Tips and Advice for Priming your HPLC PUMP (or similar pumps, FPLC or UHPLC Pump) by William Letter, Chiralizer Services, LLC (USA)

The single most important component of any HPLC system is the Pump module. We often refer to it as "the heart of the HPLC system".

- You may have the most sensitive HPLC detector, the best column, a perfect method of analysis, but none of this will matter unless the HPLC pump(s) that provide mobile phase to the system operate *perfectly*, all of the time. If you have a poor quality (or poorly maintained) system, then you will spend much of your time trying to establish reliable flow through the HPLC system, not running samples.
- Before using an HPLC system, you should prime all of the lines in your HPLC pump. This is needed to purge any air from the tubing, introduce fresh mobile phase to each line and then to VERIFY that each channel delivers the reported amount of fluid to the column (measure it).
- NOTE: This is a LONG, detailed article with lots of information, Hints and Tips. It is available in PDF format for download, **here**.

The HPLC pump's ability (*stability*) to provide reliable operation depends on:

(1) The Chemical, Physical and Miscibility properties of the Liquid(s) being pumped;

(2) The Amount of dissolved gas inside the liquid (must be minimized);

(3) The Temperature of the room (or HPLC) must be stable;

(4) The Position of the mobile phase bottles (relative to the pump, above or below);

(5) The Solvent Pickup Filters used (are clean and appropriate in material & porosity);

(6) The Fittings used are correctly installed & tightened;

(7) The types of Tubing used are chemically, temperature and pressure compatible (esp. the Inside Diameter of the tubing);

(8) The Selected Flow Rate(s) and Back-pressure are within the optimal range of the pump;

(9) All mobile phase solutions are Filtered, Freshly prepared and Degassed;

(10) How often the Pump is properly Inspected, Cleaned & Serviced.

The HPLC pump is the most important part of your HPLC system. Take care of it. Neglect or abuse it, and you may lose time and money. Almost every problem you experience using an HPLC will be related in some way to the pump. Make sure you understand the flow path of the system in detail, and have the training to setup and use it properly. Take a hands-on training class (not a video or web

based tutorial) to learn how to use the pump on *your specific HPLC system*. Learn how to run simple verification tests to check the flow rate (best done with a graduated cylinder). Never rely on the software values, check and verify everything yourself. Priming and flushing are needed any time air bubbles are present, mobile phase solutions are changed or the system has sat unused (this includes overnight). Always flush multi-channel pumps and valves (i.e. Binary, Ternary, Quaternary...) using a setting of 100% channel composition. ***Run one channel at a time at 100%, not 25% or 50% to flush channels (a common novice mistake). Flush ALL channels on a regular basis.***

OK, so what can you do to make sure your HPLC pump is properly primed with fluid and operating to the best of its ability?

Start, by reading the operator's manual for your pump. Review the procedures for connecting it to the system, become familiar with the flow path and understand the procedures to prime the pump heads. Practice these procedures.

If an inline vacuum degasser is used, become familiar with the specifications, chemical compatibility (some are not compatible with solvents such as strong acids, strong bases, THF, chloroform, fluorinated additives and so on) and internal channel volume of each chamber used. It is useful to know what the degasser chamber volume is to figure out what the total channel priming volume is. This may be different for similar systems. Check, measure, verify, do not assume.

Priming Volume: The total volume contained in each channel's low-pressure line from the mobile phase bottle to the degasser + the degasser chamber channel volume + the total volume in the line from the degasser to the pump head (or multichannel valve) = the total minimum volume you must flush out before using the system. Because flushing just the minimum of volume (1x) of fluid through the channel is unreliable, flush 2x, 3x or more times this total volume, per channel (or as much fluid as it takes), to prime each channel. *If no degasser is present, then just calculate the volume contained in the low pressure tubing from the bottle to the pump head/valve. Set the pump to direct the flow to waste and use a high initial flow rate to speed up the priming process.

Use fresh mobile phase (prepared daily and filtered). Make sure the solvent pickup filters are clean. If possible, have the bottles placed higher than the pump's inlet (once flow has been established, this will allow natural siphoning to push liquid towards the pump head). Prime all of the lines used. The pumps run on liquid, not air so try and fill any of the lines with pure mobile phase before you connect them to the pump and/or degasser (If all of the lines are prefilled with fresh liquid, you can skip this part).

There are two ways to PRIME EACH line (Flushing the Channels).

*First, open any Prime/Purge or Waste Valve so the mobile phase is directed to waste, not the injector, column or detector. Our goal is to initially fill the lines with liquid, quickly, and we do not want these fluids to go through the entire HPLC system (i.e. column), just the HPLC pump.

(1) Wet Priming use a syringe fitted with a Luer-to-threaded fitting adapter (usually 1/4-28) to draw liquid through the tubing in the mobile phase bottle and into the pump's degasser and/or pump head's inlet. Be sure to have this type of syringe available (very useful). Never push fluid, only draw fluid through the tubing, just like the pump does. Connect the syringe to the mobile phase bottle lines, degasser ports and/or pump head multichannel valve or pump head inlet, as needed, to draw liquid through until all lines are filled.

(2) Dry Priming using the HPLC pump to draw the mobile phase out of the bottles, through the lines, degasser channels and to the pump head or multichannel valve. Always do this *one channel at a time* (e.g. A = 100%). This insures no poor miscibility or mixing problems and is standard procedure. Start with a modest flow rate to get the fluid moving through the lines, then increase the flow rate to speed up the process. The low pressure Teflon tubing is transparent so you can watch this process. Repeat with each channel. Note: Some HPLC pumps will struggle to perform this type of dry priming, as they will be unable to draw the liquid up from the bottle and/or pump the air out of the system. Pre-priming the lines using a syringe (as in #1 above) will help solve this. Running the pump with just air inside the lines may result in increased wear on the system (esp. the piston seals) so if the system struggles to fill with liquid after one minute, discontinue and manually prime each line.

