LC Troubleshooting Tips

Tailing Peaks

Causes	Solution
Secondary interactions	• For bases increase pH (as permitted); for acids decrease pH; increase ionic strength of buffer (as permitted); change column type.
Dead volume	• Reconnect the column with the fitting to reduce dead volume.
Column degradation	• Replace the column.
Column void	• Fill void (previous performance unlikely to be fully recovered).
Interfering peak	• Use a longer column; further method development.
Wrong mobile phase pH	• Adjust pH (2 clear pH units from pKa recommended).
Sample chelating to active sites	• Limit interaction via ion pair reagent, modifier or sequester agent, change column or post injector wettable flow-path.
Inadequate buffering	• Use 50-100 mM buffer concentration (UV methods).
Sample loading	• Reduce sample concentration.

Extra Peaks

Causes	Solution
Other components in sample	• It is normal to see extra peaks if they are present in the sample.
Late eluting peaks from previous injection	• Increase run time or solvent strength; increase flow rate to increase the number of column volumes per unit time.
Ghost peaks	• Check purity of mobile phase; use ghost traps (if applicable).

High Pressure

Causes	Solution
Flow rate set too high	• Reduce flow rate setting.
Blocked column	• Backflush column (if permitted) or replace column.
Incompatible mobile phase (precipitated buffer or immiscible)	• Use correct mobile phase; wash column and re-equilibrate.
Improper column	• Use correct column with correct dimensions and particle geometry.
Injector blockage	• Clear blockage (review needle, loop, valve assembly and HPV outlet).
Guard column / cartridge blockage	• Replace or remove guard column.
Column in-line filter blockage	• Replace or remove in-line filter.
Column temperature too low	• Set adequate column temperature.
Sensor malfunction	• Repair or replace pressure sensor.
Pump in-line filter blockage	• Replace in-line filter.

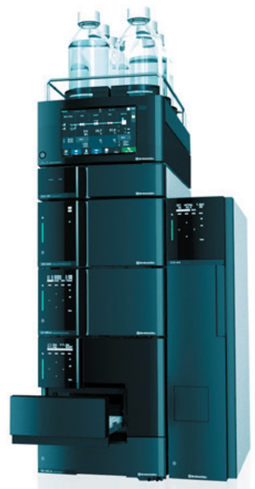
Fluctuating Pressure

Causes	Solution
Air bubbles	• Purge the solvent lines to remove the air bubbles.
Worn pump seals	• Replace seals.
Check valves	• Sonicate the check valves in isopropanol. • Change the check valves if problem persists.
Leaks	• Degradation of pump seals could cause small leaks. Replace the seals. Check connections.
Inadequate degassing	• Degas solvent; replace mobile phase frits; repair degasser (if applicable).
Using a gradient elution	• Pressure cycling caused by viscosity changes is normal but, use adequately sized mixer volume.

Basic Steps

Follow these three steps to isolate where the problems is.

Check the obvious explanations first and change only one thing at a time!



Check the Basics:

- Power supply
- Electrical connections
- Signal connections
- Sample preparation
- Analytical conditions
- Mobile phase preparation
- Seal washes primed
- Solvent flowing / no air bubbles

Identify the Cause:

- Clearly define the problem
- Review sample and maintenance logs to identify trends in the data or possible problem indicators
- Use a logical sequence of steps to isolate possible causes

Document Everything:

- Document all troubleshooting steps and results; this may help you identify and solve the next problem faster
- Always inject a known sample and compare to previous data as a reference to ensure restored performance

Still having problems?

Still struggling? Let us know.

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Changes in Sensitivity

Causes	Solution
Injector issue	• Changes in dispensing volume of injector, use a system suitability sample to determine volume changes. • Check batch / method details to ensure the correct volume was programmed. • Increase needle and loop flushing protocols to ensure no carryover from injection. • Purging the injector metering pump.
Sample	• Degradation could reduce peak signal with increases in impurity peaks. Prepare a fresh sample. • Check sample preparation to ensure the appropriate concentration was prepared.
Detector	• If all peaks have changed in sensitivity check detector for issues and parameters. • Check the lifetime of the lamp and change if above the recommended limit. • Flow cell window(s) may need replacing.
Loss of column performance	• Check the peak widths and resolution. Test the performance of the column using your standard test for loss in performance.
Instrument leaks	• Check for loose fittings post injector on the system.

Changing Retention Times

Causes	Solution
Flow rate	• Check the method uses the correct flow rate. Ensure the flow rate is accurate using a flow meter.
Insufficient equilibration	• The reversed phase column should be equilibrated using at least 10 column volumes. If 10 column volumes are insufficient, increase the equilibration time. This should be extended for other techniques such as ion exchange and HILIC.
Poor temperature control	• Check the method uses the correct temperature. Ensure the temperature in the column oven is accurate.
Change in column dimension	• Ensure the correct column including dimensions are being used.
Change in column stationary phase environment	• Do not use a column which has ion pairing reagent for other mobile phases due to memory effects. • Stationary phase 'de-wetted' (historically incorrectly termed 'phase collapse').
Improper mobile phase	• Ensure the mobile phase is accurately prepared. • If using the pump to proportionate the mobile phase, ensure the pump is accurately dispensing mobile phase. • Ensure the correct mobile phase is being used and the correct lines are being chosen on the method.
Instrument leaks	• Check for loose fittings throughoutthe system.
Air bubble in pump	• Purge pump via purge valve.

Low Pressure

Causes	Solution
Partial leak in system	• Check all connections and retighten any which have leaks.
Flow rate	• Check the method has the correct flow rate. • Test the flow rate accuracy using a calibrated flow rate meter or collect a specific volume and monitor the time required.
Method	• Check if method is using correct temperature and correct solvents.
Incorrect column	• Use correct column with correct dimensions and particle geometry.
Column temperature too high	• Set adequate column temperature and check no column damage if exceeded column temperature limit.
Sensor malfunction	• Repair or replace pressure sensor.

Broad Peaks

Causes	Solution
Sample loading	• Reduce sample concentration or injection volume.
Column issue	• Degradation of the column, column should be replaced.
Oven setting issue	• Check column oven temperature is correct. Higher column temperatures typically result in faster compound elution (NB keep under column temperature limits as described by manufacturer).
Mobile Phase	• Check correct mobile composition is being used.
Instrument settings	• Detector / sample frequency should be increased to see if this improved peak shapes. • Additional tubing or other factors have increased system dispersion volume, check tubing lengths and internal dimensions. • Check correct flow rate is being delivered / set in method correctly.

Fronting Peaks

Causes	Solution
Column degradation	• Replace the column.
Mobile phase / sample diluent incompatibility	• Adjust the mobile phase composition. Use initial mobile phase solvent (if applicable).
Sample overload	• Decrease sample concentration.

Split Peaks

Causes	Solution
Soiled guard or column inlet	• Replace guard or inline filter frit; reverse flush column (if permitted).
Sample diluent incompatible with mobile phase	• Change sample diluent. Use initial mobile phase solvent composition (if applicable). Use Co-Solvent or POISe injection function.
Analyte properties	• Possibility of isomer or analyte interconversion – alter conditions to correct for this.

Loss of Resolution

Causes	Solution
Changes in peak width	• Changes in column performance and sample load / column efficiency can result in wider peak widths. Ensure the chromatographic performance of the column is sufficient, or replace the column, and ensure the same load is consistent.
Changes in retention time	• See changes in retention time section.
Mobile phase deterioration or evaporation	• Prepare fresh mobile phases.

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