
ACQUITY UPLC® H-Class System

with TUV, PDA, ELS, or FLR Detectors

Installation Manual

Notice

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Preparing mobile phases and wash solvent for the performance tests

CAUTION: *TO PREVENT CONTAMINATION, WEAR CLEAN, CHEMICAL-RESISTANT, POWDER-FREE GLOVES.*

Tools/materials required

- 100 mL volumetric flask
- 100 mL graduated cylinder
- 1 L reservoir bottle
- Pipette
- Fisher Optima HPLC-grade acetonitrile
- Baker HPLC-grade water
- Solvent Clarification Kit (part number WAT200543)
- General-purpose laboratory vacuum pump
- ACQUITY UPLC BEH C₁₈ (2.1 x 50 mm, 1.7 µm, part number 186002350)

Solvent and glassware considerations

Due to the increased sensitivity that both the ACQUITY UPLC H-Class System and new series of detectors offer, all solvents including water and additives must be of the highest chemical purity (HPLC-grade or better). Failure to use HPLC-grade solvents will result in high background contamination, low signal-to-noise, and loss of sensitivity. Waters recommends using Fisher Optima HPLC-grade acetonitrile and Baker HPLC-grade water.

Glassware (e.g. solvent bottles) must not be washed with detergents or washed with other general glassware to prevent contamination. The glassware must be rinsed with the high purity solvents to be used.

NOTE: *For further detail on controlling contamination, refer to the document Controlling Contamination in Ultra Performance LC™/MS and HPLC/MS Systems, part number 715001307, located in the Waters Support Center on the Web (www.waters.com).*

Waters recommends that mobile phases and wash solvents be mixed as shown in Table 1.

Table 1: Mobile Phase and Solvent Concentrations

Mobile Phase and Wash Solvents	Concentration
Mobile Phase A	100% water
Mobile Phase B	100% acetonitrile
Mobile Phase C	100% water
Mobile Phase D	100% acetonitrile
Purge solvent	100% water
Seal Wash	100% water
Wash solvent	90:10 water/acetonitrile (for TUV, PDA, ELS) 100% acetonitrile (for FLR)

Preparing the mobile phases

1. Prepare a mobile phase of 100% HPLC-grade water:
 - a. Pour approximately 1 L of HPLC-grade water into a 1-L reservoir bottle.
 - b. Label the reservoir bottle: "100% water mobile phase".
2. Prepare a mobile phase of 100% acetonitrile:
 - a. Pour approximately 1 L of filtered acetonitrile into a 1-L reservoir bottle.
 - b. Label the reservoir bottle: "100% acetonitrile mobile phase".

Preparing the wash solvent (for TUV, PDA, and ELS detectors)

NOTE: The wash solvent for FLR detectors uses the "100% acetonitrile mobile phase".

1. Pour 90 mL of filtered HPLC-grade water into a reservoir bottle.
 2. Pour 10 mL of filtered acetonitrile into the same reservoir bottle.
 3. Cap the reservoir bottle and mix well.
 4. Label the reservoir bottle "90% Water/10% Acetonitrile".
-

Installing the ACQUITY UPLC H-Class System modules

Before you begin

- If the system includes an optional Flex Cart, assemble the cart according to the instructions in Assembling the Flex Cart (P/N 715001019).
- If the system includes multiple detectors, refer to "Configuring the ACQUITY UPLC System Components" (P/N 715001701) for configuration details.

CAUTION: *TO AVOID INJURY, AT LEAST TWO PEOPLE MUST LIFT THE SAMPLE ORGANIZER, QUATERNARY SOLVENT MANAGER, AND THE SAMPLE MANAGER-FTN.*

CAUTION: *TO AVOID FINGER INJURY, USE CAUTION WHEN PLACING THE QUATERNARY SOLVENT MANAGER AND SAMPLE MANAGER-FTN MODULES ON THE BENCH TOP OR FLEX CART.*

Tools/materials/software required

- ACQUITY UPLC H-Class System Start-up Kit
- Powder-free gloves
- PEEK tubing cutter
- 1/4-inch open-end wrench
- 5/8-inch open-end wrench
- Phillips head screwdriver
- EMP_HCLASS_startup project (for Empower-based systems)
- ML_HCLASS_startup project (for MassLynx-based systems)

Installing the Quaternary Solvent Manager (QSM)

1. Carefully place the QSM onto the bench top or optional flex cart.

Installing the Sample Manager - Flow Through Needle (SM - FTN)

1. Carefully place the SM-FTN on top of the QSM.
2. On the back of the SM-FTN, remove the center screw that secures the top cover to the unit.



Figure 1 - Center screw removed

Installing an optional Column Heater (CH-A)

1. Carefully place the CH-A on top of the SM-FTN.
2. Slide the unit forward until the two studs on the back of the SM-FTN align with and extend through the two slots in the back plate of the CH-A.

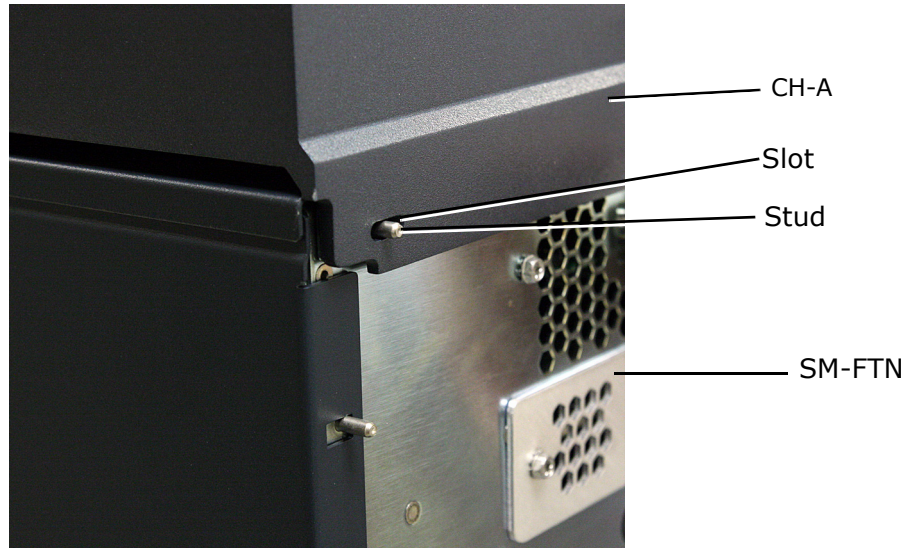


Figure 2 - Position on the SM-FTN

3. Using the Phillips head screw with nylon washer from the CH-A startup kit, secure the center of the CH-A back plate to the back of the SM-FTN.

Installing the optical detector

1. Carefully place the TUV, PDA, ELS, or FLR on top of the CH-A.

NOTE: *If the system includes multiple detectors, refer to "Configuring the ACQUITY UPLC System Components" (P/N 715001701) for configuration details.*

2. Place the Solvent Tray module on top of the detector.
-

Making the fluidic connections

CAUTION: *TO PREVENT CONTAMINATION, WEAR CLEAN, CHEMICAL-RESISTANT, POWDER-FREE GLOVES.*

WARNING: *TO AVOID RELEASING SOLVENT VAPORS INTO THE ROOM, ROUTE THE DEGASSER VENT TUBE INTO A FUME HOOD OR OTHER SUITABLE EXHAUST SYSTEM, OR TO A SUITABLE WASTE CONTAINER, ENSURING THE END OF THE TUBING IS ABOVE THE FLUID LEVEL.*

CAUTION: *TO AVOID FLUID BACKUP, ENSURE THAT THE WASTE CONTAINER IS BELOW THE STACK, ENSURE THERE ARE NO CRIMPS OR BENDS IN THE WASTE LINES, AND ENSURE WASTE AND DEGASSER LINE ENDS ARE NOT IMMERSSED IN WASTE FLUID.*

1. Release the degasser vent line from its holding clip on the QSM.

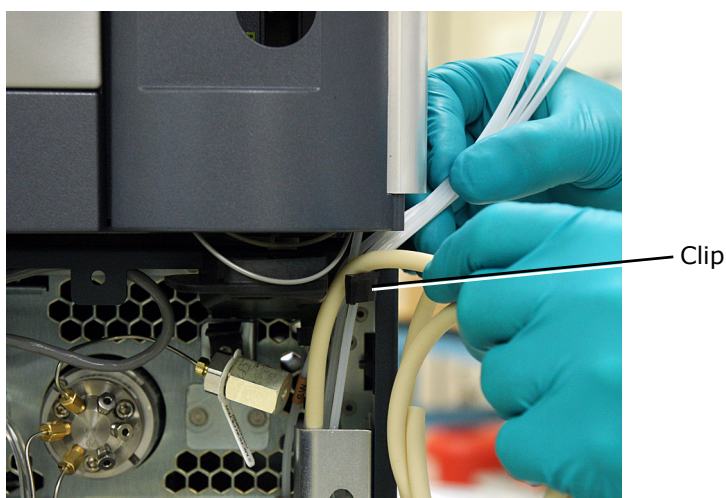


Figure 3 - Unclipping the degasser vent line

2. Locate the solvent tubing labeled "Sample Manager 2" and remove its solvent filter.
3. Route the "Sample Manager 2" solvent tubing through the tubing management channel in the SM-FTN, then reattach the solvent filter and insert the tubing into the reservoir labeled "100% Water mobile phase".

