

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.

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SYMPTOM:	Possible cause:	Corrective action:
No flow or pressure	<ul style="list-style-type: none"> a System leak b Injection valve improperly positioned c Air in the pump d Faulty pressure gauge e No mobile phase f Solvent delivery system not pumping g Pump starvation 	<ul style="list-style-type: none"> a Locate and repair b Check valve for proper rotation c Disconnect system at pump outlet and pump at maximum flow rate with MeOH until no more bubbles appear; replace mobile phase d Check for proper flow at the system outlet and insert a different gauge e Check solvent reservoir and refill f Check that the pump is plugged in and turned on; check the pressure limit switch g Check the boiling point of the solvent for potential pump cavitation
Pump pressure up but no flow through system	<ul style="list-style-type: none"> a Particulate matter clogging inlet system or head of column b Leak in system c Plugged detector line d Injection valve improperly positioned e Column inlet clogged with dirt accumulation 	<ul style="list-style-type: none"> a1 Filter mobile phase and sample a2 Check syringe for a barb(s) breaking septa pieces off into the system b1 Check all system fittings and repair b2 Check detector cell for leaks c Turn pump off immediately and carefully clean lines and cell d Check valve for proper rotation e Clean inlet and/or replace column
Baseline stepping and peaks are flat-topped; Baseline does not zero	<ul style="list-style-type: none"> a Recorder gain and damping improperly adjusted b Improper grounding c Saturated electronics 	<ul style="list-style-type: none"> a Properly adjust gain and damp b Check system ground c Reduce sample size
Baseline spiking	<ul style="list-style-type: none"> a Air bubbles passing through detector  b Improper system ground c Electronic interference from other lab equipment turning on and off d Loose electronic connections e Rf feedback 	<ul style="list-style-type: none"> 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 Check for proper grounding c Check for other equipment turning on and off on same circuit and remove (e.g., constant temperature bath) d1 Check all connections and plugs d2 Check for vibrations d3 Check for loose fitting source lamp e Properly grounded equipment

SYMPTOM:	Possible cause:	Corrective action:
Baseline drifting	<ul style="list-style-type: none"> a Dirt in detector sample or reference cells b Temperature gradient over the system c Contamination bleed in system d System leak e Bubble trapped in detector sample or reference cell f Solvent immiscibility or immiscible pools (previous solvent not thoroughly flushed out) g Mobile phase not in equilibrium with column h Mobile phase/sample vaporizing i Contamination in mobile phase j Failing detector source k Recorder problems 	<ul style="list-style-type: none"> a Flush detector cells with solvent or carefully clean cell 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 c1 Check for septum bleed and replace with proper septum c2 Check for column bleed: <ul style="list-style-type: none"> 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 Locate and repair e1 Flush out cell e2 Degas mobile phase e3 Locate and repair any system leaks e4 Add suitable back pressure to detector outlet f Flush system with compatible solvents until only the desired mobile phase is present g Continue to flush system until equilibrium is established h At elevated temperatures check boiling point of mobile phase i Change mobile phase j Replace with new source k Short out detector; if drift continues, check recorder

