HPLC Troubleshooting Mini Guide

Peak Issues

Contents

Overview

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Locating and Correcting the Problem

A systematic approach to identifying the problem is the best path to troubleshooting your HPLC system. This guide is organized by four major categories of symptoms to help you quickly identify the source of the problem(s) you are encountering:

- pressure abnormalities
- leaks
- peak problems
- baseline issues

When you have corrected the problem, record the incident in the system recordbook to help with future problems.

Prevention

Many LC problems can be prevented with routine preventive maintenance such as replacing pump seals regularly. Consistent preventive maintenance practices will enhance lab productivity, avoid system critical damage, equipment downtime and costly repairs.

Where to Get Additional Help

1. Chat with Phenomenex technical experts. Phenomenex has experienced technical consultants who can assist you with any chromatography issue in real time. To chat now go to www.phenomenex.com/chat.
2. The operator's and service manuals for the instrument should be consulted. These contain exploded diagrams, troubleshooting procedures for specific models, and part numbers to help you order replacement parts.
3. Other people in the lab may have had experience solving a problem which is giving you trouble; they can be a helpful resource.
4. The manufacturer of your instrument can help you. Most LC manufacturers offer free technical support to their customers.
5. Phenomenex offers seminars on HPLC/UHPLC. Join PhenoAcademy for specialty troubleshooting webinars, www.phenomenex.com/phenoacademy
6. Other resources:

   J.W. Dolan and L.R. Snyder, ***Troubleshooting LC Systems***, Humana Press, NJ (1989).

   L.R. Snyder and J.J. Kirkland, ***Introduction to Modern Liquid Chromatography***, 2nd ed., Wiley, NY (1979).

   D.J. Runser, ***Maintaining and Troubleshooting HPLC Systems - A User's Guide***, Wiley, NY (1981).

   J.W. Dolan, "LC Troubleshooting", LC/GC Magazine. This is a monthly column.

Peak Issues

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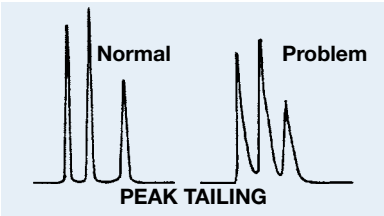
Many problems in the LC system show up as changes in the chromatogram. Some of these can be solved by changes in the equipment; however, others require modification of the assay procedure. Selecting the proper column type and mobile phase are keys to “good chromatography.”

Peak tailing

POSSIBLE CAUSE	SOLUTION
1. Blocked guard/frit or column contamination	1. a. Replace/remove guard b. Reverse flush column (if allowed) c. Replace column
2. Column void	2. Replace column; avoid sudden pressure shocks. Ensure operating pressure is within column advised operating conditions and that mobile phase pH is within operating guidelines for the column utilized.
3. Interfering peak	3. a. Use longer column b. Change mobile phase and/or column/selectivity
4. Wrong mobile phase pH	4. Adjust pH. For basic compounds, lower pH usually provides more symmetrical peaks
5. Inadequate buffer concentration	5. Increase buffer concentration
6. Sample reacting with active sites 	6. a. Use lower pH to deactivate residual silanols b. Use columns specialized in reducing secondary interactions (consult Phenomenex) c. Utilize ion-pair reagent or volatile basic modifier
7. Extra-column effect	7. a. Replumb system (shorter, narrower tubing) b. Use smaller volume detector cell c. Check for correct column connection

Peak fronting

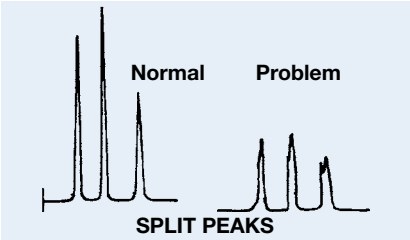
POSSIBLE CAUSE	SOLUTION
1. Sample solvent stronger than mobile phase	1. Use mobile phase for injection solvent
2. Sample overload	2. a. Decrease sample concentration or volume b. Increase column ID

