

HPLC

Troubleshooting Guide



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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.

Pressure

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A change in the operating pressure is a sign that there may be a problem. Choose the category below that best fits the symptoms that you observe, and follow the suggestions to correct the problem.

No pressure reading, no flow and/or pressure dropping to zero

POSSIBLE CAUSE	SOLUTION
1. Power off	1. Turn on power
2. Fuse blown	2. Replace fuse
3. Controller setting or failure	3. a. Verify proper settings b. Repair or replace controller
4. Broken piston	4. Replace piston
5. Air trapped in pump head	5. Degas solvents; bleed air from pump, prime pump
6. Insufficient mobile phase	6. a. Replenish reservoir b. Replace inlet frit if blocked
7. Faulty check valve(s)	7. Replace check valve(s)
8. Major leak	8. Tighten or replace fittings

No pressure reading, normal flow

POSSIBLE CAUSE	SOLUTION
1. Faulty meter	1. Replace meter
2. Faulty pressure transducer	2. Replace transducer

Pressure (continued)

Steady, high pressure and/or climbing pressure

POSSIBLE CAUSE	SOLUTION
1. Incorrect Flow Rate	1. a. Adjust flow rate b. Collect eluent to confirm system flow rate delivery
2. Blocked column frit	2. a. Backflush column (if permitted) b. Replace column
3. Improper mobile phase; precipitated buffer	3. a. Check mobile phase miscibility and viscosity. High viscosity mobile phase will result in high pressure. b. Check buffer solubility. Use high aqueous wash for precipitated salts.
4. Improper column	4. Confirm column is of compatible particle size and dimensions for system
5. Injector blockage	5. Clear blockage or replace injector
6. Column temperature too low	6. Raise temperature
7. Controller malfunction	7. Repair or replace controller
8. Blocked guard column	8. Remove/replace guard column
9. Blocked in-line filter	9. Remove/replace in-line filter

Steady, low pressure and/or pressure dropping

POSSIBLE CAUSE	SOLUTION
1. Flow rate too low	1. a. Adjust flow rate b. Collect eluent to confirm system flow rate delivery
2. Leak in system	2. Locate and correct
3. Column temperature too high	3. Lower temperature
4. Controller malfunction	4. Repair or replace controller
5. Air in solvent lines	5. Check solvent lines for visible bubbles; flush lines

Pressure cycling

POSSIBLE CAUSE	SOLUTION
1. Air in pump	1. a. Degas solvent b. Bleed air from pump
2. Faulty check valve(s)	2. Replace check valve(s)
3. Pump seal failure	3. Replace pump seal
4. Insufficient degassing	4. a. Degas solvent b. Change degassing methods
5. Leak in system	5. Locate and correct
6. Using gradient elution	6. Pressure cycling is normal due to viscosity changes

Leaks



Leaks are usually stopped by tightening or replacing a fitting. Be aware, however, that overtightened metal compression fittings can leak and plastic fingertights can wear out. If a fitting leak does not stop when the fitting is tightened a little, take the plastic fingertight fittings apart and inspect for damage (e.g. distorted ferrule, or particles on the sealing surface); damaged fittings should be discarded.

Leaky fittings

POSSIBLE CAUSE	SOLUTION
1. Loose fitting	1. Tighten
2. Stripped fitting	2. Replace
3. Overtightened* fitting	3. a. Loosen and retighten b. Replace
4. Dirty fitting	4. a. Disassemble and clean b. Replace
5. Mismatched parts	5. Use all parts from same brand

Leaks at pump

POSSIBLE CAUSE	SOLUTION
1. Loose check valves	1. a. Tighten check valve (do not overtighten) b. Replace check valve
2. Loose fittings*	2. Fingertight fittings (do not overtighten)
3. Mixer seal failure	3. a. Replace mixer seal b. Replace mixer
4. Pump seal failure	4. Repair or replace
5. Pressure transducer failure	5. Repair or replace
6. Pulse damper failure	6. Replace pulse damper
7. Proportioning valve failure	7. a. Check diaphragms, replace if leaky b. Check for fitting damage, replace
8. Purge valve	8. a. Tighten valve b. Replace purge valve

*Note: Use fingertight fittings to avoid sealing problems and the need for wrenches

Leaks (continued)

