Science with Passion

KNAUER

60 YEARS KNAUER 1962–2022

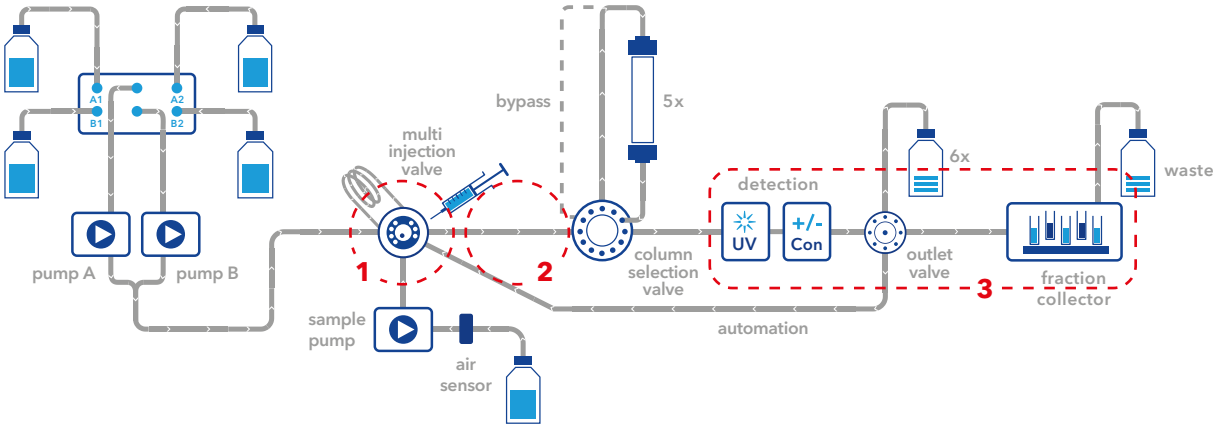
FPLC Troubleshooting Guide

Basics. Prevention. Safety tools.
Identification and problem solving.

think **LC.** think **KNAUER.**

BASICS

- Identify and resolve your FPLC problems by checking for obvious explanations.
- Use a logical sequence of steps to isolate possible causes.
- Change only one thing at a time.
- Know your system: perform a standard separation, if anything goes wrong repeat the method and compare before and after.

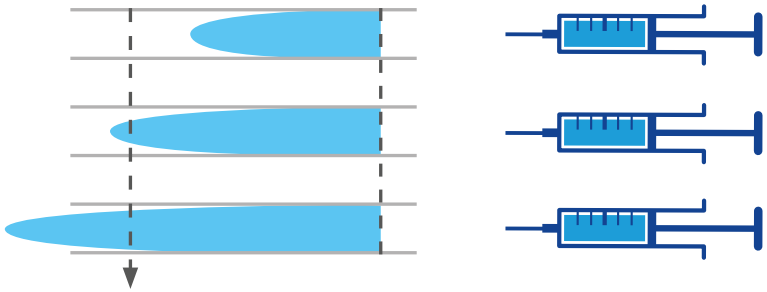


Typical flow scheme of a FPLC system and points of optimization

INJECTION WITH A SAMPLE LOOP

The flow rate of the sample entering the loop creates a parabolic flow profile in the loop. To fill the loop completely, a larger volume needs to be loaded. For small loops a 3-fold overfill is recommended. For larger sample volumes less overfill is needed.

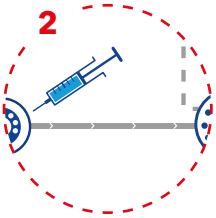
Flow during loop filling



Partial loop fill
– Low reproducibility
+ Low sample loss

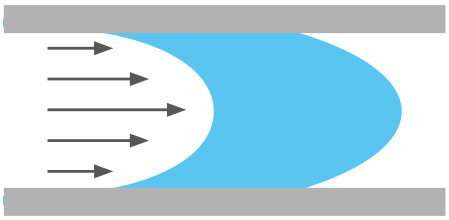
Full loop fill
+ High reproducibility
– High sample loss

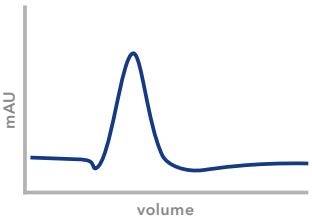
Flow during loop emptying



PEAK BROADENING

Peak broadening is the effect that a product is not staying concentrated, but is diffusing while passing through the system. This effect can impair the resolution of your separation. One can avoid peak broadening by shortening the tubing length of the overall system and reducing inner tubing diameter. But keep in mind, that smaller inner tubing diameters are also increasing the system pressure.

