HPLC Troubleshooting Mini Guide

Baseline 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).

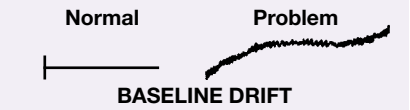
   L.R. Snyder and J.J. Kirkland, ***Introduction to Modern Liquid Chromatography***, 2nd ed., Wiley, NY (1979).

   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.

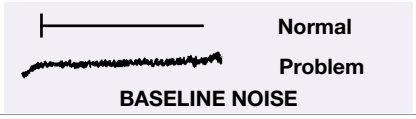
Baseline Issues 2

Baseline drift

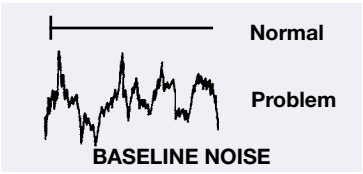
POSSIBLE CAUSE	SOLUTION
1. Column temperature fluctuation. (Even small changes cause cyclic baseline rise and fall. Most often affects refractive index and conductivity detectors, or UV detectors at high sensitivity or in direct photometric mode.)	1. Control column and mobile phase temperature, use heat 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 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, 0.5 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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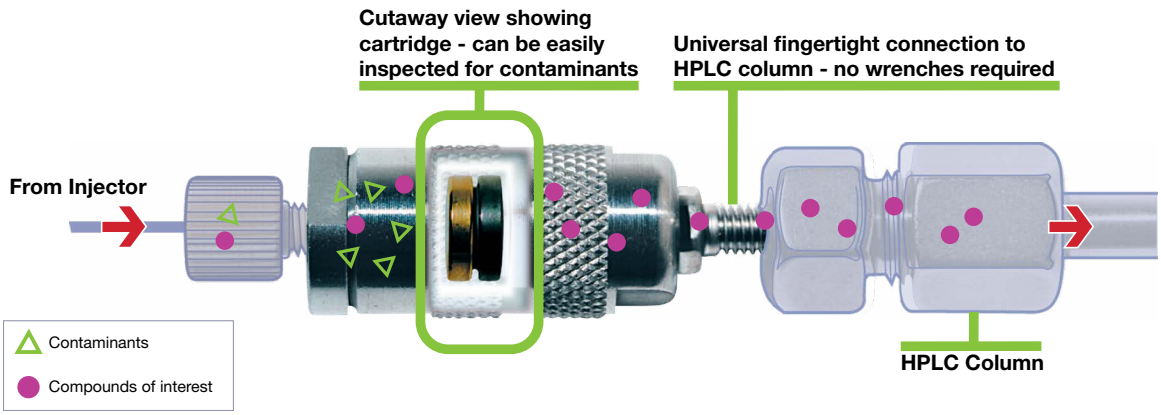
Contaminants Can Cause the Following:

- 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
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- Increased column lifetime

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

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

Phenex™ Syringe Filters

To order and find out more about this product visit **www.phenomenex.com/phenex**

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