An HPLC troubleshooting guide

Noisy baselines ruining your sleep? Negative peaks got you down? Here's what to do.

It comes down to a question of money. With the cost of a complete HPLC system ranging into the tens of thousands of dollars and service calls at $50 per hour, it is in the interest of all researchers to know something about fixing their own equipment.

The first step is locating the cause of the problem. To make that step easier, *LC* magazine is presenting an extensive troubleshooting guide in a format designed for quick reference at the bench. The guide lists many frequently encountered symptoms characteristic of HPLC system problems. Each symptom has corresponding suggestions as to the cause of the problem as well as recommendations for corrective action.

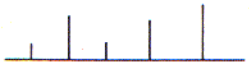
The material was prepared by Dennis J. Runser, currently Director of Corporate Chemical Affairs at Marion Laboratories, Inc. Dr. Runser, who received his PhD in analytical chemistry from the University of Iowa in 1971, has also held managerial positions in quality control and analytical research at G.D. Searle & Co. and the personal-care division of Gillette Co.

In upcoming issues, Dr. Runser will go into more detail on troubleshooting the mobile phase of your HPLC system, and ways to detect and solve problems in your solvent delivery system. Troubleshooting will be an ongoing feature in *LC*, and we welcome letters from researchers describing problems they are encountering with their equipment. Letters will be considered for inclusion in a question-and-answer column beginning later in the year.

SYMPTOM:	Possible cause:	Corrective action:
No flow or pressure	**a** System leak	**a** Locate and repair
	b Injection valve improperly positioned	**b** Check valve for proper rotation
	c Air in the pump	**c** Disconnect system at pump outlet and pump at maximum flow rate with MeOH until no more bubbles appear; replace mobile phase
	d Faulty pressure gauge	**d** Check for proper flow at the system outlet and insert a different gauge
	e No mobile phase	**e** Check solvent reservoir and refill
	f Solvent delivery system not pumping	**f** Check that the pump is plugged in and turned on; check the pressure limit switch
	g Pump starvation	**g** Check the boiling point of the solvent for potential pump cavitation

SYMPTOM:	Possible cause:	Corrective action:
Pump pressure up but no flow through system	**a** Particulate matter clogging inlet system or head of column	**a1** Filter mobile phase and sample **a2** Check syringe for a barb(s) breaking septa pieces off into the system
	b Leak in system	**b1** Check all system fittings and repair **b2** Check detector cell for leaks
	c Plugged detector line	**c** Turn pump off immediately and carefully clean lines and cell
	d Injection valve improperly positioned	**d** Check valve for proper rotation
	e Column inlet clogged with dirt accumulation	**e** Clean inlet and/or replace column

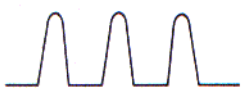
SYMPTOM:	Possible cause:	Corrective action:
Baseline stepping and peaks are flat-topped; Baseline does not zero	**a** Recorder gain and damping improperly adjusted	**a** Properly adjust gain and damp
	b Improper grounding	**b** Check system ground
	c Saturated electronics	**c** Reduce sample size

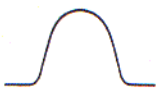
SYMPTOM:	Possible cause:	Corrective action:
Baseline spiking	**a** Air bubbles passing through detector	**a1** Degas mobile phase **a2** Locate and repair all leaks **a3** Flush air out of pump and check valves **a4** Check boiling point of mobile phase
	**b** Improper system ground	**b** Check for proper grounding
	c Electronic interference from other lab equipment turning on and off	**c** Check for other equipment turning on and off on same circuit and remove (e.g., constant temperature bath)
	d Loose electronic connections	**d1** Check all connections and plugs **d2** Check for vibrations **d3** Check for loose fitting source lamp
	e *Rf* feedback	**e** Properly grounded equipment

