HPLC Troubleshooting

1. INTRODUCTION

Locating and Correcting the Problem

A systematic approach is best to identify any problems when troubleshooting the high-performance liquid chromatography (HPLC) system. This guide is organized into five major categories of symptoms to help quickly identify the source of the problem(s).

- Pressure abnormalities
- Leaks
- Problems with the chromatogram
- Injector problems
- Other problems detected by smell, sight, and sound

When the problem has been corrected, record the incident in the HPLC system record book to help with future problems.

Prevention

Many liquid chromatography (LC) problems can be prevented with routine maintenance. For example, replacing pump seals at regular intervals should eliminate pump-seal failure and its associated problems. Section 7 lists the most common problem areas for each LC module, as well as preventive maintenance practices to reduce their frequency. These suggestions can be modified to fit any particular model of LC, and should be made a regular part of the laboratory routine.

2. ABNORMAL PRESSURE

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

A. No Pressure Reading, No Flow

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 setting 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

B. No Pressure Reading, Flow Is Normal

Possible Cause	Solution
1. Faulty meter	1. Replace meter
2. Faulty pressure transducer	2. Replace transducer

C. Steady High Pressure

Possible Cause	Solution
1. Flow rate set too high	1. Adjust setting
2. Blocked column frit	2. a. Backflush column (if permitted) b. Replace frit c. Replace column
3. Improper mobile phase; precipitated buffer	3. a. Use correct mobile phase b. Wash column
4. Improper column	4. Use proper column
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

D. Steady Low Pressure

Possible Cause	Solution
1. Flow set too low	1. Adjust flow rate
2. Leak in system	2. Locate leak and correct
3. Improper column	3. Use proper column
4. Column temperature too high	4. Lower temperature
5. Controller malfunction	5. Repair or replace controller

E. Pressure Climbing

Possible Cause	Solution
1. See Section C	1. See Section C

F. Pressure Dropping to Zero

Possible Cause	Solution
1. See Sections A and B	1. See Sections A and B

G. Pressure Dropping, But Not to Zero

Possible Cause	Solution
1. See Section D	1. See Section D

H. 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 (use Degassex on-line degasser)
5. Leak in system	5. Locate leak and correct
6. Using gradient elution	6. Pressure cycling is normal due to viscosity changes

3. 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 fitting apart and inspect for damage (e.g., distorted ferrule or particles on the sealing surface); damaged fittings should be discarded and replaced.

A. 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

B. Leaks at Pump

Possible Cause	Solution
1. Loose check valves	1. a. Tighten check valve (do not over-tighten) b. Replace check valve
2. Loose fittings	2. Tighten 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

C. Injector Leaks

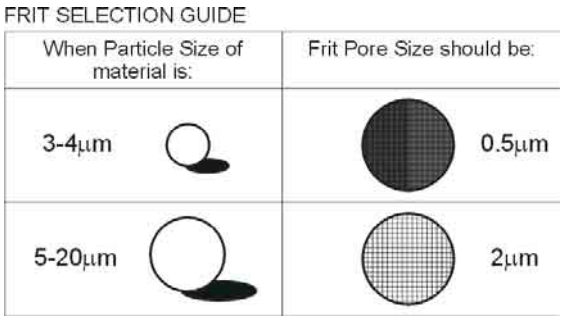
Possible Cause	Solution
1. Rotor seal failure	1. Rebuild or replace injector
2. Blocked loop	2. Replace loop
3. Loose injection-port seal	3. Adjust
4. Improper syringe-needle diameter	4. Use correct syringe
5. Waste-line siphoning	5. Keep waste line above surface waste
6. Waste-line blockage	6. Replace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

D. Column Leaks

Possible Cause	Solution
1. Loose endfitting	1. Tighten endfitting
2. Column packing in ferrule	2. Disassemble, rinse ferrule, reassemble
3. Improper frit thickness	3. Use proper frit (see Frit selection guide chart)

E. 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. Rebuild or replace

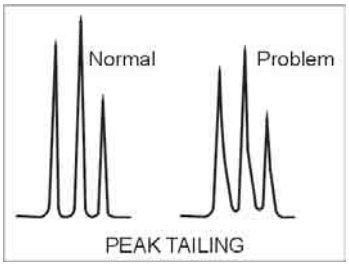


4. PROBLEMS WITH THE CHROMATOGRAM

Many problems in an 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."

