Preparative HPLC Troubleshooting Guide

Your guide to solving common problems and staying productive

Plates to start

Solute types
- 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.

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.

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.
- Use the appropriate system-conditioning method.

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.

Poor recovery

Possible Cause
- Incorrect delay volume ratio between detector and fraction collector
- Incorrect trigger combination
- Incorrect trigger signal on trigger

Solution
- Platform delayed calibration, check for correct UV delay volume/M5 delay time in method
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Check current settings using the fraction preview function of the fraction collector method

Crude
- Incorrect delay volume ratio between detector and fraction collector
- Incorrect trigger combination
- Incorrect trigger signal on trigger

Solution
- Platform delayed calibration, check for correct UV delay volume/M5 delay time in method
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Check current settings using the fraction preview function of the fraction collector method

Pure
- Incorrect delay volume ratio between detector and fraction collector
- Incorrect trigger combination
- Incorrect trigger signal on trigger

Solution
- Platform delayed calibration, check for correct UV delay volume/M5 delay time in method
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Check current settings using the fraction preview function of the fraction collector method

No trigger/late trigger MISD

Possible Cause
- Incorrect delay volume ratio between detector and fraction collector
- Incorrect trigger combination
- Incorrect trigger signal (if trigger is not visible by all detectors that are selected as triggers)

Solution
- Platform delayed calibration, check for correct UV delay volume/M5 delay time in method
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Check current settings using the fraction preview function of the fraction collector method

No trigger/late trigger other detectors

Possible Cause
- Compounds not visible in selected trigger signal
- Incorrect trigger combination
- Incorrect trigger signal (if trigger is not visible by all detectors that are selected as triggers)

Solution
- Select correct signal in the fraction collector method (independent of monitored signals)
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Check current settings using the fraction preview function of the fraction collector method

Loss of resolution

Possible Cause
- Mobile phase contaminant detected (causing retention times and/or selectivity to change)
- Column performance

Solution
- Prepare fresh mobile phase for re-chromatography
- Run a checkout sample to check the performance of your preparative LC column

Peak splitting/sample breakthrough

Possible Cause
- Sample holding too high
- Injection volume too large
- Column packing

Solution
- Reduce sample load to acceptable organic and aqueous channels and place acetonitrile organic flow path, adding aqueous channel at column head

Flow insufficient
- Detector in split flow: split too high
- Incorrect pump/flow rate

Solution
- Adjust split ratio; check number of monitored signals
- Change to prep LC sampler; adjust sampling speed to sample viscosity

Low signal intensity

Possible Cause
- Incorrect sample volume on down
- Detector in split flow: split too high
- Detector in split flow: split too low

Solution
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Select correct signal in MSD fraction collector method (independent of monitored signals)

Pressure increase

Possible Cause
- System blockage
- Water/organic system buffer precipitation

Solution
- Check flow path (leaks seals, capillaries, FLSC, CDS, XDS, and detector not properly sized for flow rate)
- Test buffer-organic mixtures for compatibility; separate aqueous and organic phase flow paths—place acetonitrile organic flow path, adding aqueous channel at column head

High column backpressure

Possible Cause
- Partially too small
- Partially too small

Solution
- Select correct signal in MSD fraction collector method (independent of monitored signals)
- Select correct signal in MSD fraction collector method (independent of monitored signals)

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