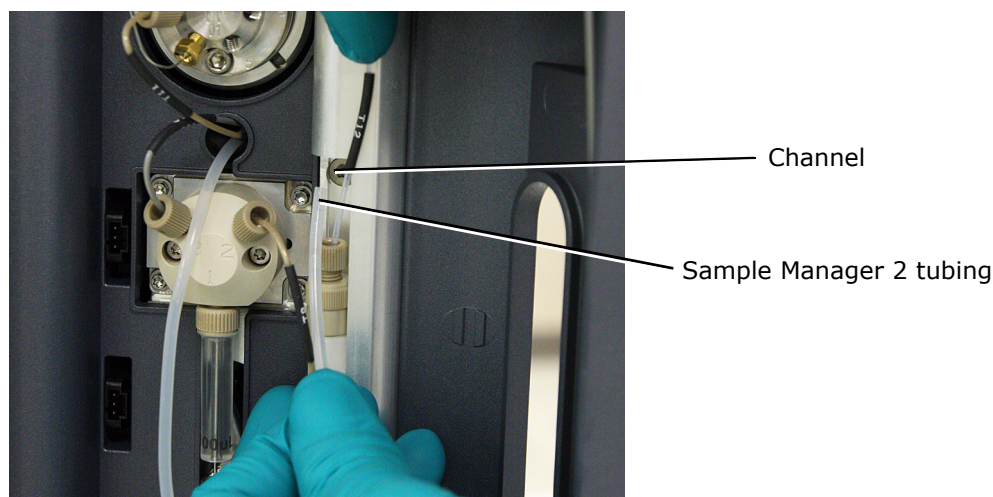


Figure 4 - Routing the Sample Manager 2 tubing

4. Route the A, B, C, and D solvent tubing through the outside of the channel.

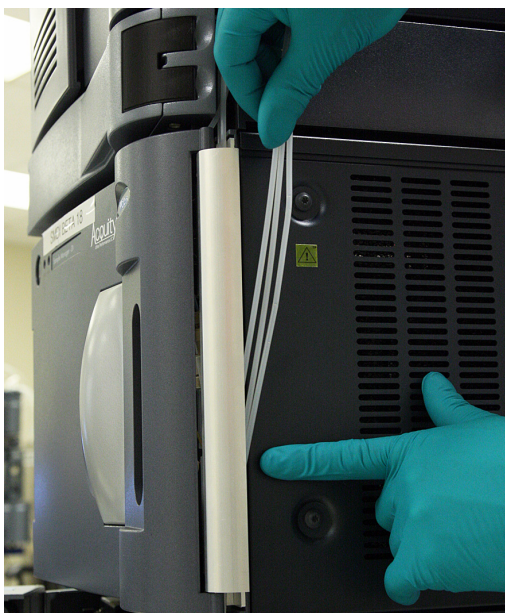


Figure 5 - Routing the solvent tubing

5. Insert these solvent lines into their appropriate reservoirs:
 - a. Insert Solvent A and Solvent C tubing into the 100% water reservoir.
 - b. Insert the Seal Wash In tubing into the 100% water reservoir.
 - c. Insert Solvent B and Solvent D tubing into the 100% acetonitrile reservoir.
6. Connect the Sample Manager 1 tubing to either of the two SM SYRINGE ports on the QSM.

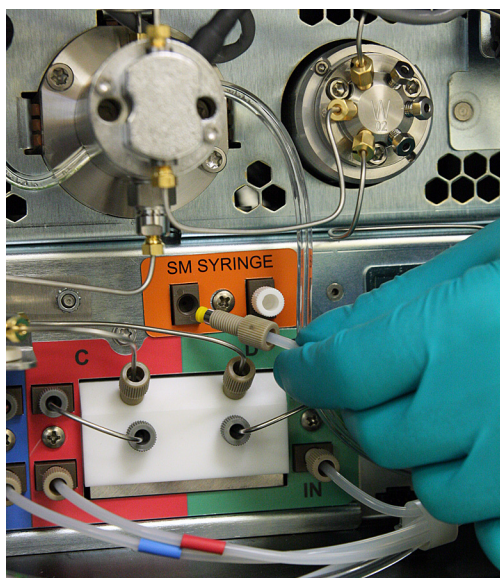


Figure 6 - Sample Manager 1 tubing connection to the QSM

7. Route the other end of the Sample Manager 1 tubing through the channel and insert it into the 90% water/10% acetonitrile reservoir (for TUV, PDA, and ELS detectors), or into the 100% acetonitrile reservoir (for FLR detectors).
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8. Unfasten the taped syringe waste and purge solvent tubing from the inside of the SM-FTN door.

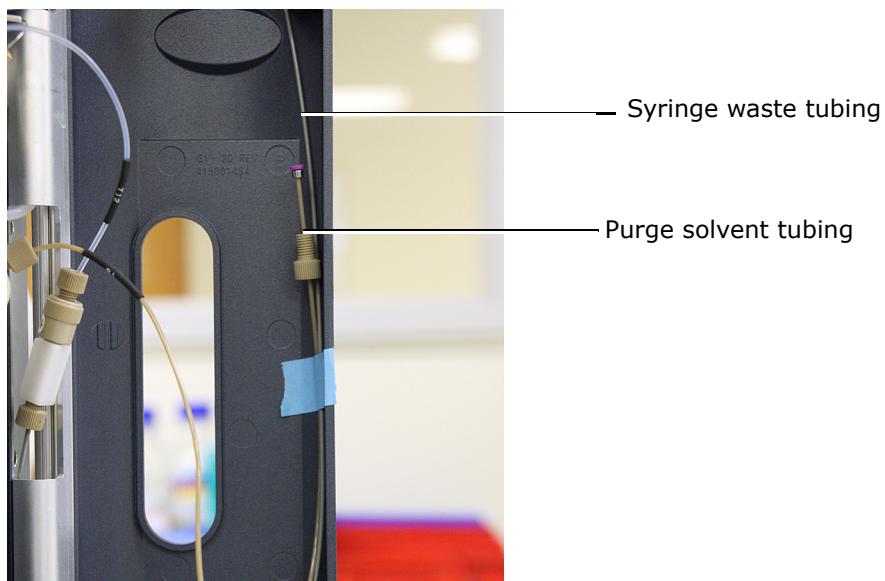


Figure 7 - Syringe waste and purge solvent tubing

9. Connect the Purge Solvent tubing to the available SM SYRINGE port on the QSM.

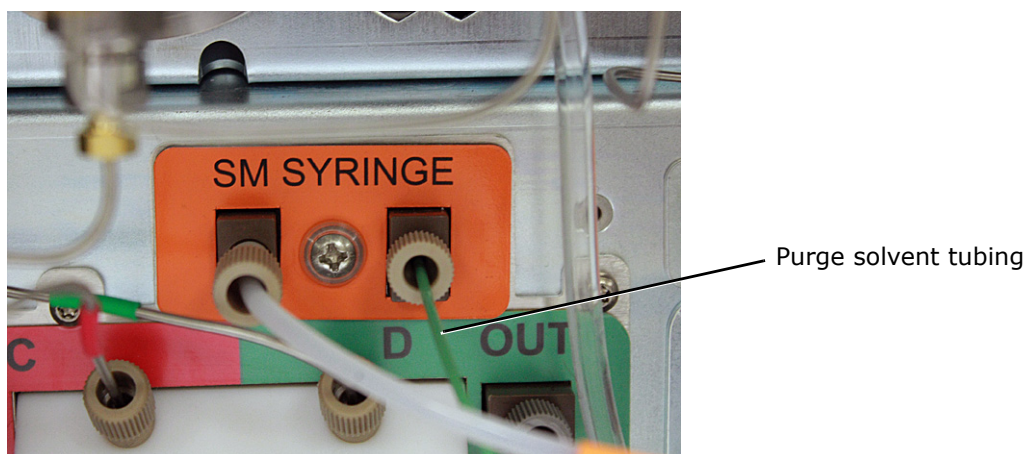


Figure 8 - Purge Solvent tubing connection to QSM

10. Route the syringe waste line behind the stainless steel clip on the QSM.

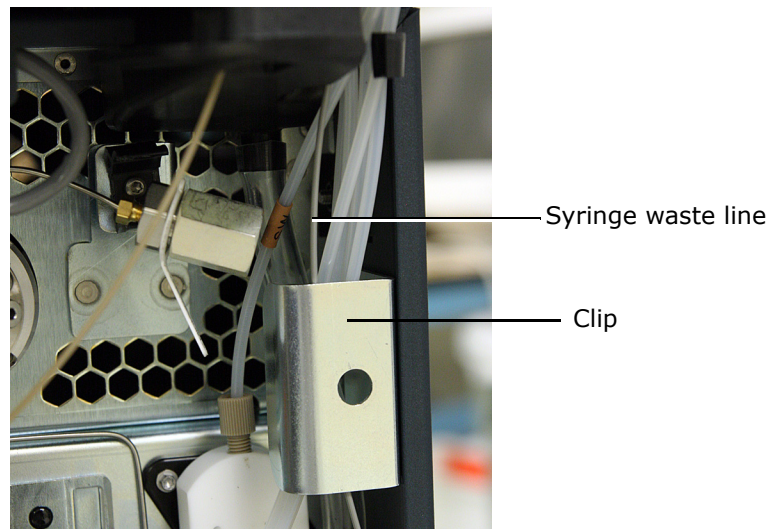


Figure 9 - Syringe waste line routing

11. Use a tubing cutter to cut the syringe waste line to allow connection to its waste port on the QSM drip tray.

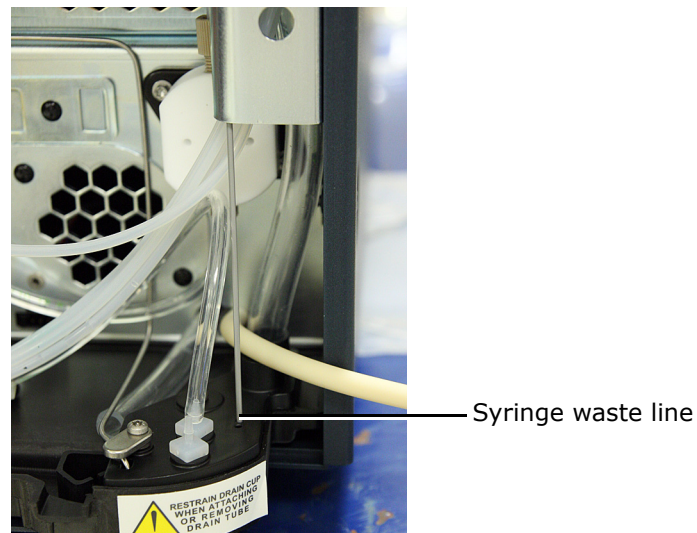


Figure 10 - Syringe waste port connection

12. Connect the convoluted waste tubing to the SM-FTN waste port, then route it through the hole in the SM-FTN drip tray.

