LC TROUBLESHOOTING

LC Problem Guide

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"My system died" is a common laboratory lament. Use this guide to find out what broke and how to fix it.

This "LC Troubleshooting" column usually focuses on a single troubleshooting problem. From time to time it is useful to step back and take a look at the big picture of troubleshooting liquid chromatography (LC) instrumentation. This month's column is designed to help isolate and correct many types of problems with LC instrumentation and separations. Because of space considerations, this guide does not cover all LC problems — it uses broad brush strokes to cover the most common ones. For more detailed information and for coverage of problems not mentioned here, consult a standard resource such as reference 1.

Many of you keep back issues of *LC•GC* or tear out this column and save it in a notebook for future use. For this reason, I've added references to past "LC Troubleshooting" columns as a simple guide to articles on the isolation and correction of specific problems. (The annual index of authors and articles in the December issue of *LC•GC* is another useful source of relevant articles.) References to previous columns take the form [**volume**(issue), starting page].* Thus, April's "LC Troubleshooting" column is referred to as [**10**(4), 294]. This will give you enough information to find the article on your bookshelf or in the library. The references imbedded within a sentence deal with the subject being discussed in that sentence or paragraph; the references at the end of a paragraph cover related topics.

As was pointed out in a recent "GC Troubleshooting" column (2), problem isolation is best approached using a "divide and conquer" technique. I divide the most common LC problems into four areas: pressure problems, leaks, chromatogram problems, and quantitation or data problems. The first three topics are discussed in this article. Quantitation and data problems are not covered here; consult reference 1 and "The Data File" column for more information on that topic.

To use this guide, find the category that most nearly fits your problem. Within that category, scan the subtopics and follow the path to the level of detail you require to solve the problem. The problem symptoms are listed more or less in order of the likelihood of occurrence, but the mode of failure varies from system to system. It is therefore useful to read each section completely before implementing any of the corrective measures. The discussion assumes that the system was working properly at one time and that the performance has deteriorated to the point where action is needed. The coverage concentrates on reversed-phase separations, but most of the advice applies to other separation modes as well.

Finally, remember the first of the "Five Rules of Thumb" for troubleshooting: Change just one thing at a time [**6**(4), 304]. Stepwise problem correction may take a little longer than a shotgun approach, but it gives you valuable information that will help you solve the problem more quickly and less expensively the next time.

For a general discussion of problem isolation procedures, see **7**(12), 960. Certain chromatographic measurements, such as capacity factor, plate number, and resolution, are very helpful in diagnosing LC problems [**9**(6), 406].

PRESSURE PROBLEMS

Pressure problems are observed as a change in the system pressure displayed on the pump or system controller. Of course, knowledge of the current pressure is of little use if you don't know the standard pressure for the method, so be sure to record this value along with other method measurements. See also **9**(9), 618 and **10**(2), 84.

Fluctuating pressure: Cycling or erratic pressure usually results from a leaky check valve or a bubble in the pump [**9**(2), 88]. A bubble is the most common cause. Check for proper degassing (helium sparging is best). Flush the pump by opening the purge valve and increasing the flow. Some pumps require special priming techniques. If degassing, flushing, and priming fail to fix the problem, replace the check valves one at a time until the problem is cured. If none of these solutions work, replace the pump seal(s). If any leakage is evident below the pump head behind the check valves, seal leakage is the likely cause [**8**(12), 916].

Prevent these problems by using filtered mobile phase, degassed solvents, an inlet-line filter in each reservoir, and a clean-solvent flush of the system after each day's use [**7**(3), 224].

High pressure: System pressure normally increases over time as samples are run. Generally the pressure increase amounts to no more than ~100 psi/day, but it will vary with the method. Pressure increases are due to a buildup of particulate matter in the system, causing a resistance to flow. Of course, an increase in the flow rate also increases the pressure, so be sure to check the flow rate setting before trying other corrective measures. Temperature changes can also affect the pressure, but unless temperature extremes are observed, it is unlikely that temperature changes will cause an alarming change in pressure.

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Particulate matter usually is trapped on the first frit that is downstream from the injector. The particles originate from the sample or mobile phase or from seal wear in the pump or injection valve. The most methodical technique for isolating the location of a blockage is to start at the detector and loosen the connecting fittings. Work upstream until the pressure drops dramatically when the fitting is loosened (remember that pressure normally changes significantly when the column is disconnected). You may find the problem faster, however, if you check for a blockage in the most likely places first, starting with the in-line filter, then the guard column, and finally the analytical column. If the in-line filter is blocked, replace the filter element. When a guard column becomes blocked, replace the entire guard column — it is likely to have other problems as well [**9**(12), 834]. For blocked analytical columns, generally it is worthwhile to replace the inlet frit or backflush the column [**8**(7), 516].