Injector leaks

POSSIBLE CAUSE	SOLUTION
1. Rotor seal failure	1. Rebuild or replace injector
2. Blocked loop	2. Replace loop
3. Loose injection-port seal	3. Adjust
4. Improper syringe-needle diameter	4. Use correct syringe
5. Waste-line siphoning	5. Keep waste line above surface waste
6. Waste-line blockage	6. Replace waste line

Column leaks

POSSIBLE CAUSE	SOLUTION
1. Loose endfitting	1. Tighten endfitting
2. Column packing in ferrule	2. Disassemble, rinse ferrule, reassemble
2. Tubing stem length longer than column port depth	2. Check column port depth; adjust or replace connecting fitting

Detector leaks

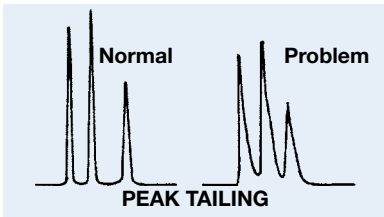
POSSIBLE CAUSE	SOLUTION
1. Cell gasket failure	1. a. Prevent excessive backpressure b. Replace gasket
2. Cracked cell window(s)	2. Replace window(s)
3. Leaky fittings	3. Tighten or replace
4. Blocked waste line	4. Replace waste line
5. Blocked flow cell	5. Clean or replace

Peak Problems

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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

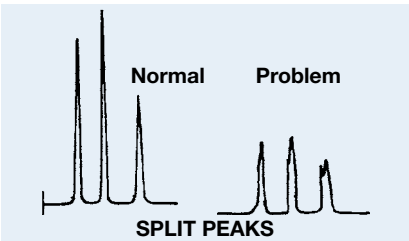
POSSIBLE CAUSE	SOLUTION
1. Sample solvent stronger than mobile phase	1. Use mobile phase for injection solvent
2. Sample overload	2. a. Decrease sample concentration or volume b. Increase column ID

Distortion of larger peaks

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

Peak Problems (continued)

All peaks splitting

POSSIBLE CAUSE	SOLUTION
1. Contamination on guard or analytical column inlet	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 pKa 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 pKa 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 Problems (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

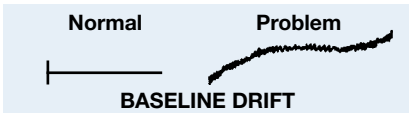
Broad Peaks

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

Baseline Issues

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Baseline drift

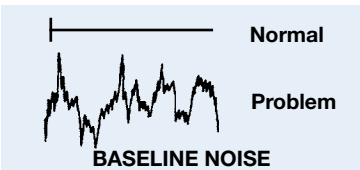
POSSIBLE CAUSE	SOLUTION
1. Column temperature fluctuation. (Even small changes cause cyclic baseline rise and fall. Most often affects refractive index and conductivity detectors, or UV detectors at high sensitivity or in direct photometric mode.)	1. Control column and mobile phase temperature, use heat exchanger before detector 
2. Nonhomogeneous mobile phase. (Drift usually to higher absorbance, rather than cyclic pattern from temperature fluctuation)	2. a. Prepare fresh mobile phase with HPLC grade reagents b. Gently swirl or mix any premixed mobile phases that have been idle for longer than a day c. Ensure mobile phase is being properly degassed; check degasser.
3. Contaminant or air buildup in detector cell	3. Flush cell with methanol or other strong solvent. If necessary, clean cell with 1N HNO ₃ (never with HCl). Add a back pressure regulator after the detector to prevent out gassing in the detector flow cell.
4. Plugged outlet line after detector. (High pressure cracks cell window, producing noisy baseline)	4. Unplug or replace line. Refer to detector manual to replace flow cell.
5. Mobile phase mixing problem or change in flow rate	5. Correct composition / flow rate. To avoid, routinely monitor composition and flow rate
6. Slow column equilibration, especially when changing mobile phase	6. a. Flush with intermediate strength solvent, run 10-20 column volumes of new mobile phase before analysis b. Ion-Pairing & HILIC mobile phases may take up to 60 column volumes for equilibration
7. Mobile phase contaminated, deteriorated, or prepared from low quality materials	7. Check make-up of mobile phase. Use highest grade chemicals and HPLC solvents. Prepare fresh mobile phase.
8. Strongly retained materials in sample (high k') can elute as very broad peaks and appear to be a rising baseline. (Gradient analyses can aggravate problem)	8. Use guard column SecurityGuard™ or SecurityGuard ULTRA is recommended. If necessary, flush column with strong solvent between injections or periodically during analysis
9. Mobile phase recycled but detector not adjusted	9. Reset baseline. Use new mobile phase when dynamic range of detector is exceeded
10. Detector (UV) not set at absorbance maximum but at slope of curve	10. Change wavelength to UV absorbance maximum

