

HPLC

TROUBLESHOOTING GUIDE



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I. INTRODUCTION

LOCATING AND CORRECTING THE PROBLEM

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

- pressure abnormalities
- leaks
- problems with the chromatogram
- injector problems
- other problems detected by the senses of smell, sight, and sound

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

PREVENTION

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

WHERE TO GET ADDITIONAL HELP

- 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.
- Other people in the lab may have had experience solving a problem which is giving you trouble; they can be a helpful resource.
- The manufacturer of your instrument can help you. Most LC manufacturers offer free technical support to their customers.
- Phenomenex has experienced technical consultants who can assist you with almost any problem. We welcome your phone calls, faxes or emails.
- Phenomenex offers seminars, as well as a complete line of reference books on HPLC.
- There are a number of reference sources that can give you guidance in problem solving:

J.W. Dolan and L.R. Snyder, ***Troubleshooting LC Systems***, Humana Press, NJ (1989). Phenomenex Order No.: AA0-1717

L.R. Snyder and J.J. Kirkland, ***Introduction to Modern Liquid Chromatography***, 2nd ed., Wiley, NY (1979). Phenomenex Order No.: AA0-1700

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

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

II. 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 that you observe, 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 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

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

* Check manufacturer's column warranty first. Removal of end-fittings may void column warranty.

II. ABNORMAL PRESSURE (continued)

D. Steady, low pressure

POSSIBLE CAUSE	SOLUTION
1. Flow set too low	1. Adjust flow rate
2. Leak in system	2. Locate 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 and correct
6. Using gradient elution	6. Pressure cycling is normal due to viscosity changes

III. 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.

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 overtighten) 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

* Use fingertight end-fittings to avoid sealing problems and the need for wrenches

III. LEAKS (continued)

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 chart below)

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

FRIT PORE SIZE SELECTION GUIDE

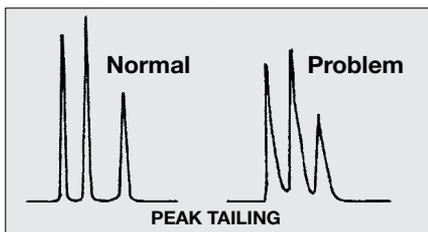
When Particle Size of material is:	Frit Pore Size should be:
3 - 4 μm 	 0.5 μm
5 - 20 μm 	 2 μm

IV. PROBLEMS WITH THE CHROMATOGRAM

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.”

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. Adjust pH. For basic compounds, 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. Remove guard column and attempt analysis. Replace guard

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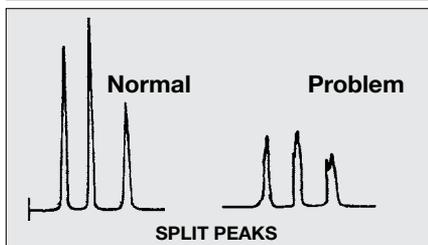
* Check manufacturer's column warranty first. Removal of end-fittings may void column warranty.

IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

C. Split peaks (continued)

POSSIBLE CAUSE

SOLUTION



if necessary. If 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. 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.
e. See page 19

2. Secondary retention effects, normal-phase mode

2. a. Add triethylamine (basic compounds)
b. Add acetic acid (acidic compounds)

IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

G. Increased tailing as k' increases (continued)

POSSIBLE CAUSE	SOLUTION
2. Secondary retention effects, normal-phase mode	2. c. Add water (poly-functional compounds). Only for normal-phase methods which use water-miscible solvents. d. Try a different LC method
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 c. See page 19

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
4. Contamination	4. Filter sample

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 flowrate
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

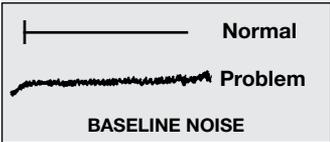
IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

L. Baseline drift

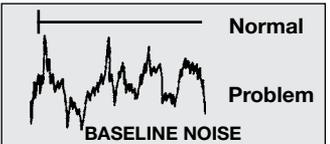
POSSIBLE CAUSE	SOLUTION
1. Column temperature fluctuation. (Even small changes cause cyclic baseline rise and fall. Most often affects refractive index and conductivity detectors, or UV detectors at high sensitivity or in direct photometric mode.)	1. Control column and mobile phase temperature, use heat exchanger before detector 
2. Nonhomogenous mobile phase. (Drift usually to higher absorbance, rather than cyclic pattern from temperature fluctuation.)	2. Use HPLC grade solvents, high purity salts, and additives. Degas mobile phase before use, sparge with helium during use.
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.)
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 window.
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. Flush with intermediate strength solvent, run 10-20 column volumes of new mobile phase before analysis
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
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. 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

IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

M. 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. See section III. Check system for loose fittings. Check pump for leaks, salt build-up, unusual noises. Change pump seals if necessary
	
3. Incomplete mobile phase mixing less viscous solvent	3. Mix mobile phase by hand or use
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

N. Baseline noise (irregular)

POSSIBLE CAUSE	SOLUTION
1. Leak	1. See section III. 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 back-pressure device after detector

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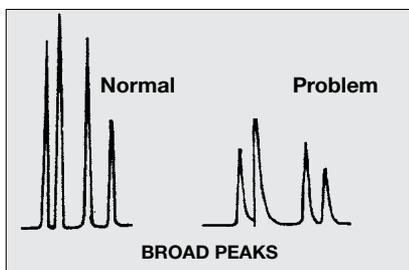
IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