SYMPTOM:	Possible cause:	Corrective action:
Baseline drifting	**a** Dirt in detector sample or reference cells	**a** Flush detector cells with solvent or carefully clean cell
	b Temperature gradient over the system	**b1** Check for drafts **b2** Insulate column and column inlet/outlet lines or use a constant temperature jacket **b3** Move instrument away from direct sunlight
	c Contamination bleed in system	**c1** Check for septum bleed and replace with proper septum **c2** Check for column bleed: i. Previous sample(s) buildup — wash the column ii. Column-mobile phase incompatibility — replace column or mobile phase **c3** Stationary phase bleed (particularly at elevated temperatures); check stationary phase solubility in mobile phase; change mobile phase and column, or add stationary phase to mobile phase, or add a heavily loaded precolumn to the system **c4** Uneluted peaks; wash column
	d System leak	**d** Locate and repair
	e Bubble trapped in detector sample or reference cell	**e1** Flush out cell **e2** Degas mobile phase **e3** Locate and repair any system leaks **e4** Add suitable back pressure to detector outlet
	f Solvent immiscibility or immiscible pools (previous solvent not thoroughly flushed out)	**f** Flush system with compatible solvents until only the desired mobile phase is present
	g Mobile phase not in equilibrium with column	**g** Continue to flush system until equilibrium is established
	h Mobile phase/sample vaporizing	**h** At elevated temperatures check boiling point of mobile phase
	i Contamination in mobile phase	**i** Change mobile phase
	j Failing detector source	**j** Replace with new source
	k Recorder problems	**k** Short out detector; if drift continues, check recorder

SYMPTOM:	Possible cause:	Corrective action:
Noisy baseline	**a** Air bubbles passing through detector	**a1** Degas mobile phase **a2** Flush pump check valves clear of air **a3** Check all fittings for air leaking into mobile phase stream; look for salt-like deposits and stains near fittings; tighten appropriately
	b Column packing passing through detector	**b** Check column outlet for proper column plug and screen
	c Leak in system	**c** Locate and repair
	d Pulses from pump	**d** Add a pulse dampener and/or restrictor
	e Pulse dampener(s) and/or restrictors not properly flushed	**e** Disconnect solvent delivery system from injector and purge with suitable solvent(s)
	f Bubbles in detector sample or reference cell	**f** Check for bubbles entering detector and flush out air
	g Dirt in detector	**g1** Disconnect detector from system and back flush with suitable solvents **g2** Clean detector cell
	h Detector source failing	**h** Check and replace source
	i Temperature effects on detector cell input tubing	**i1** Insulate inlet tubing **i2** Move instrument away from drafts and/or direct sunlight
	j Recorder improperly grounded	**j** Check recorder and properly ground
	k Noisy electronics	**k1** Check appropriate detector and recorder circuits; consult instrument manual **k2** At high detector attenuation check source lamp **k3** Check for dirty or loose electronic contacts; also check for instrument vibration

SYMPTOM:	Possible cause:	Corrective action:
Negative peaks	**a** Polarity reversed on detector or recorder	**a1** Switch polarity to other position **a2** Reverse detector leads
	b Negative peaks in UV trace	**b** Check for non-UV absorbers in system or sample impurities
	c Negative peak at V_O	**c1** Result of pressure surge due to sample introduction; don't quantitate peaks at V_O **c2** Air sampled, improve sample introduction technique

SYMPTOM:	Possible cause:	Corrective action:
Flat bottom peaks	**a** Bubbles in detector	**a** Degas mobile phase or add suitable back pressure to detector cell
	**b** Dirt buildup on detector cell windows	**b** Clean detector cell
	c Optics out of adjustment	**c** Check alignment or call service representative
	d Light output in reference beam lower than on sample side	**d** Clean cell and check with operation manual or service representative

SYMPTOM:	Possible cause:	Corrective action:
Very rounded peaks	**a** Operating beyond linear dynamic range of detector	**a** Reduce sample size
	b Recorder gain is too low	**b** Adjust recorder gain
	**c** Column-sample interaction (e.g., absorption)	**c1** Check sample chemistry and change column **c2** Increase temperature **c3** Change ionic strength or pH
	d Column dried out at ends	**d** Replace column
	e Column overload	**e** Reduce sample size
	f Contamination on detector cell windows	**f** Clean detector cell

