

CombiFlash® EZ Prep Solvent Changes

Overview

The EZ Prep has several flow paths to accommodate use of multiple solvent phases for the flash and preparative HPLC columns. The autophase change process will manage the system operation to ensure that immiscible solvents of different phases are properly flushed when changing from flash to prep separations or vice versa; however, there may be cases that don't fit this scenario. To assist the user in these cases, this technical note will cover some of the basic guidelines along with procedures that will work in most cases.

Shared Fluid Paths

The EZ Prep flow paths can be grouped into three separate sections; a shared path, a flash path, and a prep HPLC path. In figure 1, each path is shown. The prep HPLC path is the top portion of the figure, the shared path is the mid portion, while the flash path is the lower portion of the figure.

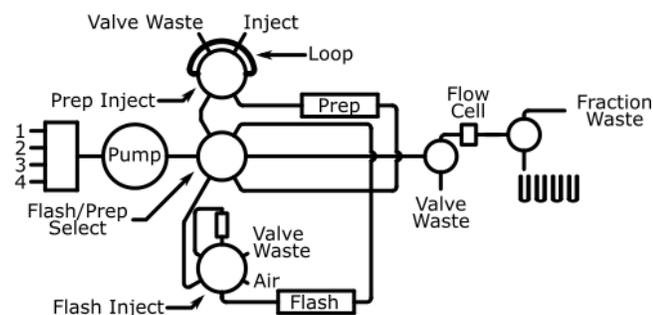


Figure 1: Flow paths

Shared Flow Path

A shared flow path includes the lines from the gradient forming valves on the back panel, through the mixing/debubbling chamber, the pump, and up to the flash/prep selection valve. The line after the flash/prep injection valve is shared and branches through the flow cell to fraction collection or waste, or through the prime pump.

Preventative Flow Path

The dedicated preparative flow path goes from the flash/prep selection valve to the prep injection valve, through the column, and back to the flash/prep selection valve.

Flash Flow Path

The dedicated flash flow path goes from the flash/prep selection valve to the flash injection valve, option-

ally through the solid load cartridge, through the column, and back to the flash/prep selection valve. The flash injection valve also includes lines for flow direct to waste for washing the valve after separations and to air purge the solid load cartridge and column.

Priming and Manual Control

The pump used in the EZ Prep is an HPLC style pump. While it works well pumping fluid, it is not an efficient air pump. To allow the user to easily prime the pump, the EZ Prep has an auxiliary prime pump that is able to efficiently pump both liquid and fluids. The autoprime function or any operation in manual control with the word "Prime" in the flow path activates the prime pump. This function should be used any time to prime the EZ prep pump when it could contain air in the inlet lines.

Miscibility and Priming Order

Typically solvents used for flash (normal phase) and reverse phase are mostly immiscible. There are a few solvents such as isopropanol and acetone that is miscible with most commonly used flash or reverse phase solvents. These solvents are very useful as a wash solvent for flushing the lines when changing solvent phases in the EZ prep. The EZ Prep is shipped from the factory with isopropanol in all lines and then air purged to eliminate dripping during shipment. Flushing all lines with a wash solvent is also a useful technique when the EZ Prep contains unknown solvents.

Autoprime

Autoprime is designed to properly prime the pump, i.e. fill the inlet lines with solvent and remove air from the main solvent pump. If you are priming all four lines, it is important to prime the lines in an order that corresponds with the solvent in the lines before beginning the process.

- If the lines have a solvent miscible with most chromatograph solvents, the order used for autoprime should be in the order of polarity ending with the solvent used for the ensuing separation.
- If you are using autoprime with both normal and reverse phase solvent, **a repeat autoprime is of no value.**

For example, if you autoprime all four solvent ending with hexane, then repeat the prime process starting with water, you are trying to mix hexane with water which is not ideal.

Typical Priming Order for New Systems

If you are configuring the system to use both normal phase and reverse phase solvents and are going to operate in normal phase for the first separation, follow the below procedure. If you want to start with a reverse phase separation, the easiest process is to follow the below steps followed by a short, dummy flash separation (select a 4g column, set length to 2 minutes and leave the prime tube in position). When you switch to reverse phase prep operation, the autophase change process will manage the flushing of the internal, shared tubing.

