Liquid Chromatography

TROUBLESHOOTING GUIDE

SHIMADZU

Excellence in Science

Contents

Introduction

Instrument downtime is often costly and time consuming, but frequently the problems can be resolved quickly with some troubleshooting knowledge.

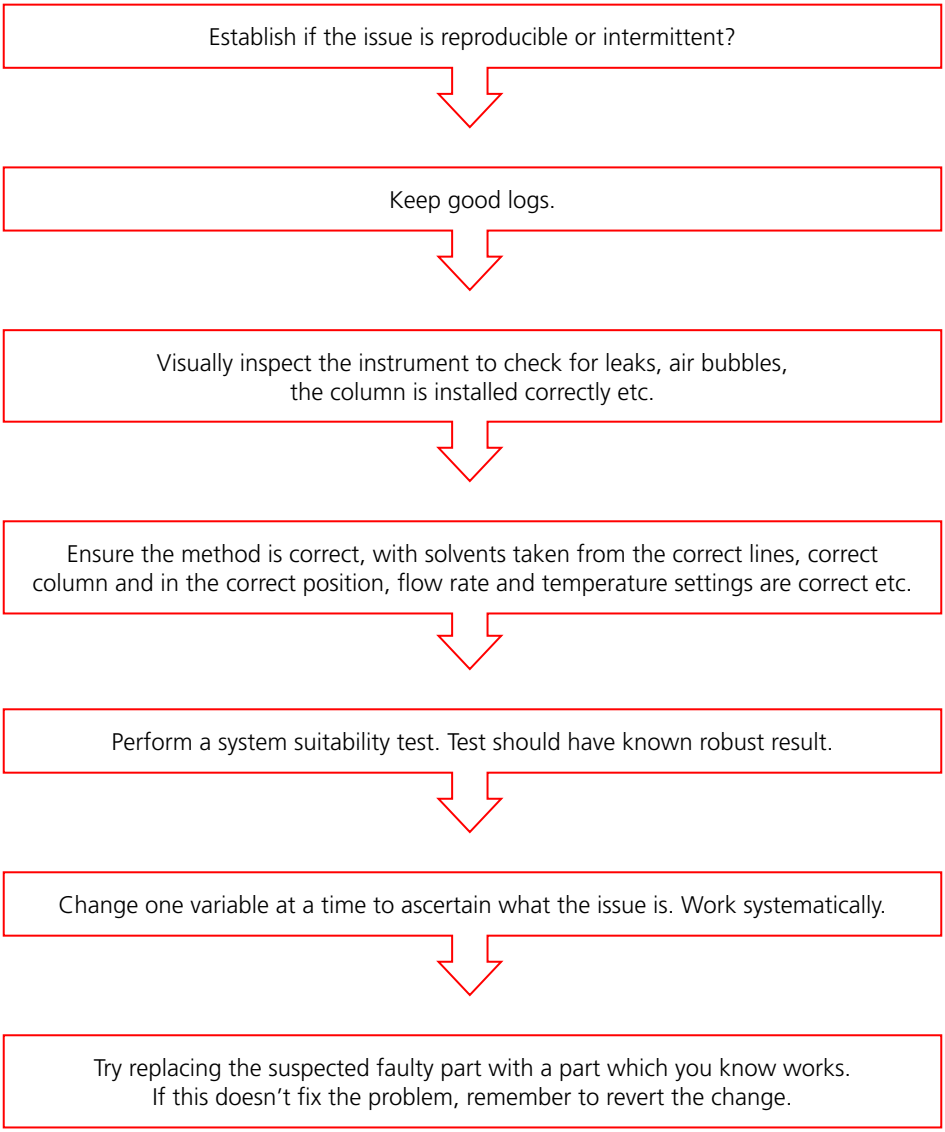
This Liquid Chromatography Troubleshooting Guide is designed to assist chromatographers assess common LC problems. The booklet includes how to effectively troubleshoot and fix these issues to allow you to get your system back up and running and continue your analyses.

