Liquid Chromatography Mass Spectrometry

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 Mass Spectrometry Troubleshooting Guide is designed to assist mass spectrometrists assess common LCMS 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 analysis.

Basic Steps

Follow these steps to isolate where the problem is. Check the obvious explanations first and change only one thing at a time:

Check the basics:

- Power supply
- Electrical connections
- Communication cables (instrument modules and PC)
- Standard preparation
- Sample preparation
- Analytical conditions
- Mobile phase preparation
- Needle rinse and seal washes
- Solvent flowing / no air bubbles
- LC pump pressures
- Ion source maintenance
- Roughing pump (oil level and gas ballast)
- MS vacuum
- Argon gas cylinder (level and pressure)
- Gas generator (pressure readings)
- LC fittings and flowpath

Identify the cause:

Clearly define the problem e.g. “loss of sensitivity” or “shift in retention times”. Review system counters to identify parts beyond their replacement schedule.

Review sample and maintenance logs to identify trends in the data or possible problem indicators.

Use a logical sequence of events to isolate possible causes.

Refer to relevant common issues section for further troubleshooting advice.

Document everything:

- During method validation, document analyte retention times and normal LC operating pressures (initial conditions). This will act as a benchmark to indicate deterioration in system performance.
- 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 with your instrument?

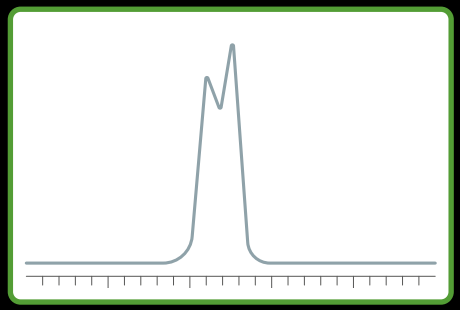
Still struggling? Let us know at **lcms_support@shimadzu.co.uk**

No Peaks

Cause	Solution
MS setting issue	- Ensure MS is a suitable technique for the physico-chemical properties of the analyte(s). - Ensure probe is at correct distance from orifice (e.g. 2 or 3 mm). - Check the method uses appropriate MS settings (ion source, acquisition mode, polarity) for the compounds of interest. - Check ion source temperatures / gas flows are as expected and stable. - Check collision gas pressure is correct and stable. - If running in scheduled events such as MRM, ensure analyte elutes within event window. To aid troubleshooting, acquire across the full method runtime to assess possible shifts in retention times. - Check protrusion of capillary from probe (0.5 - 1 mm). - Check spray from capillary. - Perform and check MS tune. - Check for adducts such as Na^+, NH_4^+, K^+. Consider different glassware, make up new mobile phases. - Check for matrix effects such as ion suppression. Consider different LC methods and sample preparation procedures. - Concentration injected is below limit of detection.

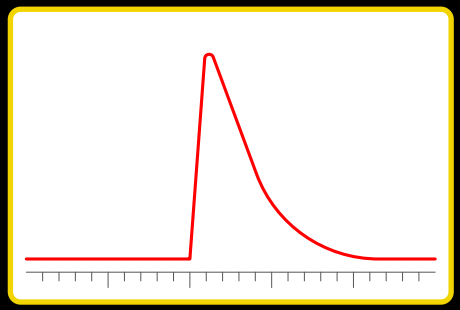
Cause	Solution
LC	- Check LC outlet tubing is connected to ion source. - No mobile phase flow, possibly purge valve left open. - Purge the system to remove possible air bubbles in pump. - Purge injector to remove air bubbles in metering pump. - Purge LC system using isopropanol to ensure check valves are working correctly. - Check for crimped or damaged tubing.
Compounds not retained or retained longer than run time in method conditions	- Check mobile phase composition is correct. - Check correct analytical column type is being used. - Increase run time. - Increase solvent strength. - Check correct flow rate is being achieved.
Sample issues	- Ensure the sample hasn't degraded. Prepare fresh samples. - Ensure correct injection volume in sequence / method. - Ensure the sample is in the correct position in the autosampler. - Sample adsorption issue. - Check for air pockets trapped in bottom of vial or well.
Blocked injection needle	- Try to clear the blockage or replace the needle. Address why the needle blocked (i.e. blocked by septa, lack of centrifugation or poor sample preparation).
Sample flowing to waste	- Check divert valve settings if applicable.