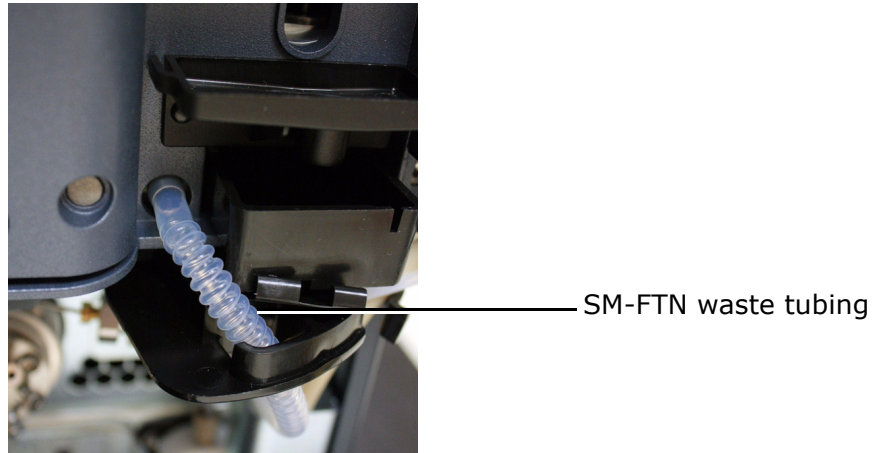


Figure 11 - SM-FTN waste tube routing

13. Route the SM-FTN waste tubing behind the stainless steel clip on the QSM, then connect it to the waste port on the QSM drip tray.

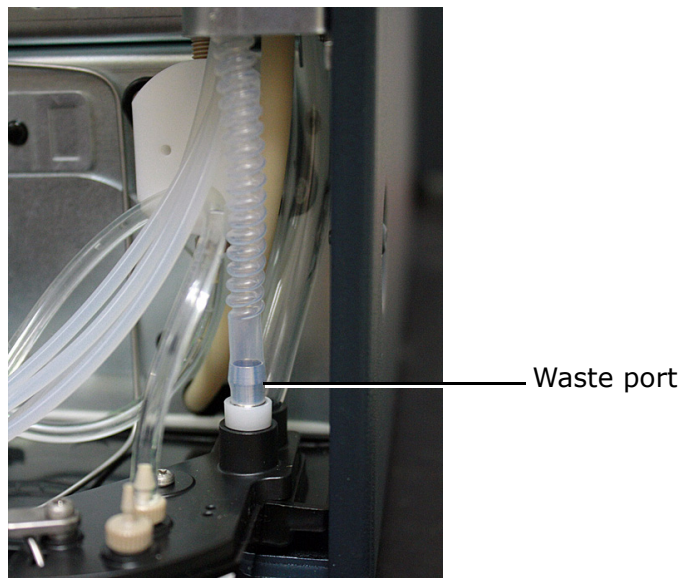


Figure 12 - SM-FTN waste port connection

14. Route the degasser vent tube as shown in [Figure 13](#), then on to a suitable container or exhaust system.

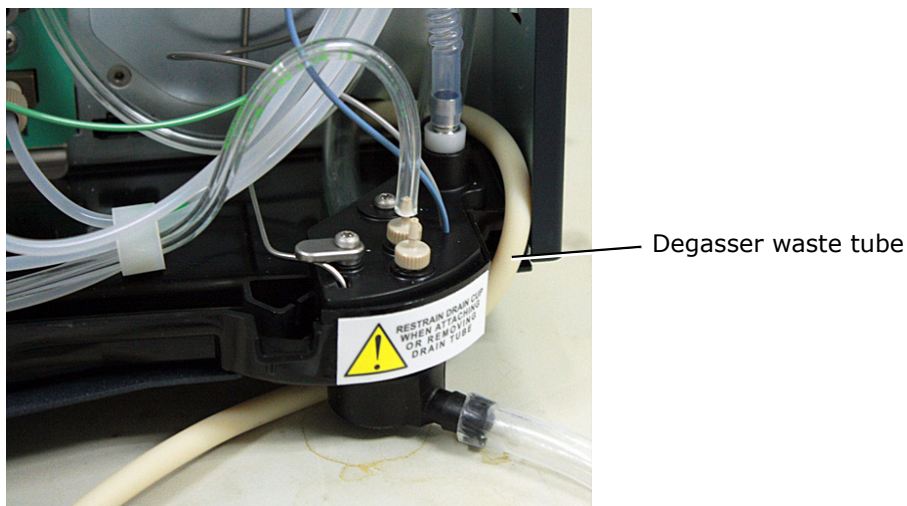


Figure 13 - Degasser waste tube routing

WARNING: TO AVOID RELEASING SOLVENT VAPORS INTO THE ROOM, ROUTE THE DEGASSER VENT TUBE INTO A FUME HOOD OR OTHER SUITABLE EXHAUST SYSTEM, OR TO A SUITABLE WASTE CONTAINER, ENSURING THE END OF THE TUBING IS ABOVE THE FLUID LEVEL.

15. Connect a section of Tygon tubing from the QSM waste outlet to a waste reservoir.
16. Remove the long compression screw and ferrules from the stainless steel PUMP OUTLET tube, then route the end of the tube through the channel in the SM-FTN.

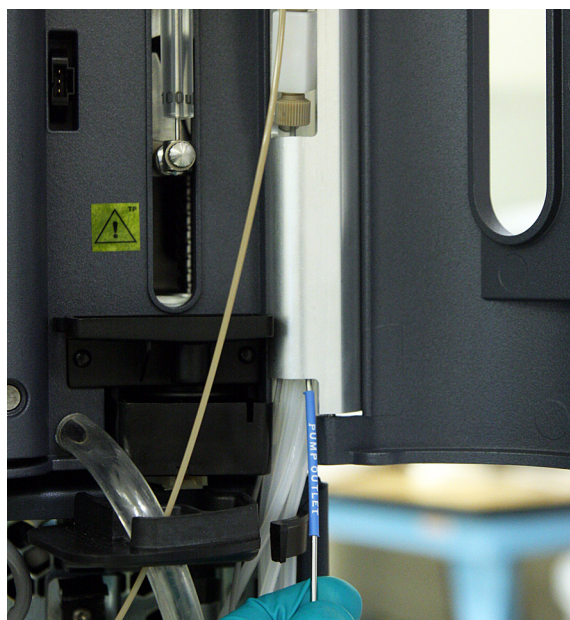
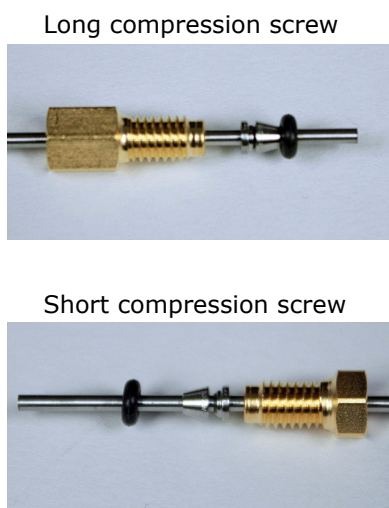


Figure 14 - Pump Outlet tube routing

17. Reattach the long compression screw and ferrules to the PUMP OUTLET TUBE, then secure to port 5 on the inject valve (finger-tight plus 3/4-turn using the 1/4-inch open-end wrench).

