

## Troubleshooting HPLC Problems

Despite advancements in HPLC methods and technology, there are still issues that can rear their ugly head when using an HPLC system. Keep reading to find out the most common HPLC problems and how to deal with them.

### HPLC Pressure Problems

The primary issues with pressure include:

- **Abnormal pressure**, which generally means there is no flow because there is no power, happens when there is a leak or air trapped in the pump head, there is an issue with the controller setting, or a piston is broken. If there is flow and pressure, the meter or pressure transducer could need replacement.
- **High backpressure** is typically caused by a flow rate that is set too high. It could also be due to blockage in the HPLC column frit, HPLC guard column, injector, or in-line filter; the use of the wrong HPLC column or the wrong mobile phase; low column temperature; or controller malfunction.
- **Low backpressure** is generally caused by a flow rate that is set too low. Using an improper HPLC column, having the column temperature set too high, a system leak, and controller malfunction can also cause low backpressure.
- **Pressure cycling** can be caused by air in the pump, faulty valves, a system leak, seal failure in the pump, insufficient degassing, or the use of gradient

elution.

## HPLC Leakage Problems

Leaks can occur anywhere within the HPLC.

- **Leaks at the fittings** typically mean the fitting needs to be tightened, cleaned, or replaced if it is stripped or damaged. Other issues include overtightening of the fitting or using parts from different manufacturers.
- **Leaks at the pump** can be due to loose fittings or loose valves that must be tightened. There could also be failure of the mixer seal, pump seal, pulse damper, or proportioning valve, in which case the faulty part needs to be repaired or replaced.
- **Leaks at the injector** can be caused by using a syringe needle with the wrong diameter. A failure of the rotor seal can cause leaks and requires repair or replacement. Blockage can occur in the loop or the waste line, requiring cleaning or replacement. If the injector port seal is loose, it should be tightened. Leaks can occur due to waste line syphoning, which can be corrected with proper slope and a waste line that is kept above the surface.
- **Leaks at the HPLC column** can be caused by a loose end-fitting that needs to be tightened.
- **Leaks in the detector** can be caused by fittings that leak and need tightening, failure of the cell gasket requiring repair or replacement, cracked cell windows

that need replacement, or blockage of the waste line or blocked flow cell requiring replacement of these parts.

## HPLC Chromatography Problems

### Possible Problems that can stem from HPLC Columns

- **Peak tailing** due to frit blockage, a column void, sample interaction with active sites, an interfering peak, the wrong mobile phase pH, or a column that needs to be replaced.
- **Peak fronting** due to low temperature, using the wrong sample solvent, overloading the sample, or a bad column that needs to be replaced.
- **Split peaks** due to contamination in the column inlet or on the guard or incompatibility of the sample solvent with the mobile phase.
- **Distortion of large peaks** due to overloading the sample.
- **Distortion of small peaks** due to using the wrong injection solvent.
- **Tailing of early peaks** due to issues outside the column requiring a smaller volume detector cell or replumbing of the system.
- **Tailing that increases as  $k'$  increases** due to issues with secondary retention effects.
- **Tailing of acidic or basic peaks** due to inadequate buffering.
- **Extra peaks** due to the presence of ghost peaks or a late eluting peak from a prior injection (carryover).

- **Retention time drifts** due to poor control of temperature or column equilibration or mobile phase changing.
- **Retention time changes** due to a change in flow rate, an air bubble in the pump, or improper mobile phase.
- **Baseline drifts** due to the presence of contaminants in the mobile phase, fluctuations in column temperature, slow column equilibration, issues with the mobile phase, strongly retained materials in the sample, or detector not set properly.
- **Baseline noise** due to leaks, air trapped in the system, contamination, incomplete mobile phase mixing, inadequate degassing of the mobile phase, detector issues, temperature issues, pulsation of the pump, or the use of other electronic equipment on the same line.
- **Broad peaks** due to issues with the mobile phase, leaks, contamination in the column or guard column, temperature issues, low buffer concentration, issues with detector settings, high detector time constant, or a void at the column inlet.
- **Loss of resolution** due to contamination of the mobile phase, obstruction of the analytical or guard column, or a column that needs to be replaced.
- **Peaks that are too small or too large** due to issues with detector attenuation, injection size, or an improper recorder connection.

### Troubleshooting HPLC Injector Problems

Problems can exist with a manual injector and an autosampler injector. When it comes to a

manual injector, the problem typically is that it is too hard to turn or too hard to load. If it's too hard to turn, the rotor seal may be damaged, or the rotor may be too tight - replace the rotor seal. If it is too hard to load, there may be valve misalignment, blockage in the loop or lines, or a dirty syringe.

The most common problem with an autosampler injector is that it won't turn, which can be due to a lack of power or pressure, valve misalignment, or a rotor that is too tight. Other issues with an autosampler injector include blockage, a faulty controller, or a jammed mechanism.

If you have any questions or would like more information, reach out to our team of technical specialists through our free online portal - **Chat Now**.



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