Split Peaks



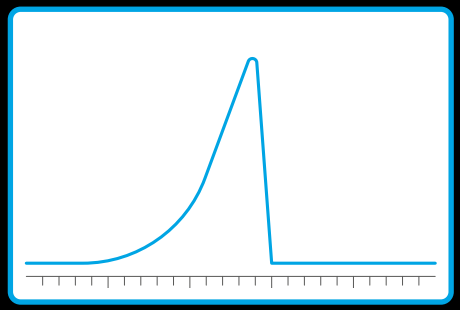
Causes	Solutions
Soiled guard or column inlet	- Replace guard or inline filter frit; reverse flush column (if permitted).
Sample diluent incompatible with mobile phase	- Change sample diluent. Use initial mobile phase solvent composition (if applicable). Use Co-Solvent or POISe injection function.
Analyte properties	- Possibility of isomer or analyte interconversion – alter conditions to correct for this.
Detector saturation	- Reduce injection volume or inject lower concentration of sample. - Set quadrupoles to "High" resolution for analytes affected in MRM method.

Tailing Peaks



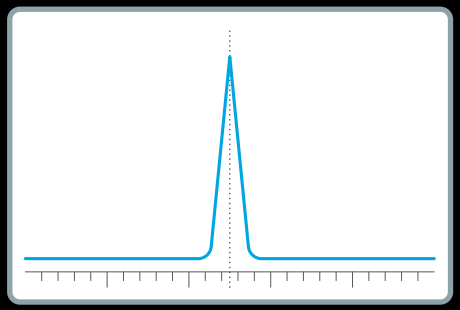
Causes	Solutions
Secondary interactions	- For bases increase pH (as permitted); for acids decrease pH; increase ionic strength of buffer (as permitted). - Change to an inert silica-based column. - Change column type.
Dead volume	- Reconnect the column with the fitting to reduce dead volume.
Column degradation	- Replace the column.
Column void	- Fill void (previous performance unlikely to be fully recovered). - Replace column.
Interfering peak	- Use a longer column; change gradient conditions; further method development. - Change column phase type.
Wrong mobile phase pH	- Adjust pH (2 clear pH units from pK_a recommended) – check column pH compatibility.
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	- Increase buffer concentration up to 10 mM.
Sample loading	- Reduce sample concentration by injecting less or dilution.

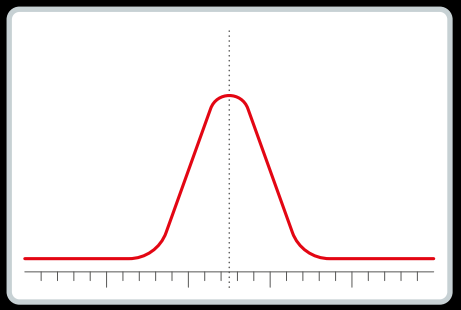
Fronting Peaks



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	- Reduce sample concentration by injecting less or dilution.