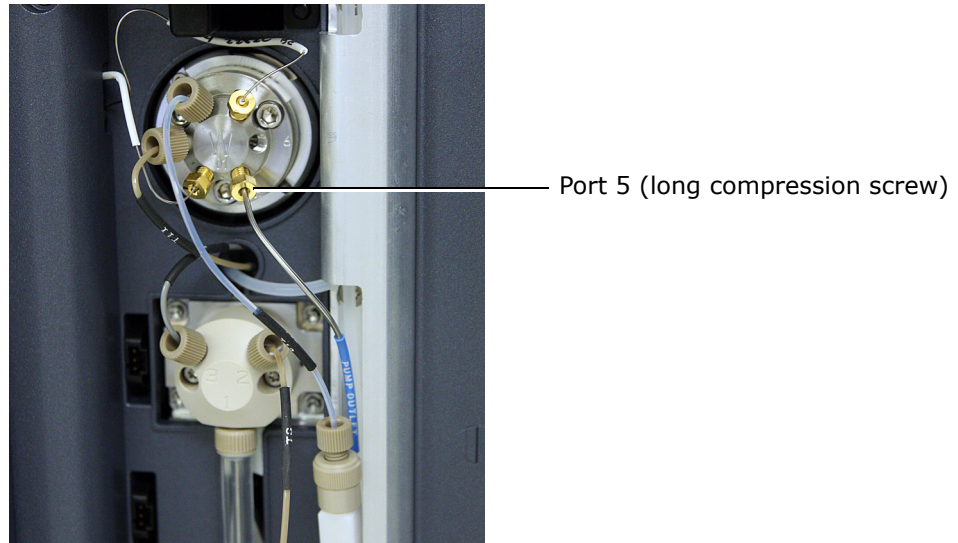


Figure 15 - Pump Outlet tube inject valve connection

18. Remove the inline filter from its clip on the QSM, then attach the other end of the PUMP OUTLET tube to the inline filter using the 5/8- and 1/4-inch open-end wrenches.

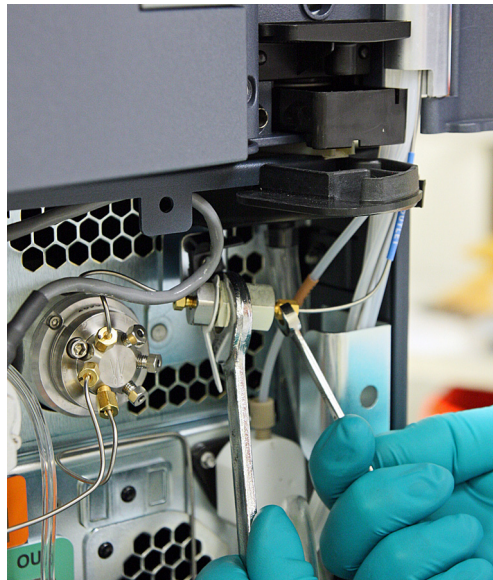


Figure 16 - Pump Outlet tube to inline filter connection

19. Connect the pre-heater tube assembly to port 6 of the inject valve, then carefully apply a bend to the tube, making sure the tube exits through the tubing channel.

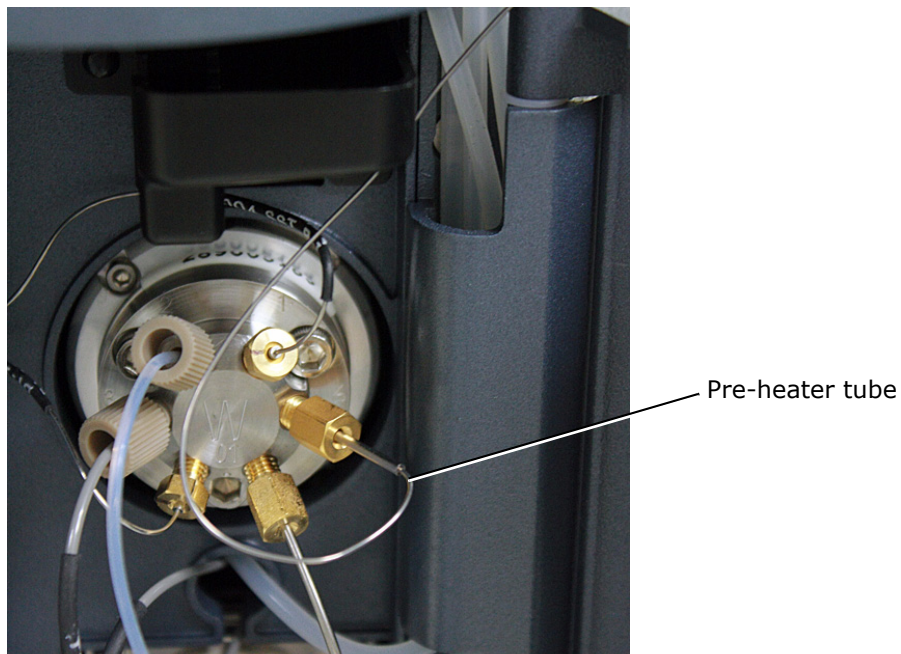


Figure 17 - Pre-heater tube inject valve connection

20. Attach the V-detail union (Start-up kit) to the outlet of the pre-heater tube assembly:

CAUTION: TO AVOID DAMAGING THE ACTIVE PREHEATER TUBING, DO NOT OVERTIGHTEN THE FITTING. OVERTIGHTENING THE FITTING COULD SEPARATE THE TUBING COLLAR FROM THE INTERNAL WELD CAUSING LEAKS.

- Before connecting the union to the fitting, make sure the ferrule is near the end of the tubing and the collet touches the back of the ferrule. See [Figure 18](#).
- Push the tubing into the union until it stops.
- Without placing any strain on the pre-heater cartridge assembly, carefully screw the union into the finger-tight fitting.
- Finger-tighten the fitting until snug, then tighten an additional 1/4-turn.



Figure 18 - Finger-tight fitting detail

- Connect a waste line to the union outlet.
- Plug the pre-heater tube assembly with attached union and waste tubing into the CH-A.
- Connect the waste line to a waste container.

NOTE: You will connect the column after priming and flushing the system in preparation for the system performance tests.

- Connect Tygon tubing from the back of the solvent module tray to waste.

Making the power and electronic connections

1. Connect Ethernet cables from the following instruments to the 6-port Ethernet block on the SM-FTN:
 - Computer
 - QSM
 - Detector(s)
2. Connect the 25-pin D-shell connector from CH-A to the QSP1 port on the SM-FTN.
3. Connect all power cords.



Figure 19 - Power and electronic connections

4. Install the two leak sensors on the SM-FTN and one on the QSM. [Figure 20](#) shows the upper and lower SM-FTN sensor connection details.

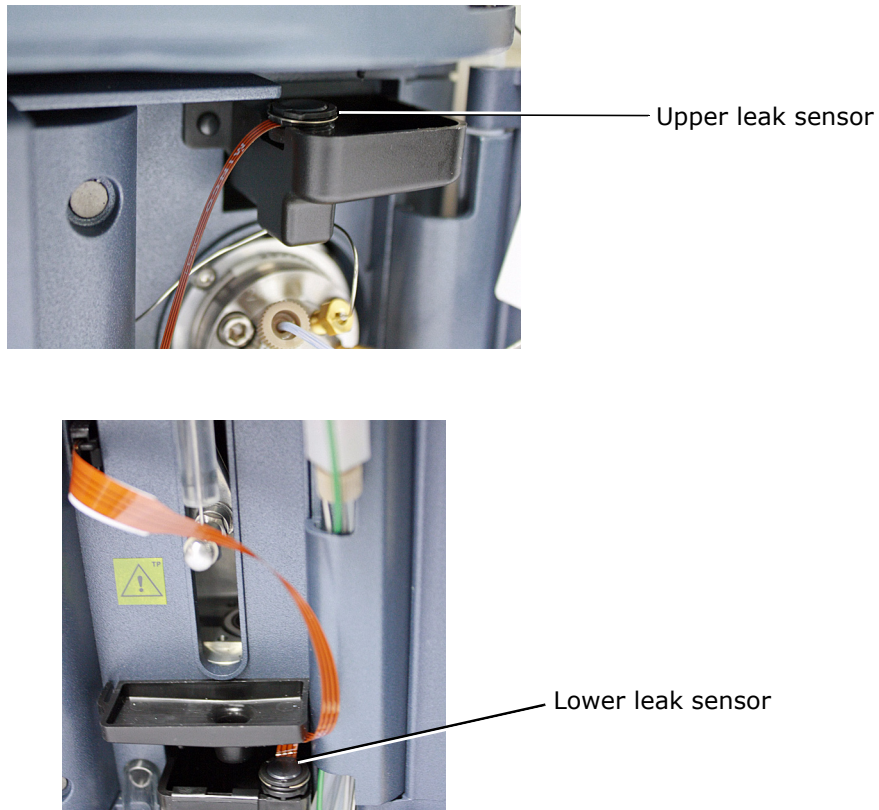


Figure 20 - SM-FTN leak sensor connections

Powering-on the system

1. Power on the PC, then install Empower or MassLynx. Ensure that the appropriate service packs/SCNs/software builds are installed.
2. Starting with the SM-FTN, power on all ACQUITY H-Class instruments (booting can take several minutes).
3. After a successful boot, launch Empower or MassLynx and ensure communication with the system instruments.
4. Do one of the following:
 - If the system is Empower-based, go to [Restoring the startup project and configuring the system \(Empower-based systems\)](#).
 - If the system is MassLynx-based, go to [Configuring the system \(MassLynx-based systems\)](#).

Restoring the startup project and configuring the system (Empower-based systems)

For Empower-based systems, restore the EMP_HCLASS_startup project to the system PC before configuring the system.

1. Download the Empower EMP_HCLASS_startup project from the *Waters Service Knowledge Center*:
 - a. Click the **Knwl Ctr - Svc & Support** tab.
 - b. Click the **Support Information** folder in the Detailed Navigation area.
 - c. Under the LC Technology heading, click **ACQUITY UPLC**.
 - d. Under the ACQUITY UPLC H-Class heading, click **Downloads**, then download the EMP_HCLASS_startup project.
2. From the Projects view of the Configuration Manager, right-click and select **Restore Project**.
3. Navigate to the EMP_HCLASS_startup project, then use the Restore Projects Wizard to restore the project.
4. Configure the system from Empower Pro.

