Best Practices Changing from Aqueous to Organic Mobile Phase While Using Advanced Polymer Chromatography

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INTRODUCTION

Gel permeation chromatography (GPC) does not easily allow for switching from aqueous mobile phases to organic mobile phases in one instrument. Some of the GPC solvent switching limitations stem from the immiscibility of mobile phases, cross-contamination of mobile phases causing poor chromatographic reproducibility, and traditional GPC column technology that requires very slow overnight mobile phase changes due to column bed swelling and packing breakdown.

The newer GPC columns used in UltraPerformance Liquid Chromatography (UPLC™) systems are extremely robust and can withstand the very high pressures of a UPLC analysis. The Waters™ ACQUITY™ Advanced Polymer Chromatography (APC™) System with ethylene bridged hybrid (BEH Technology™) columns allows for a change of solvent in just a few hours.[1] Traditional GPC column technology takes days to change solvents, and the analysis time is lengthy. The APC/BEH robust and fast mobile phase change capability, when combined with a solvent switching valve, enables multiple mobile phase changes in one sample sequence. Multiple column oven capability enables switching from aqueous columns and mobile phases to organic columns and mobile phases, and the Polymer Quaternary Solvent Manager (p-QSM) can mix multiple organic and aqueous mobile phases.

Having so much flexibility in one system requires some mobile phase best practices. For example, mixing a buffered water mobile phase with tetrahydrofuran (THF) can produce a precipitated salt or analyte; therefore, it is necessary to consult solvent miscibility tables and plan mobile phase changes and mixing responsibly.[2]

AQUEOUS TO ORGANIC MOBILE PHASE

This guide is designed for the advanced polymer chromatographer when changing mobile phases using Waters ACQUITY APC Columns or ethylene bridged hybrid (BEH) particle columns.[3,4] The APC is unique in its capability of changing mobile phases quickly, while using the same ACQUITY column bank.

Aqueous to organic is a challenging mobile phase change and some knowledge of solvent compatibility is needed (Figure 1). Two separate ACQUITY column banks will be required when using the Polymer Isocratic Solvent Manager (p-ISM) pump for aqueous and organic mobile phases. The APC is also available with a p-QSM pump for gradient elution separations that require the use of aggressive solvents, such as tetrahydrofuran, and robust BEH particle columns. Both APC pumps have the same solvent compatibility.

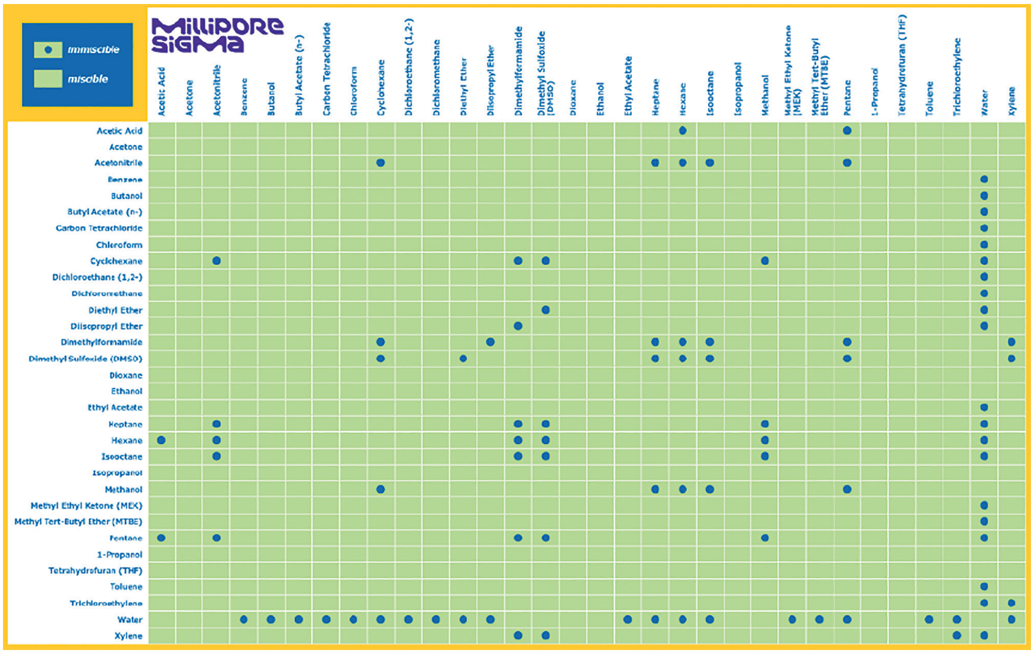
This paper will not cover using the optional recycling method. Using the recycling method is not needed or recommended with the ACQUITY APC Columns and traditional solvent choices such as THF. The APC System uses significantly less solvent than traditional GPC, and the ACQUITY APC Columns are robust. Therefore, recycling a solvent using APC is reserved for traditional GPC columns and/or advanced methods with exotic solvents such as hexafluoro isopropanol (HFIP).

