this flush with a few milliliters of methanol or isopropanol to remove the water.

A damaged piston can be another source of pump seal wear. Most pistons are made of sapphire or ceramic materials and are extremely smooth. Any roughness of the piston, such as a scratch, can directly abrade the seal and allow mobile phase to leak under the seal. For this reason, it is a good idea to examine the pistons carefully for any damage each time the pump seals are changed. Scratches on sapphire pistons can be highlighted by holding a small light to the end of the piston to cause it to act as a light pipe and glow, which accents any surface irregularities.

Pump seals don't last forever. I remember when pump seals lasted only a few weeks or months. Design improvements in today's pumps, better seal materials, and good flushing habits can provide seal lifetimes of one year or more. If you don't use a shorter replacement cycle, it is a good idea to replace pump seals during annual pump maintenance. If you wait to replace the seals until you observe leaks, you will have extra work to clean the pump, and seal fragments can cause check valve problems and frit blockage downstream.

Autosampler parts: The autosampler is a second LC system component in which component wear can cause leak problems. The most obvious component to fail is the injection valve itself. The injection valve contains a polymeric rotor and a stationary mating surface of ceramic material or stainless steel. Any leakage of buffers or salts between these two components can act as an abrasive and accelerate wear. The pressure limit of the injection valve is directly related to its useful lifetime before the rotor seal must be replaced. Although most LC systems can be operated at pressures as great as 6000 psi (400 bar), most workers like to keep the upper pressure limit in the 2500–3000 psi range. This limit results in fewer leak problems and less mechanical wear. For this reason, most injectors are not tensioned for 6000 psi operation; the tighter the two surfaces are squeezed together, the higher the pressure limit, but the more friction occurs and the shorter the seal life.

As long as the LC system is flushed regularly, chromatographers can observe injection valve lifetimes of 10,000 cycles or more. In my experience, changing the rotor seals offers no advantage as a preventive maintenance operation. Depending upon the level of use, the rotor seals can last several years before replacement is necessary.

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Another source of leakage in the autosampler is the seal between the injection needle and the valve incorporated in many autosamplers. Each autosampler design is a little different, and users should consult their operators' manuals for instructions about how to adjust the needle seal. Some systems use a graphite seal that works very well at high pressure, but it can be cracked if it is tightened incorrectly. Others use an O ring–type design that can be adjusted with a twist of a fitting. Operators' manuals should suggest replacement frequency for these seals. Remember that the needle seal can leak because it is worn or because a blockage exists somewhere else in the system.

Loose Components

Loose components are a second major source of system leaks. Generally, this problem source is the easiest to locate and correct. Loose tube fittings probably are the biggest problem. With the widespread use of finger-tightened fittings, it is possible to exceed the pressure limits of the fittings if a system blockage occurs. When they observe a leak at a fitting, chromatographers first should look at the pressure readout for the system and make sure that excessive pressure is not present. If the pressure is too high, fix the pressure problem (see the "Excessive Pressure" section) before worrying about the fitting.

If leaks occur at a stainless steel fitting, tightening the fitting one-quarter turn often will stop the leak. Unless the fitting obviously was loose, don't continue to tighten the fitting. It is possible to break the fitting or distort the mating part by overtightening. If a partial turn does not fix the leak, an internal problem is likely, and the fitting should be disassembled and cleaned or replaced before retightening. If high-salt mobile phases are used, it is wise to disassemble any leaking fitting and rinse it with clean water before tightening because it is possible for the buffer salts to cause the fitting to seize if they crystallize or corrode the surfaces. If the tightened fitting continues to leak, be sure you are inspecting the correct fitting. I recently worked on a leaky fitting on an injector valve in which all efforts to correct the leak failed. After more careful inspection, I realized that the actual leak was from a fitting above the suspected one — the mobile phase running down the face of the valve was nearly invisible. Because it dripped off a lower fitting, I was looking in the wrong place.