A. Peak Tailing

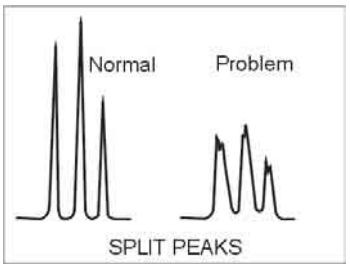
Possible Cause	Solution
1. Blocked frit	1. a. Reverse flush column (if allowed) b. Replace inlet frit c. Replace column
2. Column void	2. Fill void
3. Interfering peak	3. a. Use longer column b. Change mobile-phase and/or column/ selectivity
4. Wrong mobile-phase pH	4. a. Adjust pH b. For basic compounds, a lower pH usually provides more symmetric peaks
5. Sample reacting with active sites	5. a. Add ion pair reagent or volatile basic modifier b. Change column



B. Peak Fronting

Possible Cause	Solution
1. Low temperature	1. Increase column temperature
2. Wrong sample solvent	2. Use mobile phase for injection solvent
3. Sample overload	3. Decrease sample concentration
4. Bad column	4. See A.1. and A.2.

C. Split Peaks

Possible Cause	Solution
1. Contamination on guard or analytical column inlet  	1. a. Remove guard column and attempt analysis b. Replace guard if necessary c. If analytical column is obstructed, reverse and flush d. If problem persists, column may be fouled with strongly retained contaminants e. Use appropriate restoration procedure f. If problem persists, inlet is probably plugged g. Change frit or replace column
2. Sample solvent incompatible with mobile phase	2. Change solvent; whenever possible, inject samples in mobile phase

D. Distortion of Larger Peaks

Possible Cause	Solution
1. Sample overload	1. Reduce sample size

E. Distortion of Early Peaks

Possible Cause	Solution
1. Wrong injection solvent	1. a. Reduce injection volume b. Use weaker injection solvent

F. Tailing, Early Peaks More Than Later Ones

Possible Cause	Solution
1. Extra-column effects	1. a. Replumb system (shorter, narrower tubing) b. Use smaller volume detector cell

G. Increased Tailing as k′ Increases

Possible Cause	Solution
1. Secondary retention effects, reversed-phase mode	1. a. Add triethylamine (basic samples) b. Add acetate (acidic samples) c. Add salt or buffer (ionic samples) d. Try a different column
2. Secondary retention effects, normal-phase mode	2. a. Add triethylamine (basic compounds) b. Add acetic acid
3. Secondary retention effects, ion-pair	3. Add triethylamine (basic samples)

H. Acidic or Basic Peaks Tail

Possible Cause	Solution
1. Inadequate buffering	1. a. Use 50–100 mM buffer concentration b. Use buffer with pKa equal to pH of mobile phase

I. Extra Peaks

Possible Cause	Solution
1. Other components in sample	1. Normal
2. Late-eluting peak from previous injection	2. a. Increase run time or gradient slope b. Increase flow rate
3. Vacancy or ghost peaks	3. a. Check purity of mobile phase b. Use mobile phase as injection solvent c. Reduce injection volume

J. Retention Time Drifts

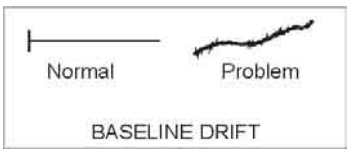
Possible Cause	Solution
1. Poor temperature control	1. Thermostat column
2. Mobile phase changing	2. Prevent change (evaporation, reaction, etc.)
3. Poor column equilibration	3. Allow more time for column equilibration between runs

K. Abrupt Retention Time Changes

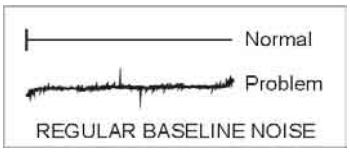
Possible Cause	Solution
1. Flow rate change	1. Reset flow rate
2. Air bubble in pump	2. Bleed air from pump
3. Improper mobile phase	3. a. Replace with proper mobile phase b. Set proper mobile phase mixture on controller