Prevention is better than cure – Basic tips prevent common LC issues

Many common issues can be prevented by replacing regularly consumed items such as seals to keep the system running smoothly. Review below tips to keep your instruments running smoothly:

- Use seal washes and replace the solution regularly to maintain the life of the pump seals.
- Don't keep aqueous buffers for too long to avoid microbial growth.
- Ensure organic mobile phases are capped to prevent compositional changes.
- Use appropriate solvent grades. Ideally, HPLC grade should be used as the minimum standard to avoid significant particulates in the mobile phase which can block parts of the LC or cause disturbances in the baseline.
- Flush the instrument (and column) with a non-buffered mobile phase such as H_2O/MeCN (1:1 v/v) to avoid salt precipitation or column degradation.
- Ensure the detector is not stored in harsh mobile phase and do not leave the lamp on when not in use to avoid diminishing the lamps lifetime.
- Have appropriate warranty or maintenance cover to help reduce downtime due to unexpected problems.

Good practice for troubleshooting



Keep records

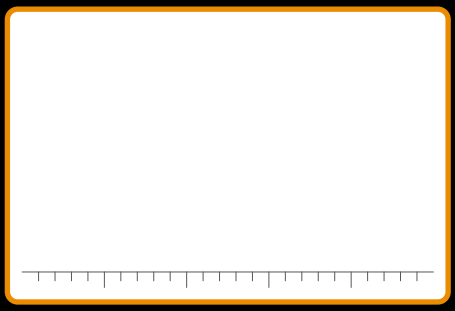
Records can be the key to locating issues as well as acting as a log of information. Recording usage of instruments, columns as well as sample and mobile phase preparation can allow the information to be used when an issue occurs. Recording something as simple as the mobile phase used with a certain column, could highlight why a blockage or pressure change has occurred.

Log Type	Type of information recorded
Sample / Mobile Phase Preparation	- How mobile phases are prepared. - Solvent batch numbers. - Calibration of equipment such as pipettes and balances. - Sample preparation including volumetric size and diluents.
Instrument	- Modules, mixer size, serial numbers, warranty information. - Maintenance log including dates of replacement parts. - LC characteristics such as dwell volume, dispersion, autosampler accuracy and reproducibility, typical pressures.
Column	- Manufacturer system suitability test / chromatographic performance. - Date first received and date first used. - Storage information. - Summary of the column usage, number of injections, pressure during runs, type of mobile phases used.

Still having problems?

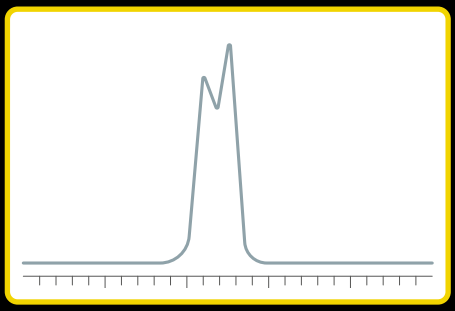
Still struggling? Let us know at **lc@shimadzu.co.uk**

No Peaks



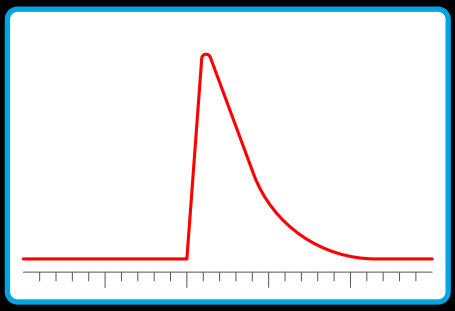
Causes	Solutions
Detector setting issue	- Check the detector lamp is turned on. - Check electrical cables are connected. - Check the life span of the lamp and replace if exceeded hours. - Ensure an appropriate detector is used for the physico-chemical properties of the analyte. - Check the method procedure uses appropriate detector settings for the compounds.
Compounds retained longer than run time in method conditions	- Check mobile phase composition is correct. - Check correct column is being used. - Increase run time. - Increase solvent strength.
Sample issues	- Ensure the sample hasn't degraded. Prepare fresh samples. - Ensure the sample is in the correct position in the autosampler. - Sample adsorption issue.
Blocked needle	- Try to clear the blockage or replace the needle. Address why the needle blocked (i.e. blocked by septa or poor sample preparation).
Instrument	- No mobile phase flow, possibly purge valve left open. - Purge the system to remove possible air bubbles in pump. - Purge injector to remove air bubbles in metering pump.

