Instrumentation of HPLC

HPLC Solvent Pumping Systems

Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.
Aims and Objectives

Aims

- To describe the role of the Solvent Delivery System (Pump) within High Pressure Liquid Chromatography
- To explain the operational principle of the most popular Solvent Delivery System types
- To describe the function of various Solvent Delivery System components and highlight potential troubleshooting and maintenance issues
- To highlight good practice in pump use, care and storage
- To outline logical troubleshooting procedures for Solvent Delivery Systems
- To describe the operating principle of alternative pump designs such as preparative and micro-flow pumping systems

Objectives

At the end of this Section you should be able to:

- Explain the working principles of the most popular Solvent Delivery Systems in HPLC
- Demonstrate knowledge of the working principles of various Solvent Delivery System components and explain maintenance procedures
- Describe the advantages of dual piston pumps over single piston pumps
- Demonstrate a knowledge of troubleshooting procedures associated with
- Recognise System problems from characteristic baseline problems
- Identify Solvent Delivery System problems associated with Retention Time variation
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In the techniques infancy, HPLC was referred to as “High Pressure Liquid Chromatography”. This was a reflection of the exacting demands of producing an accurate flow at pressures operating up to 5000 psi (approximately 350 Bar), using the pump technology of the late 1960’s. However, in the 1980’s, improvements in column technology shifted the emphasis away from “pressure” and today most analytical chemists refer to HPLC as “High Performance Liquid Chromatography”.

The purpose of the solvent delivery system is to ‘deliver a continuous pulse free flow of mobile phase to the HPLC system -regardless of the system back pressure’.

Several variations of the reciprocating piston pump are currently manufactured and will be discussed in more detail in this section.
Simple Pumping Systems

Shown opposite is a schematic representation of the single piston pump. Most modern HPLC pumping devices are based around this simple concept of a pumping chamber with two check valves to regulate the liquid flow.

The component parts are:

1. **Eccentric cam:** As the eccentric cam rotates it moves the piston into and out of the piston chamber – causing mobile phase to enter or leave the pump head chamber

2. **Spring mounted piston:** The piston moves forward to force the liquid out of the chamber and through the check valve. With the piston at the end of its stroke the spring is fully compressed. The spring pushes the piston back and liquid is drawn into the piston chamber.

3. **Liquid filled chamber**

4. **Check (ball) valves:** to regulate flow direction
   - **Outlet Check Valve (Ball Valve):** This ball and seat valve allows unidirectional flow of liquid out of the piston chamber. As the piston moves into the chamber the ball (usually made from ruby) lifts from the seat (usually made from sapphire), to allow liquid to flow out of the pump head. As the piston moves out of the chamber, the ball is forced down against the seat under the vacuum created in the pump head. This stops back flow of mobile phase into the pump head.
   - **Inlet Check Valve (Ball Valve):** This ball and seat valve allows unidirectional flow of liquid into the piston chamber. As the piston moves out of the chamber the ball (usually made from ruby) lifts from the seat (usually made from sapphire), to allow liquid to flow into the pump head. As the piston moves into the chamber, the ball is forced down against the seat under the pressure created in the pump head. This stops flow of mobile phase out of the pump head.

An electric motor is used to drive an eccentric mounted cam and spring mounted piston or ball drive piston which moves into (the compression or delivery stroke) and out of (the filling stroke) a chamber drilled into a pump head.

**The filling stroke** causes mobile phase to fill the chamber and the liquid flows through the inlet check valve as the ball is lifted from the check valve seat. No mobile phase flows from the pump during this phase of pumping cycle.

The delivery stroke compresses the liquid within the chamber and forces the liquid out through the outlet check valve and to the HPLC system.
Disadvantages of Single Piston Pumps

The basic design of a single piston pump does have one disadvantage that is related to the non-continuous manner in which the liquid is delivered. During the filling stroke of the piston the pump has no flow exiting the pump head. This results in an intermittent pulse of liquid being delivered from the pump on the delivery stroke only. This pulsed flow can lead to undesirable disturbances in the chromatogram baseline at lower limits of detection and results in a pressure ‘ripple’ unless the output is heavily damped.

The next figure presents a typical pressure or flow profile from a single piston pump.

Typical pressure or flow profile from a single piston pump

Clearly this pump design does not meet the initial requirements of a continuous pulse-free flow of mobile phase.

Most manufacturers of HPLC pumps now use a reciprocating design in which two single piston pumps operate exactly 180° out of phase – resulting in a continuous delivery of mobile phase at a constant pressure from either one of the two pump heads.

Dual Piston Reciprocating Pumps

Dual-piston reciprocating pumps consist of identical hydraulic chambers and pistons that are operated 180° out of phase. This has the benefit of delivering a virtually pulse free flow of liquid because the dual-piston system has one hydraulic chamber filling whilst the other is delivering the mobile phase to the system (usually via the injector). This design is also often used in conjunction with a pulse dampener to provide the lowest possible pressure fluctuation in mobile phase delivery – providing the most stable baseline and reproducible retention times.
The next figure presents a Dual Piston Reciprocating pump.