Configuring the system (MassLynx-based systems)

1. Download the MassLynx ML_HCLASS_startup project file from the *Waters Service Knowledge Center*. (Follow steps 1a through 1d, above, to download the project).
 2. Copy the downloaded ML_HCLASS_startup project file to the MassLynx folder.
 3. From Console, select the ACQUITY H-CLASS System, then configure the system.
-

Running the performance tests for systems with a TUV, PDA, ELS, or FLR detector

Test solutions

Table 2: Test Solutions

Part Number	Description	Quantity
700002669	Waters ACQUITY UPLC System Absorbance Startup Solution (TUV and PDA)	1
700002848	Waters ACQUITY UPLC System ELSD Test Solution (ELSD)	1
700003694	Waters ACQUITY UPLC System FLR Test Solution (FLR)	1

Tools/materials required

- Union from the Start-up kit
- Pin plug
- Business card (for calibrating the z axis)

Preparing samples

For TUV and PDA detectors

Use Waters ACQUITY UPLC Systems Absorbance Start-Up Solution (TUV and PDA only).

700002669 - 2 ampules of standard solutions containing:

- Ampule# 700002642-6: 0.100 mg/mL caffeine in HPLC-grade water/acetonitrile (90:10)
 - Ampule# 700002642-11: 4.0 µg/mL 2-acetylfuran in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL acetanilide in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL acetophenone in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL propiophenone in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL butylparaben in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL benzophenone in HPLC-grade water/acetonitrile (90:10)
4.0 µg/mL valerophenone in HPLC-grade water/acetonitrile (90:10)
-

For ELS detectors

Use Waters ACQUITY UPLC Systems ELSD Test Solution (ELSD only).

700002848 - 1 ampule of 10 mL standard solution containing:

- 0.16 mg/mL caffeine in HPLC-grade water/acetonitrile (90:10)

For FLR detectors

Use Waters ACQUITY UPLC Systems FLR Test Solution (FLR only).

700003694 - 1 ampule of 10 mL standard solution containing:

- 5 pg/μL anthracene in HPLC-grade water/acetonitrile (80:20)

Preparing diluent

1. Fill an appropriate sized sample vial with 90:10 water/acetonitrile.
2. Label the vial "Diluent".

Preparing the system precision test sample

1. Fill an appropriate sized sample vial with caffeine test solution from ampule # 700002642-6 (TUV and PDA only) or ampule # 700002848 (ELSD only), or with the anthracene test solution from ampule # 700003694 (FLR only).
2. Label the vial as System Precision Test.

Preparing the gradient performance test sample

1. Fill an appropriate sized sample vial with the 7-component mix test solution from ampule # 700002642-11.
2. Label the vial as Gradient Performance Test.

Preparing for the system performance test

1. From Console, select **ACQUITY UPLC System** in the system tree, then **Control > Start up system**.
 - a. For **Prime Solvents** (QSM tab), check all boxes and set "Duration of Prime" to **5** minutes.
 - b. For **Prime Solvents** (SM tab), prime "Wash solvent" for **200** seconds and "Purge solvent" for **40** cycles.
 - c. Click **Start**.
 2. Place a mobile phase blank (diluent) into the SM-FTN.
 3. Place the appropriate test sample into the SM-FTN for the system precision test.
 4. Place the component mix sample into the SM-FTN for the gradient performance test.
 5. Flush the system to waste using a 25/25/25/25 mixture of mobile phases A, B, C, and D at 2 mL/min for 10 minutes.
-

Running maintenance tests

NOTE: Use the default parameter settings for tests in this section.

1. Run the Quaternary Solvent Manager **Leak test** using 100% acetonitrile (solvent B or D). When the test finishes, print the results.
2. Run the Sample Manager - FTN **Calibrate needle z axis**. Print the results.

CAUTION: **TO PREVENT NEEDLE DAMAGE, DO NOT ALLOW THE TIP OF THE NEEDLE TO DIRECTLY CONTACT THE BASE.**

3. Run the Sample Manager - FTN **Characterize needle seal** test. Confirm that the test passes, then print the results.
4. Run the Sample Manager - FTN **Leak test** for the **Sample syringe**. Confirm that the test passes, then print the results.

Running a system leak test

1. Plug a V-detail pin plug into the V-detail union that is already attached to the pre-heater tube assembly, then re-install the assembly into the CH-A.
2. Ensure that column temperature control is off.
3. From Console, select the Quaternary Solvent Manager from the system tree, click **Maintain > Leak test** using 100% acetonitrile (solvent B or D).
4. In the End Point section of the Parameters tab, click the **Column** option button, then select the **Include Sample Manager Needle and Seal**.
5. Click **Start**. Confirm that the test passes, then print the results.

Connecting and equilibrating the column

1. Remove the pre-heater tube assembly from the CH-A.
2. Attach the outlet of the pre-heater tube assembly to the inlet of the ACQUITY UPLC BEH C₁₈ (2.1 x 50 mm, 1.7µm, part number 186002350) column:

CAUTION: **TO AVOID DAMAGING THE ACTIVE PREHEATER TUBING, DO NOT OVERTIGHTEN THE FITTING. OVERTIGHTENING THE FITTING COULD SEPARATE THE TUBING COLLAR FROM THE INTERNAL WELD CAUSING LEAKS.**

- a. Before connecting, make sure the ferrule is near the end of the tubing and the collet touches the back of the ferrule. See [Figure 21](#).
- b. Push the tubing into the column until it stops.
- c. Without placing any strain on the pre-heater cartridge assembly, carefully screw the column into the finger-tight fitting.
- d. Finger-tighten the fitting until snug, then tighten an additional 1/4-turn.



Figure 21 - Finger-tight fitting detail

NOTE: The direction of the flow is very important. If the column is installed in the wrong direction, the packing material will be disturbed, resulting in loss of efficiency and possible damage to the detector flow cell.

3. Plug the column with pre-heater tube assembly into the column tray of the CH-A:
 - a. Snap the column support clips, P/N 205000478, onto the body of the column (one between the reusable fitting and column). Rotate the clips so that the openings face forward.

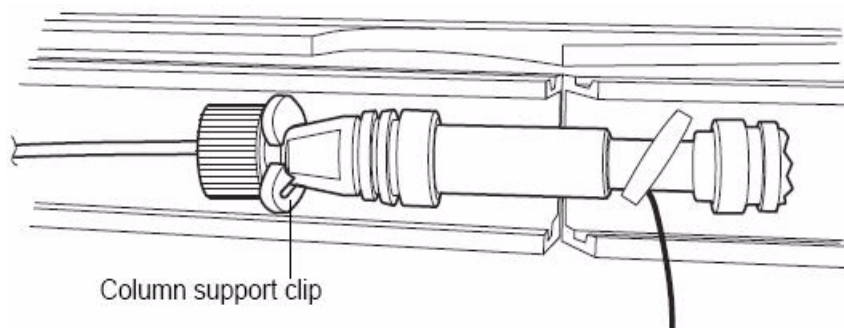


Figure 22 - Column support clip installation

- b. Place the column in the column compartment.
4. Flush the column to waste with 100% acetonitrile at 0.1 mL/min for at least one hour before connecting the column outlet to the detector.
5. Attach the eCord to the CH-A.
6. Connect the column outlet to the detector.
7. Close the column heater door, and then inspect the seal around the front panel to ensure it aligns correctly.
8. Set the CH-A temperature to 40° C and the Sample compartment to 10° C, then confirm that the temperatures are being regulated.
9. From Console, set flow to 90%A:10%B at 0.6 mL/min and equilibrate until the baseline is stable (approximately 30 minutes).
10. While equilibrating, run the Sample Manager - FTN **Needle Seal Readiness Test**. Make sure that flow is set to 0.6 mL/min. Confirm that the test passes, then print the results.

Running the system precision test

The instrument or inlet method you use to run the system precision test varies according to the type of detector (TUV, PDA, ELS, or FLR) installed on the system.

- For TUV-based systems use: **sys_prec_TUV**
- For PDA-based systems use: **sys_prec_PDA**
- For ELS-based systems use: **sys_prec_ELS**
- For FLR-based systems use: **sys_prec_FLR**

Checking the method parameters

1. Open the instrument or inlet method appropriate for the system, then verify that the parameters settings are set up as shown in [Figure 23](#) through [Figure 30](#). Parameters for both Empower- and MassLynx-based systems must match.

NOTE: The Quarternary Solvent Manager parameters are the same for the TUV, PDA, and ELSD detectors.

