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# An HPLC troubleshooting guide

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

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**I**t 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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*Excerpted from the book Maintaining and Troubleshooting HPLC Systems by Dennis J. Runser. Copyright, 1981 John Wiley & Sons, Inc. Reprinted by permission of the author.*

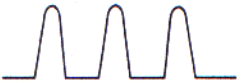
<b>SYMPTOM:</b> No flow or pressure	<b>Possible cause:</b> a System leak b Injection valve improperly positioned c Air in the pump  d Faulty pressure gauge  e No mobile phase f Solvent delivery system not pumping  g Pump starvation	<b>Corrective action:</b> a Locate and repair b Check valve for proper 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
<b>SYMPTOM:</b> Pump pressure up but no flow through system	<b>Possible cause:</b> 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	<b>Corrective action:</b> 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
<b>SYMPTOM:</b> Baseline stepping and peaks are flat-topped; Baseline does not zero	<b>Possible cause:</b> a Recorder gain and damping improperly adjusted b Improper grounding c Saturated electronics	<b>Corrective action:</b> a Properly adjust gain and damp b Check system ground c Reduce sample size
<b>SYMPTOM:</b> Baseline spiking	<b>Possible cause:</b> 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	<b>Corrective action:</b> 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

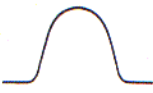
<b>SYMPTOM:</b>	<b>Possible cause:</b>	<b>Corrective action:</b>
<b>Baseline drifting</b>	<ul style="list-style-type: none"> <li>a Dirt in detector sample or reference cells</li> <li>b Temperature gradient over the system</li> <li>c Contamination bleed in system</li> <li>d System leak</li> <li>e Bubble trapped in detector sample or reference cell</li> <li>f Solvent immiscibility or immiscible pools (previous solvent not thoroughly flushed out)</li> <li>g Mobile phase not in equilibrium with column</li> <li>h Mobile phase/sample vaporizing</li> <li>i Contamination in mobile phase</li> <li>j Failing detector source</li> <li>k Recorder problems</li> </ul>	<ul style="list-style-type: none"> <li>a Flush detector cells with solvent or carefully clean cell</li> <li>b1 Check for drafts</li> <li>b2 Insulate column and column inlet/outlet lines or use a constant temperature jacket</li> <li>b3 Move instrument away from direct sunlight</li> <li>c1 Check for septum bleed and replace with proper septum</li> <li>c2 Check for column bleed: <ul style="list-style-type: none"> <li>i. Previous sample(s) buildup — wash the column</li> <li>ii. Column-mobile phase incompatibility — replace column or mobile phase</li> </ul> </li> <li>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</li> <li>c4 Uneluted peaks; wash column</li> <li>d Locate and repair</li> <li>e1 Flush out cell</li> <li>e2 Degas mobile phase</li> <li>e3 Locate and repair any system leaks</li> <li>e4 Add suitable back pressure to detector outlet</li> <li>f Flush system with compatible solvents until only the desired mobile phase is present</li> <li>g Continue to flush system until equilibrium is established</li> <li>h At elevated temperatures check boiling point of mobile phase</li> <li>i Change mobile phase</li> <li>j Replace with new source</li> <li>k Short out detector; if drift continues, check recorder</li> </ul>

<b>SYMPTOM:</b>	<b>Possible cause:</b>	<b>Corrective action:</b>
<b>Noisy baseline</b>	<ul style="list-style-type: none"> <li>a Air bubbles passing through detector</li> <li>b Column packing passing through detector</li> <li>c Leak in system</li> <li>d Pulses from pump</li> <li>e Pulse dampener(s) and/or restrictors not properly flushed</li> <li>f Bubbles in detector sample or reference cell</li> <li>g Dirt in detector</li> <li>h Detector source failing</li> <li>i Temperature effects on detector cell input tubing</li> <li>j Recorder improperly grounded</li> <li>k Noisy electronics</li> </ul>	<ul style="list-style-type: none"> <li>a1 Degas mobile phase</li> <li>a2 Flush pump check valves clear of air</li> <li>a3 Check all fittings for air leaking into mobile phase stream; look for salt-like deposits and stains near fittings; tighten appropriately</li> <li>b Check column outlet for proper column plug and screen</li> <li>c Locate and repair</li> <li>d Add a pulse dampener and/or restrictor</li> <li>e Disconnect solvent delivery system from injector and purge with suitable solvent(s)</li> <li>f Check for bubbles entering detector and flush out air</li> <li>g1 Disconnect detector from system and back flush with suitable solvents</li> <li>g2 Clean detector cell</li> <li>h Check and replace source</li> <li>i1 Insulate inlet tubing</li> <li>i2 Move instrument away from drafts and/or direct sunlight</li> <li>j Check recorder and properly ground</li> <li>k1 Check appropriate detector and recorder circuits; consult instrument manual</li> <li>k2 At high detector attenuation check source lamp</li> <li>k3 Check for dirty or loose electronic contacts; also check for instrument vibration</li> </ul>