Distortion of larger peaks

POSSIBLE CAUSE	SOLUTION
1. Sample overload	1. a. Decrease sample concentration or volume b. Increase column ID

Peak Issues (continued)

All peaks splitting

POSSIBLE CAUSE	SOLUTION
1. Contamination on guard or analytical column inlet  SPLIT PEAKS	1. Remove guard column or cartridge and attempt analysis. Replace guard if necessary. If the analytical column is obstructed, reverse and flush. If problem persists, column may be fouled with strongly retained contaminants. Use appropriate restoration procedure. If problem persists, inlet is probably plugged. Replace column.
2. Sample solvent incompatible with mobile phase	2. Change solvent. Whenever possible, inject samples in starting mobile phase.

Some peaks splitting

POSSIBLE CAUSE	SOLUTION
1. Sample solvent incompatible with mobile phase	1. Early eluting peaks may be more affected by sample solvent mismatch. Inject sample in starting mobile phase.
2. Split peak may be two coeluting peaks	2. Use a smaller injection volume to see if peaks become more distinct. Optimize method to increase resolution.
3. pH of the mobile phase is too close to the p*K*a of the functional group on the compound, or mobile phase is inadequately buffered	3. Adjust and buffer the mobile phase pH +/- 2 units above or below the p*K*a of the compound's ionizable functional group, and buffer at that pH with an appropriate.

Distortion of early peaks

POSSIBLE CAUSE	SOLUTION
1. Wrong injection solvent	1. a. Reduce injection volume b. Use weaker injection solvent

Extra peaks

POSSIBLE CAUSE	SOLUTION
1. Late-eluting peak from previous injection	1. a. Increase run time or gradient slope b. Increase flow rate
2. Negative or ghost peaks	2. a. Check purity of mobile phase. Mobile phase contaminants commonly accumulate during weak portion of a gradient and elute as strong solvent increases. b. Use mobile phase as injection solvent c. Reduce injection volume
3. Sample Contamination	3. a. Perform additional sample preparation b. Use and/or replace guard column/cartridge c. Clean column

Peak Issues (continued)

Retention time drifts

POSSIBLE CAUSE	SOLUTION
1. Poor temperature control	1. Thermostat column
2. Mobile phase changing	2. a. Prepare fresh mobile phase. Preventative measures include using SecurityCap to prevent solvent evaporation and using an effective buffer concentration. Buffer range capacity is +/- 1 pH from buffer pKa b. Ensure that mobile phase is homogenous
3. Poor column equilibration	3. Allow more time for column equilibration between runs
4. Injection Port Contamination	4. a. Clean Injection Port & flush sample loop b. Replace Needle Wash; increase needle wash strength

Abrupt retention time changes

POSSIBLE CAUSE	SOLUTION
1. Flow rate change	1. Reset flow rate, confirm pump is delivering correct flow
2. Air bubble in pump	2. Bleed air from pump
3. Improper mobile phase	3. a. Replace with proper mobile phase b. Set proper mobile phase mixture on controller
3. Degradation of stationary phase	3. Replace column; confirm pH limits of column

Broad Peaks

POSSIBLE CAUSE	SOLUTION
1. Excessive extra column volume	1. Reduce extra column volume (tubing length/ID, sample loop size, flow cell size, etc.)
2. Sample solvent incompatible with mobile phase	2. Dilute in mobile phase or weak solvent
3. Mobile phase pH incorrect	3. Use buffered mobile phase, commonly +/- 2 pH units from analyte pKa is ideal
4. Incorrect or insufficient buffer	4. Check that the correct buffer is being used. Buffer capacity is +/- 1 pH unit from buffer pKa. Increase buffer concentration.
5. High longitudinal diffusion	5. Retention time too long. Use gradient elution, faster flow rates, stronger eluent, and/or less retentive stationary phase (e.g. core-shell)
6. Column overload	6. a. Reduce sample concentration or volume. b. Use larger column ID
7. Detector response time too long	7. Increase detector scan rate

Key Problem Areas and Preventive Maintenance

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The chart below lists the most common problems that occur with each LC module. In the right-hand column are listed preventive maintenance practices that can reduce the failure rate. The numbers in parentheses are suggested intervals between maintenance. The operator's and service manuals for your LC may have additional suggestions for preventive maintenance of your model of LC.

