

HPLC Troubleshooting Mini Guide

Pressure Issues



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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 Issues

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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 Issues (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

Key Problem 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

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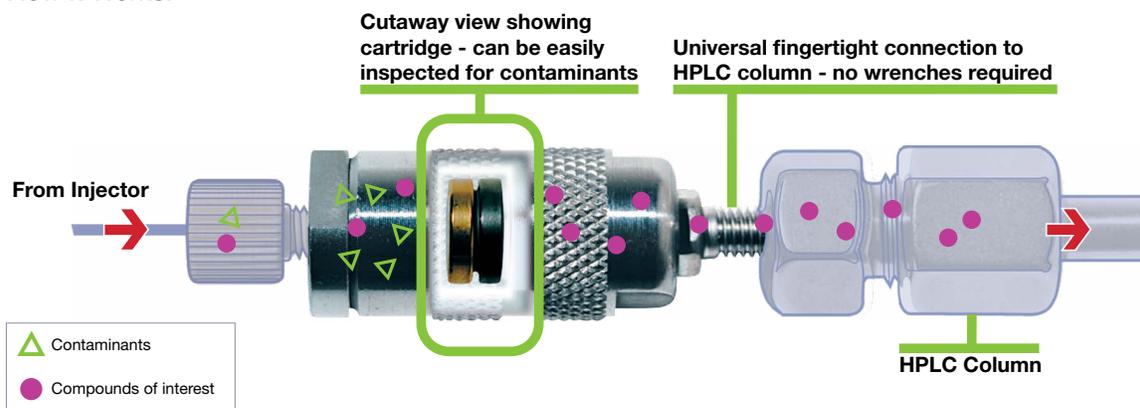
Contaminants Can Cause the Following:

- 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 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

Phenex™ Syringe Filters

For Sample and Solvent Filtration Prior to Chromatography

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- More consistent, reproducible results
- Increased column lifetime

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



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PTFE, Teflon® (Polytetrafluoroethylene)

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PVDF (Polyvinylidene Fluoride)

NY (Nylon)

CA (Cellulose Acetate)

GF (Glass Fiber)



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