HPLC Troubleshooting Guide

60 YEARS KNAUER 1962-2022 — Science with Passion

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1. BASICS AND IDENTIFICATION

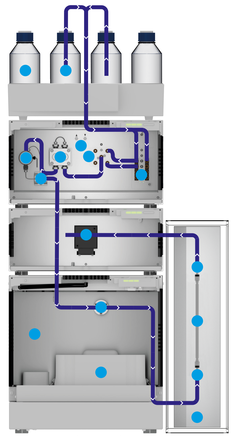
Follow the four steps of troubleshooting using this guide to identify and resolve your HPLC problems. Check obvious explanations and change only one thing at a time.

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.

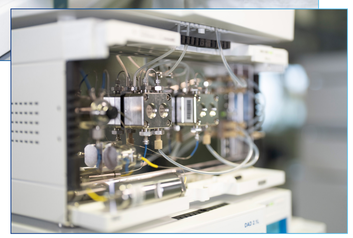
2. PREVENTION

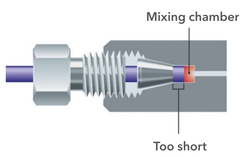
Working hygiene

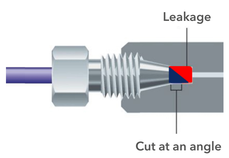
- Always use HPLC grade solvents.
- Renew / Replace water and buffers at least once a week.
- Do not forget to renew the autosampler cleaning solution as well.
- Samples have to be soluble in the mobile phase.
- You should dissolve the sample in starting eluent.
- Filter buffers and all samples.
- Degas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

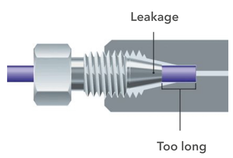
Safety Tools for a longer and better HPLC performance

- 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)

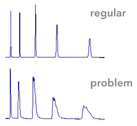
- Clean check valve with 2-Propanol and ultrasonication
- Exchange check valve
- Mix mobile phase beforehand (as a test)
- Minimize back pressure by reducing the flow
- Use a pulse damper (as a test)
- Degas mobile phase

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

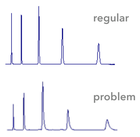
SPLIT PEAKS OR PEAK BROADENING WITH RISING t_R



- 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

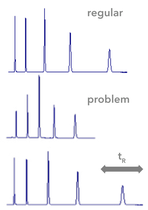
- Check column performance with a standard
- Try to add modifier (e.g. TFA or TEA) or salts
- Adjust the pH
- Dissolve sample in mobile phase
- Exchange the fittings after the column

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

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