SEAL AND NEEDLE WASH CHOICES

Once the miscibility of the mobile phases is determined, the choice of seal wash and needle wash is needed. The decision tree below guides the user through these choices (Figure 2). These choices are further explained as we follow the flow path of the APC.

Note the seal pump wash and its flow path (Figure 3a and 3b). The flow path travels behind the pump seals.

Aggressive solvents can swell the pump seals and should not be used as seal wash. Buffered water can precipitate salts behind the pump seals and should not be used as a seal wash. The seal wash solvent should be compatible with the sample solvent and mobile phase, compatible with the sample, and free of salts/additives. For organic mobile phase, use an isopropanol (IPA) seal wash, and for an aqueous mobile phase with salt, use a mixture of 50/50 water/methanol (MeOH) seal wash. Dimethyl sulfoxide (DMSO) with lithium bromide (LiBr) mobile phase uses a mixture of 50/50 water/IPA seal wash. LiBr is soluble in IPA.



● immiscible
🟩 miscible

	Acetic Acid	Acetone	Acetonitrile	Benzene	Butanol	Butyl Acetate (n-)	Carbon Tetrachloride	Chloroform	Cyclohexane	Dichloroethane (1,2-)	Dichloromethane	Diethyl Ether	Diisopropyl Ether	Dimethylformamide	Dimethyl Sulfoxide (DMSO)	Dioxane	Ethanol	Ethyl Acetate	Heptane	Hexane	Isooctane	Isopropanol	Methanol	Methyl Ethyl Ketone (MEK)	Methyl Tert-Butyl Ether (MTBE)	Pentane	1-Propanol	Tetrahydrofuran (THF)	Toluene	Trichloroethylene	Water	Xylene
Acetic Acid																				●						●						
Acetone																																
Acetonitrile									●										●	●	●					●						
Benzene																															●	
Butanol																															●	
Butyl Acetate (n-)																															●	
Carbon Tetrachloride																															●	
Chloroform																															●	
Cyclohexane			●											●	●								●								●	
Dichloroethane (1,2-)																															●	
Dichloromethane																															●	
Diethyl Ether															●																●	
Diisopropyl Ether														●																	●	
Dimethylformamide									●				●						●	●	●					●						●
Dimethyl Sulfoxide (DMSO)									●			●							●	●	●					●						●
Dioxane																																
Ethanol																																
Ethyl Acetate																															●	
Heptane			●											●	●								●								●	
Hexane	●		●											●	●								●								●	
Isooctane			●											●	●								●								●	
Isopropanol																																
Methanol									●										●	●	●					●						
Methyl Ethyl Ketone (MEK)																															●	
Methyl Tert-Butyl Ether (MTBE)																															●	
Pentane	●		●											●	●								●								●	
1-Propanol																																
Tetrahydrofuran (THF)																																
Toluene																															●	
Trichloroethylene																															●	●
Water				●	●	●	●	●	●	●	●	●	●					●	●	●	●			●	●	●			●	●		●
Xylene														●	●															●	●	

