Preparative HPLC Troubleshooting Guide

Your guide to solving common problems and staying productive

Agilent InfinityLab

Places to start

Solvents

- Use brown borosilicate bottles to avoid algal growth.
- Use only HPLC-grade solvents.

Prepare and power up the pump

- Inspect solvent bottles and inlet filters.
- Always use seal wash when installed and purge the pump.
- Use the appropriate system conditioning method.

Daily tasks

- Replace aqueous and organic mobile phases every second day.
- Check seal wash solvent.
- Flush the system with the composition of your application.

Weekly tasks

- Change seal wash solvent and bottle, and inspect solvent filters.
- Check system backpressure and change filters if necessary.

Pump shutdown

- Flush all channels to remove salt deposits and particulate matter.
- Flush the system with appropriate storage solvent and power down the system.

Daily shutdown

- Remove high and low pH solvent compositions from system.
- Purge more than three column volumes with neutral solvents.
- Leave system with 90% organic and 10% water as solvent composition to avoid clogging or faster degradation of column.

Maintenance

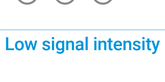
Agilent Lab Advisor software helps you manage your Agilent LC instruments to achieve high-quality chromatographic results in the most efficient way by ensuring high instrument performance, productivity, and reliability. It is available free of charge.

- Diagnostic tests to evaluate performance
- Easier maintenance of all Agilent LC modules
- Comprehensive reports generated to ease communication with Agilent service

Poor recovery

Possible Cause	Solution
Incorrect delay volume / time between detector and fraction collector	Perform delay calibration, check for correct UV delay volume / MS delay time in method
Capillary / delay coil id too large—possible peak dispersion between detector and collector	Use knitted delay coil of appropriate length and inside diameter

No trigger / late trigger: MSD



Possible Cause	Solution
Target mass not found	Ensure the correct mass, polarity, and ion species are selected
Incorrect signal selected as trigger	Select correct signal in MSD fraction collection (independent of monitored signals)
Incorrect trigger combination	Select OR combination if target is not visible by all detectors that are selected as triggers
Incorrect threshold/ slope settings	Check EIC signal of the target ion; adjust threshold if necessary
Remote cable unplugged	Ensure the fraction collection remote Y cable (p/n 5188-8057) connects the MSD with the LC

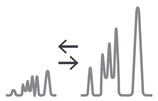
No trigger / late trigger: other detectors

Possible Cause	Solution
Compounds not visible in selected trigger signal	Select correct signal in the fraction collector method (independent of monitored signals)
Incorrect threshold/ slope settings	Check current settings using the fraction preview function of the fraction collector method
Incorrect trigger combination	Select OR combination if target is not visible in all detector signals that are selected as triggers
Incorrect pump / flow rate linked to fraction collector	Link preparative LC pump to fraction collector; if not, enter correct flow rate in fraction collector method

Lower than expected volume in tubes

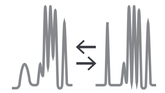
Possible Cause	Solution
Loss of pump prime	Check pressure signal recorded during the failed run
Discharge from pressure relief valve	Check for solvent in pressure relief valve waste line

Low signal intensity



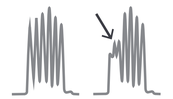
Possible Cause	Solution
Incorrect sample volume drawn	G7158B—prime injection path with Change to prep LC sampler; adjust sampling speed to sample viscosity
Detector in split flow: split too low / MS flow modulator seal worn out / make-up flow insufficient	Adjust split ratio; check number of switches and replace rotor seal if necessary; check make-up solvent flow

Loss of resolution



Possible Cause	Solution
Mobile phase contaminated/ deteriorated (causing retention times and/or selectivity to change)	Prepare fresh mobile phase
Column performance	Run a checkout sample to check the performance of your preparative LC column

Peak splitting / sample breakthrough



Possible Cause	Solution
Sample loading too high	Reduce sample load; separate organic and aqueous channels and place autosampler into organic flow path, adding aqueous channel at column head
Injection volume too large	Reduce injection volume (increase sample concentration)
Sample solvent too strong	Use weaker sample solvent
Void in column	Load & Lock column: use the packing station to recompress the column bed
Contamination	If guard column present, remove and attempt analysis; replace if needed. Reverse and flush prep column if clogged. If problem persists, retained contaminants may be present; use restoration. If persists, inlet frit may be partially plugged
Small (uneven) void at preparative LC column	If guard column present, remove and attempt analysis. If needed, replace guard/preparative LC column. Check for loose fittings
Sample solvent incompatible with mobile phase	Adjust solvent. Whenever possible, inject samples in mobile phase

Pressure increase

Possible Cause	Solution
System blockage	Check flow path (needle seat, capillaries, filter, frits). Capillaries not properly sized for flow rate
Water/organic systems: buffer precipitation	Test buffer-organic mixtures for compatibility; separate aqueous and organic phase flow paths—place autosampler in organic flow path. Mixing point with aqueous phase is shifted to T-piece, upstream of column

High column backpressure

Possible Cause	Solution
Column blockage	Better sample cleanup; use guard column
Particle size too small	Use larger d_p packing
Plugged inlet frit	Replace prep LC column or replace the inlet/outlet frit of your Load & Lock column

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