LC Troubleshooting Guide

Your guide to solving common problems and staying productive

Agilent InfinityLab

Places to Start

Solvents

- Use brown borosilicate bottles to avoid algae growth
- Prepare solvent volume to be used up within 1 to 2 days
- Use only HPLC-grade solvents filtered through 0.2 µm filters

Preparing and powering up the pump

- Inspect solvent bottles and inlet filters for damage or coloring
- 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

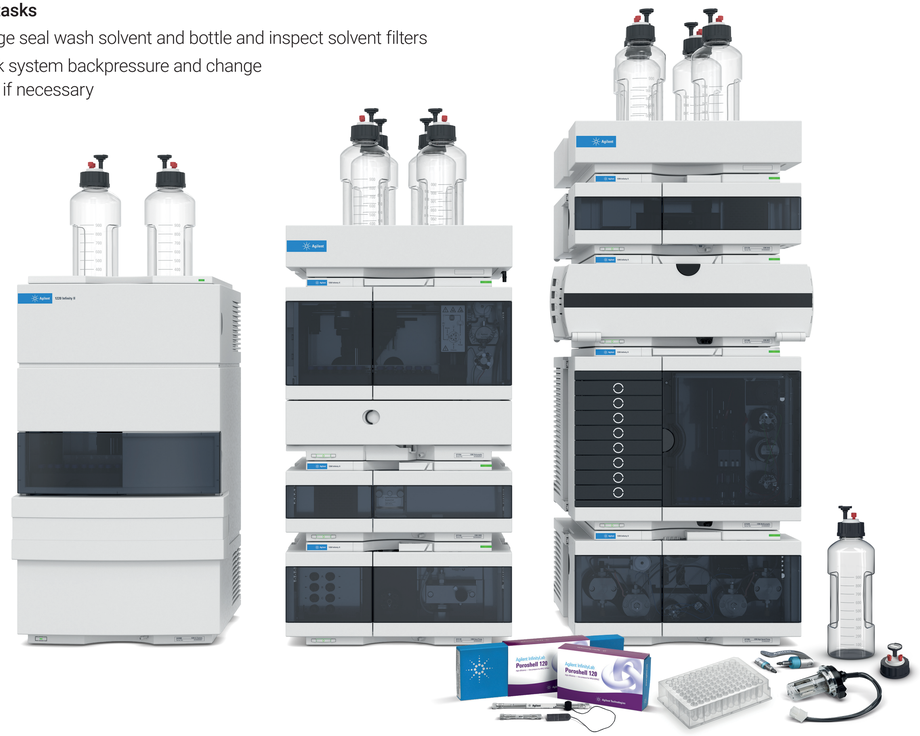
- Change seal wash solvent and bottle and inspect solvent filters
- Check system backpressure and change filters if necessary

Pump shutdown

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

Handling of acetonitrile

- If possible, use 5 to 10% of water in your mobile phase
- Be sure to avoid ACN evaporation
- Don't leave ACN on the system for more than 2 to 3 days
- Perform a periodic warm water wash (60 to 70 °C) if you face problems

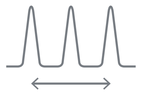


Maintenance

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

Retention Time Drift



Possible Cause	Solution
Inconsistent online mobile phase mixing	Ensure gradient system delivers constant composition; compare with manual preparation of mobile phase
Variation in column temperature	Thermostat or insulate column; ensure constant lab temperature
Insufficient equilibration time with gradient run or change in isocratic mobile phase	Make sure at least 10 column volumes pass through column after sample run
Selective evaporation of mobile phase component	Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase
Contamination buildup	Occasionally flush column with strong solvent
Column overloaded with sample	Decrease injection volume or concentration

Pressure Fluctuation

Possible Cause	Solution
Leak in the system	Identify the channel and clean or replace check valve; replace pump seals
Buildup of particulates	Filter sample and mobile phase
Bubble in pump	Perform solvent degassing; sparge solvent with helium

Pressure Increase

Possible Cause	Solution
System blockage	Check flowpath (needle seat, capillaries, filter and frits)
Water/organic systems: buffer precipitation	Test buffer-organic mixtures to ensure compatibility

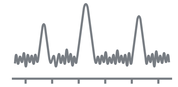
High Column Backpressure

Possible Cause	Solution
Column blockage	Better sample cleanup; use guard column
Mobile phase viscosity too high	Use lower viscosity solvents or higher temperature
Particle size too small	Use larger d_p packing
Plugged inlet frit	Replace column

Drifting Baseline

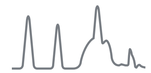
Possible Cause	Solution
Positive/negative direction: contaminant buildup/elution	Flush column; clean up sample; use pure solvents
Positive/negative: difference in refractive index of injection solvent	Use mobile phase for sample solvent
Temperature changes	Insulate and thermostat column and tubing

Noisy Baseline



Possible Cause	Solution
Contamination	Use degassed HPLC-grade solvents; flush system; clean up sample
Detector problems	Check number of hours of UV lamp; replace UV lamp or flow cell

Ghost Peaks



Possible Cause	Solution
Peaks from previous injection	Flush column to remove contaminants; check with blank injection
Contamination; unknown interferences in samples	Proper sample cleanup
Ion pair: disequilibrium	Prepare sample in actual mobile phase to minimize disturbance
Contaminated mobile phase	Check your mobile phase
Bubbles in solvent	Check and degas your solvents

Peak Tailing

Possible Cause	Solution
Unswept dead volumes	Minimize number of connections; ensure injector seal is tight; ensure fittings are properly seated
Column performance	Change mobile phase; replace column
Silica-based: column degradation	Use specialty, polymeric, or sterically protected column
Silica-based: basic interactions with stationary phase	Use stronger mobile phase or add appropriate base (e.g., TEA)

Peak Broadening

Possible Cause	Solution
Injection volume too large	Decrease injection volume or solvent strength of injection solvent; use gradient methods
Low sampling rate of data system	Increase data rate
Detector cell volume too large	Use smallest possible cell volume
Injection volume too large	Decrease injection volume

Sensitivity Problems

Possible Cause	Solution
Peaks are outside of sensitivity range of detector	Dilute/concentrate sample to bring into linear region
Sample-related losses during preparation	Use internal standard during sample preparation; optimize sample preparation method

Leaks

Possible Cause	Solution
White powder at fitting/ loose fitting	Tighten fittings; replace capillaries
System leak	Identify location checking leak sensors/errors; check flow cell

Discover more best practices for using an Agilent LC system:
https://www.agilent.com/chem/lc-best-practices

Training courses are available at:
https://www.agilent.com/crosslab/university

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