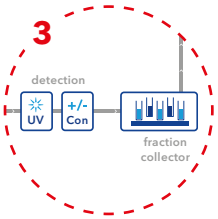




Smaller ID – higher back pressure!

Capillary between column outlet and UV detector should be as short as possible!

mm ID	Colour coding	Dead volume (µl/m)	Recommended flow rate	Article No.	Max. pressure (bar)
0.13	Red striped	13	Up to 0.25 ml/min	A2522	420
0.25	Blue striped	49	0.25–2 ml/min	A2524	385
0.50	Orange striped	196	2–10 ml/min	A2525	240
0.75	Green striped	442	10–50 ml/min	A2526	240



FRACTION DELAY VOLUME / DEAD VOLUME

The fraction delay volume of a system is the volume between the UV detector flow cell and the fraction collector. It is important to know this volume for collecting peaks accurately. The software will take this volume into account and start fractionation in a delayed manner accordingly.

Peak is detected

Fraction delay = dead volume/time

Peak reaches the fraction collector

PREVENTION

- Always degass and filter your buffers
- Filter your sample
- Refresh buffers on a regular basis
- Never leave system overnight on high salt buffer
- Regular clean up routine
- Check system performance with system test
- Clean columns according to manufacturers recommendation on a regular basis

Storage of your system	Overnight	Over the weekend	Longer then a week
Buffer high salt	👎	👎	👎
Buffer low salt	👍	👎	👎
Water	👍	👍	👎
20 % Ethanol	👍	👍	👍

1. START UP ROUTINE

- Switch on detector/system
- Degass and filter buffers
- Temper buffers
- Purge pump
- Wash system
- Equilibrate column
- Prepare sample

2. SYSTEM CHECK UP

- Check for leakage
- Check for stable pressure
- Check for stable UV signal
- Exchange tubes in fraction collector
- Check waste level
- Check buffer levels
- Check or renew back piston rinsing solution

4. SHUT DOWN ROUTINE

- Flush system with suitable solution for storage
- Flush injection port and sample loop
- Rinse fraction collector valve
- Optional: switch of detector lamp
- Rinse pH electrode and store in storage solution
- Never switch of system at 4°C!

3. CLEANING OF THE SYSTEM

- Remove pH electrode from the flow path
- Flush system with water
- Flush system with 0.5–1M NaOH
- Flush with water
- Store in 20 % Ethanol

SAFETY TOOLS

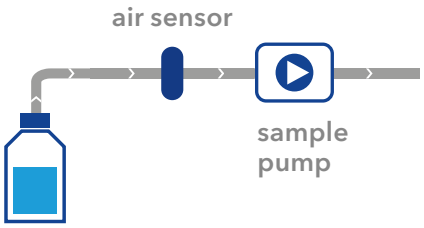
AIR SENSOR

Air sensors are very useful tools to protect columns from damage by air and supports automatic sample loading.

Using an air sensor for column protection.

Upon air detection, various functions can be programmed. The run can be paused or stopped to prevent air from entering the system.

