SHIMADZU
Excellence in Science

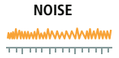
LC TROUBLESHOOTING TIPS

Shop for consumables now at ShopShimadzu.com

BASELINE ISSUES

Noisy Baseline

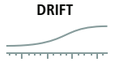
NOISE



Causes	Solutions
Mobile phase degrade	- Degraded mobile phase can cause noisy baseline. - Prepare fresh mobile phase. Flush system and column with fresh mobile phase.
Inappropriate grade mobile phase	- Use HPLC/UHPLC or LC-MS grade solvent.
Air in mobile phase flow line	- Air in flow line can cause noisy baseline or random spikes. - Degas mobile phase. - Purged pump using degassed mobile phase.
Insufficient mobile phase mixing	- Use mixer with appropriate volume. Consult your manufacturer for more details on therecommended mixer volume.

Baseline Drift

DRIFT



Causes	Solutions
Mobile phase absorption	- Base line will drift when using UV absorbing solvent as mobile phase, particularly in gradient elution. - Ensure detection wavelength is above UV cut-off of solvent. - Use alternative solvent that does not absorbs UV at detection wavelength.
Contaminant in mobile phase	- Prepare fresh mobile phase

Spikes

Causes	Solutions
Air in mobile phase flow line	- Air in flow line can cause noisy baseline or random spikes. - Degas mobile phase. - Purged pump using degassed mobile phase.
Pump Issue	- Contact service engineer for assistance.

PRESSURE ISSUES

High Pressure

Causes	Solutions
Buffer precipitation	- Ensure compatibility of buffer-organic mixture to prevent buffer precipitation. - In the event of accidental buffer precipitation, flush system and column with low organic content mobile phase to dissolve the salt.
Sample precipitation	- Ensure sample is soluble in method's initial mobile phase composition to prevent precipitation of sample. - Contact service engineer if system clogged. - Flush column at half the flow rate with stronger solvent that can dissolve the sample if column is clogged.
Contaminated or clogged column	- Check if system is clogged by disconnecting the column. Contact service engineer if system is clogged. - If using guard column, check if guard column is clogged. Replace the guard column if clogged. - Check if main column is clogged or contaminated. Flush column at half the flow rate with stronger eluting solvent to remove contaminants. If pressure does not return to normal, reverse flush the column (if allowed) to remove particulate clogging the column inlet. If pressure still does not return to normal, replace the column. - Install an in-line filter in front of analytical column to prevent clogging of column due to particulates. - Use a guard column to prevent contamination of column by strong binding compounds.

Low or No Pressure

Causes	Solutions
Insufficient mobile phase	- Replace mobile phase and ensure it is sufficient for analysis. - Purge pump and system with mobile phase to remove air in flow line. - Flush column with mobile phase. - If column performance is affected, replace column.
Mobile phase leak	- Check for leak in mobile phase flow line. - Ensure fitting is sufficiently tight to prevent leakage. - Use fitting with appropriate pressure rating to prevent leakage.

Unstable Pressure

Causes	Solutions
Bubbles in mobile phase flow line	- Degas mobile phase. - Purge pump to remove bubbles.
Faulty pump seal or check vale.	- Contact service engineer for assistance.

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 • Analytical conditions • Electrical connections
- Mobile phase volume • Mobile phase leakage

Identify the Cause:

- Define the problem clearly; for example, "Peak tailing occurs after changing to a new column."
- Review sample preparation procedure and maintenance records to identify trends in the data or problem indicators, such as pressure increasing over time.
- 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 test mix and compare to previous data to ensure restored performance.

Still having problems?
Let us help. Contact us at:
consumablesap@shimadzu.com.sg

PEAK ISSUES

No Peak

Causes	Solutions
Mobile Phase Leak	- Check for leak in mobile phase flow line. - Ensure fitting is sufficiently tight to prevent leakage - Use fitting with appropriate pressure rating to prevent leakage.
Wrong Detector Setting	- Ensure correct data acquisition time setting. - Ensure correct wavelength setting for UV or florescence detector. - Ensure correct mass setting for MS detector.
No Injection	- Ensure correct vial number setting. - Ensure sufficient sample volume in vial. - Ensure injector needle not clogged.
Sample Concentration Too Low	- Increase sample concentration or injection volume.
Sample Not Eluted	- Increase elution strength of mobile phase to elute compound.

Peak Tailing

Causes	Solutions
Sample interacting with silica silanol group	- Use endcapped reversed phase columns to reduce silanol activity. - Adjust pH of mobile phase to reduce silanol activity.
Inappropriate mobile phase pH	- Adjust mobile phase pH (do not exceed column pH limit).
Clogged column filter frit	- Flush column in reversed direction if allowed (check with column manufacturer for details). - Replace column if flushing cannot recover original performance.
Column damaged or worn out	- Replace column.

