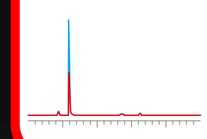
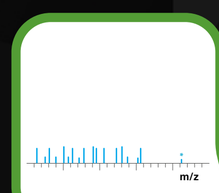
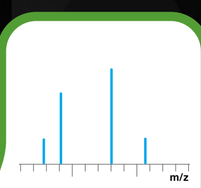
LCMS Troubleshooting Tips



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 accurate acquisition parameters are being used. - Check for adducts such as Na+, NH4+, 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. - Include an internal standard with known concentration during sample preparation. - 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.



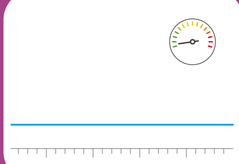


Undesired Fragmentation

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

No Peaks

Causes	Solution
MS setting issue	- Check the method uses appropriate MS settings for the compounds of interest. - Ensure probe is at correct distance from orifice. - Check protrusion of capillary from probe. - Check spray from capillary. - Check ion source temperatures / gas flows are as expected and stable. - Check collision gas pressure is correct and stable. - Ensure analyte elutes within event window set in method. - 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. - Perform and check MS tune.
LC	- Check LC outlet tubing is connected to ion source. - No mobile phase flow, possibly purge valve left open. - Purge the system including injector to remove possible air bubbles in 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	- Prepare fresh samples. - Review injection volume in sequence / method. - Ensure the sample is in the correct position in the autosampler. - Check for sample adsorption issue. - Check for air pockets trapped in bottom of vial or well.
Sample flowing to waste	- Check divert valve settings if applicable.



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.

Basic Steps

Follow these steps to isolate where the problems is.

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

Check the Basics:

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

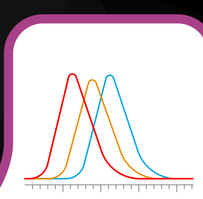
Identify the Cause:

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

Document Everything:

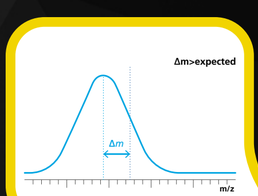
- Document typical values such as analyte retention times and normal initial LC operating pressures. Use as a benchmark to indicate deterioration in system performance
- Document all troubleshooting steps and results. This may help you identify and solve the next problem faster
- Always inject a known sample and compare to previous data as a reference to ensure restored performance

Still having problems with your instrument?
Click Here to Contact Tech Support



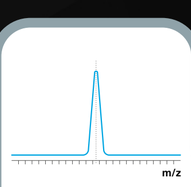
Changing Retention Times

Causes	Solutions
Flow rate	- Check the method uses the correct flow rate.
Insufficient equilibration	- The column should be equilibrated using at least 10 column volumes (check with manufacturer's manual). - Allow more time or column volumes to equilibrate column between injections.
Poor temperature control	- Ensure column oven temperature 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'.
Improper mobile phase	- Ensure the mobile phase is accurately prepared and the correct lines are being chosen on the method. - Ensure the pump is accurately dispensing mobile phase.
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.



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.



Carry Over

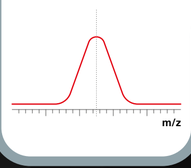
Causes	Solutions
Inappropriate wash settings	- Check wash solution and wash settings.
Sample concentration	- Use lower concentrated sample or inject less.
Column contamination	- Flush column; replace guard columns and analytical column if applicable.
Injector issue	- Changes in dispensing volume of injector, 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.

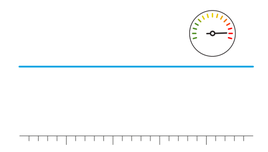
High Pressure

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.

Changes in MS Resolution

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





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