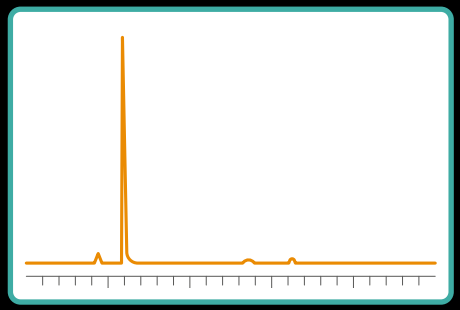
Broad Peaks



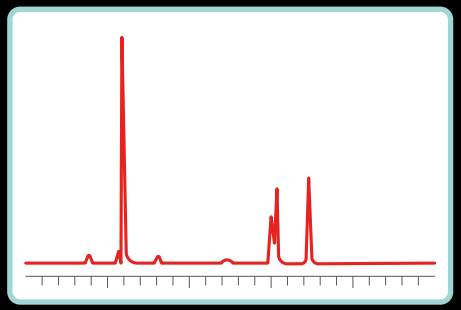


Causes	Solutions
Sample loading	- Reduce sample concentration, % organic content in diluent or injection volume.
Column issue	- Degradation of the column, column should be replaced.
Oven setting issue	- Check column oven temperature is correct. Higher column temperatures typically result in faster compound elution (NB keep under column temperature limits as described by manufacturer).
Mobile phase	- Check correct mobile composition is being used. Re-prepare if necessary.
Slipped ferrule	- Check all connections are correctly fitted.
LC settings	- Additional tubing or other factors have increased system dispersion volume. Check tubing lengths, valve / column connections and internal dimensions. - Check correct flow rate is being delivered / set in method correctly.

Extra Peaks



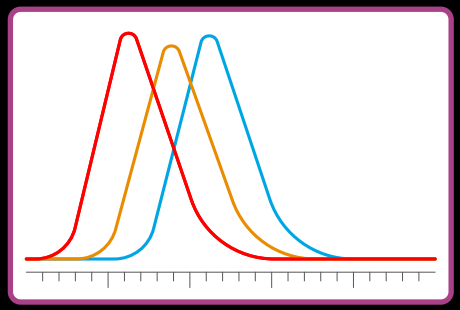
Injection 1



Injection 2

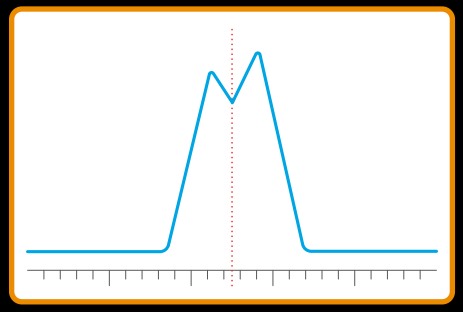
Causes	Solutions
Other components in sample	- It is normal to see extra peaks if they are present in the sample.
Late eluting peaks from previous injection	- Increase run time or solvent strength; increase flow rate to increase the number of column volumes per unit time.
Ghost peaks	- Check purity of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

Changing Retention Times



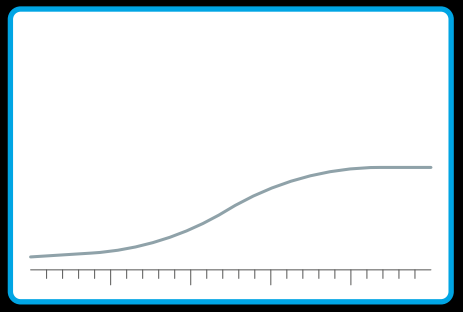
Causes	Solutions
Flow rate	- Check the method uses the correct flow rate. Ensure the flow rate is accurate using a flow meter.
Insufficient equilibration	- The reversed phase column should be equilibrated using at least 10 column volumes. If 10 column volumes are insufficient, increase the equilibration 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.
Poor column equilibration	- Allow more time or column volumes to equilibrate column between runs.
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.
Sample diluent	- Inappropriate sample diluent for column.
Needle rinse	- Needle rinse solvent reaching the column.
Faulty or 'sticky' check valve	- Purge LC system using isopropanol to ensure check valves are working correctly.

Loss of Chromatographic Resolution



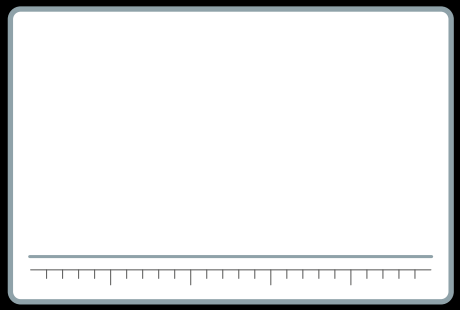
Causes	Solutions
Changes in peak width	- Changes in column performance and sample load / column efficiency can result in wider peaks. Ensure chromatographic performance of the column is sufficient i.e peak resolution and asymmetry. Check sample injection volumes or consider replacing column.
Changes in retention time	- See changes in retention time section.
Mobile phase deterioration or evaporation	- Prepare fresh mobile phases.