Peak Fronting

Causes	Solutions
Sample overload	- Decrease sample concentration or reduce injection volume.
Inappropriate diluent	- Use mobile phase as diluent. - Reduce injection volume.
Column damaged	- Flush column in reversed direction if allowed (check with column manufacturer for details). - Replace column if flushing cannot recover original performance.

Broad Peak

Causes	Solutions
Inappropriate diluent	- Use mobile phase as diluent.
Injection volume too high	- Reduce injection volume.
Inappropriate data acquisition rate and time constant setting	- Adjust data acquisition rate to ensure there is at least 10 data points across a peak. - Reduce time constant.
Void volume at column inlet	- Ensure inlet tubing has flat, right angle cut. - Ensure installation depth of the tubing match the mounting depth of the column.
Column temperature too low	- Increase column temperature (ensure temperature is below column max temperature limit).
Contaminated or damaged column	- Flush column with stronger eluting solvent to remove contaminants. - Replace column if column flushing does not recover the performance.

Change in Response

Causes	Solutions
Sample issue	- Check sample concentration. - Check sample preparation procedure. - Check sample decomposition/shelf life.
Mobile phase leak	- Check for leakage in flow line after injection port. - Ensure fitting is sufficiently tight to prevent leakage. - Use fitting with appropriate pressure rating to prevent leakage.
Detector lamp worn out	- Check that detector lamp does not exceed recommended operating hours. - Replace detector lamp if exceed recommended operating hours.

Split Peak

Causes	Solutions
Inappropriate diluent	- Use mobile phase as diluent.
Contaminated Column	- Flush column with stronger eluting solvent to remove contaminants. - If problem persist, replace column.
Column Partially Clogged	- Reverse flush column with lower flowrate if allowed (check with column manufacturer for details).
Column Damaged	- Replace column.

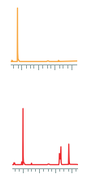
Varying Retention Time

Causes	Solutions
Mobile phase leak	- Mobile phase leak will cause retention time to increase. - Check for leak in mobile phase flow line. - Ensure fitting is sufficiently tight to prevent leakage - Use fitting with appropriate pressure rating to prevent leakage.
Variation in column temperature	- Use a column oven to ensure consistent column temperature.
Inadequate column equilibration	- Increase equilibration time.
Evaporation of mobile phase	- Evaporation of mobile phase can cause change in the mobile phase composition. - Use solvent bottle cap to reduce solvent evaporation.
Sample overload	- Decrease sample concentration or reduce injection volume.
Column stationary phase collapse/ dewetting	- Phase collapse/dewetting will cause retention time to decrease. - Rewet column using high organic content mobile phase. - Replace column if rewetting fails. - If analysis require use of low organic content mobile phase, ensure the column used is compatible with 100% aqueous condition.
Degradation of stationary phase	- Degradation of stationary phase will cause retention time to decrease. - Replace damaged column. - Ensure mobile phase pH within recommended pH limit.
Pump issue	- Check flow rate of each pump. - If flow rate is lower than setting, contact service engineer for assistance.

Poor Resolution

Causes	Solutions
Non-selective stationary phase	- Choose appropriate stationary phase and column dimension.
Poor efficiency	- Increase column length. - Decrease column particle size.
Sample overload	- Optimise sample concentration or sample injection volume.
Incorrect analysis conditions	- Check and optimise gradient program, flow rates and column temperature etc.

Carryover/ Ghost Peak



Causes	Solutions
Previous analysis ended too soon	- Increase analysis run time. - Increase elution strength of mobile phase to elute stronger retained compounds.
Contaminated mobile phase	- Perform analysis without injection. If peak is observed, contaminant originated from mobile phase. - Use appropriate grade solvent (Organic: HPLC/ UHPLC/LC-MS grade, aqueous: ultrapure water). - Prepare fresh mobile phase daily. - Use Ghost Trap (For non-MS detector) or GLC Suction filter 2. (contact Shimadzu for more information.)
Contaminated diluent	- Perform diluent blank analysis. If peak is present, contaminant originated from diluent. - Use appropriate grade diluent (Organic: HPLC/ UHPLC/LC-MS grade, aqueous: ultrapure water).
Contaminated injection port/	- Perform needle and injection port rinse. - Use appropriate solvent for needle and injection.

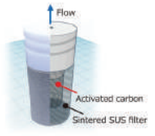
Guard column is essential to extend column service life by protecting the analytical column from contaminants

Have pressure issues?

Install **Inline filter** between auto-sampler and column to remove pump seal debris and sample particles. This will prevent **column back pressure problems.**

Have limited budget?

Use economical **direct connect filter** to protect analytical column from from the harmful effects of sample and mobile phase particulates.



Want to eliminate Ghost Peaks?

Use **Ghost Trap DS** to effectively adsorb impurities in the mobile phase in order to reduce the time required for method development and impurity analysis.

Use **GLC Suction Filter 2** to remove contaminants in mobile phase to minimizes chromatographic interferences. It is suitable for LC/MS analysis due to its low bleed.