When polyetheretherketone (PEEK) or other finger-tightened fittings are used, I advise using a different procedure after observing a leak. First, shut off the pump flow and loosen the fitting. Next, push the tube end into the fitting to be sure it is securely seated before tightening the fitting again. This procedure is necessary because sometimes the tube end will slip in the PEEK ferrule during the tightening process if the system is pressurized and will cause unintentional addition of extracolumn volume to the system.

Leaks on the low-pressure portion of an LC system most commonly occur at the fittings that connect tubing to other system components. Although these fittings often are designed so that workers can use a wrench on them, I advise chromatographers to be very careful when tightening these fittings any more than finger-tight — the threads are easy to strip if they are overtightened. If an additional one-half turn doesn't correct a leak at one of these fittings, disas-

semble it and clean or replace the components.

Here's a trick I learned years ago that will help determine if a leak has been stopped. Cut a pointed strip from a piece of thermal printer paper that is approximately the size of the sharpened end of a pencil. Touch the tip of this paper to the suspected leak. Any trace of organic solvent will turn the paper black instantly. This technique can be handy to isolate a small leak or confirm that one has been eliminated.

Excessive Pressure

Excessive system pressure is the most common cause of LC system leaks. Components such as pump seals, injector rotor seals, and tube fittings can provide satisfactory performance at pressures less than 2500 psi, but when a system blockage occurs either the pump upper pressure shutoff will be triggered or a leak will occur to release the excessive pressure. Because any leak of buffer or salt represents a secondary problem of wear or corrosion, it is a good idea to set the upper pressure shutoff so that the pump shuts off at 3000–3500 psi to minimize pressure-caused leaks.

The most common source of excessive pressure is blockage of the frit at the inlet end of the column. Changing this frit or backflushing the column to remove debris on the frit can be time-consuming and might ruin the column. For this reason, I recommend using a 0.5-µm-porosity in-line filter between the injector and the column. This frit will catch anything that would be trapped on the column frit, and it is easier and less expensive to change.

The isolation of an excessive pressure problem usually is quite simple. Remember that the observed leak is upstream from the blockage. Just start loosening fittings and connections downstream from the leak until you can identify the source. After you have identified the problem source, clean out the blockage or replace the component, and the problem should disappear. If you are using buffered or high-salt mobile phases, it is wise to disassemble a leaky fitting and rinse it with clean water to remove any potential salt deposits.

Excessive pressure almost always happens when particulate matter collects on a frit or in a narrow passage in the flow stream of an LC system. Common sources of particulates are the mobile phase, dirty samples, and seal wear debris. Mobile phases should be filtered if they are the problem source. Always use a frit on the inlet line of the tubing to deliver solvent from the reservoir to the pump. Sample cleanup can be simple or extensive, but injected samples should be free of particulate matter.

Some workers filter each sample, but I find this time-consuming, expensive, and a potential source of error in a method. Instead, I like to centrifuge all samples before placing them in autosampler vials. If I use an in-line filter after the autosampler, it will help catch particulate matter from the occasional unfiltered sample.

Particulate matter from worn components should be avoided. As was discussed above, the pump seal is a prime source of system-generated particulate matter; replace the seal before it becomes severely worn.

Conclusions

I don't think you can eliminate leaks from LC systems. However, good maintenance practices will help eliminate leaks as a common problem. Keep the system clean, tighten (but don't overtighten) fittings, replace the pump seals now and then, and avoid injecting samples that contain particulate matter. These simple practices can go a long way toward minimizing the problem of system leaks.

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

Erratum

The influence of flow rate on average retention factor (k^*) was stated incorrectly during the discussion of equation 3 in April's "LC Troubleshooting" (J.W. Dolan, "Flow Rate and Peak Spacing," *LCGC* **21**[4], 352 [2003]). The text should have stated "If the flow rate is halved from 2 mL/min to 1 mL/min, k^* also is halved."