

Ardent Scientific Chromatography Newsletter



Welcome to the first Ardent Scientific Newsletter of 2007. This month we have a Tech Tip on How HPLC Pumps Work.

On-going technical tips explained in these Newsletters will cover both liquid and gas chromatography areas and will focus on areas which are pertinent to the practicing chromatographer. The tips will be always focus on simple modifications you can make to further optimize your instrument.

Tech Tip

How HPLC Pumps Work

The aim of a modern HPLC pump is:

- to maintain a constant flow of solvent through the column regardless of the resistance encountered by the mobile phase
- to maintain this constant flow regardless of variation in resistance (back pressure)
- to change the proportion of solvents flowing to the column

If the pump consisted of a constant head pressure system then the flow would decrease as the resistance in the system increased. Resistance increase can be due to buildup of:

- particulates produced by pump wear and tear which can block filters and connecting tubing
- particulates in the mobile phase or sample due to inadequate filtering
- breakdown of column material to smaller particles which can block frits or connecting tubing

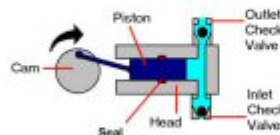
A constant flow rate in HPLC is very important because there is much reliance on retention time matching between peaks in a sample and peaks in a standard. A change in the column flow rate will change retention times. Therefore, the system has to be robust enough to withstand flow changes due to an increase in resistance in the system. The measure of this increase in resistance is the backpressure. This is the pressure which is generated behind (back) of the point

of resistance when the pump is trying to maintain constant flow. Backpressure is one of the main diagnostics used in HPLC for system performance. The main type of pump used in HPLC is a reciprocating piston pump.

Single Piston Pump

A single piston reciprocating pump is shown here. A cam causes the piston to move back and forth and thereby either withdrawing solvent from a container or delivering solvent to the system. Two valves are needed on either side of the piston. The bottom valve shown here is present to ensure that once the solvent is inside the chamber and the piston is returning to pump this solvent towards the column, there is no piston solvent which is returned to the mobile phase container. Additionally, the top valve is present to ensure that once the solvent has been delivered to the column, this valve is closed to ensure no downstream solvent is pulled back into the system.

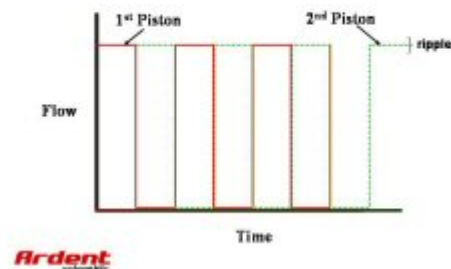
Single Piston Pump



Dual Piston Pump

For single piston pumps, once the piston is pulling back solvent into the piston, there is no delivery to the column. Single piston pumps handle this deficiency in two ways. Firstly, by minimizing the time between dispensing flow to the system and refilling the piston. This is often simply achieved by very large springs, which pull back the piston very quickly and thereby reduce the time between piston strokes. The second technique is to dampen the flow after the pump has dispensed the solvent and thereby the amount of ripple is minimized. Single piston pumps however are not suitable for pumping at low flows.

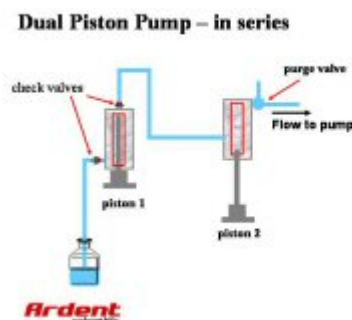
Dual Piston Pumps and Flow



A better, albeit a more expensive, solution is to introduce a second piston which is 180° out of phase with the first piston. As shown in this slide, the second piston (dotted line) is dispensing solvent to the system while the first piston is pulling solvent into the pump. This results in a more continuous flow to the system compared with a single piston pump. The relative amount of flow variation resulting from a difference in flow from the pump is known as the percentage ripple. Modern pumps usually have a specification of 2% maximum for this value i.e. 0.2mL variation in output flow in 1.0mL/min flow rate.

