FAQs on HPLC troubleshooting

How should I store my HPLC column?

The right storage of a column is an important requirement for saving the column life time.

Silica-based normal phase columns should be stored with heptane or another inert solvent.

Reversed phase columns are best stored in mixtures of organic compounds (acetonitril, methanol) and water, where the water content must not be higher than 50%. When using buffered mobile phases it is necessary to flush the silica-based column with pure solvents to avoid precipitation of buffer salt.

After usage all columns have to be plugged to avoid the stationary phase to dry out. Ambient tempartures of 18°C-26°C are convenient for a typical silica column.

Polymer columns, like Eurokat, have to be kept cool (4°C) because they are operated organic solvent free. Furthermore a modifier (< 10%) can be added to the mobile phase to inhibit bacterial growth.

How to deal with a baseline drift?

a.) Fluctuation of column temperature. (Even small changes cause cyclic baseline rise and fall. RI-, conductivity and UV detectors at high sensitivity are most often affected.)

Solution: Control temperature of column and mobile phase, use heat exchanger before detector.

b.) Mobile phase is inhomogeneous. (Drift usually to higher absorbance, rather than cyclic pattern from temperature fluctuation.)

Solution: Use HPLC grade solvents, high purity salts and additives. Degas mobile phase before use and apply a degasser or sparge solvents with helium during use.

c.) Contaminant or air buildup in detector cell.

Solution: Flush cell with methanol or other strong solvent. If necessary clean cell with 1 N HNO3 (never with HCl).

d.) Plugged outlet line after detector. (High pressure cracks cell window, producing noisy baseline.)

Solution: Unplug or replace line. Refer to detector manual to replace window.

e.) Mobile phase mixing problem or change in flow rate.

Solution: Correct composition/flow rate. Routinely monitor composition and flow rate to avoid problem.

f.) Slow column equilibration, especially when changing mobile phase.

Solution: Flush column with intermediate strength solvent, run 10-20 column volumes of new mobile phase through column before analysis.

g.) Mobile phase contaminated, deteriorated or prepared from low quality materials.

Solution: Check make-up of mobile phase.

h.) Strongly retained materials in sample (high k´) can elute as very broad peaks and appear to be a rising baseline. (Gradient analyses can aggravate problem.)

Solution: Use guard column. If necessary flush column with strong solvent between injections or periodically during analysis.

i.) Mobile phase recycled but detector not adjusted.

Solution: Reset baseline. Use new materials when dynamic range of detector is exceeded.

j.) Detector (UV) not set at absorbance maximum but at slope of curve.

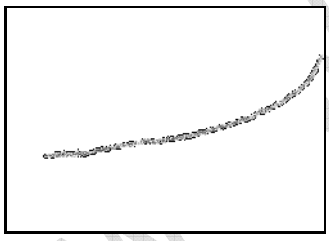
Solution: Change wavelength to UV absorbance maximum.

k.) At higher lab temperatures (28°C) more baseline instabilities comparing to lower lab temperatures (22°C) when using ACN/Water or –buffer gradients and mixtures.

Solution: Higher temperatures can enhance the polymerization of ACN resulting in building of polymers. Filtration of ACN-eluent with Empore SDB-XC Polystyroldivinylbenzol filter.

Regular:

Problem:



Why does the height of one or more peaks change between different runs?

a.) One or more sample components deteriorated or column activity changed.

Solution: Use fresh sample or standard to confirm sample as source of problem. If some or all peaks are still smaller than expected, replace column. If new column improves analysis, try to restore the old column, following appropriate procedure. If performance does not improve, discard old column.

b.) Changes in the sample preparation process. Differences in the matrix can affect peak heights.

Solution: Check sample preparation process and eliminate matrix effects as cause of problem.

c.) Leak, especially between injector port and column inlet. (Retention also would change.)

Solution: Check system for loose fitting. Check pump for leaks, salt build-up, and unusual noises. Change pump seals if necessary.

d.) Inconsistent sample volume.

Solution: Be sure samples are consistent. For fixed volume sample loop, use 2-3 times loop volume to ensure the loop is completely filled. Be sure automatic sampler vials contain sufficient sample. Check syringe-type injectors for air. In systems with wash or flushing step, be sure wash solution does not precipitate sample components.

e.) Detector or detector setting changed.

Solution: Please check the settings.

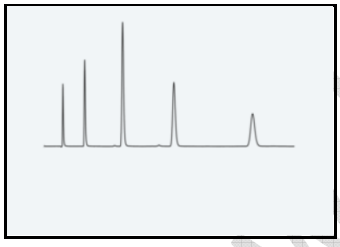
f.) Weak detector lamp.

Solution: Please replace the lamp.

g.) Contamination in detector cell.

Solution: Please clean the cell.

Regular:



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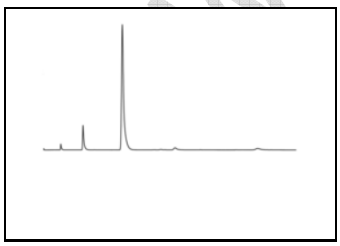


Why do I detect no peaks or only very small peaks?

a.) Detector lamp off.

Solution: Turn on lamp.

b.) Loose/broken wire between detector and Computer.

Solution: Check electrical connections.

c.) No mobile phase flow.

Solution: Start pump. Check mobile phase level in reservoir(s). Check flow throughout system. Examine sample loop for obstruction or air lock. Make sure mobile phase components are miscible and mobile phase is properly degassed. Check system for loose fittings. Check pump for leaks, salt buildup, unusual noises. Change pump seals if necessary. Disconnect tubing at column inlet. Check for flow. Purge pump at high flow rate (e.g. 5 ml/min), prime system if necessary (prime each pump head separately). If system has check valve, loosen valve to allow air to escape. If problem persists, flush system with 100% methanol or isopropanol. If problem still persists, contact system manufacturer.