One disadvantage of this pump design is the higher number of moving parts than a simple single piston design which results in higher running costs. Typically, mechanical check valves and pistons / piston seals are the cause of most mechanical / hydraulic failures in an HPLC pump. Increasing the complexity of the pump increases the maintenance requirements.

**Mixing Solvents, Binary Pumps**

In Gradient HPLC –a more strongly eluting (usually organic) solvent is gradually introduced into the mobile phase to progressively elute more strongly elute analytes within a reasonable timeframe. To meet the requirements for gradient HPLC there is a need to employ a more sophisticated pump design that is capable of pumping and mixing exact proportions of more than one liquid simultaneously and reproducibly. There are two ways of achieving a gradient profile using HPLC pumping equipment:

- Two pumps working in unison but each delivering a different volume fraction of the total flow –this is called a Binary Gradient Pump
- One pump fitted with a proportioning value to allow exact volumes of liquids to be mixed prior to the pump –this design is called a Ternary or Quaternary Gradient Pump
Binary Pumps consist of two ‘channels’ or pumps – each channel has an identical reciprocating pump, both channels being connected to a low volume mixing chamber and to a single pulse damper, which in turn may be connected to a second mixing chamber. Computing firmware within the pump, or an external computer, controls the pumping speed (or volume) of each reciprocating pump so that the solvent volume fraction from each channel will be ‘proportioned’ to give the desired total flow. That is, if a gradient composition of 50% solvent A and 50% solvent B is required the pumps will each pump at an equal flow rate. If the total desired pump output is 1mL/min, each of the reciprocating pumps will deliver solvent at 0.5 mL/min.

Where:

1. **Low Volume Mixing Chamber**: Primary mixing of the mobile phase from the two pumps heads prior to pulse damping

2. **Pulse Damper**: Removes pressure fluctuations in the mobile phase flow caused by the operation of the two pump heads

3. **Second Mixing Chamber**: A large volume mixing component which also acts to further reduce pressure ripple to ensure the most stable baselines and lowest pressure ripple

When working at lower flow rates (less than 1.0mL/min) binary pump systems are more accurate than other pumping designs such as ternary or quaternary systems.

Also when there is a large difference in the proportion of mixing, for example 98% of solvent A and 2% solvent B, it is generally more reproducible when metered (measured) from a binary pump system than a ternary or quaternary system using a proportioning valve.

Advantages and disadvantages of binary pumps are presented next
Advantages

- The binary pump is said to be a ‘High Pressure Mixing System’ – that is, the mobile phase constituents are mixed on the ‘high pressure’ side of the system after compression by the pump.
- These systems are generally recognised to give the most reproducible gradient profile, especially at extremes of flow or gradient composition.
- Binary Pumping systems generally have the lowest internal mixing volumes and as such can mix and deliver gradients more quickly than other systems – they are said to have the lowest – ‘Gradient Dwell Volume’.

Disadvantages

- The binary pump system is more complex and more expensive to purchase and maintain than a quaternary pump as it has approximately twice as many moving parts as the quaternary pump.
- Some solvent compositions may ‘cavitate’ at high flow rates under high pressure – this is rare.

Mixing Solvents, Quaternary Pumps

The quaternary pump will deliver up to four different solvents simultaneously via a mixing device located prior to the pump(s). Rather than use four pumps, which would be prohibitively expensive, the quaternary pump uses one dual-piston reciprocating pump and a solenoid controlled portioning valve located in-line between the solvent degasser and the pump head.

The design of the quaternary pump dictates that all mixing of solvents is done prior to liquid compression – these pumps are therefore known as low pressure mixing devices.

The solenoid controllers of the proportioning valve open to control the volume fraction of each mobile phase component at any instant. The time cycle of the proportioning valve are usually based on the pump ‘duty cycle’. If the pump duty cycle (one full pump cycle) is around one second – then a 25:25:25:25 A:B:C:D mobile phase composition would be achieved by opening each solenoid valve for one quarter of a second each. The proportioning valve is usually controlled by firmware resident with the pump module which is in turn programmed by a computer data acquisition system. The use of a PC make this whole process relatively simple and the end users needs only concern themselves with entering the gradient conditions correctly in the pump set-up screen of the operator software.
Where:

1. **Proportioning Valve:** Each solenoid valve will be open for a proportion of what is called the ‘duty cycle’ of the pump (often based on the piston stroke time, typically around one second) – i.e. when mixing four solvents at equal proportion, each solenoid will be open for one quarter of the duty cycle (0.25 seconds)

2. **Inlet Valve:** Provides unidirectional flow into the piston chamber

3. **Damper:** Removes pressure fluctuations in the mobile phase flow caused by the cyclical operation of the two pistons

