Problem	Reason	Resolution
System Related		
Low/unsteady system pressure.	Leak.	Check all connections and tighten connections, replace seals.
	Air in pump head.	Degas mobile phase and purge system.
	Dirt in check valve (check whether valve cannot close).	Firstly try purging system at high flow rate to dislodge contamination. Secondly, disassemble check valve and sonicate.
High system pressure.	Blockage (contamination).	Open connections sequentially from the detector back to the pump to locate blockage. Flush capillaries, replace in-line filters or guard columns, clean injector valve, reverse column flow (without detector in-line!) depending on where the blockage was located.
	Blockage (precipitated buffer salts) can happen when the system or user suddenly changes mobile phase composition from high organic to aqueous buffer or vice versa.	Disconnect column and flush with pure water at low flow rate to dissolve buffer salts again.
	High viscosity mobile phase.	Increase temperature, change mobile phase, or decrease flow rate.
	Small stationary phase particles.	Increase temperature, reduce flow rate, use shorter column.
	Crushed particles (sudden pressure spikes can cause porous silica to fracture and generate "fines").	Replace the column (see ProteCol™ HPLC columns pages 208-210).
Noisy, fluctuating, drifting baseline.	System contamination.	Disconnect column and rinse system with a combination of acid (10% nitric acid or 15% phosphoric acid for a short period of time followed by water and a organic wash of 75% acetonitrile/25% IPA over night) Do NOT run the acid through the column!
	Age of the UV lamp.	Replace the UV lamp.
	Temperature fluctuations.	Use column oven.
	Higher UV absorption of either mobile phase A or B causes drift in gradient elution.	Use HPLC grade solvents, check UV cut-off values for mobile phase components, change to higher wavelength.
Regular pulsing of the baseline.	Air in pump head (also causes pulsing of the back pressure).	Degas mobile phase and purge system.
	Dirt in check valve (also causes pulsing of the back pressure).	First try purging system at high flow rate to dislodge contamination. Second disassemble check valve and sonicate.
	Bubble trapped in the flow cell – the detector response changes dramatically when the detector outlet is temporarily blocked with a finger.	Degas mobile phase and purge system.
The Chromatogram		
Tailing peaks.	Wrong pH (some peaks are tailing while others are symmetrical).	The pH of the mobile phase should be 1.5 units or more above or below the pKa value of the analyte to have all molecules either in the charged or in the neutral state.
	Void volumes (all peaks are tailing).	Check connections, replace guard column, replace column.
	Non-specific interactions (some/all sample components can interact with active sites in the flowpath - silanol groups, metal surfaces of tubes and frits).	Replace column with an inert column, replace metal tubing (see inert PEEKsil™ tubing pages 238-239), add additives (e.g. EDTA) into mobile phase, lower pH to <2.5 in order to protonate silanol groups.
Fronting/tailing peaks.	Channeling.	Channeling indicates a serious problem with the column and the column needs replacing. For the interim one can try to reverse the column flow direction.
	"Viscous fingering" – happens when there is a large difference between the viscosity of the sample and the viscosity of the mobile phase.	Try to match the viscosity of the sample with the mobile phase. Ideally, always use mobile phase as diluent.
	Stationary phase degradation.	Loss of ligands when the column is exposed to extreme pH or when the column is very old can lead to peak fronting. Replace the column.
	Column over loading.	Reduce the amount of sample injected or use a column with a larger ID.

Problem	Reason	Resolution
Broad but symmetrical peaks.	Column over loading / sample volume too large.	Reduce the amount of sample injected or use a column with a larger ID.
	Poor column efficiency.	Optimize running conditions (flow rate, temperature) use column with smaller particles, reduce extra column volumes.
	Late eluting sample components from the previous injection.	Use double injections – late eluters only appear in the second run. Extend run time, use strong eluting wash step, use gradient.
Ghost peaks.	Carry-over from contaminated injector.	Clean system/injector until obtaining a clean blank.
	Contaminated mobile phase A in a gradient elution.	Make fresh mobile phase. Use only HPLC grade solvents.
	Air bubbles.	Air bubble cause very sharp spikes. Degas solvents.
	Electronic interference.	Check for source of interference. Use independent power source.
Shifting retention times.	Change in temperature.	Use column oven or operate in a temperature controlled laboratory.
	Mobile phase not mixed properly.	Make sure the mobile phase is well mixed (isocratic) or the solvent mixer (proportioning valve or pump heads A and B) is working correctly (gradient).
	Solvent evaporation.	Make sure solvent bottles are capped.
	Column contamination.	Build-up of non-eluting sample components change the selectivity of the column. Introduce washing procedure at regular intervals.
Low sensitivity.	Wrong wavelength/weak chromophore.	Use photodiode array detector, change detection mode (for example to to fluorescence, RI or electrochemical etc).
	Broad peaks.	Optimize running conditions (flow rate, temperature) use column with smaller particles, reduce extra column volumes. Use stronger eluent, use gradient elution.
	Sample loss due to non-specific binding.	Use inert HPLC system; use inert HPLC columns, use mobile phase additives to reduce non-specific binding.