

Normal Phase Purifications with the ACCQPrep 125 Solvent Changes

Abstract

On occasion, there is a need to run normal phase columns on the ACCQPrep HP125. This system can easily perform these purifications. However, if not running HILIC columns, some care must be taken to change solvent so that the column remains in non-polar solvents. There are two methods of changing solvents, the advantages of each will be discussed.

Preparing the ACCQPrep HP 125

Inappropriate solvents will affect the chromatography on normal phase columns. Although the column may not be damaged by these solvents, there may be solvent miscibility issues, or an extended column equilibration may be required to restore consistent chromatography. To avoid this, follow the steps below before attaching your column (Read the instructions for your column to determine which solvents are compatible with your column):

- Flush modifiers (such as trifluoroacetic acid) from the column being removed, and flush with a storage solvent, if recommended in the Use and Care Instructions.
- If any buffers were used, make sure they are washed from the ACCQPrep.
- When configuring the new column, set the maximum pressure limit as per the instructions that came with the column. Setting this pressure prevents possible damage to the column.
- Remove the Reverse Phase column and replace it with a simple union so entire fluid path can be flushed, including the loop. If using a column selection valve, remember to choose the correct position containing the union in all steps below.
- If the system is equipped with an AutoSampler, place the line for the probe wash station into methanol or 2-propanol. This extends the life of the peristaltic pump tubing. The injection procedure used by the AutoSampler does not allow these solvents to enter the sample loop or contact the sample. The probe wash solvent is only used to fill the space inside the injection mechanism to improve the injection accuracy.
- Verify the Bracketed Injection Tube station, if used, contains a solvent compatible with the column used (system equipped with the AutoSampler only).
- If the system has a solvent Selector Valve, choose an A and B solvent line for all the steps below. You will use these lines for the solvent change and the final solvents used.
- It is a good idea to update the solvents used for the solvent lines. The solvents can be entered using the System Configuration screen. Although

the system will pump solvents correctly no matter what name is displayed, it avoids confusion if the solvents used are displayed on the system.

Using an Intermediate Solvent

The use of an intermediate solvent such as 2-propanol or acetone allows a faster solvent changeover. It is also easier to perform since acetone and 2-propanol are miscible with nearly all other solvents. However, it also requires these solvents to be available in the lab. Acetone is commonly used for washing glassware, but is less commonly used for chromatography due to its UV absorbance. The viscosity of 2-propanol limits its use in chromatography.

1. Remove and dry the solvent lines from the reservoirs and then place both the lines into the intermediate solvent reservoir.
2. Prime the system with the intermediate solvent that was chosen.
3. Use MANUAL CONTROL to flush the loop and tubing leading to the column with the intermediate solvent; 50 mL is sufficient; 50 mL/minute allows a fast wash of the system.
4. Remove and dry the solvent lines from the reservoir and then place the lines into the solvents that will be used for the normal phase purification.
5. Prime the ACCQPrep with the solvents used for the purification.
6. Flush the system once more with the “weak” solvent through the column flow path using MANUAL CONTROL; set the FLOW PATH through the HPLC Loop and Column.
7. Replace the union with the normal phase column.

To go back to reverse phase, flush the system as described in steps 1 through 3 above. For Step 4, place the solvent lines in the reverse phase solvents and prime as described in step 2 and 3. Flush with the reverse phase solvent as described in steps 5 and 6. In step 7, replace the union with the reverse phase column.

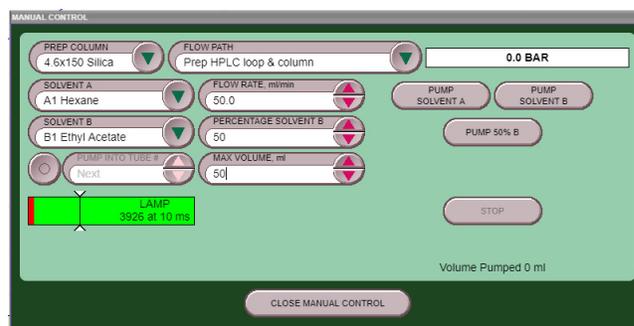


Figure 1: Manual Control Screen

In Figure 1 the FLOW PATH is set to “Prep HPLC loop and column” to flush the sample loop. The MAX VOLUME is set to 50 mL; sufficient to flush the loop and system. The FLOW RATE is set to 50 mL/min. These settings are used for all manual control settings in this note.

Changing Solvent Using Only Normal Phase/Reverse Phase Solvents

This method is a little more complicated because one needs to pay attention to the order of solvents used. It also requires more steps to change the solvent. However, its advantage is that only the solvents employed on the system are required. Essentially, consider the type of chromatography that will be used after the solvent change, and change the solvents in order of “strong” to “weak” solvents for that chromatography. The B solvent lines are assumed to be the strong solvent for all chromatography.

Reverse Phase to Normal Phase

1. As described in preparing the system above, replace the reverse phase column with a simple union to allow flushing the system.
2. Place both solvent lines in the reverse phase B solvent (usually methanol or acetonitrile) and prime the system.
3. Use manual control to flush the loop and tubing leading to the column with the solvent used for the reverse phase B solvent. This ensures the loop contains a compatible solvent for the next step. Remove the lines and dry the solvent inlets.
4. Place both solvent lines in the normal phase B solvent (usually methanol or ethyl acetate) and prime the system.
5. Use MANUAL CONTROL to flush the loop and tubing leading to the column with the solvent used for the normal phase B solvent. This flush makes the loop solvent compatible with normal phase solvents.
6. Place the A-solvent line into the weak normal phase solvent; dry the line before placing into the solvent bottle. Prime the system again. This second prime is required because the ACCQPrep primes both A and B solvent lines at the same time; this prime allows the A solvent to be primed.
7. Flush the system once more with the “weak” solvent through the column flow path, including the loop, using MANUAL CONTROL.
8. Replace the union with the normal phase column.

Normal Phase to Reverse Phase

1. As described in preparing the system above, replace the normal phase column with a simple union to allow flushing the system.
2. Place both solvent lines in the normal phase B solvent (usually methanol or ethyl acetate) and prime.
3. Use MANUAL CONTROL to flush the loop and tubing leading to the column with the solvent used for the normal phase B solvent. This ensures the loop contains a compatible solvent for the next step. Remove the lines and dry the solvent inlets.
4. Place both solvent lines in the reverse phase B solvent (usually methanol or acetonitrile) and prime.
5. Use MANUAL CONTROL to flush the loop and tubing leading to the column with the solvent used for the reverse phase B solvent. This flush makes the loop solvent compatible with reverse phase solvents.
6. Place the A-solvent line into the weak reverse phase solvent; dry the line before placing into the solvent bottle. Prime the system again.
7. Flush the system once more with the “weak” solvent through the loop and column flow path using MANUAL CONTROL.
8. Replace the union with the reverse phase column.

Conclusion

Either of the procedures above will allow one to switch between normal phase and reverse phase with ease. The use of a union insures that all the solvent from the loop, column flow path, and flow cell is flushed from the system.

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