TROUBLESHOOTING

Problem Isolation: Shortening Troubleshooting Time

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The key to solving any problem that occurs with a liquid chromatograph, whether it is chromatographic, mechanical, or electrical, is to find out which portion of the instrument is not operating properly. The tools available for initial detection of the problem are the analog and digital readout devices on the liquid chromatograph, and your eyes (and sometimes your nose and ears). If you have a second liquid chromatograph in the lab, substitution of components may be the fastest way to isolate a hardware problem if a cursory examination fails. By far the most common problems encountered are pressure-related, or involve either hydraulic leaks or changes in the chromatogram. Each of these will be discussed briefly.

PRESSURE READOUT

Most liquid chromatographs are equipped with either a digital or analog pressure-readout meter on the face of the pump or control unit. Some instruments are also equipped with an electrical output to display the pressure reading on a strip-chart recorder. If the unit you are using does not include a pressure gauge of some kind, one should be added. Bourdon-tube gauges with an analog readout resembling a clockface are available from a number of vendors of chromatography supplies. These gauges have the added advantage of acting as pulse dampeners. Be sure to buy a gauge with an adequate range (0–6000 psi, for example) for your work.

Pressure-related problems appear on the pressure readout as either a steady pressure that deviates significantly from the typical operating pressure or as a fluctuation in pressure. Steady but abnormal pressure readings usually can be traced to hydraulic restrictions in the case of increased pressure, and to leaks in the case of low pressure. Another obvious but often overlooked cause is a change in operating conditions.

PRESSURE TOO HIGH: LOOK FOR OBSTRUCTIONS

Extreme hydraulic restrictions will cause the pump to overpressurize and shut down if a maximum pressure shutoff is part of the system. More often, though, an increase of several hundred psi will be observed. The easiest way to locate the blockage is to loosen fittings until a pressure drop is seen. *Warning:* Eye protection should be worn at all times when using a liquid chromatograph. Do not attempt to disassemble the system under high pressure. Reduce the flow rate so that less than 500 psi of pressure is obtained. Systematically loosen the hydraulic fittings, working from the detector upstream toward the pump. When the fitting just upstream of the blocked portion is loosened, the pressure will drop significantly. The faulty part should be cleaned or replaced, as appropriate. Extreme cases of blockage may require calling for service or even returning the unit to the vendor for repair. Obstructions often recur at the same place; therefore, if you clear a blockage and then observe it again a few days later, you should examine the problem more closely.

One common point of blockage is the frit at the head of the analytical column. The frit or column may need to be replaced. An excellent discussion of the repair of the inlet end of the column may be found in an earlier issue of *LC* (July/August 1983, p. 270). A gradual pressure increase during the life of a column is generally an unavoidable result of the collection of foreign material at the head of the column. This tendency is harmless as long as the pressure limits of the system are not exceeded. Premature pressure increases are generally caused by poor laboratory technique. This source of blockage may be minimized by properly pretreating samples, using a column prefilter or guard column, and using the best solvents available for mobile phases.

A second common point of blockage is located just beyond the silica-saturator column, which is used in many systems to keep the analytical column from dissolving. (Details of the use of a saturator column in a "highly protected" LC system will be discussed in a future article.) As the saturator-column packing dissolves, microparticles are released, blocking the frit at the end of the silica saturator column. Blockage frequently is catastrophic if temperature, pH, or solvent composition is changed abruptly, or if 10 L of aqueous mobile phase have already passed through the system. A simple method for avoiding these problems is to have a backup saturator column.

A third point of blockage is in the tubing connecting system components. Tubing blockage often can be traced to a burr at the cut end of the tubing. Tiny burrs can stop otherwise insignificant particles and cause blockage; deburr and backflush or replace such a piece of tubing. Tubing blockages may also be a result of physical degradation of the system. A deteriorating pump seal may block tubing between the pump and injector, or may block the injector itself. Contaminated samples or damaged injectors can cause valve, tubing, or column frit blockages. Leakage of column packing may block the tubing leading to the detector or the detector itself. Once the problem is corrected, retighten all of the fittings and run a standard chromatogram to ensure that no more problems exist. Record this in your logbook along with a description of the incident.

PRESSURE TOO LOW: LOOK FOR LEAKS

A reduced but steady pressure is often the result of a hydraulic leak, which can usually be located visually. The most likely areas of leakage are worn pump seals, a leaky injector, or loose fittings. Fittings with plastic or fluorocarbon ferrules are particularly susceptible to loosening or slipping. Trace the hydraulic path of the instrument and look for solvent dripping or collecting at the tubing connections. Be sure not to overlook places where the mobile phase may leak inside the instrument, such as in the detectors and pumps. Once the leak is located, tighten or replace the fitting, or service the seals or other components that are causing the problem. *Warning:* Use proper safety precautions when dealing with solvent leaks. Volatile, toxic, or flammable solvents should be cleaned up with care before resuming operation of the liquid chromatograph.