Advantages and disadvantages of quaternary pumps are presented next

**Advantages**

- Quaternary Systems use only one pump head. Compared to Binary systems the purchase price is lower as are the maintenance costs
- The ability to mix 4 different solvents gives great flexibility when developing gradient separations – especially with complex separations
- Four ‘channels’ also lends the possibility of establishing automatic instrument flushing methods, as well as automated method development routines

**Disadvantages**

- Does not tend to perform as well as the Binary Pump when working at extremes of flow or gradient composition
- Low pressure solvent mixing is inherently less accurate and reproducible than high pressure mixing (i.e. Binary Pumps)
- A problem associated with low pressure mixing is that solvent mixing is more prone to out-gassing and necessitates the use of an in-line degasser unit
Mixing Solvents, Ternary Pumps

The ternary pump will deliver up to three solvents simultaneously - thus allowing gradients to be more sophisticated, perhaps using an aqueous component with two organic modifiers to give increased selectivity options. The ternary pump is less common in the modern laboratory as they are not now commonly manufactured; isocratic, binary or quaternary pumps being the most popular, the latter also being capable of operating as a ternary pump. The ternary pump can in many ways be seen as the evolutionary predecessor of the more sophisticated quaternary pump commonly found in today's analytical laboratory.

Both ternary and quaternary pumps mix the solvents prior to compression of the solvent within the piston chamber and as such are termed low pressure mixing devices. A problem associated with low pressure mixing is that of mobile phase out-gassing in the mixing chamber and necessitates the use of an in-line degasser unit.

Furthermore the slightly larger mixing volume of a low pressure mixing ternary pump compared with a binary pump results in gradient profiles that are slightly different for the two pumps. Ultimately this can produce a different chromatographic separation under identical gradient conditions. This arises due to the complex nature of gradient analyte elution and the inherent differences in system ‘dwell volume’ between binary and quaternary systems. When transferring gradient elution methods between high and low pressure mixing systems – differences in system dwell volume must be accounted for.

Where:

1. **Proportioning Valve:** Each solenoid valve will be open for a proportion of what is called the ‘duty cycle’ of the pump (often based on the piston stroke time) – i.e. when mixing three solvents at equal proportion, each solenoid will be open for one third of the duty cycle (0.33 seconds)

2. **Inlet Valve:** Provides unidirectional flow into the piston chamber
3. Outlet Valve: Provides unidirectional flow out of the piston chamber

4. Damper: Removes pressure fluctuations in the mobile phase flow caused by the cyclical operation of the two pistons

Advantages and disadvantages of ternary pumps are presented next

Advantages

- Ternary Systems use only one pump head. Compared to Binary systems the purchase price is lower as are the maintenance costs
- The ability to mix 3 different solvents gives added flexibility when developing gradient separations – especially with complex separations
- Three ‘channels’ also lends the possibility of establishing automatic instrument flushing methods, as well as automated method development routines

Disadvantages

- Ternary and Quaternary Pumps do not tend to perform as well as the Binary Pump when working at extremes of flow or in terms of the reproducibility of the gradient composition
- Low pressure solvent mixing is inherently less accurate and reproducible than high pressure mixing (i.e. Binary Pumps)
- A problem associated with low pressure mixing is that solvent mixing is more prone to out-gassing and necessitates the use of an in-line degasser unit

Check Valves

The ‘check’ or ‘ball and seat’ valve may be positioned in-line before and / or after the piston chamber. The valve is designed to allow flow of mobile phase in one direction only.

Constructed from inert materials such as ceramic or ruby, the ball sits in a small cylinder or seat made of similar material (sapphire being another popular material for seat manufacture). Depending on the stroke of the piston the ball is either positioned against the cylindrical seat, thus stopping mobile phase flow, or in the main body of the valve where it offers little resistance to the flowing liquid.

Check valves are designed to give uninterrupted flow of liquid over many thousands of hours. However, check valves and their constituent components are classed as consumable items and will need to be replaced after extended use. The use of highly corrosive (mineral acids or acid halides), un-filtered, or high buffer concentration mobile phases may considerably shorten the lifetime of the check valve.

The check valve should offer trouble free operation for thousands of hours - however their lifetime will be shortened if particulate matter is present in the mobile phase.

A failing check valve will give symptoms similar to that of air bubbles in the system with a systematically erratic flow being observed. If a failing check valve is suspected then investigate by replacing with a new or known good check valve. If replacing both the inlet and outlet check valves to avoid inadvertent swapping place identifying marks on the check valves prior to removal.
Check valves can be cleaned in a sonic bath using solvent (typically methanol or isopropyl alcohol) or using a dilute solution of nitric acid. However before attempting this, the manufacturers manual should be consulted.