SYMPTOM:	Possible cause:	Corrective action:
Loss of resolution	**a** Column overload	**a** Reduce sample size
	b Loss of column efficiency	**b** Adjust mobile phase or replace/regenerate column
	c Loss of column liquid phase	**c** Replace column
	d Dirty column	**d** Wash column with suitable solvents or replace
	e Distorted column bed (cracked, compressed)	**e** Repack or replace column
	f Used wrong column or mobile phase	**f** Change system

SYMPTOM:	Possible cause:	Corrective action:
Increased retention volume	**a** System flow rate decreased	**a** Check and increase flow rate; if flow rate decreasing, check and repair any leaks
	b Column temperature too low	**b** Insulate or jacket column
	c Column activity changing	**c** Solvent stripping H_2O or stationary phase off of column; add H_2O or liquid phase to mobile phase

SYMPTOM:	Possible cause:	Corrective action:
Decreased retention time	**a** System flow rate increased	**a** Check pump for proper setting
	b Column activity changing	**b** Change column
	c Wrong mobile phase	**c1** Change mobile phase **c2** Check for changes in mobile phase ratio

SYMPTOM:	Possible cause:	Corrective action:
Recorder will not zero	**a** Bubbles in UV reference cell or wrong solvent in RI reference cell	**a1** Flush cell out and replace with air or proper solvent **a2** Degas mobile phase **a3** Add suitable back pressure to detector outlet
	b Mobile phase has not equilibrated with column	**b** Flush system longer
	c Mobile phase has not equilibrated with detector or is not compatible with detector	**c1** The mobile phase's refractive index or UV is not compatible with the detector; adjust detector or change mobile phase **c2** Contaminant in mobile phase; prepare fresh and change, allowing time to equilibrate
	d Column bleed	**d1** Add H_2O or stationary phase to mobile phase **d2** Check solubility of stationary phase in mobile phase; if working above ambient, reduce temperature to check effect
	e Contamination bleed from column	**e** Thoroughly wash column with proper solvents or replace column
	f Previous mobile phase still in system	**f** Properly flush entire system with compatible solvents
	g Detector not connected to recorder	**g** Check detector lines to recorder
	h Detector source lamp failing/faulty	**h** Replace source
	i Dirty detector cell windows	**i** Clean cell windows
	j Particulates in detector cell	**j** Flush and clean detector cell
	k Electronic problem with detector	**k** Check service manual
	l Recorder or detector not plugged in or turned on	**l** Check power cord and on/off switch
	m Recorder improperly zeroed	**m** Rezero recorder
	n Recorder calibration knob out of position	**n** Readjust calibration knob

SYMPTOM:	Possible cause:	Corrective action:
Low sensitivity	**a** Inadequate flow rate	**a** Adjust flow rate
	b Sample not compatible with detector	**b** Check sample chemistry and adjust detector or change it
	c Insufficient sample	**c** Increase sample size
	d Sample not eluting from column	**d** Check sample chemistry; change mobile phase and/or column
	e Dirty detector cell windows	**e** Clean cell
	f Gas bubble(s) in detector cell	**f1** Degas mobile phase **f2** Apply suitable back pressure to detector output
	g Detector attenuation too high	**g** Adjust attenuation
	h Detector and/or recorder out of calibration	**h** Check detector and recorder calibration; recalibrate if necessary
	i Failing/faulty detector source	**i** Change detector source
	j Recorder in wrong millivolt range	**j** Check setting and adjust

SYMPTOM:	Possible cause:	Corrective action:
No peaks; no response	**a** Detector/recorder not on	**a** Check and turn on
	b Detector/recorder not plugged in	**b** Check and plug in
	c No sample injected	**c** Check injection/injector for complete sample introduction; clean or change syringe or valve
	d Failure in the electronics	**d1** Check and replace fuse **d2** Check service manual

REFERENCES:

(1) E.L. Johnson and R. Stevenson, *Basic Liquid Chromatography*, Varian Associates, Palo Alto, California 1978.

(2) L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed., John Wiley & Sons, Inc., New York, 1979.

(3) J. Q. Walker, M. T. Jackson Jr., and J. B. Maynard, *Chromatography Systems — Maintenance and Troubleshooting*, 2nd ed., Academic Press, New York, 1977. ■