HPLC Troubleshooting

Before you start any troubleshooting, it is essential to observe safe laboratory practices. Know the chemical and physical properties of any solvents used and have the appropriate Material Safety Data Sheets (MSDSs) readily available. All electrically powered instruments should be shut down and unplugged before starting. Eye protection should also be worn.

The following table lists common HPLC problems encountered, the possible causes and solutions for your quick reference.

Symptom	Cause	Action
Pressure Related Problems		
Low Pressure	Low viscosity mobile phase.	Confirm expected pressure using the Kozeny-Carmen or similar equation.
	Piston seals leaking.	Check for evidence of leaking or wear and replace where necessary.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Air in solvent lines or pump.	Ensure that the reservoirs and solvent lines are fully primed and the purge valve is fully closed.
High Pressure	High viscosity mobile phase.	Confirm expected pressure using the Kozeny-Carmen or similar equation.
	Pump flow-rate malfunction.	Contact manufacturer.
	Tubing blocked.	Working backwards from detector outlet, check source of blockage and replace item as necessary.
	Guard blocked.	Replace guard cartridge.
	Sample precipitation.	Consider sample clarification steps such as filtration or SPE.
	Detector blockage.	Clean the flow cell according to the manufacturer's instructions.
Baseline Related Problems		
Fluctuating Baseline	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Bubbles in flow cell.	Degas the mobile phase and pass degassed solvent through the flow-cell. Do not exceed the cell's pressure limit.
	Contaminated guard.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Detector contamination.	Clean the flow cell according to the manufacturer's instructions.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Sloping Baseline	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Gradient mobile phase.	Consider purer solvents or higher wavelengths. Otherwise, this is normal.
	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Temperature fluctuations.	Use a thermostatically controlled column oven.
	Contaminated column.	Wash the column using an appropriate solvent. Ensure that a gradient method has a wash period at the end.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Blocked solvent reservoir frits.	Ultrasonicate the reservoir frits in water and then methanol.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Peak Shape Problems		
Broad Peaks	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Injection solvent too strong.	Ensure that the injection solvent is the same or weaker strength than the mobile phase.
	Injection volume too high.	Reduce the injection volume to avoid overload. Typically injection volumes of < 40% of the expected peak width should be used.
	Injected mass too high.	Reduce the sample concentration to avoid mass overload.
	Extra column volume too high.	Reduce diameter and length of connecting tubing. Reduce the volume of the flow cell where possible.
	Temperature fluctuations.	Use a thermostatically controlled column oven. Higher temperatures will produce sharper peaks.
	Old guard cartridge.	Replace the guard cartridge.
	Old column.	Do not use columns that have been used with ion-pair reagents for reverse-phase methods. Old columns give much lower efficiencies than new columns. Replace the column if necessary.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Double Peaks	Old guard cartridge.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Negative Peaks	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Wrongly wired detector.	Check the signal polarity from the detector to the recording device.
	Unbalanced RI detector optics.	Refer to manufacturer's instructions.
	Ion pair method.	Inject the sample in the mobile phase.

Symptom	Cause	Action
Peak Shape Problems		
Flat topped Peaks	Detector overload.	Reduce the sample concentration.
	Detector set-up.	Check the detector attenuation and re-zero.
Tailing Peaks	Old guard cartridge.	Replace the guard cartridge.
	Injection solvent too strong.	Ensure that the injection solvent is the same or weaker strength than the mobile phase.
	Injection volume too high.	Reduce the injection volume to avoid overload. Typically injection volumes of < 40% of the expected peak width should be used.
	Injected mass too high.	Reduce the sample concentration to avoid mass overload.
	Old column.	Do not use columns that have been used with ion-pair reagents for reversed phase methods. Old columns give much lower efficiencies than new columns. Replace the column if necessary.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Fronting Peaks	Old or damaged column.	Replace the column.
Peak Size and Retention Problems		
Small Peaks	Degraded sample.	Inject a fresh sample.
	Low analyte concentration.	Increase the analyte concentration.
	Detector set-up.	Check the detector attenuation and re-zero.
	No wash solvent.	Check that the solvent wash reservoir is filled with a miscible solvent and that the injector is set to wash between injections.
	Damaged or blocked syringe.	Replace the syringe.
	Incorrect amount injected.	Check injector loop size and that no more than 50% of this volume is used for partial loop injections.
	Viscous injection solvent.	Reduce syringe draw-time.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
No Peaks	Sample vial empty.	Fill sample vial.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Damaged or blocked syringe.	Replace the syringe.
	Different dwell volume.	For gradient methods, check that the dwell volume of any new system is not very different from any previous system. Apply a final hold time if necessary.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Missing Peaks	Degraded sample.	Inject a fresh sample.
	Immiscible mobile phase.	Check that any solvent already in the column is miscible with the mobile phase. Flush with propan-2-ol or ethanol where necessary.
	Fluctuations in pH.	Buffer the mobile phase so that retention of ionizable compounds is controlled.
Extra Peaks	Degraded sample.	Inject a fresh sample.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade. Gradient methods often show 'ghost-peaks'.
	Immiscible mobile phase.	Check that any solvent already in the column is miscible with the mobile phase. Flush with propan-2-ol or ethanol where necessary.
	Fluctuations in pH.	Buffer the mobile phase so that retention of ionizable compounds is controlled.
	Contaminated guard cartridge.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem , replace the column.
Varying Retention	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Temperature fluctuations.	Use a thermostatically controlled column oven.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Blocked solvent reservoir frits.	Ultrasonicate the reservoir frits in water and then methanol.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Different dwell volume.	For gradient methods, check that the dwell volume of any new system is not very different from any previous system. Apply a final hold time if necessary.
	Piston seals leaking.	Check for evidence of leaking or wear and replace where necessary.
	Air in solvent lines or pump.	Ensure that the reservoirs and solvent lines are fully primed and that the purge valve is fully closed.

For more information, please request Successful HPLC Operation – A Troubleshooting Guide, TG20094.