HPLC Troubleshooting Guide

60 YEARS KNAUER 1962-2022

Science with Passion

KNAUER

1. BASICS AND IDENTIFICATION

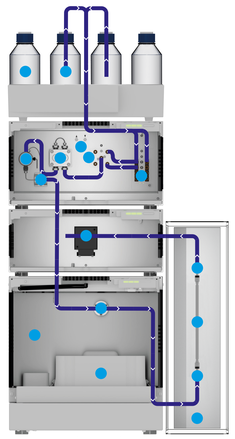
Follow the four steps of troubleshooting using this guide to identify and resolve your HPLC problems. Check obvious explanations and change only one thing at a time.

Document work and stay alert

- Document all important changes within the system.
- Restore system performance parameters and regular perfomance verification runs.
- Compare the relevant parameters to previous tests.
- Document troubleshooting information to solve the next problem faster.

Basic approach

- If there is an obvious problem, identify all indicators and combine them into an overall pattern.
- If there is an undefined problem, use a logical sequence of steps to isolate possible causes.



The blue spots depict possible sources of nonconformances in a HPLC system.

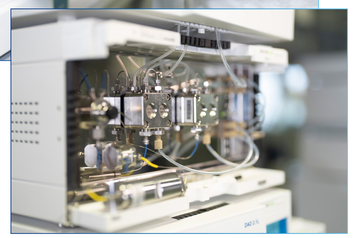
2. PREVENTION

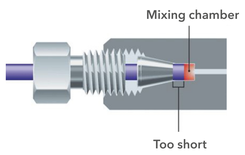
Working hygiene

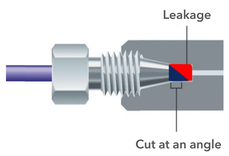
- Always use HPLC grade solvents.
- Renew / Replace water and buffers at least once a week.
- Do not forget to renew the autosampler cleaning solution as well.
- Samples have to be soluble in the mobile phase.
- You should dissolve the sample in starting eluent.
- Filter buffers and all samples.
- Degas the eluents with ultrasonication or other suitable method.
- Do not store columns and the HPLC system in 100 % water/buffer.

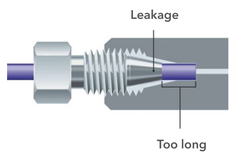
Safety Tools for a longer and better HPLC performance

- Use a precolumn to protect your column.
- Back piston flushing increases the life time of the pump.
- Eluent filters elongate the life time of the system.
- Use precut capillaries and correct fitting ferrules.
- Use a new ferrule for a new position of a capillary.
- Use variable K-Connect fingertight fittings to be flexible with the position (column).
- Eluent pre-heating prevents a temperature gradient in the column.









3. DIAGNOSTIC

4. PROBLEM SOLVING

DRIFT

- Temperature gradient in column
- Warm up phase detector
- Contamination in eluent
- Mobile phase mixing problem
- Column equilibration too slow for the gradient

- Eluent preheating for higher temperature analysis
- Use freshly prepared HPLC grade solvents
- Blank to identify contamination or carry over
- Use a larger mixing chamber (as a test)
- Change wavelength to UV absorbance maximum

REGULAR NOISE

- Check valve blocked (pressure jumps)
- Mobile phase mixing problem
- Pump pulsation / pump broken
- Pressure close to maximum
- Less flow → less pulsation? (pump problem)

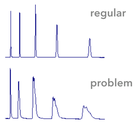
- Clean check valve with 2-Propanol and ultrasonication
- Exchange check valve
- Mix mobile phase beforehand (as a test)
- Minimize back pressure by reducing the flow
- Use a pulse damper (as a test)
- Degas mobile phase

IRREGULAR NOISE

- Leakage
- Mobile phase or flow cell contaminated
- Air trapped in flow cell or pump head
- Detector electronics problem
- Weak detector lamp or in warm up time
- Column bleeding

- Check for loose fittings and tighten them
- Check the quality of the mobile phase
- Flush the system with higher flow (without column)
- Clean the flow cell
- Check the detector lamp life time, running hours or similar and replace if necessary
- Check with another column and replace if necessary

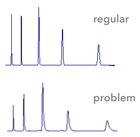
SPLIT PEAKS OR PEAK BROADENING WITH RISING t_R



- Contamination on column or precolumn
- Column bed is broken
- Partially blocked frit
- Sample solvent incompatible with mobile phase
- Small void at column inlet

- Replace precolumn
- Replace frit
- Test the solubility of the sample in the eluent
- If possible reverse flush the column with low flow rates
- Replace column

TAILING



- Sample reacting with active sites
- Wrong column type
- Wrong mobile phase
- Wrong injection solvent
- Small void at column inlet
- Bad fitting ferrule after column

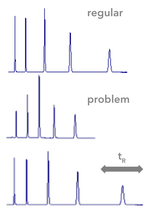
- Check column performance with a standard
- Try to add modifier (e.g. TFA or TEA) or salts
- Adjust the pH
- Dissolve sample in mobile phase
- Exchange the fittings after the column

LOSS OF SIGNAL / NO PEAKS

- Detector lamp too old / not warm
- Loose / broken wire with resulting leakage
- No mobile phase flow or purge valve open
- Change in sample preparation
- Injection needle blocked
- Air trapped in autosampler syringe

- Check lamp energy in diagnostic tool
- Check for leaks and replace leaking wire
- Close the purge valve
- Check the flow rate at the outlet with a graduated vessel
- Perform needle wash with appropriate solvents (e.g. 2-Propanol)

VARIABLE RETENTION TIME



t_R too short:
- Leakage
- Flow of eluent A (water) too low
- Column temperature fluctuations

t_R too long:
- Flow of eluent B too low
- Column problem
- Air trapped in pump head
- Column temperature fluctuations

- Check the flow rate at the outlet with a graduated vessel
- Open the purge valve and check the flow rate of each pump head
- Check the pressure and compare with previous measurements values
- Purge the system with 100 % A or B and higher flow rates
- Replace precolumn
- Renew and adjust the solvents
- Degas eluents and dissolve sample in mobile phase