High Background Signal



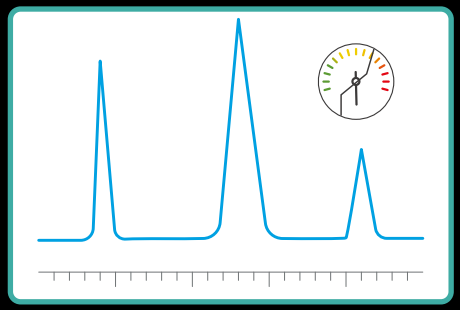
Causes	Solutions
Low quality mobile phase	- Use MS grade solvents, high purity salts and additives.
Strongly retained materials with high capacity factor eluting in subsequent injections	- Use a strong flush procedure between injections or if permitted backflush column with strong solvent between injections for more challenging strongly retained contaminants.
Mobile phase contaminated or deteriorated	- Check mobile phase. - Ensure solvent bottles are rinsed thoroughly with high purity water before use. - Replace stock bottles if required. - Ensure mobile phase components are replaced regularly. - Use of designated glassware is recommended for LCMS analysis and maintenance.
Air trapped in system	- Flush and purge flow path.
Column leaking silica or packing material	- Replace column.
New column	- Flush new column with at least 10-20 column volumes of mobile phase. Ensure flow is diverted to waste.
MS	- Clean ion source and ion optics. If necessary, replace capillary and desolvation line.

No Flow



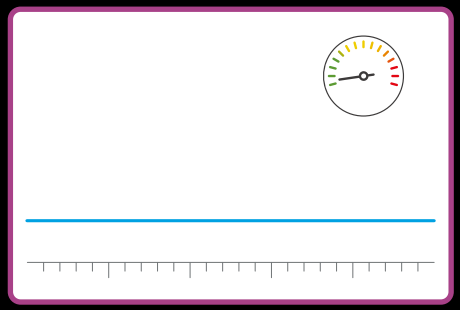
Causes	Solutions
Mobile phase	- Check mobile phase line and volume.
Purge valves are open	- Ensure purge valves are closed.
Leaks	- Check for leaks in the flow line.

No Pressure Reading, but Flow is Normal



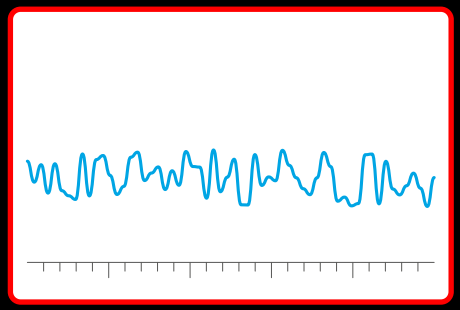
Causes	Solutions
Sensor malfunction	- Repair or replace or repair pressure sensor.
Incorrect units	- Check pressure units are correct.
Software incompatibility	- Use alternative software which records pressure data.

Low Pressure



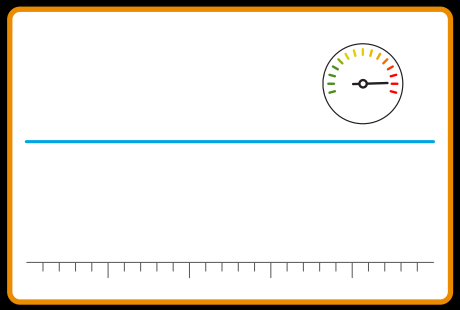
Causes	Solutions
Partial leak in system	- Check all connections and retighten any which have leaks.
Flow rate	- Check the method has the correct flow rate. - Test the flow rate accuracy using a calibrated flow rate meter or collect a specific volume and monitor the time required. - Replace worn out or damaged pump seals.
Method	- Check if method is using correct temperature and correct solvents. - If a column section valve is used, check correct column selected.
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.
Airlock in LC tubing	- Remove tubing from degasser and ensure flow under gravity. Reconnect and purge pumps in isopropanol.
Stuck check valve	- Purge LC system using isopropanol and ensure check valves are working correctly. - Sonicate the check valves in isopropanol.
Sensor malfunction	- Repair or replace pressure sensor.