<b>SYMPTOM:</b> Negative peaks	<b>Possible cause:</b> a Polarity reversed on detector or recorder b Negative peaks in UV trace c Negative peak at $V_0$	<b>Corrective action:</b> 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
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<b>SYMPTOM:</b> Flat bottom peaks 	<b>Possible cause:</b> 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	<b>Corrective action:</b> 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
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<b>SYMPTOM:</b> Very rounded peaks 	<b>Possible cause:</b> 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	<b>Corrective action:</b> 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
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<b>SYMPTOM:</b> Loss of resolution	<b>Possible cause:</b> 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	<b>Corrective action:</b> 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
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<b>SYMPTOM:</b> Increased retention volume	<b>Possible cause:</b> a System flow rate decreased b Column temperature too low c Column activity changing	<b>Corrective action:</b> 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
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<b>SYMPTOM:</b> Decreased retention time	<b>Possible cause:</b> a System flow rate increased b Column activity changing c Wrong mobile phase	<b>Corrective action:</b> a Check pump for proper setting b Change column c1 Change mobile phase c2 Check for changes in mobile phase ratio
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<b>SYMPTOM:</b>	<b>Possible cause:</b>	<b>Corrective action:</b>
<b>Recorder will not zero</b>	<p><b>a</b> Bubbles in UV reference cell or wrong solvent in RI reference cell</p> <p><b>b</b> Mobile phase has not equilibrated with column</p> <p><b>c</b> Mobile phase has not equilibrated with detector or is not compatible with detector</p> <p><b>d</b> Column bleed</p> <p><b>e</b> Contamination bleed from column</p> <p><b>f</b> Previous mobile phase still in system</p> <p><b>g</b> Detector not connected to recorder</p> <p><b>h</b> Detector source lamp failing/faulty</p> <p><b>i</b> Dirty detector cell windows</p> <p><b>j</b> Particulates in detector cell</p> <p><b>k</b> Electronic problem with detector</p> <p><b>l</b> Recorder or detector not plugged in or turned on</p> <p><b>m</b> Recorder improperly zeroed</p> <p><b>n</b> Recorder calibration knob out of position</p>	<p><b>a1</b> Flush cell out and replace with air or proper solvent</p> <p><b>a2</b> Degas mobile phase</p> <p><b>a3</b> Add suitable back pressure to detector outlet</p> <p><b>b</b> Flush system longer</p> <p><b>c1</b> The mobile phase's refractive index or UV is not compatible with the detector; adjust detector or change mobile phase</p> <p><b>c2</b> Contaminant in mobile phase; prepare fresh and change, allowing time to equilibrate</p> <p><b>d1</b> Add H<sub>2</sub>O or stationary phase to mobile phase</p> <p><b>d2</b> Check solubility of stationary phase in mobile phase; if working above ambient, reduce temperature to check effect</p> <p><b>e</b> Thoroughly wash column with proper solvents or replace column</p> <p><b>f</b> Properly flush entire system with compatible solvents</p> <p><b>g</b> Check detector lines to recorder</p> <p><b>h</b> Replace source</p> <p><b>i</b> Clean cell windows</p> <p><b>j</b> Flush and clean detector cell</p> <p><b>k</b> Check service manual</p> <p><b>l</b> Check power cord and on/off switch</p> <p><b>m</b> Rezero recorder</p> <p><b>n</b> Readjust calibration knob</p>

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

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

#### REFERENCES:

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- (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. ■