Reservoir

POSSIBLE CAUSE	SOLUTION
1. Blocked inlet frit	1. a. Replace (3-6 mo.) b. Filter mobile phase, 0.5 µm filter
2. Gas bubbles	2. Degas mobile phase

Pump

POSSIBLE CAUSE	SOLUTION
1. Air bubbles	1. Degas mobile phase
2. Pump seal failure	2. Replace (3 mo.)
3. Check valve failure	3. Filter mobile phase, use inlet-line frit. Keep spare.

Injector

POSSIBLE CAUSE	SOLUTION
1. Rotor seal wear	1. a. Don't overtighten b. Filter samples

Column

POSSIBLE CAUSE	SOLUTION
1. Blocked frit	1. a. Filter mobile phase b. Filter samples c. Use in-line filter and/or guard column
2. Void at head of column	2. a. Avoid mobile phase pH at or near column max pH limit b. Install column at low flow rates to avoid pressure shock c. Use guard column

Protect Your HPLC Column

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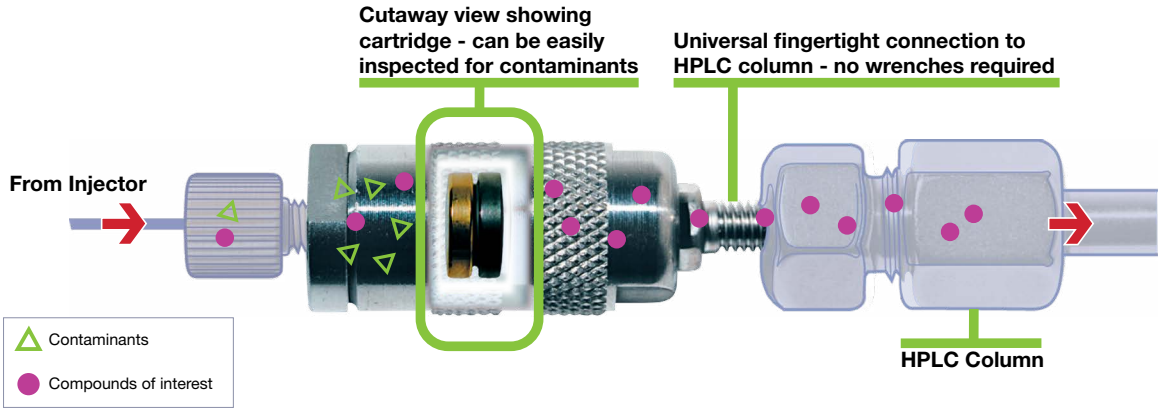
Contaminants Can Cause the Following:

- High Backpressure
- Split Peaks
- Broad Peaks
- Baseline Noise
- Baseline Drift
- Loss of Resolution
- Irreversible Column Damage
- System Damage

Protect Your HPLC Column. Protect Your Results.

The SecurityGuard™ and SecurityGuard ULTRA cartridge systems effectively protects analytical columns from the damaging effect of contaminants that could impact results and data quality. Either cartridge system is designed to trap contaminants without altering your chromatography.

How It Works:



SecurityGuard and SecurityGuard ULTRA standard can adjust to fit any manufacturer's female/ inverted endfitting.

SecurityGuard STANDARD™ Column Protection

Additional information can be found at
www.phenomenex.com/securityguard

Phenex™ Syringe Filters

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- Less system downtime
- More consistent, reproducible results
- Increased column lifetime

Features and Benefits

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- Excellent flow rate
- High total throughput
- Low hold-up volume
- Certified quality
- 100 % integrity tested

MEMBRANE TYPES
RC (Regenerated Cellulose)
PTFE, Teflon® (Polytetrafluoroethylene)
PES (Polyethersulfone)
PVDF (Polyvinylidene Fluoride)
NY (Nylon)
CA (Cellulose Acetate)
GF (Glass Fiber)

Phenex™ Syringe Filters

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