**Pistons**

The piston is used to compress the mobile phase in the ‘analytical volume’ – i.e. the piston chamber within the pump head. It should be physically robust and chemically inert to withstand the rigors of pumping potentially corrosive solvents at high pressures (up to approx. 5000psi). Typically pistons are constructed from sapphire rods with rounded ends –mounted onto a passivated metal holder. Depending upon the pump design the piston may fit exactly into the piston chamber, however, it is much more usual for the piston have a small tolerance between itself and the inner surface of the piston chamber and a small amount of solvent will flow between these surfaces for purposes of lubrication.

The piston needs to move through a seal, which will isolate the liquid in the piston chamber from the pump mechanism. Piston seals are typically constructed using a metallic spring encased in a solvent resistant plastic material -PEEK and CALRES are commonly used plastics. The seal must fit the piston plunger tightly enough to avoid leaks at high pressure but must also avoid excessive wear on the piston. Both the piston and the seals are consumable items, however it is reasonable to expect a longer lifetime from the piston if the pump is properly maintained. Extended periods of inactivity with buffered mobile phases, or poorly filtered mobile phases are likely to reduce the lifetime of both the piston and piston seal.
Troubleshooting

The piston is commonly made of sapphire, which offers an extremely hard, durable and inert surface to the flowing mobile phase. Precise alignment of the piston should enable thousands of hours of continuous use. Problems will arise generally from poor use or maintenance of the pump. Crystallisation of buffer solutions is the most common cause of damage to the piston. If the mobile phase within the pump head is allowed to evaporate on standing – the buffer salts will crystallise. When the pump is restarted the buffer crystals will score the piston and seal – causing a leak. Similarly, if solvent system are used which result in precipitation of buffers at high concentration (i.e. high organic phases containing acetonitrile for example) the piston and pump seal will also be scored – again causing a leak.

Pulse Dampers

Even when using reciprocating piston pumps a slight pulse can be detected in the baseline – especially at higher attenuation. In order to achieve the lowest possible pressure flow / ripple characteristics, modern HPLC pumps use pulse damping units. The damping unit is filled with a compressible liquid, and is separated from the flowing mobile phase via a flexible membrane. The mobile phase is allowed to enter the pulse damper after leaving the liquid filled chamber of the pump head. The pulse damper absorbs energy fluctuations of the pulsed flow and can mechanically smooth the flow output from the pump. Typically a greater than 98% reduction on the observable pulse of a reciprocating piston pump will be observed when using a pulse damping unit.
It is usual for the flexible membrane within the pulse damping unit to be attached to a tension or strain gauge - this is used to measure and report pressure and pressure ripple via the software control system.

Pulse dampening units require no maintenance and rarely fail. Particulate matter is always a possible cause of a blockage and care should be taken when using and changing mobile phase composition.

**Diaphragm Type:** the most usual damper type is based on a membrane or diaphragm, usually having a very low internal volume (< 0.5 mL). The compressibility of the filling liquid is enough to compensate for the pulsations of the dual piston pump with piston volume up to around 100 μL. Pressure ripple should be reduced to less than 2% of the system pressure with this type of unit. Greater pressure ripple indicates problems with the piston / piston seal combination.

**Coil Type:** as the pump strokes, the coil flexes, absorbing the energy of the pulsations. This type of pulse damper holds a large amount of liquid which must be purged during solvent changes or when performing gradient elution.

**Purge Valves**

The purge valve allows solvent to be primed (drawn) into the pump head. Without this – the mere pumping action of the pump head itself might not be enough to draw solvent from the eluent reservoir, through any online degassing equipment that might be present and into the pump. Purge valves can be used to prime the HPLC pump in two definitive ways:
1. The purge valve is opened and a higher than usual pump flow selected (~5 – 10 mL/min. typically) in order to effectively ‘draw’ the solvent into the pump head.

2. The draw off valve has a port to which a syringe might be attached – once the valve is open the user may manually draw solvent from the reservoir into the pump head.

The purge valve is located after the liquid filled chamber of the pump outlet and before the injection valve in the HPLC system and can be either fully open or fully closed. When open the flow of the mobile phase will bi-pass the injector, column and detector and flow to waste – a useful feature when work on the system is required but when stopping the mobile phase flow would be inconvenient – whilst inserting a column into the system for example. In routine use it is common to open the purge valve only when changing solvents or priming the system. When priming the system it should be noted that at least 2x the system volume up to the pump head should be drawn through to eliminate the previous phase, avoid precipitation issues and reduce column / mobile phase incompatibility issues. Some systems may have up to 20mL system volume prior to the pump head if online degassers are included in the configuration.

Depending upon the instrument manufacturer, the instrument may have one or more in-line filters between the eluent reservoir and the pump head. Indeed there may also be filters between the pump and the autosampler.

In general these filters are used to trap particulate materials which have not been filtered out of the eluent or precipitated buffer salts in the instance where involatile buffers have been used.

The purge valve should operate without maintenance for many thousands of hours. The two common problems associated with purge valves are particulate matter accumulating from worn pump seals and particulate material in the mobile phase or by overenthusiastic closing of the valve.