Figure 1. Solvent miscibility table from Sigma Aldrich.[2]

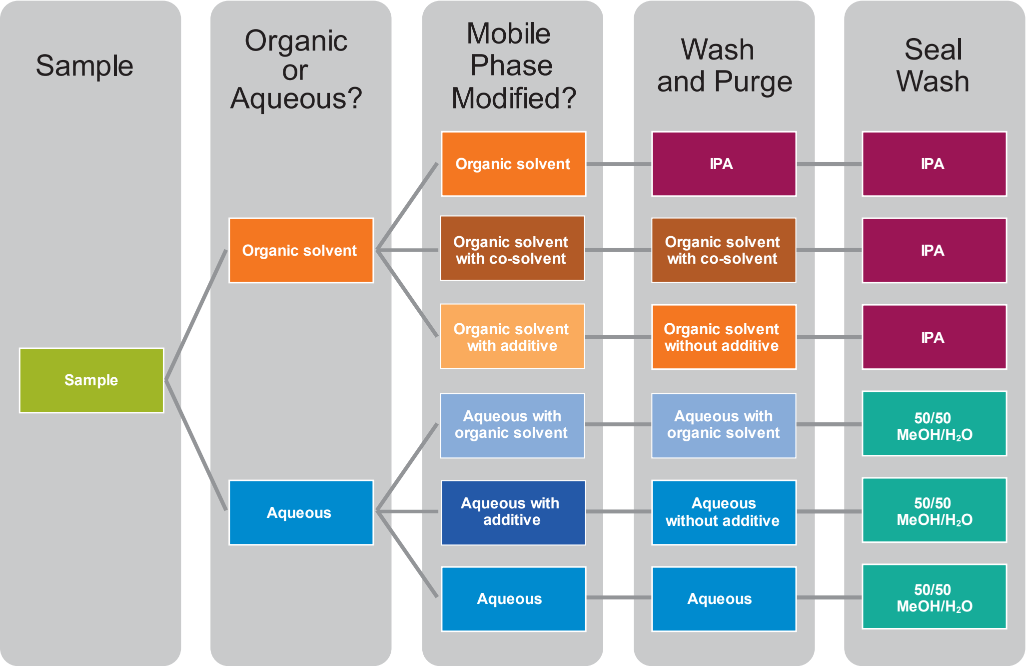


Figure 2. Mobile phase decision tree.

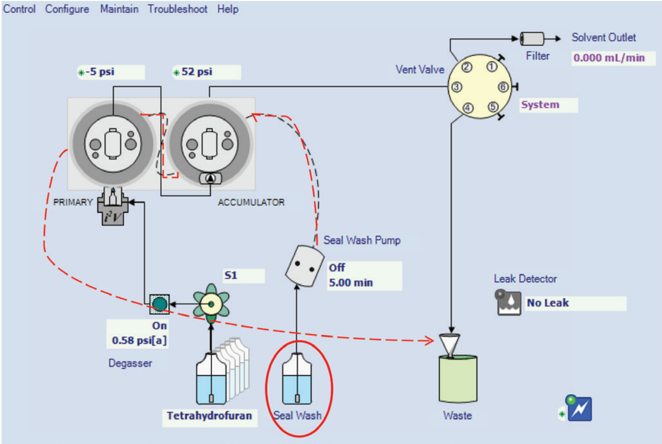


Figure 3a. APC polymer isocratic solvent manager (p-ISM) pump interactive display.