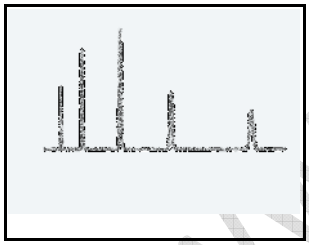
d.) No sample/deteriorated sample.

Solution: Be sure automatic sampler vials have sufficient liquid and injector valve works well. Evaluate system performance with fresh standard to confirm sample as source of problem.

e.) Settings too high on detector/Software.

Solution: Check attenuation or gain settings.

Regular:



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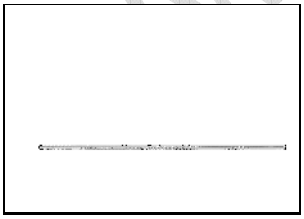


How to lower the column back pressure?

If the column back pressure is too high or higher than usual, please check the following suggestions.

a.) Problem in pump, injector, in-line-filter or tubing.

Solution: Disconnect column from system and replace with unions 0.010'' ID or larger tubing to reconnect the injector to the detector. Run pump at high flow rate (2 - 5 ml/min). If pressure is minimal, see cause 2. If not, isolate cause by systematically eliminating system components. Start with the detector and work back to the pump.

b.) Obstructed column.

Solution: Remove guard column if present and check pressure. Replace guard column if necessary. If column is obstructed, reverse and flush the column while disconnected from the detector. If problem persists, use appropriate restoration procedure. If problem still persists, replace column.

c.) Wrong mobile phase.

Solution: Check mobile phase. Check make-up of mobile phase: Even small changes in composition can affect back pressure.

How to raise the column back pressure?

If the column back pressure is too low or lower than usual, please check the following suggestions.

a.) Leak.

Solution: Check the system for loose fittings. Check the pump for leaks, salt build up and unusual noises. If necessary, change the pump seals.

b.) Mobile phase flow interrupted or obstructed.

Solution: Check mobile phase level in reservoirs. Check flow throughout the system. Especially examine sample loop for obstruction or air lock. Make sure that mobile phase components are miscible and that the mobile phase is degassed.

c.) Air trapped in pump head, revealed by pressure fluctuations.

Solution: Disconnect tubing at column inlet and check for flow. Purge pump at high flow rate (e.g. 10 ml/min), prime system if necessary.

d.) Leak at column inlet end fitting.

Solution: Reconnect column and pump solvent through column. If pressure is still low check for leaks at column inlet and end fitting.

e.) Air trapped elsewhere in system.

Solution: Disconnect column and purge system. Reconnect column. If problem still persists, flush system with 100 % methanol or isopropanol.

f.) Worn pump seal causing leaks around pump head.

Solution: Replace seal. If problem persists, replace piston and seal.

g.) Wrong mobile phase.

Solution: Check mobile phase. Check make-up of mobile phase: Even small changes in composition can affect back pressure.

How to deal with fronting peaks?

The problem with fronting peaks can occur due to following reasons:

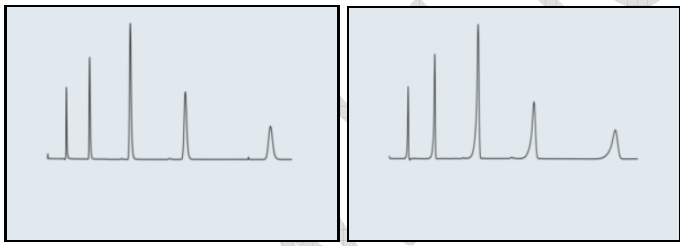
a) Interference in sample. Solution: Check column performance with standards.

b) Shoulder or gradual baseline rise before a main peak may be another sample component. Solution: Increase efficiency or change selectivity of system to improve resolution. Try another column type if necessary.

c) Column overloaded. Solution: Inject smaller volume or dilutions (e.g. 1:10 or 1:100) of sample.

d) Sample solvent incompatible with mobile phase. Solution: Adjust solvent: Whenever possible, inject samples solved in mobile phase. Flush polar bonded phase column with 200 ml HPLC grade ethyl acetate, then with intermediate polarity solvent prior analyses.

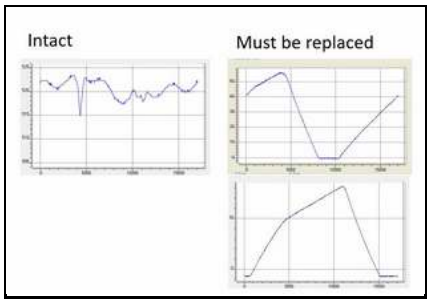
Regular Problem



Where can I arrange my bottles for buffers, eluents and samples?

The bottles for buffers, eluents and samples have to be arranged above or aside of the pumps. Otherwise the pumps will have problems to pump the eluent. Use our eluent trays (AZZ00) which perfectly fit on top of our AZURA housing.

How can I identify check valve malfunction ?



Pressure traces of intact and defective check valves

When your HPLC or FPLC system is unable to reach the given flow rate, system pump service is required. In many cases, a faulty check valve is responsible for the malfunction. Trained personnel with access to a special Service Software (eg. KNAUER Service Tool), can record the pressure profile of the pump for diagnostic purposes. In most cases, check valve malfunction can easily be detected.

The figure shows the pressure profile of an intact pump head (left) and a pump head with faulty check valves (right). KNAUER recommends regular service intervals to avoid any unscheduled downtime of your laboratory equipment.