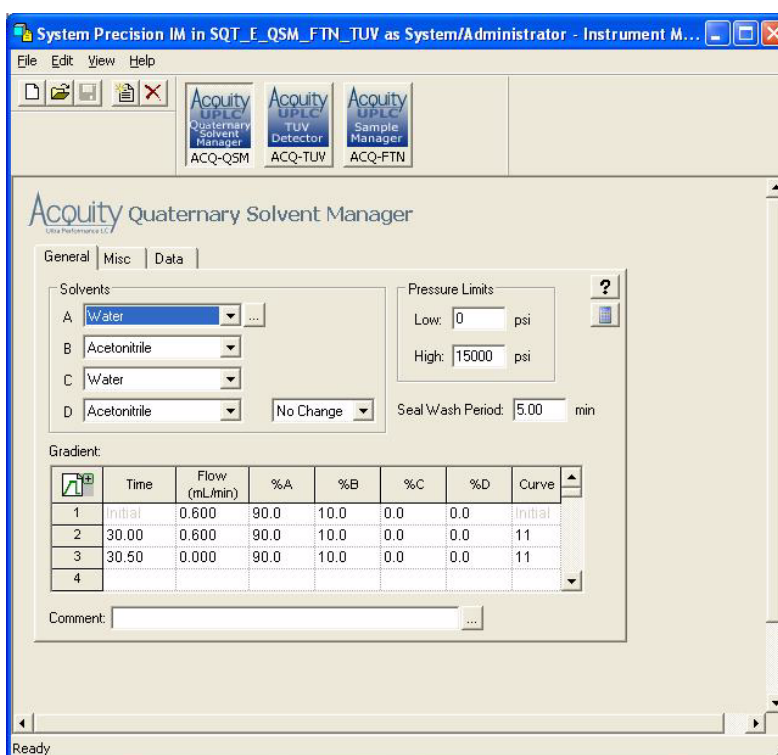


Figure 23 - System Precision Parameters for the Quarternary Solvent Manager for the TUV, PDA, or ELSD

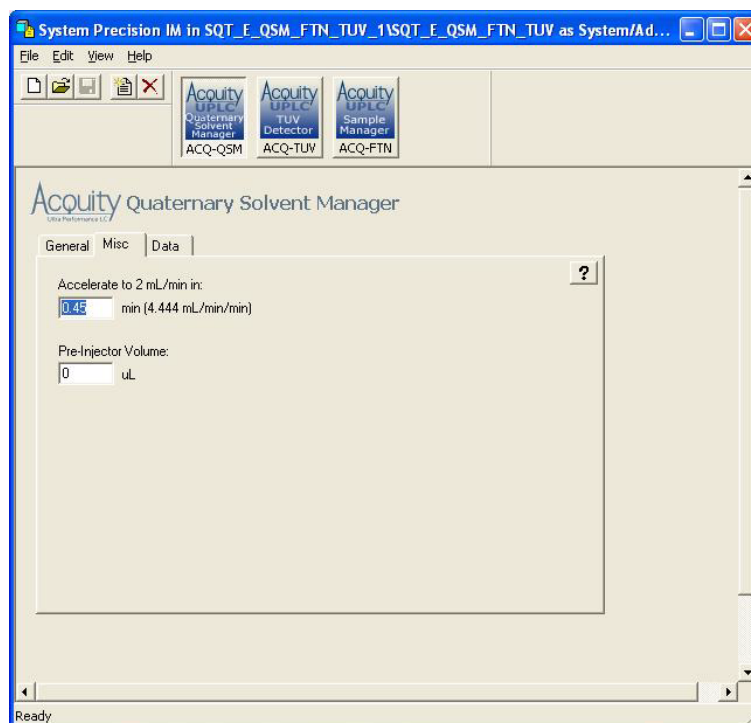


Figure 24 - System Precision Parameters for the Quarternary Solvent Manager for the TUV

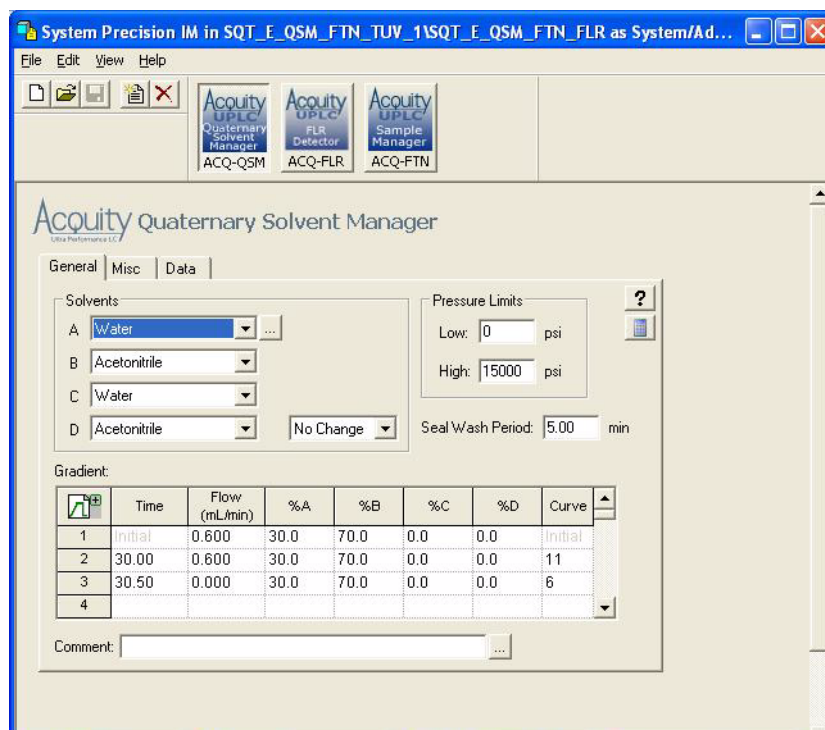


Figure 25 - System Precision Parameters for the Quarternary Solvent Manager for the FLR

NOTE: The Sample Manager - FTN parameters are the same for the TUV, PDA, and ELSD detectors (see Note about FLR detectors, below).

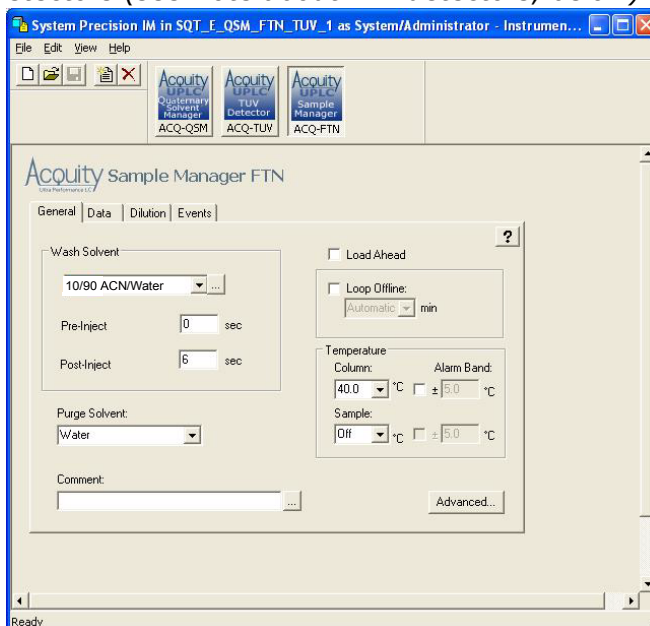


Figure 26 - System Precision Parameters for the Sample Manager - FTN

NOTE: For systems with an FLR detector, Wash Solvent is 100% acetonitrile.