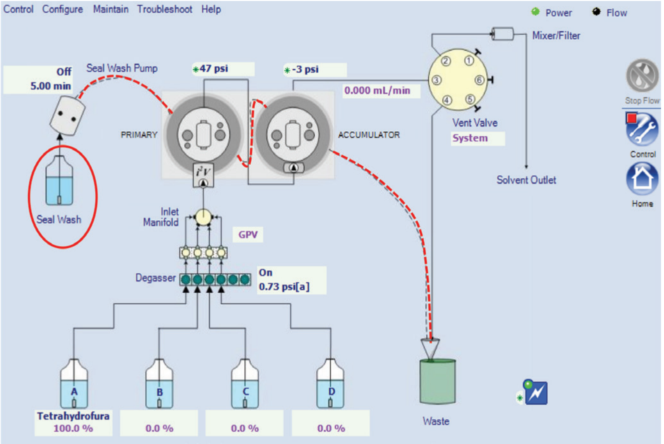


Figure 3b. APC polymer quaternary solvent manager (p-QSM) pump interactive display.

SOLVENT FLOW PATHS

Note the purge solvent flow path and the wash solvent flow path (Figure 4). All these flow paths have components that are tested with the approved solvents for APC. Examples of approved solvents are acetone, acetonitrile, chloroform, dimethylformamide, DMSO, ethyl acetate, hexane, MeOH, methylene chloride, THF, toluene, water, and HFIP (remove degasser for HFIP).

When only a co-solvent is used as an additive in the mobile phase, the wash solvent and purge solvent tubing can be in the same bottle as the mobile phase. Examples of co-solvents include MeOH, water, IPA, ethanol, acetonitrile, and THF.

When a chemical additive is used in the mobile phase, the wash solvent and purge solvent tubing should be free of additives. Therefore, a mobile phase bottle without additives is used for the wash solvent and purge solvent tubing. Examples of additives include formic acid, phosphoric acid, trifluoroacetic acid, sodium nitrate, sodium chloride, sodium borate, triethyl amine, ammonium formate, ammonium acetate, and LiBr.

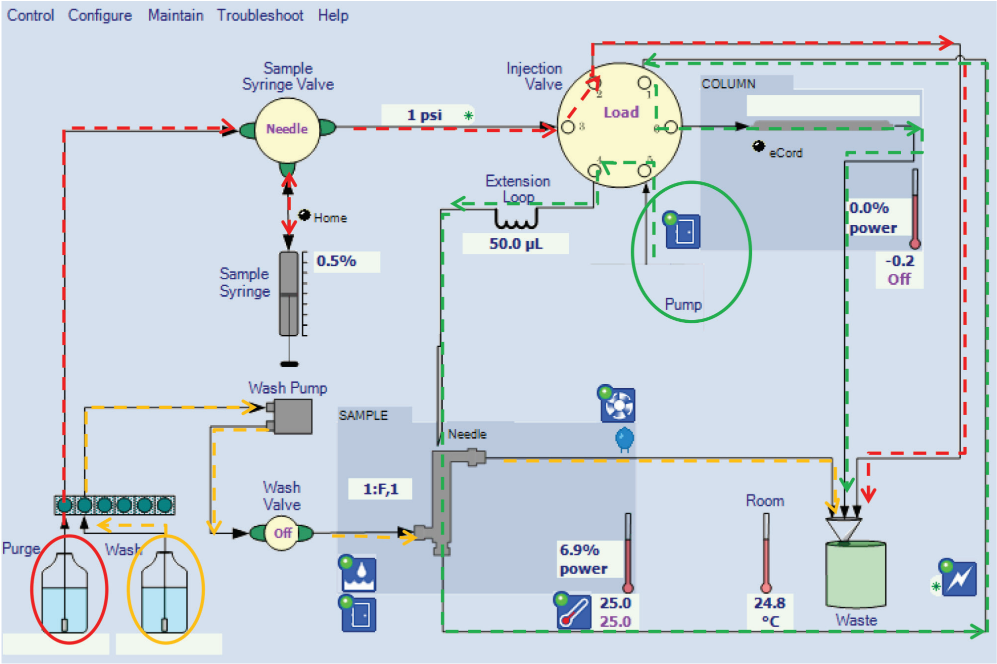


Figure 4. APC Flow-Through Needle Sample Manager (FTN-SM) interactive display.