NOTES:

- The back-pressure shown on the system readout should be very low during this initial priming process (e.g. < 15 bars) as the HPLC system should not be plumbed with the column or detector inline, during the priming process (it should be by-passing those parts). Only the viscosity of the solution, the selected flow rate and the internal diameter of the tubing going into and out of the pump will contribute to the observed back-pressure, and this should be very low value.

- Once you have verified that liquid is exiting through the pump head waste port, you can increase the flow rate to speed up the priming process, but pay attention to the back-pressure. It should increase as the flow rate increases and drop as the flow rate drops. Continue to prime each channel in this way, one-at-a-time, until all channels are primed and flushed with liquid. You can gradually slow the flow rate down as you stop, to transition from one channel to another.

- If liquid has been drawn to the pump head, but the pump head still is not pumping liquid through it, it may be experiencing cavitation (air locked). If there is an outlet port on top of the pump head, try drawing liquid through this port, while it is running, to gently fill the pump head chamber and remove the air. Alternatively, the outlet fitting above the pump head can sometimes be briefly loosened allowing the system to push the air out more easily (open it slightly with a wrench, then quickly close it). Have a towel ready to soak up any fluid that comes out. Keep the area clean and dry.

- In some case, the inlet or especially the outlet check valve(s) can also become "stuck" open. When buffers are left in the system (they should be flushed out with water), crystals and particulate matter may deposit on the valve resulting in poor sealing, leaks or air being drawn through. Drawing liquid out of the pump head's outlet port with a syringe (or gently pushing it through the pump head) may remove the air bubble, debris and prime the valve, restoring function. Note: If needed, shut down the pump and clean/replace any contaminated or worn check valves before proceeding.
- In more extreme cases, you can change the mobile phase going into the pump head to a more viscous intermediate solvent to get things moving (an alcohol such as IPA might work well. If buffers have been used, then always first flush with pure water).
- Degas all eluents / mobile phase solutions used. All of them. Degassing will help reduce the formation of bubbles inside the pump head. Failure to properly degas the solutions used may result in loss of prime, baseline and/or pressure instability. Make sure your degasser is operating properly (electronic vacuum degassers only last ~ 5 years at most. Be sure to have them professionally serviced). Sonicating fluids at the bench or using vacuum filtration to initially remove gas from the solution will only degas the solution for a short time (minutes). Gas will slowly diffuse back into the solution resulting in baseline noise, drift and pump problems (for HPLC, only use inline degassing or Helium sparging).
- Verify the flow rate. It may be unwise to rely on the indicated flow rate shown on the instrument screen or display. It is wise to measure the flow rate of each channel, separately, using a graduated cylinder and a timer. This is the most reliable way to determine what the actual flow rate is through the system (and is also the method we use during performance verification or qualification testing too). To check the flow, make sure the system has been primed and flushed. Install a flow restriction capillary in place of the column (to provide the required back pressure). Set the flow rate to a value which is appropriate for the pump and measure/record the volume delivered vs. time. *Example: Using a flow rate of 1.000 mL/min obtain a 10 mL volume, glass laboratory grade graduated cylinder. At time zero, direct the flow from the restrictor's outlet into the graduated cylinder. Measure the volume of fluid collected in 8 minutes. *It should be 8.00 mLs.*

If you continue to have priming problems and/or air bubbles disrupting the flow there are three more things you can check.

1. Make sure the solvent pickup filters/frits are clean and unobstructed (these are maintenance items). If the filters are obstructed, then a vacuum may form on the line resulting in pump cavitation and loss of prime. One quick way to check if this might be a problem is to remove the suspect solvent pickup filter from the tubing, then try again. If flow is restored w/o the filter in place, then the filter may have been clogged. Install a new solvent filter as soon as possible. *Never run the HPLC without solvent filters installed. Those filters perform a very important job and protect the flow path of the system.
2. Service the Pump Heads. Regular cleaning, inspection and *replacement of worn parts* must be done to maintain the function of the pump. Worn parts will result in failures, instability, lost time, plus invalid data. The pump has many mechanical parts which wear out and require replacement. Most pumps should be inspected/serviced every 6 months. Keep the pumps clean and fully serviced (replace: piston seals, pistons, frits, check valves as needed). Depending on the brand, model and applications, the types of parts needed and the frequency of repairs varies widely. *This is discussed in another article.

3. If your HPLC system has an inline vacuum degasser (either a standalone or integrated module), it may be damaged, contaminated or broken. The typical service life of an electronic inline vacuum degasser is only five years (some models have even shorter lifespans). Degasser's with internal damage may result in contamination of the mobile phase. A failing or damaged HPLC vacuum degasser may directly contribute to analysis problems (ghost peaks, pressure instability, poor baseline stability...). Have your degasser professionally diagnostically tested and serviced often.
4. Clean and/or replace any worn or damaged inlet or outlet pump head valves. Not flushing buffers out of the HPLC system on a regular basis or remain in contact with the solution for long periods of time can damage the valves. In some cases, cleaning is all that is needed, but in others, replacement is required to restore function. Be sure to have the system professionally serviced on a regular basis.

- **Additional Troubleshooting Info can be found here:**

  Diagnosing & Troubleshooting HPLC Pressure Fluctuation Problems (Unstable Baseline)