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If the pressure increases abruptly, it may be because of precipitation of buffer within the system [**9**(3), 204]. This is more common with acetonitrile mobile phases and at organic concentrations > 50%, where buffer solubility may be a problem. Premixing all or part of the mobile phase may help prevent buffer precipitation.

Prevent or minimize high-pressure problems by using high-quality filtered mobile phases, filtering your samples, and changing the pump seals before they deteriorate completely [**9**(12), 834]. I strongly endorse the use of a 0.5-µm porosity in-line filter just downstream from the injector. This filter will stop most particles that can block columns and is much easier to change than a column frit or guard column.

Low pressure: Low pressure indicates that the flow of mobile phase to the column is lower than expected or that there is a major problem with loss of column packing. First, check the flow rate to be sure that it was not inadvertently lowered. Bubble and check-valve problems can lower the flow rate, but these problems usually manifest as fluctuating pressures (see above).

If the pressure is steady, check for leaks throughout the system (see below). If no leaks are found and the pump settings are OK, make sure the pump is not starved for mobile phase. Insufficient flow of mobile phase can result from blocked inlet frits in the reservoirs or from reservoirs that are sealed too tightly. Replace the frit or loosen the reservoir cap to correct the problem.

LEAKS

When you find a leak in the system, the location and the cause are usually obvious from the liquid dripping from the part. Sometimes, the only sign of a leak may be a bit of white crystalline "fuzz" at a fitting connection, which results from a slight leak of mobile phase that evaporates before it drips, leaving a buffer residue. When leaks are observed, the system pressure may or may not be affected.

Fittings leaks often can be fixed by tightening the fitting ¼ turn or so. If buffers are used in the system, disassemble and rinse the internal fitting surfaces before tightening the fittings so that no buffers are trapped between the sealing surfaces. If tightening does not fix the problem, replace the fitting [**9**(2), 88].

Leaks at the pump head may be due to worn pump seals, so try replacing these next. Other locations for leaks are autosamplers, injection valves, and detector cells. Correcting these problems will require rebuilding or replacing the defective part. Check the particular module's operation and service manual for instructions. If the system exhibits high system pressure as well as leaks, fix the pressure problem first (see above) — the leak may disappear when normal pressure is restored.

CHROMATOGRAM PROBLEMS

Problems that manifest as changes in the chromatogram probably are the most common LC problems and certainly are the most frustrating to fix. The problem you encounter may fit into more than one of the categories below, so be sure to check for useful information in more than one place.

Baseline problems: Baseline problems are defined as changes in the appearance of the baseline, even when no sample is injected. Allow the system to run a blank baseline for at least 30 min to see if a pattern to the problem emerges.

Baseline noise. Excessive baseline noise can result from problems within the separation process, from the LC equipment, or from external environmental sources. The first job is to determine the problem's origin. Setting the flow rate to 0.0 mL/min (or shutting off the pump) is the easiest way to separate chromatographic problems from equipment or environmental problems. If the problem is chromatographic, the baseline should be straight when the flow is off; if the problem is an equipment malfunction or environmental problem, the noise will continue. Select the appropriate symptom from the following paragraphs. See also **8**(5), 358.

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Noise stops when the flow is off. This problem relates to the flow of mobile phase and sample through the system. Noise spikes and sharp, fronted peaks may be caused by bubbles in the detector cell. Degassing the solvents may fix this problem. Another alternative is to use a back-pressure regulator on the flow cell. A wavy or undulating baseline can be the result of contaminant buildup on the column, which can be caused by insufficiently pure mobile phase (be sure to use HPLC-grade solvents) or by late-eluted components in the sample. Try flushing the column with strong solvent to remove accumulated contaminants (see "Column problems," below). A daily flush with strong solvent will reduce contaminant buildup [**7**(7), 556] and improve system reliability.

Noise continues when pump is off. Sharp noise spikes or intermittent rapid pen movement may indicate a detector lamp problem [**7**(2), 102]. If the detector lamp has a usage meter on one of the leads, check it. UV lamps can be expected to fail after ~1000 h of use. If your lamp has >700 h on the meter, replacing the lamp is a good first choice when these symptoms appear.

Noise spikes can also be caused by external environmental influences [**6**(11), 978]. If the spikes have a regular interval (for example, once every 20–30 s), check for the presence of heating baths, drying ovens, or other heating devices on the same electrical circuit or in close proximity to the LC system. Turn these devices off to see if the problem goes away. Other, less common sources of noise spikes are centrifuges, vacuum pumps, and other devices that put a noticeable load on the electrical circuit when starting.