If particulate matter blocks the Purge Valve Frit a significant (i.e. above 5 Bar) back pressure with the purge valve open will be observed. In such cases the frit must be replaced.
Generally if a purge valve has been over-tightened a poor seal will be produced. The symptoms of a damaged purge valve are a pressure drop or leak from the valve.

**Flushing, Gradients and Gradient Dwell Time**

If buffers are used it is important to flush the whole HPLC system, especially the pump, when the instrument is not in use. If the pump is not cleaned buffers will tend to crystallise over time and can cause serious problems with the pistons, piston seals and the check valves. When high concentrations of involatile buffers are used, (ion paring reagents are involatile and are often used at high concentration), the buffer or reagent can easily precipitate at high mobile phase organic concentrations or if the instrument stands and the mobile phase components evaporate. Scratched or broken pistons and premature wearing of pump seals are the common problems observed when pumps are not properly maintained when using buffered solvents.

To avoid premature pump failure always pump through with a flush solvent before turning off the pump. This can be used to clean the analytical column at the same time thus extending the lifetime of both column and pump.

The critical parts of the pumps susceptible to buffer crystallisation are shown below. A 50:50 solution of acetonitrile and water is a suitable wash solvent.
**Important:**

**Buffers such as:** Potassium Phosphate, Trifluoracetic acid, or Ammonium acetate are commonly used to maintain ionic strength in the mobile phase thus allowing ionic analyte molecules to behave in a more reproducible manner. Common concentrations are 10-20mM.

**Ion pairing reagents such as:** Alkylsulphonate salts or Tetrabutylammonium salts are used for the retention of strong acids and bases and are typically used at concentrations 40-60mM.
Solvent Flushing

A typical flushing program achieved using a gradient mixing system is demonstrated next.

Where

1. Switch to water / organic – to wash the column and system free of buffers which may precipitate
2. 95% H₂O – to remove all traces of buffer without risking buffer precipitation prior to flushing the column at high organic strength (never run reverse phase columns at 100% H₂O or they may become deactivated through phase collapse – unless column is designated or recommended by the supplier as being compatible with 100% (aq) mobile phases)
3. 5% H₂O – to remove all contaminants by running at very high organic strength, including strongly absorbed matrix materials etc
4. 40% H₂O / 60% Organic (5 column volumes)
5. 10% Organic / min.
6. 95% H₂O / 5% Organic (5 column volumes)

Calculating System Dwell Volume

Gradient dwell volume is the total system volume in a gradient system between the point where the gradient is formed and the inlet of the column. In a high-pressure-mixing system, this includes the mixing chamber, connecting tubing, and injector. In a low pressure mixing system this ALSO includes the volumes of the pump head, pulse damper and other connective tubing. Typical dwell volumes range from 1 mL to 5 mL, but values can be as low as 0.5 ml or higher than 13 mL.

The dwell volume can be converted into a dwell time by multiplying by the eluent flow rate. The dwell volume has a significant impact when transferring methods between different HPLC systems. It is important to account for differences in dwell volume (time) between systems in order to accurately reproduce a separation. This is usually achieved by having an ‘isocratic hold’ section at the beginning of the gradient profile which can lengthened or shortened according to the differences in dwell time between the two HPLC systems.
Dwell volume can be easily measured using your UV detector to trace the gradient profile generated by your system – this is explained in more detail next.

**STEP 1:** Remove the column from the system and connect the injector to the detector by means of a short piece of 0.010 inch i.d. (or smaller) tubing and ensure the injector is in the inject position.

**STEP 2:** For solvent A, use HPLC-grade water; for solvent B, add about 0.1% acetone to water (methanol or acetonitrile can be used instead of water if required). Set the detector wavelength to 265 nm.

**STEP 3:** Run 100% B, and adjust the scale on your data system so that an on-scale signal is produced.

**STEP 4:** Program a 0–100% B linear gradient in 10 min at 2 mL/min (the exact conditions are not critical; just make sure the gradient volume is at least 20 mL) with a hold at 100% B.

**STEP 5:** The resulting plot should look something like the figure shown here. You will see an isocratic portion at the beginning that represents the dwell volume ($V_D$), the gradient portion, and another isocratic hold at the end. The curvature should be minimal at the ends of the gradient, and it should be linear except at the start and end.

Determine the dwell time by first locating the time at the midpoint of the gradient ($t_{1/2}$) (the time corresponding to the ‘average’ detector response; 9.2 mins in our example). Then, subtract half the gradient time (10 min/2 = 5 min for the present example) from $t_{1/2}$. The result is the dwell time ($t_D$) [9.2 min – 5 min = 4.2 min]. Convert the dwell time ($t_D$) to the dwell volume ($V_D$) by multiplying by the flow rate (F) [4.2 min x 2 mL/min = 8.4 mL].
Effects of $V_o$ differences

System 1 has been determined to have a dwell volume of approximately 0.5 min. at 1 mL/min. The gradient profile used contains an initial isocratic hold equal to the system dwell time.