N. Baseline noise (irregular) *continued*

POSSIBLE CAUSE	SOLUTION
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. Mobil 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. See section III. Check for loose fittings. Check pump for leaks, salt build-up, and unusual noises. 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 volume (e.g., 10 μ L vs. 100 μ L) 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 in. ID tubing as practical d. Reduce response time



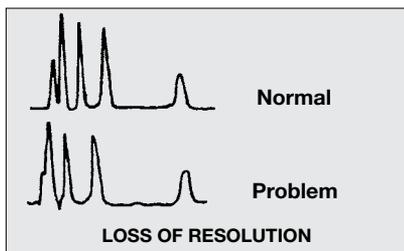
IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

O. Broad peaks (continued)

POSSIBLE CAUSE	SOLUTION
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. Replace column with new one of same type
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 60 °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. Remove guard column and attempt analysis. Replace guard if necessary. If 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. Change frit or replace column.



IV. PROBLEMS WITH THE CHROMATOGRAM (continued)

Q. *All peaks too small*

POSSIBLE CAUSE	SOLUTION
1. Detector attenuation too high	1. Reduce attenuation
2. Detector time constant too large	2. Use smaller time constant
3. Injection size too small	3. a. Increase sample concentration b. Increase injection volume, if column size allows
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. a. Reduce sample concentration b. Decrease injection volume, use a smaller sample loop or use partial loop filling
3. Improper recorder connection	3. Use correct connection

V. PROBLEMS WITH THE INJECTOR

These problems are usually detected while you are using the injection valve. Leaky injection valves are discussed in Section III (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, won't 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

VI. PROBLEMS DETECTED BY SMELL, SIGHT OR SOUND

You need to use all your senses to identify LC problems. You should get in the habit of taking a few minutes each day to expose all of your senses (except taste!) to the LC so that you can get a “feel” for how the LC performs normally. This will help you to quickly locate problems. For example, often you can smell a leak before you see it. The majority of problems are identified by sight; most of these are included in the preceding section.

A. Solvent smell

POSSIBLE CAUSE	SOLUTION
1. Leak	1. See section III
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 II
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

VI. PROBLEMS DETECTED BY SMELL, SIGHT OR SOUND

(continued)

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

VII. 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

PROBLEM	PREVENTIVE MAINTENANCE
1. Blocked inlet frit	1. a. Replace (3-6 mo.) b. Filter mobile phase, 0.5 μ filter
2. Gas bubbles	2. Degas mobile phase

Pump

PROBLEM	PREVENTIVE MAINTENANCE
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

PROBLEM	PREVENTIVE MAINTENANCE
1. Rotor seal wear	1. a. Don't overtighten b. Filter samples

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. (Most silica-based columns) b. Use guard column c. Use precolumn (saturation column)

VII. KEY PROBLEM AREAS AND PREVENTIVE MAINTENANCE (continued)

Detector

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

General

PROBLEM	PREVENTIVE MAINTENANCE
1. Corrosive/abrasive damage	1. Flush buffer from LC and clean when not in use

WARNING: CONTAMINANTS CAN CAUSE



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

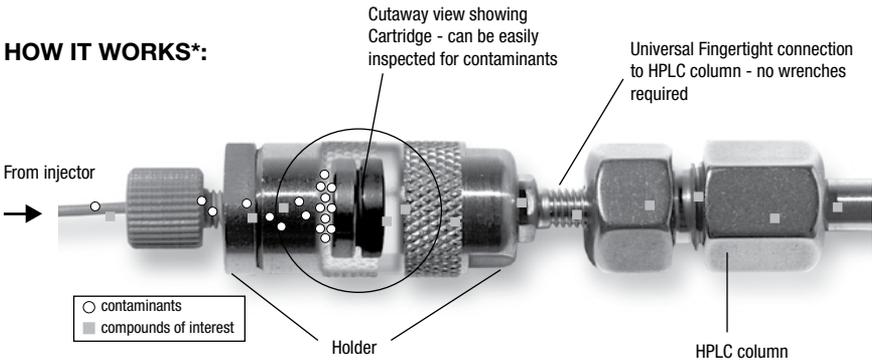
PROTECT YOUR HPLC COLUMN AND RESULTS



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

A universal HPLC guard cartridge system designed to effectively protect your valuable analytical columns and results from the damaging effect of contaminants. Trap contaminants without altering your chromatography.

HOW IT WORKS*:

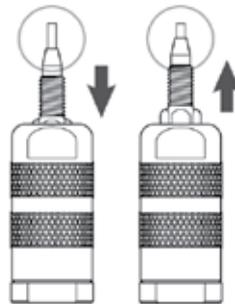


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Patented Design

*Feature applies to analytical-sized guard system only, and does not apply to SemiPrep or PREP guard cartridges.

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For Sample and Solvent Filtration Prior to Chromatography

- Less system down time
- Consistent, reproducible results
- Increased column lifetime



Phenex Offers:

- » Low absorption
- » Maximum chemical compatibility
- » Minimum extractables
- » Excellent flow rate
- » High total throughput
- » Low hold-up volume
- » Certified quality
- » 100 % integrity tested
- » Easy pore identification
- » Bi-directional use

Membrane Types	
RC (Regenerated Cellulose)	NY (Nylon)
PTFE (Polytetrafluoroethylene)	CA (Cellulose Acetate)
PES (Polyethersulfone)	GF (Glass Fiber)



Above syringe filters are non-sterile.
Housing is made of medical-grade polypropylene (PP).

Tip: Try a Sample Pack!

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