Baseline drift. A drifting baseline generally results from one of three causes. If the drift is cyclic with each chromatographic run, the likely cause is the mobile phase. With gradient runs, baseline rise during the run is common, especially at detection wavelengths below ~230 nm. This is due to differences in absorbance between the A and B solvents. Sometimes you can compensate for the difference by balancing the absorbance of the solvents [**8**(10), 768]. If the drift is cyclic within the day, the problem may be caused by temperature changes, a common problem in laboratories that use air conditioning or heating to maintain a comfortable temperature during the day but let the temperature drift at night. Be sure that the column and transfer tubing are thermostated or, at minimum, are insulated (see "Temperature problems," below). If the baseline gradually drifts one day and continues drifting with each day, the drift may be caused by contaminants building up on the column. Try a strong solvent flush at the end of each day as a curative measure. See also "Column problems" below and **7**(7), 556.

Retention-time problems: The retention time of peaks can change randomly or regularly. When you observe an anomaly, rerun the sample to see if the problem is reproducible. Compare the retention times of several runs to see if there is a pattern to the retention variation.

Column dead time has changed. If the column dead time, t_0, has changed, the problem is related to the flow rate (assuming that the column dimensions have not changed). Longer retention times can result from leaks (see "Leaks," above) or air bubbles in the pump. Shorter retention times occur when the flow rate setting is too high. This problem is easily corrected by resetting the pump.

If t_0 has not changed but the retention time has changed, one of the following causes is likely: changes in the mobile phase, column, temperature, or sometimes the sample [**8**(3), 212; **8**(11), 842].

Mobile-phase problems. To achieve constant retention times, the column must be at equilibrium with the mobile phase. Be sure to allow adequate equilibration time when you change the mobile phase (isocratic runs) or between samples (gradient runs). The adequacy of the equilibration time is easy to check by allowing longer equilibration and seeing if the problem improves or disappears. When in doubt, allow at least 10 column volumes of solvent (~25 mL for a 25 cm × 4.6 mm column) to pass through the column before injecting the sample. If longer equilibration improves the situation, adjust the method accordingly.

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Mobile-phase changes tend to cause retention to drift in one direction [**9**(3), 204]. Most of the time, mobile-phase changes will cause all peaks to shift in one direction or another, but this is not a hard-and-fast rule. Mobile-phase changes are caused by the loss of one component of the mobile phase leaving a preponderance of other components because of evaporation, decomposition, or reaction [**8**(11), 842; **9**(11), 762]. If you suspect a change in the mobile phase, it is best to mix a new batch of mobile phase to see if it corrects the problem. If you confirm a change in the mobile phase as the source of the problem, adjust the method to prevent future problems. Required adjustments may include less-vigorous helium sparging, replacing the mobile phase with a fresh batch each day, or protecting the reservoirs from light [**9**(11), 762].

When retention changes occur for a subset of the sample peaks, look for a compound-specific change in the mobile phase. The most common sources of such problems are changes in the mobile phase pH or insufficient buffer concentration. Check for the correct pH value. In general, buffers should be at least 20 mM in concentration; lower levels may give irreproducible results [**8**(3), 212].

If retention-time changes correlate with changes in the mobile phase (as determined by mixing a new batch, for example), check the mobile-phase preparation procedures to be sure that the method does not include an irreproducible step. Two common errors occur during mobile-phase preparation. The first is measuring mobile-phase components by adding a known volume of one solvent to a volumetric flask, then filling the flask to the mark with another component. This technique is error-prone because the volumes can change when mobile-phase components are mixed. The second error is measuring the pH after the organic solvent has been added. Measure pH only in aqueous solutions — measuring after the addition of organic solvents will give inaccurate readings [**7**(4), 316; **7**(10), 822].

Finally, if you use on-line mixing and the mixing hardware is suspect, compare the chromatographic results obtained when mobile phases are mixed on-line and manually (isocratic runs). Alternatively, or if gradients are used, exchange the solvent inlet lines between reservoirs and make the appropriate program adjustments. If the hardware is working properly, the results should be the same in either configuration [**7**(10), 822].

Column problems. The deterioration of an LC column or the gradual buildup of contaminants on the column can cause retention time to drift for a single compound, but it is more common for all components in the sample to drift in the same direction. A general reduction in the column plate number, *N*, may also occur, resulting in broader peaks with poorer resolution. Compared with mobile-phase problems (which cycle with the preparation of fresh mobile phase) or with temperature problems (which cycle on a daily basis), column contamination shows up as drift over a longer period, often over several days or weeks and often accompanied by changes in relative peak spacing [**9**(11), 762].