In order to match the chromatography created by this system on another, it is crucial that the dwell volume (time) of the second system is accurately known. To ‘match’ the actual gradient profile of the two systems we can adjust the gradient isocratic hold to match the ‘effective’ gradient isocratic hold periods by altering the initial hold time.

Lets suppose the dwell time of System 2 has been determined as being 0.8 minute. The adjustment to the initial hold time would be:

Similarly – another system may have dwell time only 0.2 min. – in this case we would extend the initial hold on this system to match the actual gradient profile with our original system:
Troubleshooting

Large differences in dwell volume can have a dramatic effect on both retention time and resolution of peaks throughout the chromatogram.

![Graph (a)](image)

**Troubleshooting – High Back Pressure**

High back-pressure in the HPLC system results in the pump working under greater resistance – usually due to a blockage in the system. High back pressure will lead to an increase in the need for maintenance: pump seals and pistons will need to be replaced more regularly and the lifetime of the pump will be reduced. There are a number of potential contributing factors to high back-pressure in an HPLC system and most are due to blockage in the fluid (hydraulic) path.

It is important to know (and perhaps record) the normal working back-pressure for the column, flow and mobile phase conditions you are using in order to correctly identify high system back-pressure.

The pump may be investigated as the cause of blockage by isolating it from the rest of the system. This might be achieved by disconnecting from the autosampler or by opening the purge (priming) valve. Pressure higher than 1-2 bar (5-15 psi) after isolation indicates the blockage is within the pump unit. The next figure presents the areas on the binary pump that are susceptible to blockage.
Where:

1. Partially blocked inlet filter or tubing from solvent reservoir – soak the filter in 0.1N Nitric acid for 30 mins, rinse with water / methanol and replace. Alternatively replace the filter.

2. Partial blockage in the inlet check valve (high back pressure will be accompanied by a noisy / cycling baseline) – remove a sonicate in 50:50 Methanol:Water for 15 minutes. Shake to ensure the ball is free within the valve. If required soak in 0.1N Nitric acid for 30 minutes rinse with water / methanol and replace. Alternatively replace the filter.

3. Partial blockage in the outlet check valve (high back pressure will be accompanied by a noisy / cycling baseline) – remove a sonicate in 50:50 Methanol:Water for 15 minutes. Shake to ensure the ball is free within the valve. If required soak in 0.1N Nitric acid for 30 minutes rinse with water / methanol and replace. Alternatively replace the filter.

4. Partially blocked purge valve frit – if possible change the purge valve filter or sonicate the frit in 50:50 Methanol:Water for 15 minutes. Alternatively replace the valve.

5. Blockage in the damping unit / defective damper – flush (at least 10 mL) with 0.1N Nitric acid followed by isopropanol. Flush (at least 10 mL) with 50:50 Methanol:Water. Alternatively replace the damping unit (may require engineer intervention).
Pump Troubleshooting

- Filters present the highest possibility of blocking (it's the reason they are there!!). If the pump has a purge (priming) valve filter – consider changing this first
- After checking / changing and filters test each component in the hydraulic path as the possible source of the blockage
- For example inspect the tubing from the eluent reservoir for blockages or restrictions, in particular the connections to the inlet check valve, if the visual inspection looks good then replace / maintain the solvent inlet filter
- If high back-pressure is still observed then the problem must be elsewhere – move onto the next component in the hydraulic path which is the inlet check valve
- Repeat the process with the inlet check valve, outlet check valve, mixing chamber and pulse damper unit until the source of the blockage is isolated

Troubleshooting – Check Valves, Pistons and Pistons

The check valve, pistons and pistons seal components of the pump are all considered consumable parts. It is recommended that stocks of these items are kept on-site for immediate maintenance requirements. This will reduce instrument down time due to stock orders from manufactures.

Check valves can be damaged by particulate matter accumulating in the flow path of the ball and seat mechanism of the check valve. This can have the effect of blocking the check valve from either closing or opening properly. Particulate matter is normally deposited from the mobile phase and can be reduced by the use of mobile phase filtration. Of particular importance is the need to avoid precipitation of buffers within the pump due to do mismatch of phases or drying out.

Seals should be changed immediately if a visible leak is detected from the underside of the pump head. A common symptom of a failing leak seal is the formation of crystallised buffer on the underside of the pump head that is indicative of a small leak that might not be detected by the instruments leak detection system or visual inspection of the chromatographic baseline. If there are large pressure fluctuations in the system, examine the pump seals and pistons. Seal wear is caused when particulate material is trapped between the seal and the piston – which causes the piston to be scratched and this in turn produces greater seal wear. Again great care must be taken to avoid particulate or precipitate in the pump head.
Pistons should be examined whenever the piston seals are replaced. Any signs of scratching of the piston will mean that the piston must be replaced. Although piston replacement is considered routine maintenance it can be time consuming and can be easily avoided by simple good housekeeping. Rinse solvents should always be used when buffered mobile phases have been used, to minimise the risk of buffer precipitation. Filtration of mobile phase reduces the particulate matter and therefore the chances of premature piston wear.

