

Under Pressure?

Reduce System Stress by Backflushing Your HPLC Column

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Experiencing a higher pump pressure than usual? Or perhaps a complete pressure shut-down of the system has occurred, even after replacing the in-line frit and guard column. High pump pressures can be caused by heavily retained impurities building up within the head of the analytical column. Such contamination can cause poor chromatography, usually in the form of broad, split, or misshapen peaks, and ultimately can compromise results. Backflushing a contaminated analytical column using the following procedure can help restore column performance and reduce pump pressure and system strain.

If back pressure is abnormally high, first take the column out of the equation by disconnecting it from the system altogether. Install a union and run the pumps to verify that the back pressure problem is due to the column, and not to the HPLC system. If the pressure is normal, then the column is most likely the cause of the high back pressure. To address this, reverse the column flow and rinse (backflush) the column to remove the contaminants from the inlet frit and column head. This will move the contaminants down the path of least resistance, instead of forcing them further into the analytical column. Reverse rinse into a waste beaker at low flow (e.g. 0.5mL/min. for a 4.6mm ID column) for 10-15 minutes initially, and then increase the flow to 1.5-2 times the optimal flow (1.5 to 2.0mL/min. for a 4.6mm ID column). Do not reconnect to the detector when backflushing the column. Rather, flush the waste stream into a beaker so that the detector cell is not contaminated by impurities or obstructed by particulate build-up.

Solubility is a key issue when backflushing columns, so remember the old adage, “like dissolves like”. For example, if the contaminants are suspected to be oily or hydrophobic in nature, then backflush with a strong, nonpolar solvent such as hexane. If the contamination is polar (a salt for instance), then use a polar solvent, such as water or methanol. Solvent miscibility also needs to be considered, so be sure to use solvents that are miscible with one another. If in doubt, use isopropanol (IPA) as an intermediary solvent between solvent wash steps, as it is miscible with all common solvents. This is particularly true when switching from typical normal phase solvents (such as hexane) to reverse phase solvents (such as methanol, acetonitrile, or water) and vice versa. Note that 10 to 15 column volumes are generally necessary at each step to remove all traces of immiscible solvents prior to the next step.

If the contaminants are unknown, or vary in chemistry, a series of solvent washes will provide an array of differing chemical interactions and maximize the removal most types of contamination. The solvent order presented in Table I considers miscibility, polarity, and eluotropic strength and is a very effective series for removing most contaminants. Column backflushing, with proper solvent selection, is a simple way to regenerate analytical columns, improving column performance and reducing system stress.

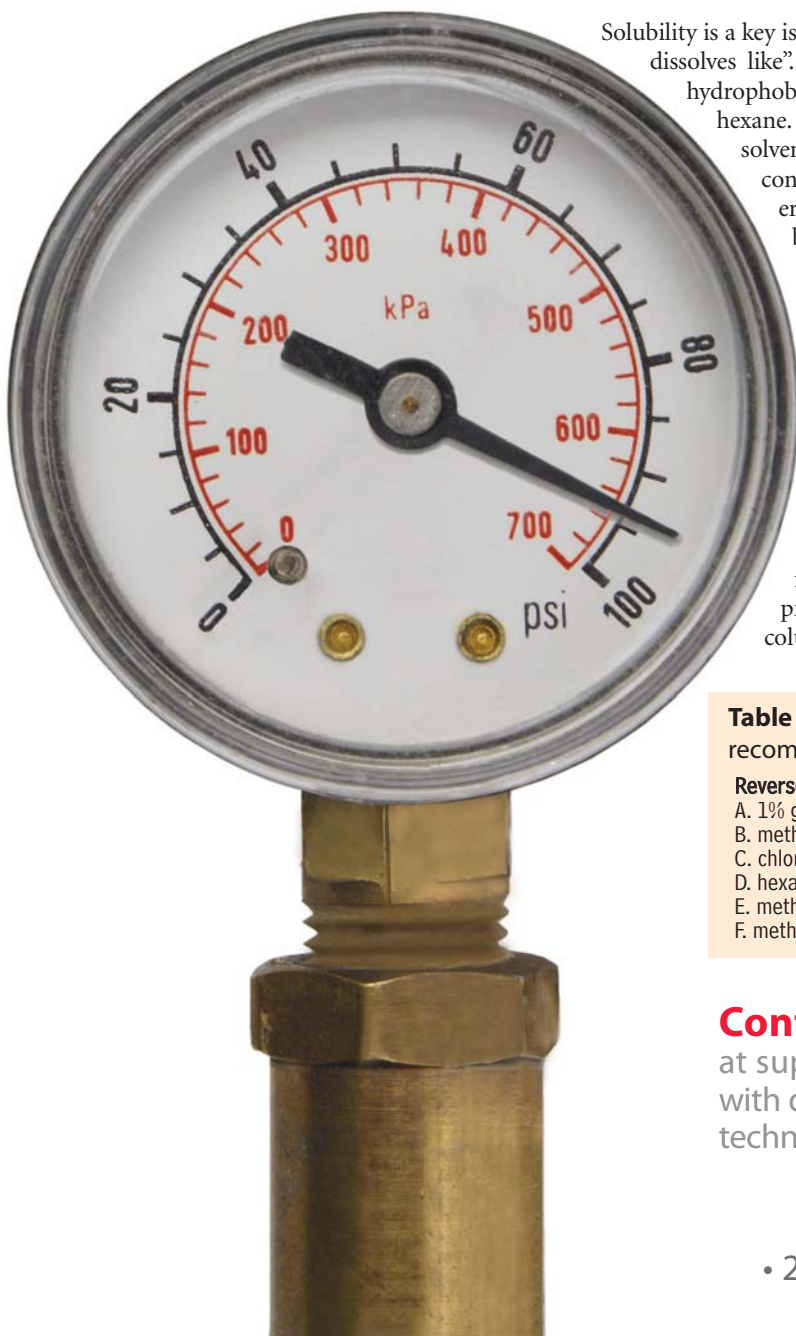


Table I Restore column performance by backflushing with recommended solvent washes.

Reversed phase series:	Normal phase:
A. 1% glacial acetic acid in methanol and water (50:50)	A. isopropanol
B. methanol	
C. chloroform	
D. hexane (or heptane)	
E. methylene chloride (dichloromethane)	
F. methanol	

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