Split Peaks



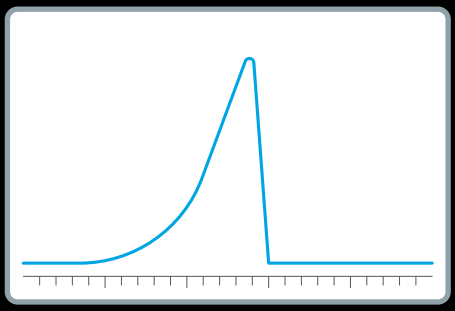
Causes	Solutions
Soiled guard or column inlet	- Replace guard or inline filter frit; reverse flush column (if permitted).
Sample diluent incompatible with mobile phase	- Change sample diluent. Use initial mobile phase solvent composition (if applicable). Use Co-Solvent or POISe injection function.
Analyte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

Tailing Peaks



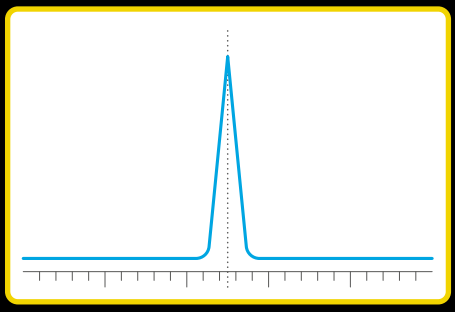
Causes	Solutions
Secondary interactions	- For bases increase pH (as permitted); for acids decrease pH; increase ionic strength of buffer (as permitted); change column type.
Dead volume	- Reconnect the column with the fitting to reduce dead volume.
Column degradation	- Replace the column.
Column void	- 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.

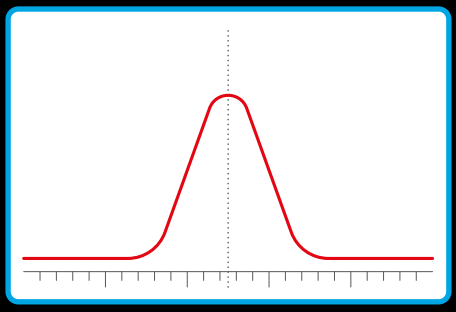
Fronting Peaks



Causes	Solutions
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.

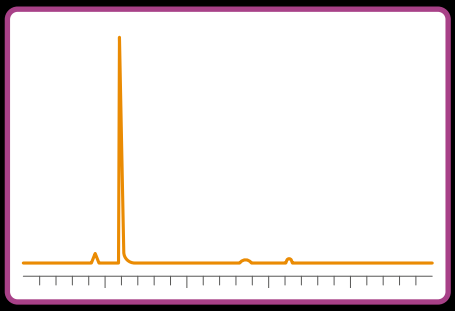
Broad Peaks



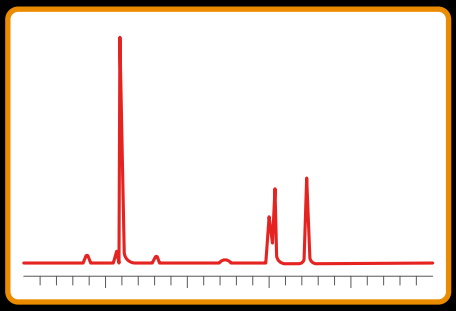


Causes	Solutions
Sample loading	- Reduce sample concentration or injection volume.
Column issue	- Degradation of the column, column should be replaced.
Oven setting issue	- Check column oven temperature is correct. Higher column temperatures typically result in faster compound elution (NB keep under 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.