L. Baseline Drift

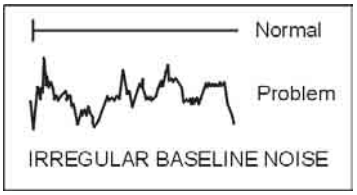
Possible Cause	Solution
1. Column temperature fluctuation (even small changes cause cyclic baseline rise and fall; most often affects refractive index and conductivity detectors, or UV detectors at high sensitivity or in direct photometric mode)	1. a. Control column and mobile phase temperature b. Use heat exchanger before detector
2. Nonhomogeneous mobile phase (drift usually to higher absorbance, rather than cyclic pattern from temperature fluctuation)	2. a. Use HPLC-grade solvents, high-purity salts, and additives b. Degas mobile phase before use c. Sparge with helium during use
3. Contaminant or air buildup in detector cell	3. a. Flush cell with methanol or other strong solvent b. If necessary, clean cell with 1 N HNO_3 (never with HCl)
4. Plugged outlet line after detector (high-pressure cracks cell window, producing noisy baseline)	4. a. Unplug or replace line b. Refer to detector manual to replace window
5. Mobile-phase mixing problem or change in flow rate	5. a. Correct composition/flow rate b. To avoid, routinely monitor composition and flow rate
6. Slow column equilibration, especially when changing mobile phase	6. a. Flush with intermediate strength solvent b. Run 10–20 column volumes of new mobile phase before analysis
7. Mobile phase contaminated, deteriorated, or prepared from low-quality materials	7. a. Check make-up of mobile phase b. Use highest grade chemicals and HPLC solvents
8. Strongly retained materials in sample (high k) can elute as very broad peaks and appear to be a problem	8. a. Use guard column b. If necessary, flush column with strong solvent between injections or periodically during analysis
9. Mobile phase recycled but detector not adjusted	9. a. Reset baseline b. 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



M. Baseline Noise (Regular)

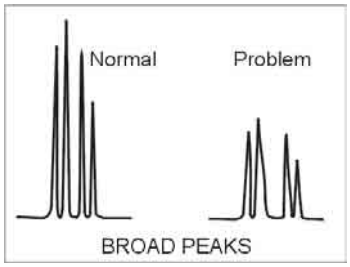
Possible Cause	Solution
1. Air in mobile phase, detector cell, or pump	1. a. Degas mobile phase b. Flush system to remove air from detector cell or pump
2. Leak  	2. a. See Section 3 b. Check system for loose fittings c. Check pump for leaks, salt build-up, unusual noises d. Change pump seals if necessary
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 head 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

N. Baseline Noise (Irregular)

Possible Cause	Solution
1. Leak  	1. a. See Section 3 b. Check for loose fittings c. Check pump for leaks, salt build-up, unusual noises d. Change seals if necessary e. 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. a. Isolate detector and recorder electronically b. Refer to instruction manual to correct problem
5. Air trapped in system	5. Flush system with strong solvent
6. Air bubbles in detector	6. a. Purge detector b. Install back-pressure device after detector
7. Detector cell contaminated (even small amounts of contaminants can cause noise)	7. Clean cell by flushing with 1 N 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

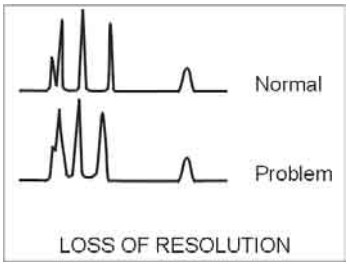
O. Broad Peaks

Possible Cause	Solution
1. Mobile-phase composition changed	1. Prepare new mobile phase
2. Mobile-phase flow rate too low	2. Adjust flow rate
3. Leaks (especially between column and detector)	3. a. See Section 3 b. Check for loose fittings c. Check pump for leaks, salt build-up, and unusual noises d. Change seals if necessary
4. Detector settings incorrect	4. Adjust settings
5. Extra-column effects: a. Column overloaded b. Detector response time or cell volume too large c. Tubing between column and detector too long or ID too large d. Recorder response time too high	5. a. Inject smaller column (e.g., 10 µl vs. 100 µl) or 1:10 and 1:100 dilutions of sample b. Reduce response time or use smaller cell c. Use as short a piece of 0.007–0.010-inch ID tubing as practical d. Reduce response time
6. Buffer concentration too low	6. Increase concentration
7. Guard column contaminated/worn out	7. Replace guard column
8. Column contaminated/worn out; low plate number	8. a. Replace column with new one of same type b. If new column provides symmetrical peaks, flush old column with strong solvent
9. Void at column inlet	9. Open inlet end and fill void or replace column
10. Peak represents two or more poorly resolved compounds	10. Change column type to improve separation
11. Column temperature too low	11. Increase temperature; do not exceed 75°C unless higher temperatures are acceptable to column manufacturer
12. Detector time constant too large	12. Use smaller time constant