1. Place the lines in the reservoirs:
 - a. Line 1 in the weak normal phase solvent, such as hexane.
 - b. Line 2 in the strong normal phase solvent, such as ethyl acetate.
 - c. Line 3 in the weak reverse phase solvent, such as water.
 - d. Line 4 in the strong reverse phase solvent, such as acetonitrile.
2. Enable AUTO PHASE CHANGE in the configuration menu.
3. Go to the TOOLS drop down and select AUTOPRIME. Prime the solvents in the following order to flush the shared solvent lines. The solvents listed follow the examples given in #1 above:
 - a. Water (3)
 - b. Acetonitrile (4)
 - c. Ethyl acetate (2)
 - d. Hexane (1)
4. Place the prime tube in the flash column position.
5. Under tools, select manual control and the "Through flash column & flow cell" flow path. Set flow rate to 50 mL/min and set MAX VOLUME to 50 mL. Pump hexane (or the weak normal phase solvent) for 1 minute.
6. Place the solid load line in a small container, select the "Through cartridge & flash column" flow path. Set the MAX VOLUME to 20 mL and pump the weak normal phase solvent at 50 mL/min.

Flush the Reverse Phase Lines (optional)

At this point you can perform a normal phase separation or you can flush the Prep lines. Typically the isopropanol remaining in the lines has a minimal effect on reverse phase chromatography so this step can be skipped.

1. Perform an autoprime in the order listed below to get the proper fluid into the pump for flushing the reverse phase lines.

- a. Strong normal phase (weak normal phase is typically less compatible with reverse phase solvents than the strong phase).
 - b. Strong reverse phase solvent. This is miscible with strong normal phase.
 - c. Weak reverse phase.
2. With a union installed on the prep side, under tools select PREP HPLC LOOP & COLUMN and pump 50 mL of strong normal phase at 50 mL/min.
 3. Perform the reverse phase separation.

EZ Prep with Unknown Solvents or Changing Phases for Flash or Prep

The most robust process is to get the EZ Prep to a known state, such as all solvent lines containing a wash solvent such as isopropanol or acetone which are miscible with both normal phase and reverse phase solvents. Then proceed with setting up the EZ Prep as you would with a new system. To flush all lines with wash solvent, use the following procedure:

1. Place all inlet lines in a container with the wash solvent and perform an autoprime in any order.
 - a. If your container doesn't have room for all four lines, place each line individually into the container and perform an autoprime on that line only before moving to the next inlet line. The final solvent used will be used for the steps following
2. Place the prime tube in the flash column position. Place a union on the prep side in place of a column.
3. Under tools, select MANUAL CONTROL and the THROUGH FLASH COLUMN & FLOW CELL flow path. Set flow rate to 50 mL/min. Enter 50 mL into MAX VOLUME.
4. Place the solid load line in a small container, select the THROUGH CARTRIDGE & FLASH COLUMN flow path. Set the MAX VOLUME to 20 mL and pump the weak normal phase solvent at 50 mL/min.
5. Under tools, select MANUAL CONTROL and the PREP HPLC LOOP & COLUMN flow paths. Set flow rate to 50 mL/min and pump solvent for 1 minute.

Troubleshooting and What If's

- I set up only solvent 1 & 2 with normal phase and now I want to add reverse phase solvents.
 1. Enable the auto phase change feature.
 2. Place lines 3 & 4 into a miscible solvent such as isopropanol or acetone.
 3. Autoprime lines 3 & 4.
 4. Place lines 3 & 4 into the reverse phase solvents.
 5. Autoprime lines 3 & 4 starting with the strong reverse phase solvent (organic

- phase) followed by the weak solvent (aqueous phase)
- My peaks come out too early with poor separation.
 - You probably have mixed phases in the system. This is typically due to use of the manual control screen without considering what solvent are in each line and are mixable.
 - Refer to the section titled “EZ Prep with Unknown Solvents or Changing Phases for Flash or Prep” on page 2 to get the system to a known condition. Then follow the procedure for initially setting up a system, “Typical Priming Order for New Systems” on page 2.
 - I see fluid coming from the waste line even when collecting all.
 - The EZ Prep uses a debubbling system instead of degassing the solvents. There is a bubble trap ahead of the pump. Whenever water is used as a solvent, the system assumes bubbles could be forming and periodically empties the air from the trap. To ensure all the air is removed, a small amount of solvent is also pumped out.
 - I see mixed phases in the collection tube.
 - You probably have mixed phases in the system. If you are unsure what solvents are in the system, refer to the section titled “EZ Prep with Unknown Solvents or Changing Phases for Flash or Prep” on page 2 to get the system to a known condition. Then follow the procedure for initially setting up a system, “Typical Priming Order for New Systems” on page 2.
 - The pressure is fluctuating.
 - If you have the pressure trace on and see spikes:
 - a. This may be due to small air bubbles going through the pump.
 - b. The gas may still be coming from the mixture after the bubble trap. Typically these bubbles are not large enough to cause a problem with the system operation or chromatography.
 - c. This could also be due to check valve contamination. If there are excessive spikes, the check valves may require cleaning.
 - If there are large scale fluctuations instead of sharp spike, it may be due to mixed phase solvents in the system. See “Mixed phases in the collection tube” in the above section for details to correct.

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