Baseline Issues (continued)

Baseline noise (regular)

POSSIBLE CAUSE	SOLUTION
1. Air in mobile phase, detector cell, or pump	1. Degas mobile phase. Flush system to remove air from detector cell or pump
2. Leak	2. Check system for loose fittings. Check pump for leaks, salt build-up, unusual noises. Change pump seals if necessary
	
3. Incomplete mobile phase mixing	3. Mix mobile phase by hand or use less viscous solvent
4. Temperature effect (column at high temperature, detector unheated)	4. Reduce differential or add heat exchanger
5. Other electronic equipment on same line	5. Isolate LC, detector or recorder to determine if source of problem is external. Correct as necessary
6. Pump pulsations	6. Incorporate pulse dampener into system

Baseline noise (irregular)

POSSIBLE CAUSE	SOLUTION
1. Leak	1. Check for loose fittings. Check pump for leaks, salt build-up, unusual noises. Change seals if necessary. Check for detector cell leak
	
2. Mobile phase contaminated, deteriorated, or prepared from low quality materials	2. Check make-up of mobile phase
3. Mobile phase solvents immiscible	3. Select and use only miscible solvents
4. Detector/recorder electronics	4. Isolate detector and recorder electronically. Refer to instruction manual to correct problem
5. Air trapped in system	5. Flush system with strong solvent
6. Air bubbles in detector	6. Purge detector. Install backpressure device after detector
7. Detector cell contaminated (even small amounts of contaminants can cause noise)	7. Clean cell by flushing with 1N HNO ₃ (never with HCl)
8. Weak detector lamp	8. Replace lamp
9. Column leaking silica or packing material	9. Replace column
10. Mobile phase mixer inadequate or malfunctioning	10. Repair or replace the mixer or mix off-line if isocratic

Key Problems Areas and Preventive Maintenance



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, 0.5µm 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

7

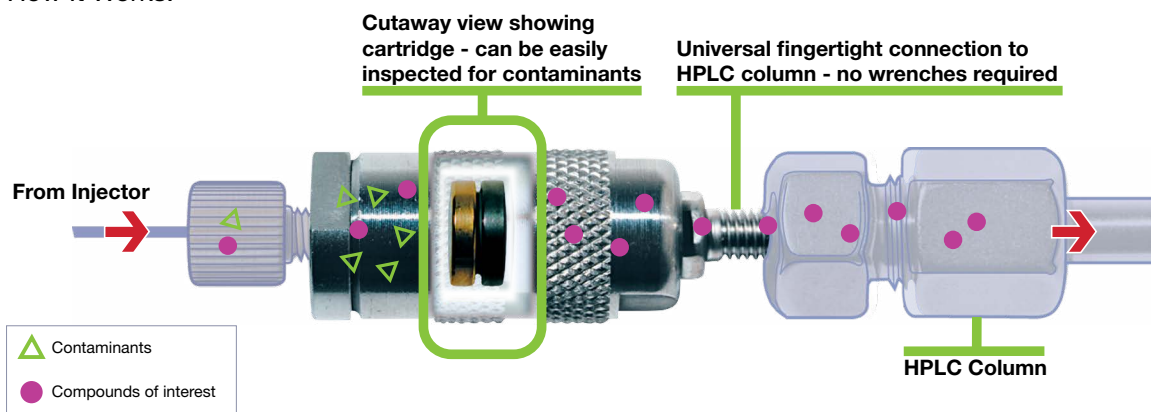
Contaminants Can Cause the Following:

- 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 protect 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.



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

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- More consistent, reproducible results
- Increased column lifetime

Features and Benefits

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- Broad chemical compatibility
- Minimized extractables
- Excellent flow rate
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- Low hold-up volume
- Certified quality
- 100 % integrity tested



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HPLC

Troubleshooting Guide

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Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 3952 5747
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 51 02 180
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: 800-852-3944
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
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