Dual Piston Pump - In Series

It is common in modern dual piston HPLC pumps to have the pistons in series rather than in parallel. In this example shown here, the first piston is twice the volume of the second piston. Thus, as the first piston is pushing solvent out, half of this solvent is dispensed to the system (through the purge valve) and the other half is dispensed to the second piston. Once the second piston chamber is full, this piston delivers the solvent to the system while the first piston has to fill the contents of its chamber by pulling back at twice the stroke speed of the second piston.

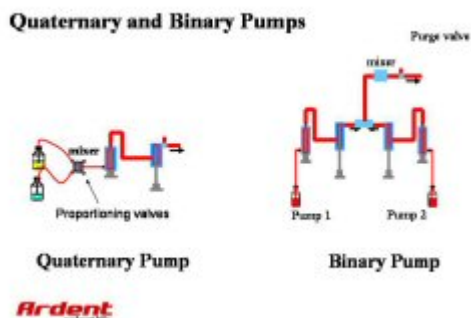


The advantage of the piston-in-series design is that only two check valves are needed, rather than four in a piston-in-parallel design. A dampener may be located between the two pistons.

Quaternary and Binary Pumps

It is common in HPLC to have the pumps do the mixing of solvents as this is more reproducible than mixing off-line. Of the dual piston pumps, there are essentially two types available in HPLC - the binary pump and the quaternary pump. The binary pump consists of two pumps each with two pistons.

Each pump is capable of delivering a solvent to the system. If a HPLC method requires a mobile phase mix of 50% water and 50% methanol, this can be achieved by having one bottle of water on pump 1 and one bottle of methanol on pump 2. If each pump is running at the same speed, there will be equal flow delivered to the system i.e. a 50 / 50 mix. Good mixing of the water and methanol needs to be carried out before delivery to the column. The ratio of the water and methanol can be changed during the analysis by changing the speed of the pistons from each pump. For example, in this case, changing the pistons speed on pump 1 to twice the speed of pump two will result in a 66:33 mix of water : methanol. The mixing ratio is precisely controlled through the software. The pump can be retrofitted with a proportioning valve on each pump which converts the binary solvent selection to quaternary solvent selection.



A quaternary pump consists of only one dual piston pump. Solvents from different bottles can be drawn into the pump through a proportioning valve system before the pistons. There are usually four valves able to choose from four different solvents hence the name. For the example described above, to achieve

50 / 50 mix of water and methanol, the valve for bottle 1 is opened 50% of time and then closed followed by the valve for bottle 2 opening. The valve opening time is defined by the time for one piston stroke.

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Chromatography Training

The 2007 training program is about to begin. The first course for 2007 will be a two-day practical hands-on Beginner HPLC course held in Melbourne.

Courses in Melbourne for March

- HPLC Beginner Course - Thurs 22nd and Friday 23rd March 2007

Places are limited to eight people so book early to reserve a place.



Click on the links below for further information and prices and the modules from each course can be customised and packaged for on-site courses e.g. a mixture of GC and HPLC training.

- [GC Beginner Course](#)
- [GC Intermediate Course](#)
- [GC-MS Beginner Course](#)
- [HPLC Beginner Course](#)

For more details and course objectives etc, [click here](#).

On-site Training

Alternatively, for three or more staff, on-site training can be a very cost-effective option. The benefit of on-site training is that the focus is your application on your instrument and the training modules can be customized to suit your needs. On-site courses are offered across Australia. [For more information click here](#)

Featured Products

[GC inlet liner recycling service](#)



Ardent Scientific offers a complete GC inlet liner recycling service including cleaning, re-packing with quartz wool (where applicable) and in-situ deactivation. Price is \$5 / liner (minimum of 30 liners) + \$15 freight.

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[2mL Autosampler Vial](#)



2mm HPLC and GC clear autosampler vial (12 x 32mm) - pkt100.

Product Code 50100