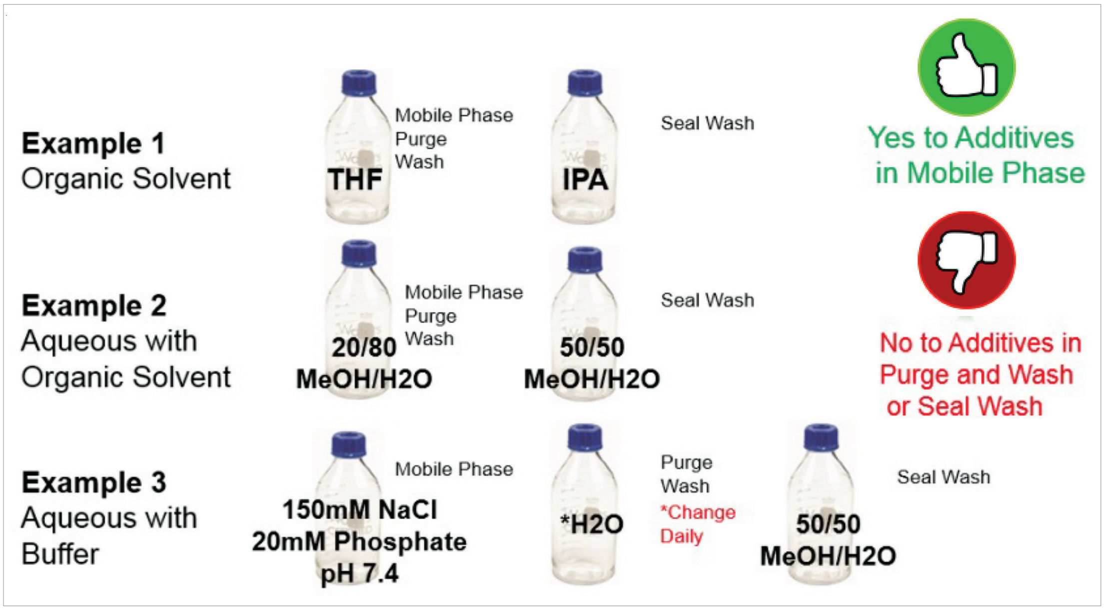


Figure 5. Three APC mobile phase examples.

These three examples of mobile phases and wash reservoirs are typical for APC applications (Figure 5). The appropriately labeled tubing with sinkers will be placed manually in each of these bottles. Before changing to a new mobile phase, the system will need to be cleared of the previous mobile phase and any additives used.

Figure 6 uses a diagram to explain the need for an intermediate solvent to clear the previous mobile phase from the system. This flow pattern depicts the methods changing from organic mobile phase to aqueous mobile phase, and aqueous mobile phase to organic mobile phase. The tubing and sinkers will need to be manually placed into the new bottles of mobile phase. IPA is the intermediate step between THF and water. Water is the intermediate step between buffered water and IPA. These intermediate step mobile phases are miscible with the previous choice and the future choice.