PRESSURE TOO LOW: INSUFFICIENT MOBILE PHASE

Next to hydraulic leaks, the most probable cause of a pressure drop is the lack of sufficient amounts of mobile phase at the pump. Check to be sure the reservoirs are not empty. An insidious form of this problem is what is known as *solvent starvation* of the pump, which may result from cavitation or vaporization of solvent in either the pump head or the prepump tubing. This may happen if the solvent reservoir is not properly vented, thereby causing a partial vacuum to form in the reservoir, or if the prepump tubing is too small in diameter. Venting or loosening the cap on the reservoir usually solves this problem. Volatile solvents like methylene chloride are more susceptible to cavitation problems than aqueous mobile phases. Because many LC pumps do not have enough suction to prime themselves, it is often necessary to draw solvent through the inlet lines and pump heads to prime the pump after running out of solvent. Operating the liquid chromatograph with the reservoirs elevated above the pump inlet will minimize the problems of pump starvation.

Another source of pump starvation is blockage of the inlet frit on the end of the solvent tubing in the reservoir. This can become blocked by accumulation of foreign matter or dust from improperly filtered or covered solvents. Bacterial growth in buffer bottles can also block these frits. Because most frit-cleaning agents leave UV-absorbing residues, the frit should be replaced for best results.

CHANGES IN OPERATING CONDITIONS

Changes in the operating conditions of the liquid chromatograph can, of course, change the pressure of the system. These may be caused by operator errors such as setting the wrong flow rate or mobile phase composition. Column replacement usually results in a different system operating pressure. Mechanical changes in the system such as replacing a section of tubing or a fitting may be easily identified if a careful logbook is kept. Once again, if all else fails, return the system to standardization conditions and check it against your previous records.

NORMAL PRESSURE FLUCTUATIONS

Observing and recording normal operating conditions will allow you to separate normal pressure fluctuations from ones that indicate a system problem. Small cyclic fluctuations in pressure are a normal part of the pump operation unless some form of pulse dampening is incorporated. This is a result of the suction-delivery stroke cycle of a reciprocating piston pump. Many manufacturers provide some means of minimizing pump pulsations. Depending on your analytical needs, these normal pump pulses may be insignificant or may make the desired analysis impossible.

The other common cyclic fluctuation in pressure is encountered during gradient elution. Although pump pulsations have cycles every few seconds, the gradient pressure cycle has the same period as the gradient — often 15–30 min. As the mobile-phase composition changes during a gradient run, the viscosity also changes, resulting in a change in pressure by as much as a factor of two or more during a single analysis.

UNWANTED PULSATIONS

Abnormal pressure fluctuations are most often caused by air bubbles in the pump chambers or check valves. With multipiston or multipump systems, a dip in pressure is usually seen when one of the pistons is not pumping properly. With air in a single-piston pump the operating pressure will be too low or, if an air bubble is trapped, the pump may not deliver any mobile phase to the instrument. In systems with an analog output of pressure to a strip-chart recorder, isolation of this type of problem is simplified by comparing sample recorder traces in the operator's manual with your trace. Correction of the air-entrapment problem may require solvent degassing, check-valve or liquid-end replacement, or simply loosening a check valve or fitting to release the air and then retightening. First, try to purge the air by opening the purge/prime valve if your instrument has one, and then raise the flow rate to about 10 ml/min. After pumping for several minutes, lower the flow rate and close the valve. To vent air from a check valve, carefully loosen the compression fitting, opening the outlet check valve in question while the pump is operating. Generally, for several pump cycles you will observe bubbles in the liquid escaping. When a bubble-free stream is observed, tighten the fitting and resume operation. Care should be taken to prevent deforming nonmetallic ferrules or twisting the pump head enough to break a piston; a wrench held on the check valve to steady it while adjusting the fitting is usually sufficient. If purging air from the system does not eliminate the pressure fluctuations, the problem may be caused by worn seals or other faulty pump parts. These factors will be discussed in a future column.

CHROMATOGRAPHIC PROBLEMS

Changes that are observed in the chromatogram itself may be isolated in much the same way as in the isolation of hydraulic problems. There are a fixed number of variables that influence the chromatographic separation; systematic checking of the parameters will usually identify the problem area. Once again, a logbook containing either a standard chromatogram for the system or a daily reference chromatogram for your method is invaluable. Before returning to your system standardization conditions, duplicate the reference chromatogram to eliminate specific sample influences. Then check each of the operating parameters: mobile-phase composition, flow rate, pressure, injection volume, column type, detector wavelength and attenuation, and recorder or data system conditions. If your analysis is sensitive to temperature, check for changes in temperature or an improperly operating temperature controller.

If the problem is not isolated by a cursory check of the parameters discussed above, return the system to the standardization conditions. Replacing the mobile phase or column, or substituting the detector may be necessary before the problem can be isolated to a specific cause. Once the problem is isolated, correct it and then restandardize the equipment. Return to work only after you have recorded the problem and its cause in sufficient detail in your logbook. By following this procedure, you may be able to shorten your next troubleshooting episode by reviewing the last one. ■

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