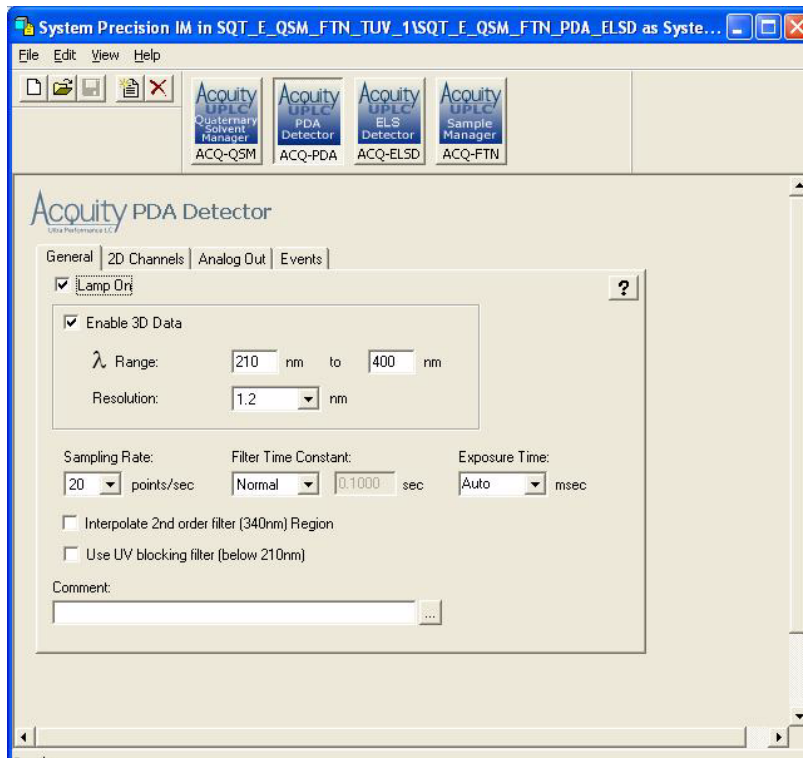


Figure 27 - System Precision Parameters for the PDA

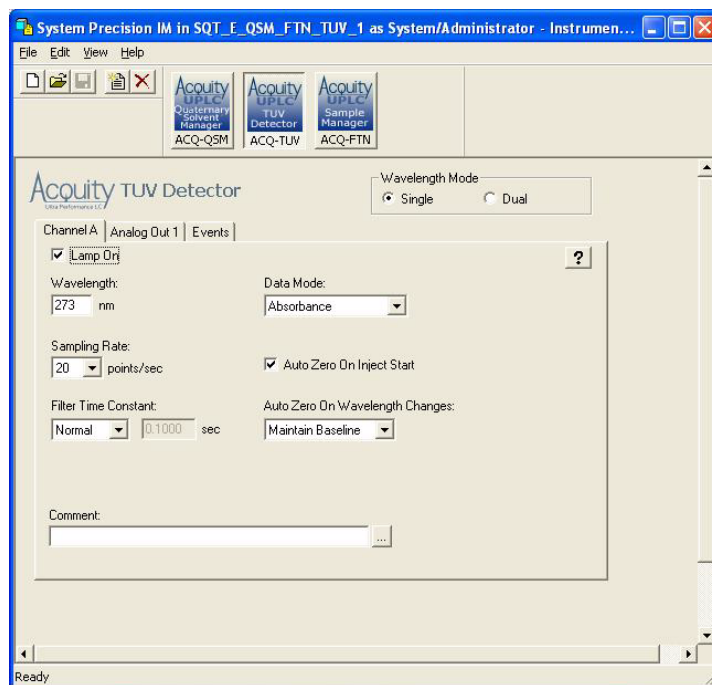


Figure 28 - System Precision Parameters for the TUV

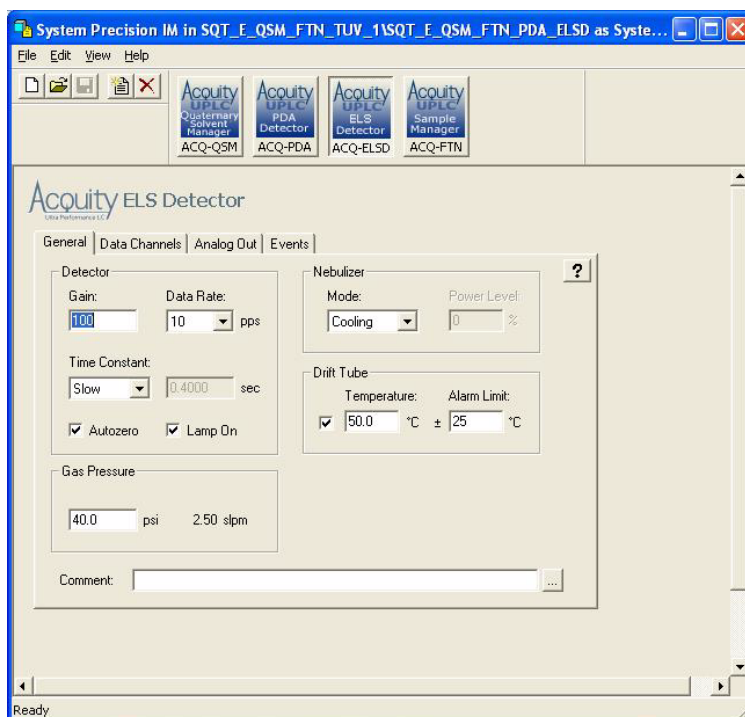


Figure 29 - System Precision Parameters for the ELSD

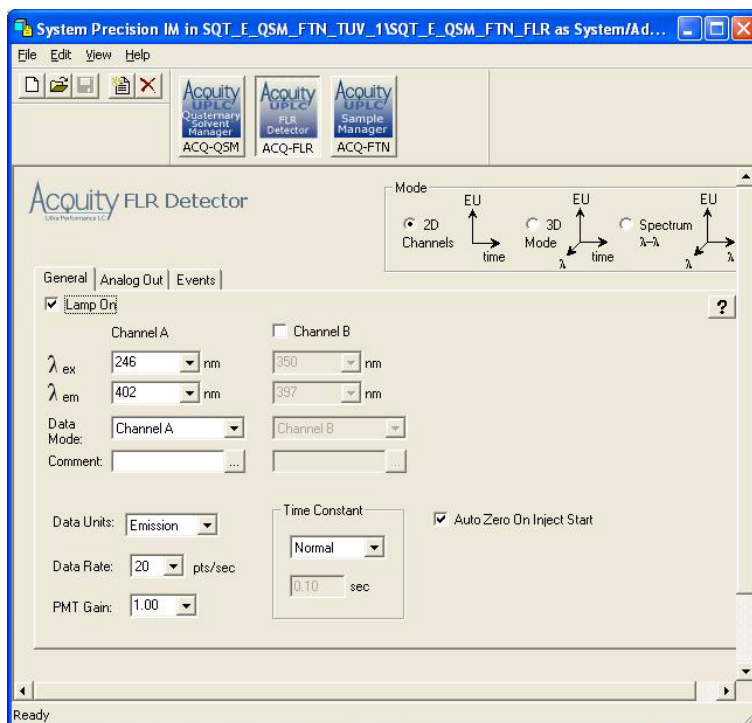


Figure 30 - System Precision Parameters for the FLR

2. Open the appropriate sample set method for the system precision test and confirm the following:
 - A 30-minute equilibrate step that uses initial conditions
 - One blank injection
 - Six sample injections
 - Injection volume = 5 μ L (using 10-mm flow cells for PDA and TUV detectors); 1 μ L (using 25-mm flow cells for PDA and TUV detectors); 5 μ L for ELS and FLR detectors
 - Run time = 1.0 minute
3. Run the sample set.
4. When the run finishes, process the samples, then analyze the results and view the reports.

System precision test results

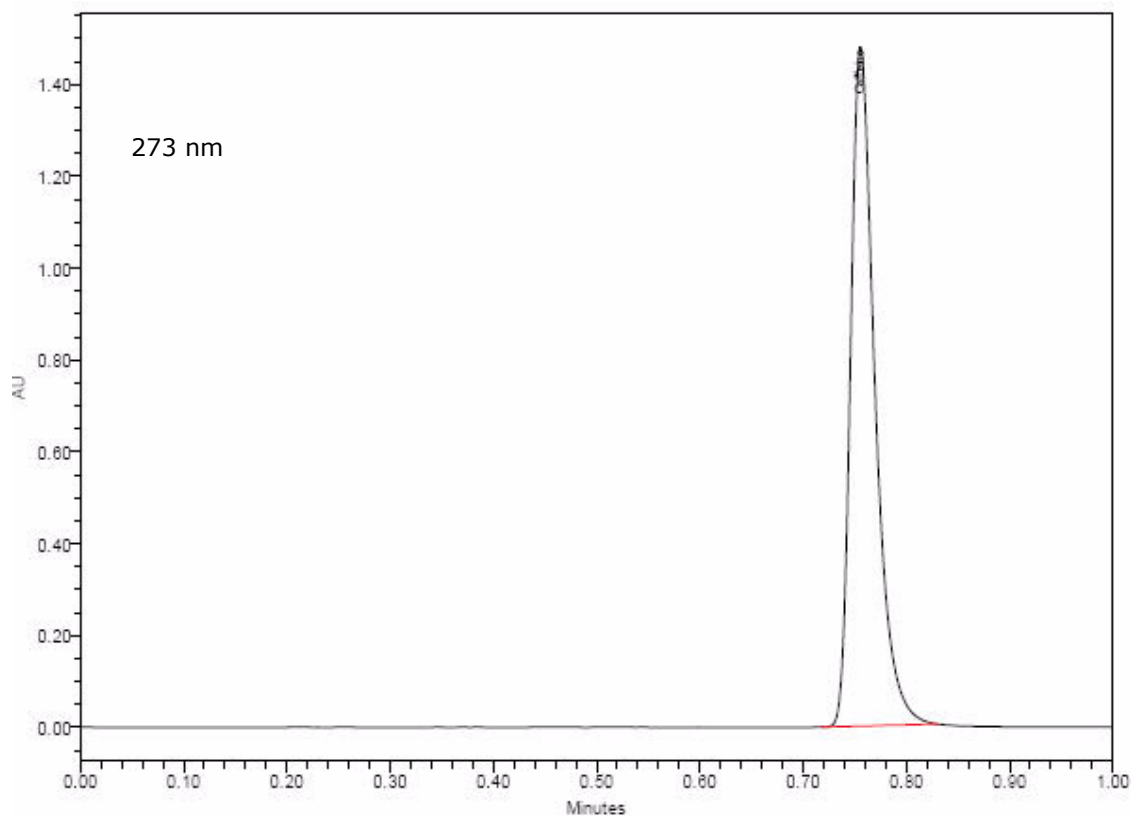


Figure 31 - System Precision Chromatogram for TUV and PDA (10-mm flow cell)

NOTE: If using the 25-mm flow cell, the peak area for the caffeine peak will be approximately 4x larger.