Fluctuating Pressure



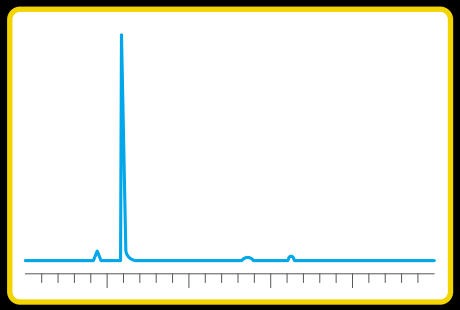
Causes	Solutions
Air bubbles	- Purge the solvent lines to remove the air bubbles.
Worn pump seals	- Replace seals.
Check valves	- Sonicate the check valves in isopropanol. - Change the check valves if problem persists.
Leaks	- Degradation of pump seals could cause small leaks. Replace the seals. Check connections.
Inadequate degassing	- Replace mobile phase filters; repair degasser (if applicable).

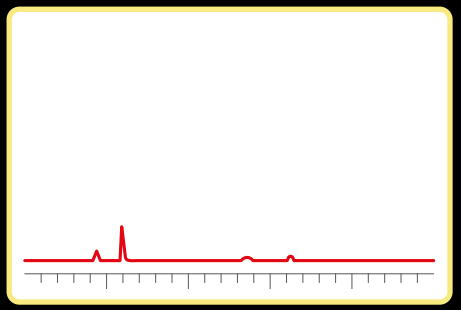
High Pressure



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.
Blocked tubing	- Replace blocked tubing as necessary.

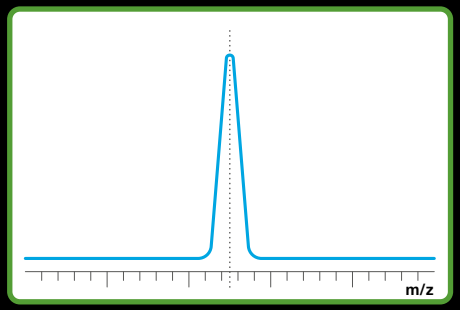
Carry Over

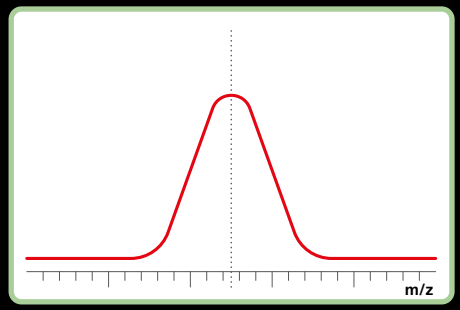




Causes	Solutions
Inappropriate wash settings	- Check wash solution and wash settings.
Sample concentration	- Use lower concentrated sample or inject less.
Column contamination	- Flush column; replace guard columns and analytical column if applicable.
Injector issue	- Changes in dispensing volume of injector, 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.
LC Gradient	- Insufficient time at strong solvent conditions during gradient program. Increase based on column dimensions.