P. Loss of Resolution

Possible Cause	Solution
1. Mobile phase contaminated/deteriorated (causing retention time to change)	1. Prepare new mobile phase
2. Obstructed guard or analytical column	2. a. Remove guard column and attempt analysis b. Replace guard if necessary c. If analytical column is obstructed, reverse and flush; if problem persists, column may be fouled with strongly retained contaminants d. Use appropriate restoration procedure; if problem persists, inlet is probably plugged e. Change frit or replace column



Q. All Peaks Too Small

Possible Cause	Solution
1. Detector attentuation too high	1. Reduce attenuation
2. Detector time constant too large	2. Use smaller time constant
3. Injection size too small	3. Use larger sample loop
4. Improper recorder connection	4. Use correct connection

R. All Peaks Too large

Possible Cause	Solution
1. Detector attenuation too low	1. Use larger attenuation
2. Injection size too large	2. Use smaller sample loop
3. Improper recorder connection	3. Use correct connection

5. PROBLEMS WITH THE INJECTOR

These problems are usually detected while using the injection valve. Leaky injection valves are discussed in Section 3 (Leaks).

A. Manual Injector, Hard to Turn

Possible Cause	Solution
1. Damaged rotor seal	1. Rebuild or replace valve
2. Rotor too tight	2. Adjust rotor tension

B. Manual Injector, Hard to Load

Possible Cause	Solution
1. Valve misaligned	1. Adjust alignment
2. Blocked loop	2. Replace loop
3. Dirty syringe	3. Clean or replace syringe
4. Blocked lines	4. Clear or replace lines

C. Autoinjector, Will Not Turn

Possible Cause	Solution
1. No air pressure (or power)	1. Supply proper pressure (power)
2. Rotor too tight	2. Adjust
3. Valve misaligned	3. Adjust alignment

D. Autoinjector, Other Problems

Possible Cause	Solution
1. Blockage	1. Clear or replace blocked portion
2. Jammed mechanism	2. See service manual
3. Faulty controller	3. Repair or replace controller

6. PROBLEMS DETECTED BY SMELL, SIGHT, OR SOUND

All senses must be used to identify LC problems. Get in the habit of taking a few minutes each day to expose all senses (except taste!) to the LC to get a "feel" for how the LC performs normally. This will help to locate problems quickly. For example, often a leak can be detected by smell before it is seen. The majority of problems are identified by sight; most of these are included in the proceeding section.

A. Solvent Smell

Possible Cause	Solution
1. Leak	1. See Section 3
2. Spill	2. a. Check for overflowing waste container b. Locate spill and clean up

B. "Hot" Smell

Possible Cause	Solution
1. Overheating module	1. a. Check for proper ventilation, adjust b. Check temperature setting, adjust c. Shut module off, see service manual

C. Abnormal Meter Readings

Possible Cause	Solution
1. Pressure abnormality	1. See Section 2
2. Column oven problem	2. a. Check settings, adjust b. See service manual
3. Detector lamp failing	3. Replace lamp

D. Warning Lamps

Possible Cause	Solution
1. Pressure limit exceeded	1. a. Check for blockage b. Check limit setting, adjust
2. Other warning lamps	2. See service manual

E. Warning Buzzers

Possible Cause	Solution
1. Solvent leak/spill	1. Locate and correct
2. Other warning buzzers	2. See service manual

F. Squeaks and Squeals

Possible Cause	Solution
1. Bearing failure	1. See service manual
2. Poor lubrication	2. Lubricate as necessary
3. Mechanical wear	3. See service manual

7. 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 LC's operator and service manuals may have additional suggestions for preventive maintenance.

A. Reservoir

Problem	Preventive Maintenance
1. Blocked inlet frit	1. a. Replace (3–6 months) b. Filter mobile phase, 0.5 µm filter
2. Gas bubbles	2. Degas mobile phase

B. Pump

Problem	Preventive Maintenance
1. Air bubbles	1. Degas mobile phase
2. Pump seal failure	2. Replace (3 months)
3. Check valve failure	3. Filter mobile phase; use inlet-line frit; keep spare

C. Injector

Problem	Preventive Maintenance
1. Rotor seal wear	1. a. Do not overtighten b. Filter samples

D. Column

Problem	Preventive Maintenance
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 >8 b. Use guard column c. Use precolumn (saturator column)

E. Detector

Problem	Preventive Maintenance
1. Lamp failure; decreased detector response; increased detector noise	1. Replace (6 months) or keep spare lamp
2. Bubbles in cell	2. a. Keep cell clean b. Use restrictor after cell c. Degas mobile phase

F. General

Problem	Preventive Maintenance
1. Corrosive/abrasive damage	1. Flush buffer from LC and clean when not in use