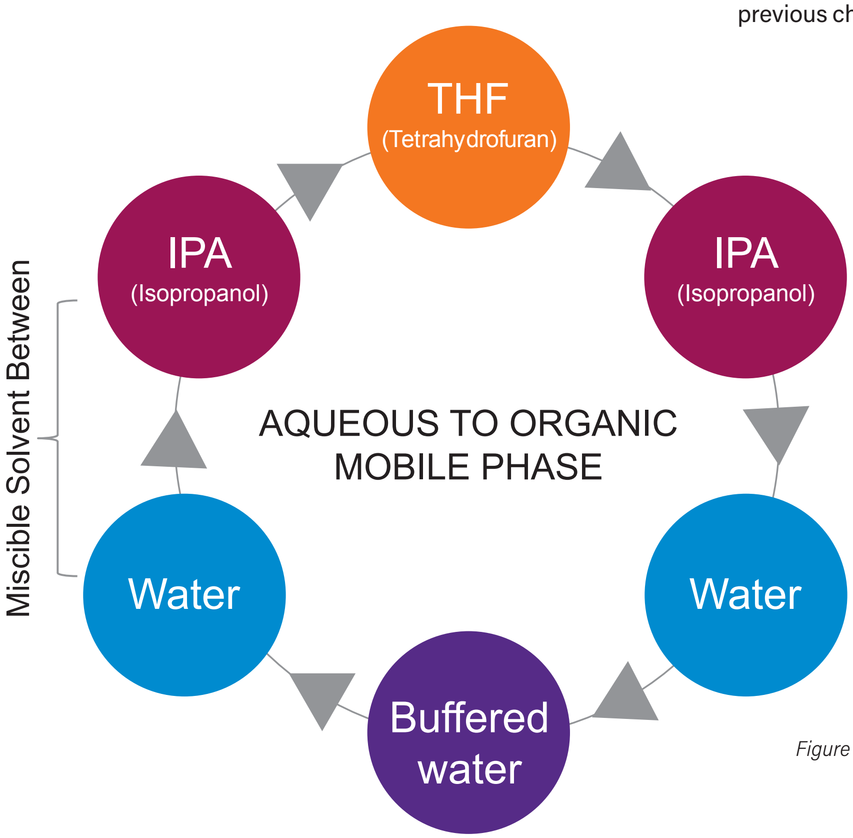
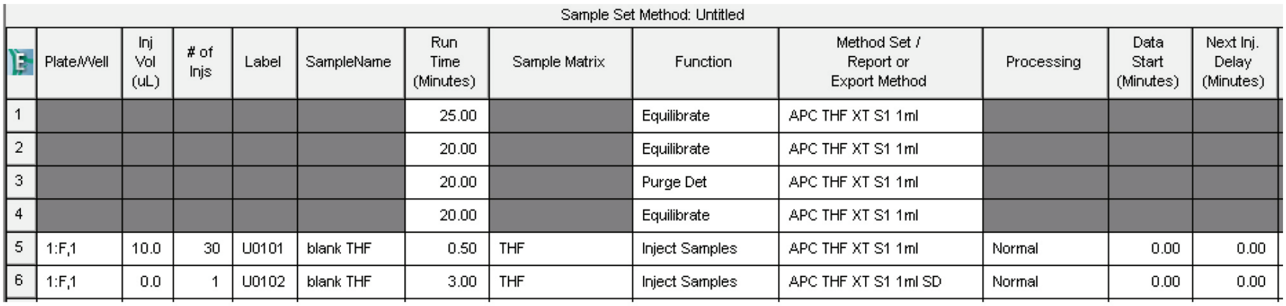


Figure 6. Using an intermediate miscible solvent.



Sample Set Method: Untitled

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Run Time (Minutes)	Sample Matrix	Function	Method Set / Report or Export Method	Processing	Data Start (Minutes)	Next Inj. Delay (Minutes)
1						25.00		Equilibrate	APC THF XT S1 1ml			
2						20.00		Equilibrate	APC THF XT S1 1ml			
3						20.00		Purge Det	APC THF XT S1 1ml			
4						20.00		Equilibrate	APC THF XT S1 1ml			
5	1:F,1	10.0	30	U0101	blank THF	0.50	THF	Inject Samples	APC THF XT S1 1ml	Normal	0.00	0.00
6	1:F,1	0.0	1	U0102	blank THF	3.00	THF	Inject Samples	APC THF XT S1 1ml SD	Normal	0.00	0.00

Figure 7. Example of Empower™ Chromatography Data Software (CDS) sample set method for cleaning the system.