**Check valve:** A check valve that is failing can often do so in an intermittent manner. A typical symptom of a failing check valve will be a pressure fluctuation sometimes called a “pressure ripple”. This is consistent with a check valve that is not properly closing – perhaps due to precipitated buffer on the seat of the check valve.

![Check valve](image)

![Cyclical Noisy Baseline](chart)

Cyclical Noisy Baseline – often associated with a ‘sticking’ check valve.

The symptoms are very similar to those encountered when one pump head contains air in the system and check valve would be the next component to check if you were satisfied that air was not the cause of the problem. Sticking check valves often show a noisier cyclical baseline than those associated with an air bubble problem.

**Pistons:** Pistons will usually fail due to scratches or pits caused when particulate material becomes trapped between the piston and the piston seal in the pump.

![Piston](image)

A failing piston will usually result in a leak of mobile phase. Initially the leak may be so small that it does not appear to affect the delivery of the pump. However left unattended the result will be gradual deterioration of the performance of the pump. Small leaks can allow small amounts of air to be introduced to the system – resulting in noisy baselines.
Ultimately the piston seal will fail - causing a cyclical baseline and corresponding pressure ripple. Catastrophic failure of the pump seal will result in loss of system pressure.

**Piston maintain**

To extend the lifetime of the pistons avoid particulate matter in the mobile phase, involatile buffers at high concentration and employ a flush method at the end of a sequence of injections.

If buffers are to be used at high concentration then it is recommended that a piston seal wash is utilised to help extend the lifetime of the pistons.

Whenever the seals are replaced the piston should be examined for scratches and/or deposits of crystallised buffer. Any deposits can be removed with a lint free cloth dipped in alcohol. Always clean from the base of the piston forwards.

Any signs of scratching or wear on the piston will require the piston to be replaced.

**Piston seal**: A failing piston seal will usually result in a leak of mobile phase. Often the leak is so small that it does not appear to affect the delivery of the pump. However left unattended the results will be gradual deterioration in the performance of the pump. Small leaks can allow air to be introduced to the system. The result might be a noisy baseline of the form:

Uneven seal ware on the pistons in a Dual Piston Reciprocating Pump is common. In such cases an increase in pressure ripple maybe observed.
Maintenance

A simple preventative maintenance (PM) schedule should be implemented which includes changing the piston seals. The duration between PM's will be determined by the user and will vary depending on the instrument usage.

Typically either 3, 6, 9 or 12 months between PM's is normal. With a good PM strategy it is common not to require any major maintenance of the instruments outside these pre-determined dates.

Troubleshooting – Solvent Mixing Issues

Mixing solvents on-line using a gradient pump can sometimes cause problems in HPLC. The different approaches to on-line solvent mixing (high pressure mixing using a binary pump, or low pressure mixing using a quaternary pump), and problems with the hardware used can lead to variability in retention time, sometimes accompanied by spurious peak shapes. Crucially – when performing gradient analysis, the selectivity of the separation may be altered if the gradient composition is irreproducible between subsequent injections. If variability in retention time from injection to injection is encountered – the following potential causes should be investigated:

Binary Pump Systems

- The flow rate from one or other of the pump heads is inaccurate (check using a flow meter running one pump head at a time, at a flow of 1mL/min. using a calibrated electronic flow meter)
- There is a blockage between one of the pump heads and the solvent mixing device – check as above

Occasionally the mixing units can block or fail. Symptoms are usually a high pressure, a noisy baseline or a combination of both.
Where

Region 1.
- Blockages in the inlet filter may cause cavitation on the solvent lines leading to the pump system. This may cause irreproducible flows from either of the pump heads.
- Retention times may vary and the selectivity of gradient analyses may alter from one injection to the next.

Regions 2 and 4.
- Problems with the inlet or outlet check valves of each of the pumps heads in a binary system can lead to problems with the flow rate – and lead to inaccurate and/or irreproducible retention times due to poor gradient composition reproducibility.
- The flow controller may be causing problems – check the flow from each pump head using an electronic flow meter.

Region 3.
- Blockages between either of the pumps and the gradient mixing device will lead to an irreproducible gradient composition.
- This might be investigated by disconnecting the tubing and measuring the flow output directly from each pump head.

Region 5.
- Blockages in the pulse damping device or any subsequent mixers may lead to irreproducible flow rates – however they are unlikely to give rise to irreproducible gradient composition.
- Gradient analyses are likely to retain the same selectivity even in retention times changes.
Quaternary Pump Systems:

- The gradient former or active inlet is not delivering an accurate mixture of solvents. This can be investigated by setting a 50:50 gradient composition and lifting (momentarily) the eluent lines out of the solvents. If the air which you introduce chases through the tubing at approximately the same rate – then the composition of the solvent should be fairly accurate (don’t forget to purge the air before starting your analysis!)
- For more accurate diagnosis, measurement of UV absorbance of a 50:50 mix of Water : 0.1% Acetone (aq) should give a constant UV absorbance at 265nm

Occasionally the mixing units can block or fail. Symptoms are usually a high pressure, a noisy baseline or a combination of both.