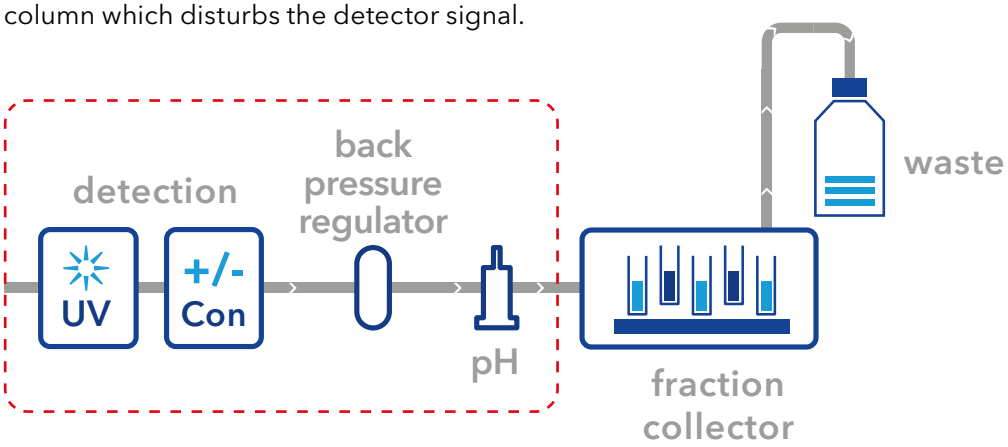
Using an air sensor for automated sample injection.



When the end of the sample is detected, the software allows the run to start or continue automatically.

BACK PRESSURE REGULATOR

A back pressure regulator (BPR) applies a constant pressure to the system. It prevents the formation of air bubbles due to the pressure drop after the column which disturbs the detector signal.



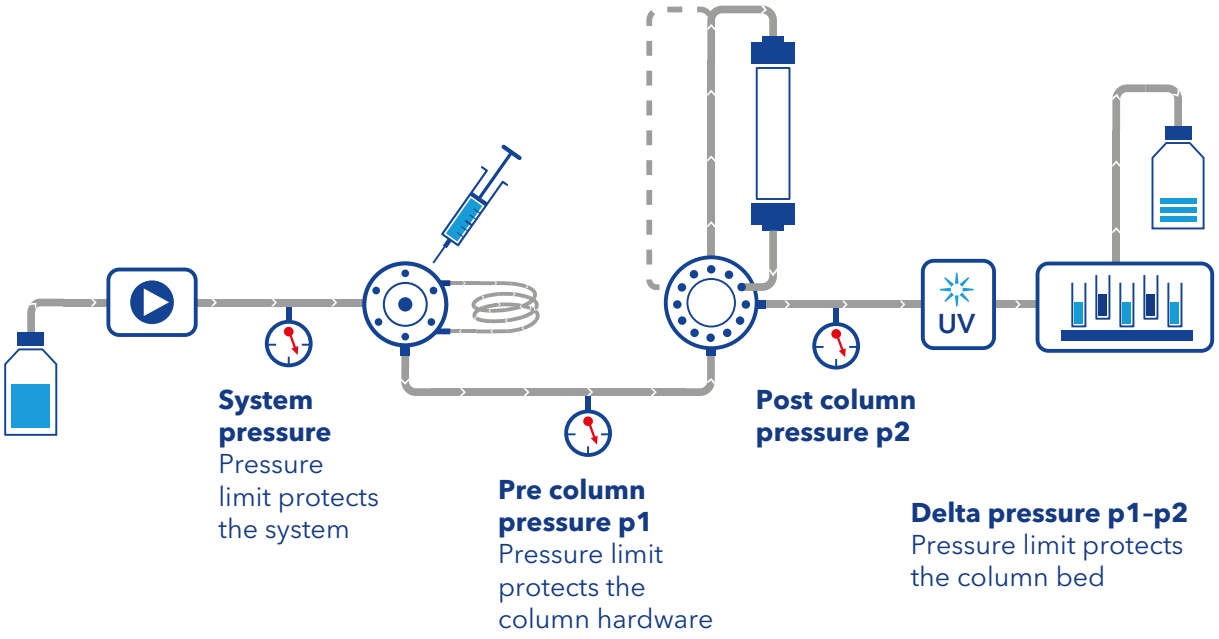
Place BPR **after** the UV detector and conductivity monitor.

Place BPR **before** the pressure sensitive pH electrode.