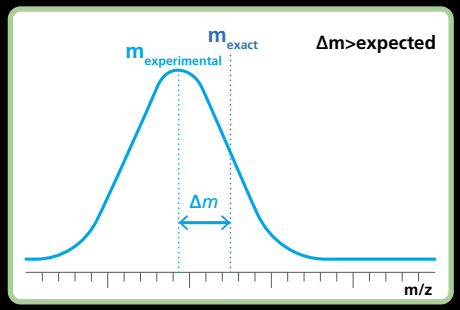
Changes in MS Resolution





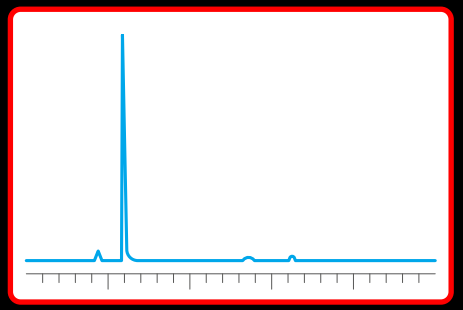
Causes	Solutions
MS out of tune	- Perform and check tune.

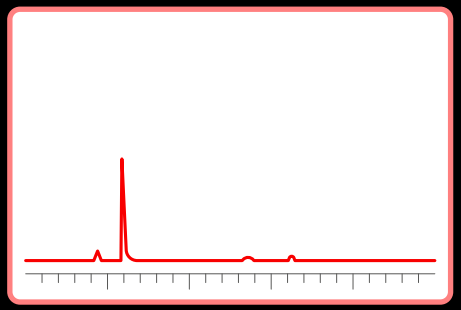
Poor Mass Accuracy, (HRAM Instruments)



Causes	Solutions
MS out of tune	- Perform and check system tune.
TOF Calibration	- Perform TOF calibration.
Calibration performed incorrectly	- Ensure sample analytes are within calibration range and adjust if required.
Detector saturation	- Dilute sample or adjust injection volume.

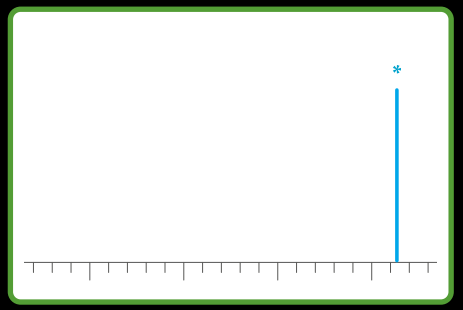
Changes in Sensitivity

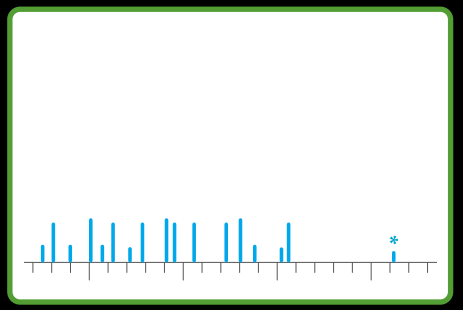




Causes	Solutions
MS	- If all peaks have changed in sensitivity check maintenance requirements; and clean the ion source and if required ion optics. - Ensure appropriate and correct acquisition parameters are being used. - Check for adducts such as Na^+, NH_4^+, K^+. Consider different glassware, make up new mobile phases. - Increase additive or buffer concentration if required. - Ensure probe is at correct distance from orifice. - Check for matrix effects such as ion suppression. Consider different LC methods and sample preparation procedures. - Replace capillary and desolvation line. - Perform and check MS tune.
Sample	- Check for sample degradation. Prepare a fresh sample. - Check sample preparation and standard/QC concentrations. - Incorrect sample diluent used. - Check injection volume.
Loss of column performance	- Check the peak widths and resolution. Test the performance of the column using your standard test for loss in performance. - Replace analytical column.
LC leaks	- Check for loose fittings post injector on the system.
Mobile phase	- Check concentration of additives. - If suppressing mobile phase components were used in previous method, clean MS source and flush out LC system.

Undesired Fragmentation





Causes	Solutions
Ion source setting too harsh	- Check source temperatures are appropriate for analyte. - Check ionisation voltage is appropriate for analyte.
Collision energy too low / high	- Check and optimise collision cell gas pressure and collision energy.
Ion optics	- Ensure correct voltage is applied to desolvation line and QArray.

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