To isolate a suspected change in the column, use the following procedure: If a guard column is being used, remove (or replace) it and rerun several samples. If the problem does not reappear, use a new guard column and replace it earlier in the use cycle. If no guard column is used or if a new guard column fails to help, flush the analytical column with strong solvent to remove any contaminants [**7**(7), 556]. If this helps, institute a regular cleaning cycle on a daily or weekly basis. If the problem remains, replace the column.

Retention drift (and sometimes sensitivity drift) can also be observed during the first few runs of the day. If retention times move in one direction and then stabilize at the start of each day, you may be observing a column loading phenomenon in which the first few samples deactivate the column [**8**(10), 768]. Often this problem is not worth correcting — just run several dummy samples or standards each day to condition the system.

Temperature problems. As mentioned above (see "Baseline drift"), temperature problems often cycle with the heating and air conditioning cycle of the laboratory. Retention will decrease 1–2% per 1 °C increase in temperature. Because it generally is difficult to control the temperature of the laboratory, you must thermostat the column and insulate the transfer lines between the injector and column and between the column and detector. If a heating or cooling vent is directed toward your instrument, you may need to block it or divert it so that the LC system is in a draft-free area. See also **8**(9), 696; **8**(11), 842; **9**(11), 762; and **10**(5), 364.

Sample problems. The sample and the injection solvent can affect the retention time. If too large a volume of strong solvent is injected, irreproducible retention times and poor peak shape can result, especially for early peaks [**8**(1), 18]. It is best to inject mobile phase or a solvent that is weaker than the mobile phase. Check for problems by injecting sample in a dilute solvent or a smaller sample — if the results improve, adjust the method accordingly. Sample overload can affect one or more peaks in the chromatogram (see "Peak shape," below). Samples that have had insufficient sample pretreatment may cause rapid contaminant buildup on the column and may result in shorter lifetimes for guard and analytical columns.

Sensitivity problems: If changes in the heights of one or more peaks are accompanied by changes in retention time, correct the retention-time problem first (see "Retention-time problems," above). Changes in peak height that do not correlate with changes in retention should be confirmed by rerunning the sample or several samples. If the problem persists, run several injections of a standard to isolate whether the problem is related to the sample or the method [**7**(5), 384]. If all the peaks change in proportion, an injection problem is likely. For changes in one or a few peaks in the chromatogram, sample preparation problems may be the cause.

Sample-related problems are beyond the scope of this brief discussion, but check for losses that may occur during extraction, evaporation, solubilization, solid-phase extraction, or other sample preparation steps. Use of an internal standard during sample pretreatment may help you track down the source of error.

If peak-height problems continue to appear when pure standards are injected, sample injection is the most likely cause. If manual injection is used, be sure to overfill the sample loop by at least three times (for example, inject 75 µL of sample into a 25-µL loop) to ensure that the loop is fully flushed. If the partially filled loop technique is used, use less than half of the loop volume for best results [**7**(11), 898]. For autosamplers, be sure that the injector needle is not partially or completely blocked with a piece of septum and that the vials contain enough sample. In all cases, check for proper flushing of the loop between samples and clear any restrictions in the waste lines. See also **7**(10), 822.

If the sensitivity changes for the first few samples and then stabilizes, suspect a column loading problem.

If peak-height problems occur only for large or small peaks, you may be outside the linear range of the method or detector. Adjust the sample mass to see if the problem is corrected with intermediate-size peaks. If this corrects the problem, adjust the method accordingly.

If the sensitivity changes for the first few samples and then stabilizes, suspect a column loading problem (see "Column problems," above) [**8**(10), 768]. Adsorption of sample on the sample loop also can cause apparent changes in sensitivity from run to run or carryover from sample to sample [**9**(1), 22].

Peak shape: Changes in the shape of one or more peaks in the chromatogram can be a difficult problem to analyze and correct. Peak shape problems fall on a continuum, so it is difficult to decide when the peak shape is bad enough to warrant corrective action. The major problems are covered here. See also **10**(4), 294.