Extra Peaks



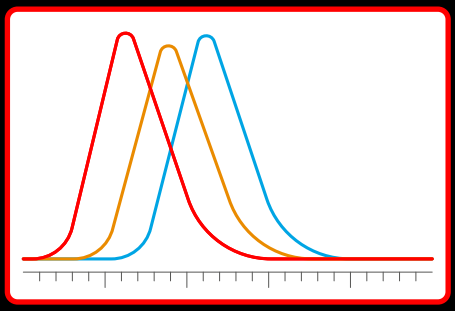
Injection 1



Injection 2

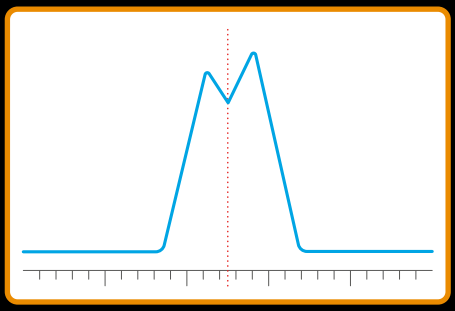
Causes	Solutions
Other components in sample	- It is normal to see extra peaks if they are present in the sample.
Late eluting peaks from previous injection	- Increase run time or solvent strength; increase flow rate to increase the number of column volumes per unit time.
Ghost peaks	- Check purity of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

Changing Retention Times



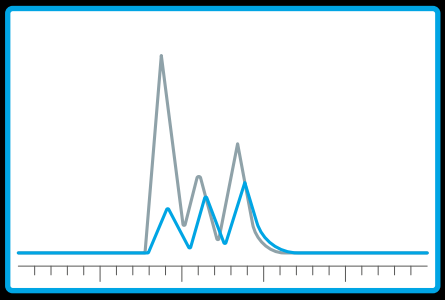
Causes	Solutions
Flow rate	- Check the method uses the correct flow rate. Ensure the flow rate is accurate using a flow meter.
Insufficient equilibration	- The reversed phase column should be equilibrated using at least 10 column volumes. If 10 column volumes are insufficient, increase the equilibration 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 throughout the system.
Air bubble in pump	- Purge pump via purge valve.

Loss of Resolution



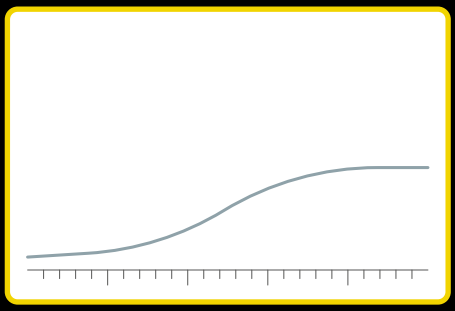
Causes	Solutions
Changes in peak width	- Changes in column performance and sample load / column efficiency can result in wider peak widths. Ensure the chromatographic performance of the column is sufficient, or replace the column, and ensure the same load is consistent.
Changes in retention time	- See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

Changes in Sensitivity



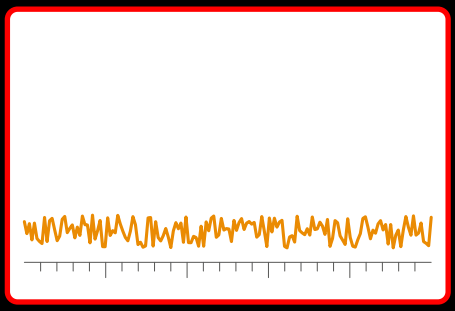
Causes	Solutions
Injector issue	- Changes in dispensing volume of injector, use a system suitability sample to determine volume changes. - Check batch / method details to ensure the correct volume was programmed. - Increase needle and loop flushing protocols to ensure no carryover from injection. - Purging 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.