BEFORE PURGING THE SYSTEM WITH IPA

Before the system is purged with IPA, follow the steps below:

1. In order to avoid precipitating any polymer or other analyte in the APC system with an IPA wash, it is necessary to use the sample set method with the last solvent used in the instrument (Figure 7).
   - If the last sample run in the APC was polystyrene in THF, the system must be cleaned with THF first.
   - If the last sample run in the APC was pullulan in buffered water, the system must be cleaned with buffered water first, and then water without buffer.
2. The cleaning process is carried out by removing the columns (possibly containing contaminate or sample), placing a union in the column oven, CM and RI at 40 °C, flow of 1 mL/min, 30 injections of 10 µL, 0.5 minute run time.
3. This cleaning will help to ensure that the previously analyzed samples are not captured in the injection valves.

SWITCHING FROM BUFFERED WATER TO THF

The APC can be switched from buffered water to THF by the following:

1. Place a one-liter bottle of both UHPLC-grade IPA and UPLC non-inhibited grade THF on the instrument bottle tray. Remove the tubing from the buffered water reservoir bottle and place them into the **water only** bottle (Solvent 1, Sample Wash, Purge). Cap the buffered water bottle. *(**Note:** The p-QSM will have four mobile phase bottles, a sample manager/needle wash bottle, and a seal wash bottle. The tubing and sinkers may be left in the appropriate mobile phase bottles. Be cautious of mobile phase changes in the gradient table of the instrument method.)*
2. Using the same temperature settings from the previously used sample set, prime the system with Solvent 1:water (five minutes).
3. After priming, start the p-ISM flow for Solvent 1 at 0.1 mL/min. Keep an eye on the back pressure, and raise the flow until the back pressure is near 10,000 psi.
4. Prime the FTN (100 cycles/100 washes) and purge the detector for 45 minutes.
5. After the RI has purged, wait 15 minutes and observe the drift. If the drift is +/- 0.5 µRI, then the system is equilibrated. For other detectors, observe baseline drift and flow until acceptably flat baseline is achieved.
6. Set the flow to zero and remove the tubing from the **water only** bottle and place them in the IPA bottle. Cap the water bottle. IPA has a higher viscosity than water, so use the 40 °C temperature for the column oven and the RI detector. *(**Note:** For the p-QSM, the tubing and sinkers may be left in the appropriate mobile phase bottles. Be cautious of mobile phase changes in the gradient table of the instrument method.)*
7. Pull the tubing from the 50/50 methanol/water seal wash bottle. Prime the seal wash for a couple of seconds to create an air bubble. Place the seal wash tubing into the IPA seal wash bottle and cap the 50/50 methanol/water seal wash bottle. Prime the seal wash until the air bubble passes into the liquid hazardous waste bottle.
8. Repeat steps 3–5 using IPA as the mobile phase. Keep an eye on the back pressure and raise the flow until the back pressure is near 10,000 psi.
9. Set the flow to zero and remove the tubing from the IPA bottle and place it into the THF bottle. Cap the IPA bottle. *(**Note:** For the p-QSM, the tubing and sinkers may be left in the appropriate mobile phase bottles: be cautious of mobile phase changes in the gradient table of the instrument method.)*

10. Remove the columns from the column oven and place a union in the column oven. Storing the aqueous columns in IPA will prevent bacteria from growing.
11. Repeat steps 3–5 using THF as the mobile phase.
12. Once the system is equilibrated with the APC XT column bank and has a consistent RI baseline, run a few blanks to determine the system readiness. For other detectors, observe baseline drift and flow until acceptably flat baseline is achieved.

SWITCHING FROM THF TO BUFFERED WATER

The APC can be switched from THF to buffered water by the following:

1. Prepare the buffered water the night before using, filter (0.2 µm), and then degas the solution by placing in a sonic bath for five minutes with the cap loosened. Tighten the cap after sonication. Also, prepare a 50/50 v/v MeOH/water (250 mL) reservoir bottle for the seal wash.
2. Place a one-liter bottle of both UHPLC-grade IPA and UHPLC-grade water on the instrument bottle tray. Remove the tubing from the THF reservoir bottle and place it into the IPA bottle (Solvent 1, Sample Wash, Purge). Cap the THF bottle. If solvent cross-contamination is a concern, rinse the sinkers and tubing ends with IPA before inserting into the IPA bottle.
3. Using the temperature settings from the THF application, prime the system with Solvent 1:IPA (five minutes at 4 mL/min). IPA has a higher viscosity than THF, hence using the THF application temperature for the column oven and the RI detector.
4. After priming, start the p-ISM flow for Solvent 1 at 0.1 mL/min. Keep an eye on the back pressure, and raise the flow until the back pressure is near 10,000 psi. The option to remove the columns and replace them with a union can be used instead, but do not exceed 0.2 mL/min, due to the viscosity of IPA.
5. Prime the FTN with IPA (50 cycles/50 washes) and purge the detector for 45 minutes.
6. After the RI has purged, wait 15 minutes and observe the drift. If the drift is +/- 0.5 µRI, then the system is equilibrated with IPA. If your analysis is more tolerant of baseline drift, +/- 1.0 µRI is acceptable. This process can take up to two hours. For other detectors, observe baseline drift and flow until acceptably flat baseline is achieved.
7. Set the flow to zero and remove the tubing from the IPA bottle and place it in the water bottle. Cap the IPA bottle. If solvent cross-contamination is a concern, rinse the sinkers and tubing ends with water before inserting into the water bottle. *(**Note:** For the p-QSM, the tubing and sinkers may be left in the appropriate mobile phase bottles. Be cautious of mobile phase changes in the gradient table of the instrument method.)*
8. Pull the tubing from the IPA seal wash bottle. Prime the seal wash for a couple of seconds to create an air bubble. Place the seal wash tubing into the 50/50 methanol/water bottle and cap the IPA seal wash bottle. Prime the seal wash until the air bubble passes into the liquid hazardous waste bottle.
9. Install the aqueous columns in the column oven. They should be stored in IPA in order to prevent bacterial growth. The water mobile phase is compatible with IPA and any residual buffer that may be in the stored columns, therefore the risk of precipitated buffer is reduced.
10. Repeat steps 3–6 using water as the mobile phase.
11. Set the flow to zero and place the **Solvent 1** tubing into the buffered water. Leave the sample wash tubing and purge tubing in the **water only** mobile phase. *(**Note:** For the p-QSM, the tubing and sinkers may be left in the appropriate mobile phase bottles. Be cautious of mobile phase changes in the gradient table of the instrument method.)*
12. Repeat steps 3-6 using buffered water as the mobile phase.
13. Create the aqueous methods in Empower CDS the day before using the system. Set the oven and detector temperature.
14. Prepare the samples the night before the analysis with the filtered and degassed buffered water. Prepare a blank vial with the buffered water.
15. Once the system is equilibrated with the aqueous column bank and has a consistent RI baseline, run a few blanks to determine the system readiness. For other detectors, observe baseline drift and flow until acceptably flat baseline is achieved.

CONCLUSION

The Advanced Polymer Chromatography (APC) System offers mobile phase flexibility by enabling both aqueous and aggressive solvent use. This system capability requires mobile phase best practices, and this document will support the scientist in successfully making the transition. The detailed instructions and supporting references will guide the user through the process of changing mobile phases, while lowering the risk of precipitating non-miscible polymer solutions and solvents. The liquid chromatographic analysis of polymers and polymer matrices can be challenging, and the APC with p-ISM or p-QSM offers greater solvent flexibility for a wide variety of polymer chemistry and solubility.

References

1. Waters ACQUITY APC Columns, https://www.waters.com/webassets/cms/library/docs/720004580en.pdf.
2. Sigma Aldrich solvent miscibility table, https://www.sigmaaldrich.com/chemistry/solvents/solvent-miscibility-table.html.
3. Waters ACQUITY APC Columns, https://www.waters.com/waters/nav.htm?cid=134722533.
4. Waters XBridge BEH Columns, https://www.waters.com/waters/nav.htm?cid=513767.

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