FPLC Tutorial: Back pressure regulator

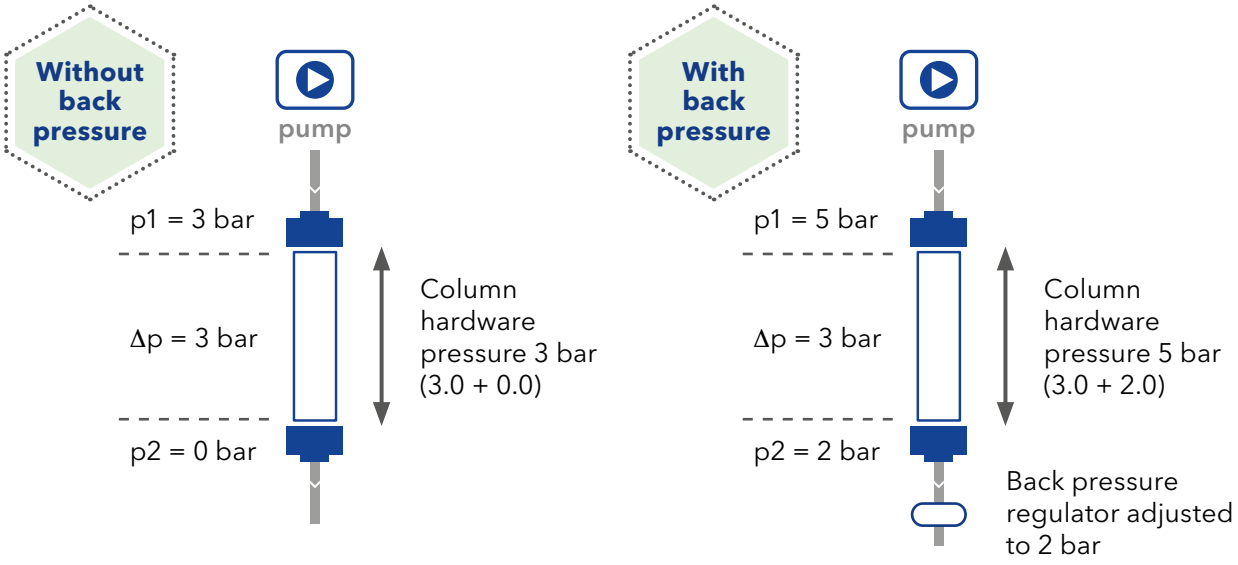
PRESSURE MEASUREMENT

Several FPLC columns and resins are very pressure sensitive, consequently pressure control is very important. The pressure before and after the column is monitored and the software calculates automatically the pressure difference over the column resin. If the pressure differential value (DP) exceeds the pressure limit, the pump stops or pauses.



PRESSURE MEASUREMENT AND BACK PRESSURE REGULATOR

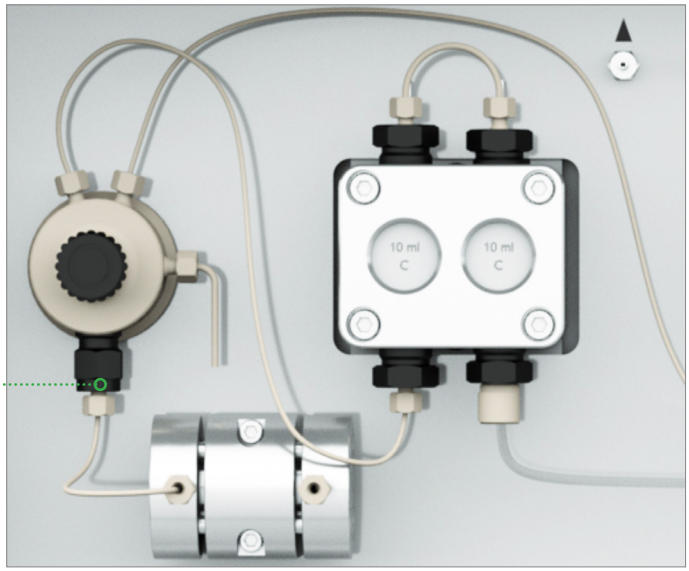
The additional pressure behind the column affects only the column hardware. The column bed is not affected.



FILTER

Injection of samples via the system pumps is possible. Always filter samples before injection. When using the pump P 6.1L for injection of sample the filter located in the pressure sensor must be removed to avoid blockage. Insert the adapter, which you can find in the accessory kit of the pump. The adapter has no filter effect. Use an inline filter for column protection which you can simply install in front of your column.

If using the pump P 6.1L for sample application, replace the filter cartridge by dummy cartridge.