Baseline Drift



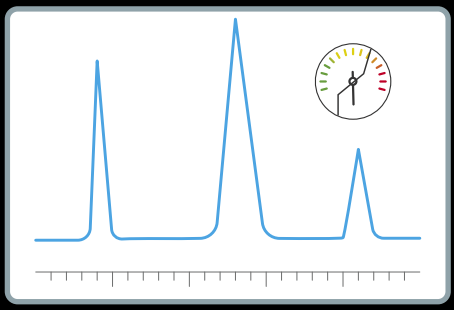
Causes	Solutions
Column temperature fluctuation	- Control column temperature.
Low quality mobile phase	- Use HPLC gradient grade solvents, high purity salts and additives. Ensure mobile phases are degassed sufficiently.
Contaminant or air bubble in detector flow cell	- Flush flow cell with isopropanol. If necessary, clean cell with 1N nitric acid.
Cracked cell window	- Replace flow cell window.
Mobile phase mixing issue	- Use larger or more efficient mixer.
Slow column equilibration	- Flush column with at least 10-20 column volumes with new mobile phase.
Strongly retained materials with high capacity factor eluting in subsequent injections	- Use a strong flush procedure between injections or if permitted backflush column with strong solvent between injections for more challenging strongly retained contaminants.
Mobile phase recycled but, detector not auto-zeroed correctly	- Auto-zero detector.
Detector (UV) not set at absorbance maxima but, on slope of curve	- Change wavelength to UV absorbance maxima.

Noisy Baseline



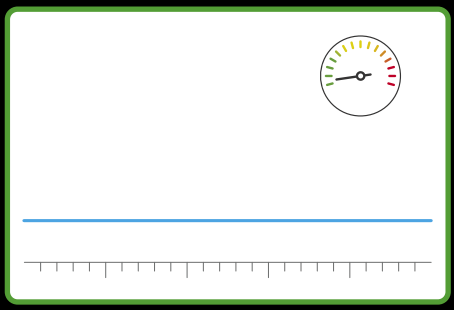
Causes	Solutions
Air in detector cell or pump	- Degas mobile phase sufficiently and purge lines and flow path.
Leak	- Check wettable flow path and fittings.
Incomplete mobile phase mixing	- Pre-mix mobile phase or use larger mixer.
Temperature variance at detector	- Use temperature-controlled detector flow cell.
Mobile phase contaminated or deteriorated	- Check mobile phase.
Mobile phase solvents immiscible (Pressure spikes can also be observed)	- Use miscible mobile phases.
Air trapped in system	- Flush and purge flow path.
Weak detector lamp	- Replace lamp.
Column leaking silica or packing material	- Replace column.

No Pressure Reading, but flow is normal



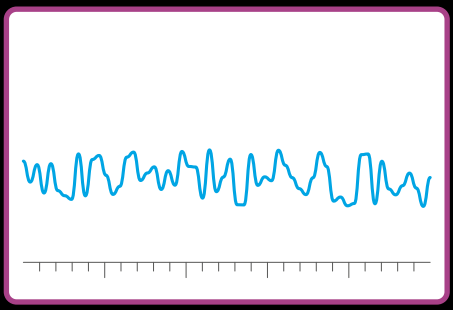
Causes	Solutions
Sensor malfunction	- Repair or replace or repair pressure sensor.
Software incompatibility	- Use alternative software which records pressure data.
Purge valve left open	- Close purge valve.

Low Pressure



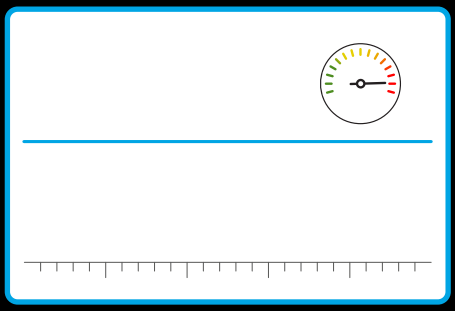
Causes	Solutions
Partial leak in system	- Check all connections and retighten any which have leaks.
Flow rate	- Check the method has the correct flow rate. - Test the flow rate accuracy using a calibrated flow rate meter or collect a specific volume and monitor the time required.
Method	- Check if method 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.

Fluctuating Pressure



Causes	Solutions
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.

High Pressure



Causes	Solutions
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.

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