Where

Region 1.
- Most low-pressure mixing valves in quaternary pump systems use solenoid valves to proportion the solvents in the on-line mix
- If these valves fail or are sticking – retention times will be irreproducible and gradient analyses may show changes in selectivity
- Check using an air bubble introduced into the solvent line or by using an UV eluent at a fixed wavelength (see main text for details)
Preparative Pumps

Preparative HPLC requires high eluent flow rates and uses large internal diameter columns (21.2mm i.d. being typical from laboratory scale work). The eluent associated with peaks of interest in the chromatogram is collected and evaporated to obtain the compound of interest. Usually this is for further testing or for use as a reference standard etc.

For preparative HPLC, the challenge is to obtain high flow rates, accurate gradient formation and all without pulsed flow. The highest flow rate required for the column used will determine the type of pump system required.

With required flow rates between 10 and 100mL/min. semi-preparative and preparative pumps are modified analytical scale pumps. A larger piston head with a higher volume liquid filled chamber is the main modification that is required to work at flows of 10 -100 mL/min. In addition, an increase in the i.d of the connecting tubing may also be required to work in the semi-preparative or preparative scale to avoid the formation large system back pressures.

- Dual preparative pump from Agilent Technologies
- 1mL/min – 100mL/min without the need for pump head change
- No pulse damper as the LC column acts as a damper at high mobile phase flow rates
Preparative scale HPLC uses columns of 10mm to 50mm i.d. and can require pumps to deliver up to 100mL per minute of solvent. At these higher flow rates the conventional reciprocating pump may not suitable and different designs can be used. This Agilent preparative pump uses the conventional reciprocating design but with large volume components.

**Capillary (Low Volume) Pumps**

For low flow applications accurate pumps capable of delivering 0.1μl to 100 μL / min are required. Capillary LC is used for the determination of very low concentrations of material in solution. A typical common example is the use in proteomics for protein sequencing. The capillary LC system may be used with an MS detector fitted with a nano-flow probe.

Such applications have encouraged manufacturers to develop highly accurate pumps with negligible dwell volumes that are capable of delivering highly accurate gradients at very low flows.

The small scale of capillary LC means that effects of cavitation and pulsed flow can be very significant, and therefore must be reduced to a minimum.

Most capillary LC systems use 'micro-components' to mix the eluent and use on-line splitting of a larger volume to achieve the very smallest flow rates.
Where

1. All tubing is narrow and as short as possible to minimise dead volume.
2. Low volume vacuum degassers or helium sparging can be used to reduce any effects of cavitations.
3. Capillary LC systems use high-pressure piston pumps to deliver gradients.
4. As a result of the absolute need to keep dead volume negligible the mixing of mobile phase cannot use a conventional mixing tube. Tiny electromagnetic proportioning valves can be used to effect mixing and flow splitting using feedback from a mass flow sensitive meter.
5. As with other pumping systems dampers are used to give a pulse free flow.
6. A higher primary flow is generated and a proportion is split to waste using the electromagnetic proportioning valve. This type of sophisticated computer controlled design can result in an accurate gradient flow at 0.1μL/min.
7. Splitter to reduce flow volume –these can be either fixed volume (ratio) or the flow may be selectable from the software in which case a micro-solenoid valve may be used.
Calibration and Testing

Performance characteristics of the HPLC pump output can be used to monitor and diagnose faults with the system. Monitoring the pressure, the flow rate stability and even the sound of the pump will alert the user to potential problems.

Computer control has improved the level of feedback available, and modern HPLC pumps can often alert the user of potential problems in advance of any failure. To minimise the system down time due to pump failure a calibration and maintenance regime should be employed. Certain components within the HPLC pump (such as piston seals etc.) should be replaced according to a schedule to ensure maintenance is pro-active rather than reactive after, a perhaps very expensive, failure.

Performance Verification (PV) is commonly used to calibrate and test an HPLC system. Depending on the working environment PV may be regulated by external regulators. In such situations high levels of traceability of the calibration, including time and date verification, will be required. The validity of results generated using non-calibrated system will be questionable.

In externally regulated environments such as the pharmaceutical manufacturing or environmental analysis there may be clear guidelines to follow regarding the calibration and testing of HPLC pumps and the information should be sought from the appropriate regulatory body.

Calibration Guidelines for Pumps

- Schedule Performance Verification (PV)
- Monitor system feedback for potential problems
- Follow appropriate guidelines of auditors
- Follow internal standard operating procedures

Test Guidelines for Pumps

- Flow stability
- Flow accuracy
- Gradient formation test
- Noise test (performed with detector)
- Drift test (performed with detector)