SYMPTOM:	Possible cause:	Corrective action:
Noisy baseline	<ul style="list-style-type: none"> a Air bubbles passing through detector b Column packing passing through detector c Leak in system d Pulses from pump e Pulse dampener(s) and/or restrictors not properly flushed f Bubbles in detector sample or reference cell g Dirt in detector h Detector source failing i Temperature effects on detector cell input tubing j Recorder improperly grounded k Noisy electronics 	<ul style="list-style-type: none"> 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 Check column outlet for proper column plug and screen c Locate and repair d Add a pulse dampener and/or restrictor e Disconnect solvent delivery system from injector and purge with suitable solvent(s) f Check for bubbles entering detector and flush out air g1 Disconnect detector from system and back flush with suitable solvents g2 Clean detector cell h Check and replace source i1 Insulate inlet tubing i2 Move instrument away from drafts and/or direct sunlight j Check recorder and properly ground 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: Negative peaks	Possible cause: <ul style="list-style-type: none"> a Polarity reversed on detector or recorder b Negative peaks in UV trace c Negative peak at V_0 	Corrective action: <ul style="list-style-type: none"> a1 Switch polarity to other position a2 Reverse detector leads b Check for non-UV absorbers in system or sample impurities c1 Result of pressure surge due to sample introduction; don't quantitate peaks at V_0 c2 Air sampled, improve sample introduction technique
SYMPTOM: Flat bottom peaks	Possible cause: <ul style="list-style-type: none"> a Bubbles in detector b Dirt buildup on detector cell windows c Optics out of adjustment d Light output in reference beam lower than on sample side 	Corrective action: <ul style="list-style-type: none"> a Degas mobile phase or add suitable back pressure to detector cell b Clean detector cell c Check alignment or call service representative d Clean cell and check with operation manual or service representative
SYMPTOM: Very rounded peaks	Possible cause: <ul style="list-style-type: none"> a Operating beyond linear dynamic range of detector b Recorder gain is too low c Column-sample interaction (e.g., absorption) d Column dried out at ends e Column overload f Contamination on detector cell windows 	Corrective action: <ul style="list-style-type: none"> a Reduce sample size b Adjust recorder gain c1 Check sample chemistry and change column c2 Increase temperature c3 Change ionic strength or pH d Replace column e Reduce sample size f Clean detector cell
SYMPTOM: Loss of resolution	Possible cause: <ul style="list-style-type: none"> a Column overload b Loss of column efficiency c Loss of column liquid phase d Dirty column e Distorted column bed (cracked, compressed) f Used wrong column or mobile phase 	Corrective action: <ul style="list-style-type: none"> a Reduce sample size b Adjust mobile phase or replace/regenerate column c Replace column d Wash column with suitable solvents or replace e Repack or replace column f Change system
SYMPTOM: Increased retention volume	Possible cause: <ul style="list-style-type: none"> a System flow rate decreased b Column temperature too low c Column activity changing 	Corrective action: <ul style="list-style-type: none"> a Check and increase flow rate; if flow rate decreasing, check and repair any leaks b Insulate or jacket column c Solvent stripping H_2O or stationary phase off of column; add H_2O or liquid phase to mobile phase
SYMPTOM: Decreased retention time	Possible cause: <ul style="list-style-type: none"> a System flow rate increased b Column activity changing c Wrong mobile phase 	Corrective action: <ul style="list-style-type: none"> a Check pump for proper setting b Change column c1 Change mobile phase c2 Check for changes in mobile phase ratio

SYMPTOM:	Possible cause:	Corrective action:
Recorder will not zero	<ul style="list-style-type: none"> a Bubbles in UV reference cell or wrong solvent in RI reference cell b Mobile phase has not equilibrated with column c Mobile phase has not equilibrated with detector or is not compatible with detector d Column bleed e Contamination bleed from column f Previous mobile phase still in system g Detector not connected to recorder h Detector source lamp failing/faulty i Dirty detector cell windows j Particulates in detector cell k Electronic problem with detector l Recorder or detector not plugged in or turned on m Recorder improperly zeroed n Recorder calibration knob out of position 	<ul style="list-style-type: none"> a1 Flush cell out and replace with air or proper solvent a2 Degas mobile phase a3 Add suitable back pressure to detector outlet b Flush system longer 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 d1 Add H₂O 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 Thoroughly wash column with proper solvents or replace column f Properly flush entire system with compatible solvents g Check detector lines to recorder h Replace source i Clean cell windows j Flush and clean detector cell k Check service manual l Check power cord and on/off switch m Rezero recorder n Readjust calibration knob
SYMPTOM: Low sensitivity	Possible cause: <ul style="list-style-type: none">a Inadequate flow rateb Sample not compatible with detectorc Insufficient sampled Sample not eluting from columne Dirty detector cell windowsf Gas bubble(s) in detector cellg Detector attenuation too highh Detector and/or recorder out of calibrationi Failing/faulty detector sourcej Recorder in wrong millivolt range	Corrective action: <ul style="list-style-type: none">a Adjust flow rateb Check sample chemistry and adjust detector or change itc Increase sample sized Check sample chemistry; change mobile phase and/or columne Clean cellf1 Degas mobile phasef2 Apply suitable back pressure to detector outputg Adjust attenuationh Check detector and recorder calibration; recalibrate if necessaryi Change detector sourcej Check setting and adjust
SYMPTOM: No peaks; no response	Possible cause: <ul style="list-style-type: none">a Detector/recorder not onb Detector/recorder not plugged inc No sample injectedd Failure in the electronics	Corrective action: <ul style="list-style-type: none">a Check and turn onb Check and plug inc Check injection/injector for complete sample introduction; clean or change syringe or valved1 Check and replace fused2 Check service manual

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