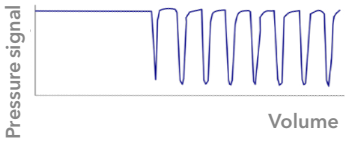
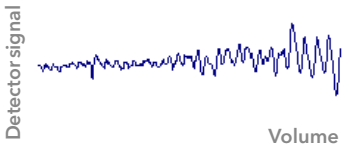
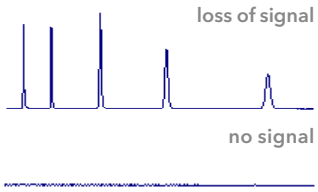
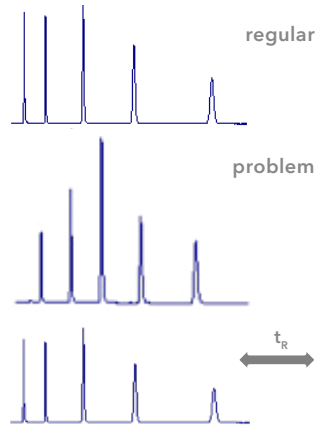
Instead use an inline filter for column protection.

FPLC Tutorial:
Pressure sensor

FPLC Tutorial:
Exchange of inline filter frit

FPLC Tutorial:
Inline filter

IDENTIFICATION AND PROBLEM SOLVING

Problem	Cause	Solution
System pressure to high	**1.** Column clogged **2.** Filter of pressure sensor clogged when applying sample via the major pump **3.** ID of tubing to small **4.** Viscositiy of buffer or sample to high (especially working at 4°C)	**1.+2.** Clean the system and/or column with Ethanol or 0.5–1M NaOH **2.** Exchange filter of the pressure sensor by dummy or use a sample pump **3.** Use capillaries with ID according to the recommended flow rate **4.** Dilute and/or filter sample and decrease the flow rate
Pressure fluctuation 	**1.** Air bubbles in buffer **2.** Air bubbles in pump head **3.** Positioning of buffer bottles	**1.** Degas buffer or/and use degasser in the system. Adjust temperature of the buffer **2.** Check for prefilled inlets and purge pump with 50 % of maximum flow rate **3.** Remove persistent air bubbles by flushing the system with 20 % isopropanol **4.** Place bottles on top or at the same heigth of the system/pump
UV signal fluctuation 	**1.** Dirty flow cell **2.** Air in flow cell	**1.** Clean the flow cell according to manual **2.** Flush flow cell and use back pressure regulator
Loss of signal/No peaks 	**1.** Detector off **2.** Leakage **3.** No flow **4.** Problem during injection	**1.** Switch on detector **2.** Check for leakage and tighten capillary connection of renew capillary **3.** Check the purge valve, check the flow rate at the outlet with a graduated vessel, exchange check valve **4.** Check position of injection valve
Retention time delay/shift 	**1.** Leakage **2.** Gradient mixing not precise **3.** Column problem **4.** Pump/flow problem	**1.** Check for leakage and thighten capillary connection or renew capillary, Check the flow rate at the outlet with a graduated vessel **2.** Check the flow rate of each pump head, Check buffer composition. Purge the system with 100 % A or B and higher flow rates. Renew and adjust the solvents **3.** Clean column, Check for column performance, if mandatory renew **4.** Check the flow rate at the outlet with a graduated vessel, exchange check valve

THE FLEXIBLE PROTEIN PURIFICATION PLATFORM

Design your FPLC system according to your purification task!

Complete solutions for FPLC (Fast Protein Liquid Chromatography) on a small footprint: AZURA® FPLC systems combine flexibility and reliability. The biocompatible/metal-free AZURA® FPLC is the perfect choice for your protein purification task.

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Questions about our products? Get your answer.

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Application Support and on-demand Method Development

KNAUER is the proud winner of the German Innovation Award 2022 in the category of medium-sized businesses.

(U)HPLC • FPLC • SMB • Osmometry and units for the production of lipid nanoparticles (LNP)

Innovation

Own hardware and software development

Customized solutions

Pumps, detectors, valves and systems adapted to your needs

Made in Germany

Independent and family-owned since 1962

think **LC.** think **KNAUER.**

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