Tailing peaks. Peak tailing is most commonly the result of secondary retention interactions between the unbonded silanol groups on the surface of the column packing and basic sample components — amine-containing sample compounds are the worst offenders. As columns age, they tend to produce more peak tailing. At some point, the column must be replaced. Although peak tailing generally cannot be eliminated, it can be minimized by the use of mobile-phase additives or specialty

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columns. Adding triethylamine to the mobile phase at a 20 mM concentration will sharpen the peaks in most separations [**7**(6), 476; **9**(11), 762]. With most columns, keep the pH of the mobile phase in the 2.5–7.0 region to prevent premature column degradation and increased peak tailing [**8**(11), 842]. Several companies offer specialty columns that use sterically protected, polymeric, or other proprietary modifications of the packing particle or bonded phase to decrease peak tailing. Other possible changes to explore when attempting to reduce peak tailing are the use of ion-pair reagents, sufficiently strong buffers (≥20 mM) [**9**(2), 88; **9**(3), 204], and sample derivatization.

Split peaks. Chromatographic peaks are supposed to be sharp and symmetrical. When peaks double, first check for the presence of an interfering compound (for example, inject a standard to see if the problem persists). A single doubled peak in the chromatogram is often caused by an interference. Sample cleanup procedures or the mobile-phase composition may need to be modified to correct problems caused by interferences.

When all the peaks are split or have shoulders, suspect a blocked frit or a column void [**8**(5), 358]. A partially blocked frit may or may not also produce high system pressure. Because the first frit in the system is the most likely to become blocked, work downstream from the injector to locate the problem. I recommend using a 0.5-μm porosity in-line filter just downstream from the injector to trap particulates. Replace the frit in this filter, and the problem should be corrected. If the problem persists, replace the guard column if one is being used. If a new guard column does not help, remove the analytical column inlet frit and inspect the packing bed. Any void or channeling calls for discarding the column. Otherwise, replace the frit with a new one and continue operation. If the problem persists, replace the column.

Early peaks affected more than later peaks. In this case, excessive extracolumn effects or injection problems are the likely cause. Check to be sure no one has inadvertently added a piece of large-bore tubing to the system, reset a time constant or data-sampling rate, replaced the detector flow cell with a larger one, or otherwise changed the system's extracolumn volume. If you have eliminated these causes, it is likely that the injection solvent is too strong or too much sample is being injected (see "Sample problems" in the discussion of retention time, above). See also **8**(1), 18; **8**(2), 98; **9**(2), 88; **9**(11), 762; and **10**(2), 84.

Broad peaks. Excessively broad peaks accompanied by shifts to shorter retention times indicate sample overload, which can occur for one or more peaks in the sample. Check for sample overload by diluting the sample by a factor of 10 and reinjecting (adjust the detector or data system settings accordingly). A sharper peak and possible shift to somewhat longer retention times confirms sample overload. Adjust the method to prevent the problem from recurring in the future [**8**(1), 18].

Negative peaks. When a UV detector is used, peaks will dip below the baseline only if the sample component has less absorbance than the mobile phase [**7**(4), 316; **7**(5), 384; **7**(10), 822]. You can begin with a properly functioning method and then develop this problem if subsequent contamination of the mobile phase causes the background absorbance to rise. When mobile phase is recycled, as can be the case with high-volume isocratic analyses, the background absorbance gradually increases. This change may not be noticed because the data system autozeros just before each injection. To check for this problem, make a new batch of mobile phase, flush the column with strong solvent, and make the run again. Generally this will solve the problem.

Other peak problems. Because most detectors are sensitive to refractive index changes, baseline disturbances can occur when a pulse of injection solvent passes through the detector. Extra peaks in the chromatogram or in a subsequent chromatogram can occur if you inject the next sample before the previous one has been fully eluted [**7**(10), 822]. Extra peaks may be caused by the presence of real interferences [**10**(4), 294] or "ghost" peaks that seem to appear from nowhere [**7**(5), 384; **8**(7), 516; **10**(4), 294].

SUMMARY

This brief troubleshooting guide is designed to help you isolate the most common LC problems. Remember to isolate the problem in a stepwise fashion so that you are sure of the real problem source. Substituting a known good part for a questionable one often is the fastest way to isolate a defective part. Once the problem is corrected, record the solution in the system record book so you will be able to solve it more quickly next time. Throw away any failed parts and order replacements.

There's nothing magic about troubleshooting — it's just common sense. By adding some commonsense preventive maintenance to logical problem isolation techniques, you'll further reduce the amount of time you spend troubleshooting your system. Degas your mobile phase, flush the system daily with clean solvent, and filter the mobile phase before use [**9**(4), 270] — these simple practices, when used on a daily basis, will greatly reduce the incidence of LC problems.

REFERENCES

(1) J.W. Dolan and L.R. Snyder, *Troubleshooting LC Systems* (Humana Press, Clifton, New Jersey, 1989).

(2) J.V. Hinshaw, *LC•GC* **10**(4), 298–302 (1992).

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