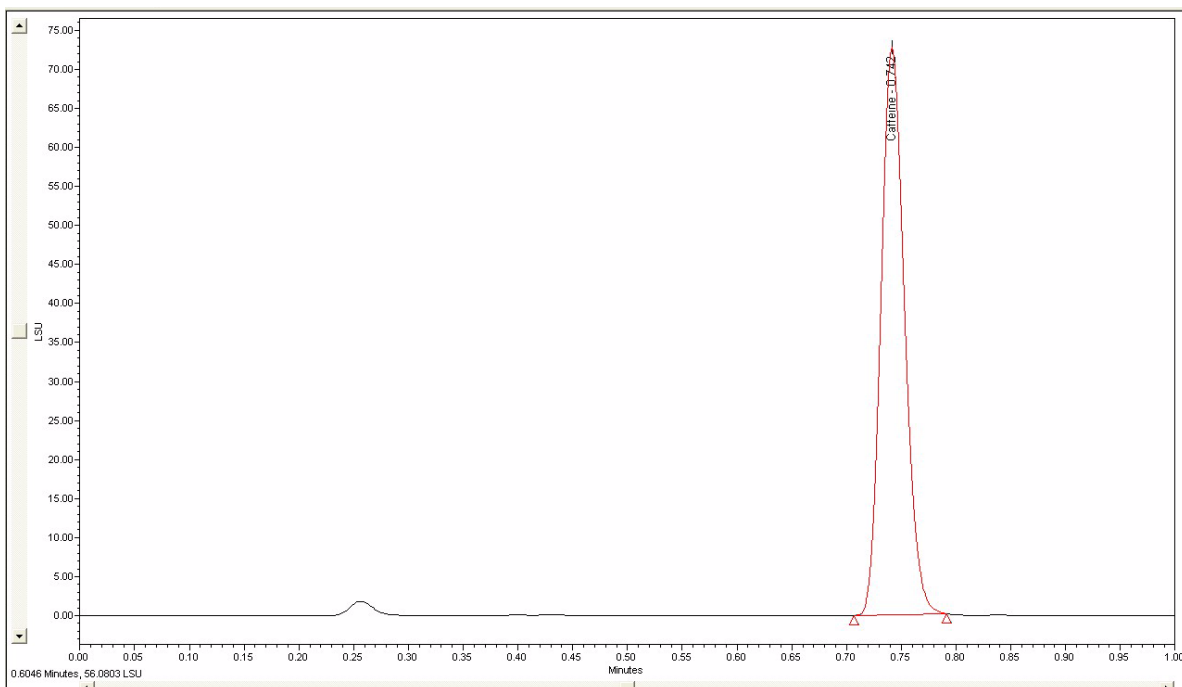


Figure 32 - System Precision Chromatogram for ELSD

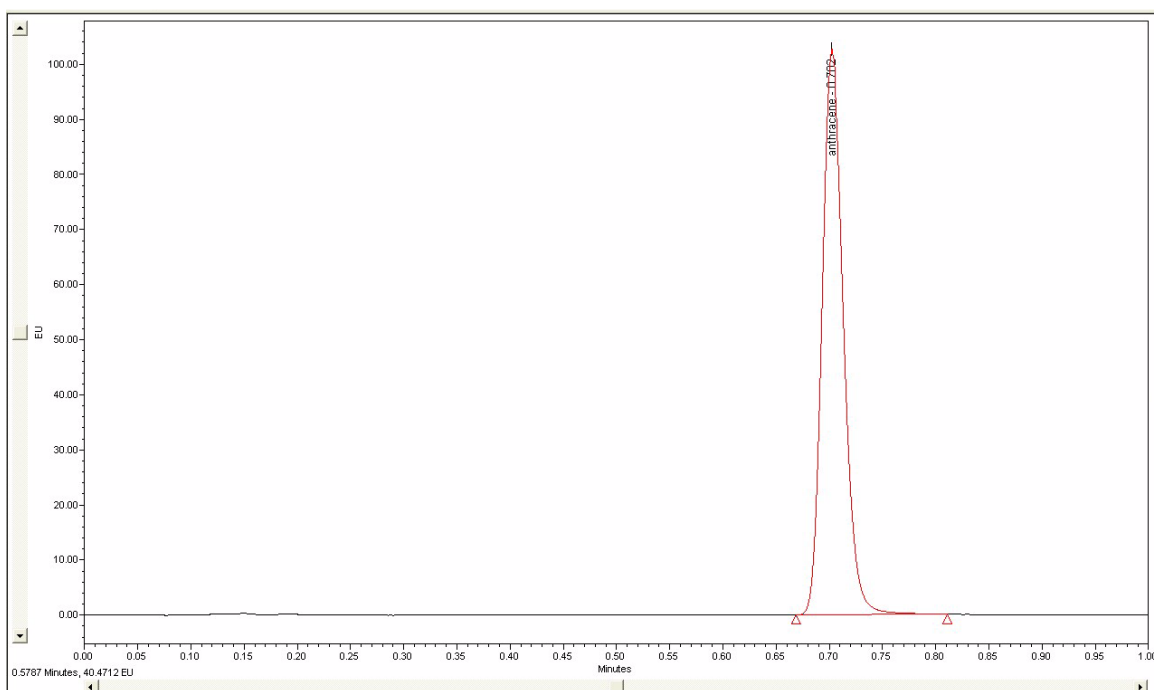


Figure 33 - System Precision Chromatogram for FLR

1. Verify that the peak is symmetrical, integrated, and identified correctly.
2. Standard deviation for peak retention over six replicate injections must be ≤ 1.0 second for caffeine and ≤ 2.8 seconds for anthracene.
3. If needed, follow these steps to use the Windows® scientific calculator:

- a. Click the Windows **Start** button and select **Programs > Accessories > Calculator**. The Windows calculator appears.
 - b. Select **Scientific** from the View menu. The calculator switches to scientific view.
 - c. Enter the first value by clicking the appropriate numbers on the Windows calculator (you can also use the numeric keypad to enter values if Num Lock is on).
 - d. Click **Sta** in the left column of the calculator. The statistics box appears.
 - e. Click **Dat** in the left column of the calculator, and then click **Sta** again to display the statistics box. Confirm that the statistics box displays the value entered in Step 2c.
 - f. Enter each of the remaining values by clicking the appropriate numbers on the calculator, and then click **Dat** to enter each value in the statistics box.
 - g. After you enter all values in the statistics box, click the **s** button in the left column of the calculator to calculate the standard deviation.
4. The %RSD for peak areas over six replicate injections must be $\leq 0.5\%$ (TUV and PDA), $\leq 5.0\%$ (ELSD), and $\leq 3.0\%$ (FLR).
 5. The %RSD for peak heights over six replicate injections must be $\leq 0.9\%$ (TUV and PDA), $\leq 5.0\%$ (ELSD), and $\leq 4.5\%$ (FLR).
 6. Record the results in the appropriate tables in the *Waters ACQUITY UPLC H-Class System with TUV, PDA, ELS, or FLR Detectors Installation Checklist*.

Running the gradient performance test

The instrument or inlet methods you use to run the gradient tests vary according to the type of detector (TUV or PDA) installed on the system.

- For TUV-based systems use: **gradient_AB_TUV** and **gradient_CD_TUV**
- For PDA-based systems use: **gradient_AB_PDA** and **gradient_CD_PDA**

NOTE: The Gradient Performance Test is **not** performed for the ACQUITY ELS or FLR detector.

1. Open the instrument or inlet method appropriate for the system (start with the method for solvents A and B), then verify that the parameters settings are set up as shown in [Figure 34](#) through [Figure 39](#). Parameters for both Empower- and MassLynx-based systems must match.
-

NOTE: Use the same Quarternary Solvent Manager parameters for the TUV or the PDA.

Gradient AB IM in SQT_E_QSM_FTN_TUV as System/Administrator - Instrument Metho...

File Edit View Help

Acquity UPLC Quaternary Solvent Manager ACQ-QSM

Acquity UPLC TUV Detector ACQ-TUV

Acquity UPLC Sample Manager ACQ-FTN

Acquity Quaternary Solvent Manager

General Misc Data

Solvents:

A: Water

B: Acetonitrile

C: Water

D: Acetonitrile

No Change

Pressure Limits:

Low: 0 psi

High: 15000 psi

Seal/Wash Period: 5.00 min

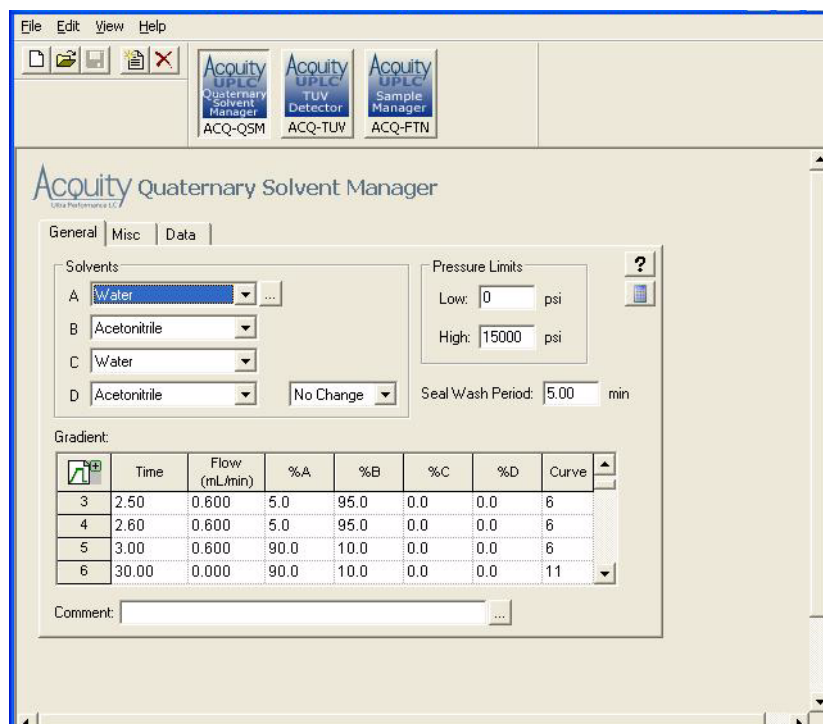
Gradient:

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	initial	0.600	90.0	10.0	0.0	0.0	initial
2	0.25	0.600	90.0	10.0	0.0	0.0	6
3	2.50	0.600	5.0	95.0	0.0	0.0	6
4	2.60	0.600	5.0	95.0	0.0	0.0	6

Comment:

Ready

Figure 34 - Gradient Performance Parameters for the Quarternary Solvent Manager (solvents A and B; rows 1 to 4)



Acquity Quaternary Solvent Manager

General Misc Data

Solvents:

A: Water

B: Acetonitrile

C: Water

D: Acetonitrile

No Change

Pressure Limits:

Low: 0 psi

High: 15000 psi

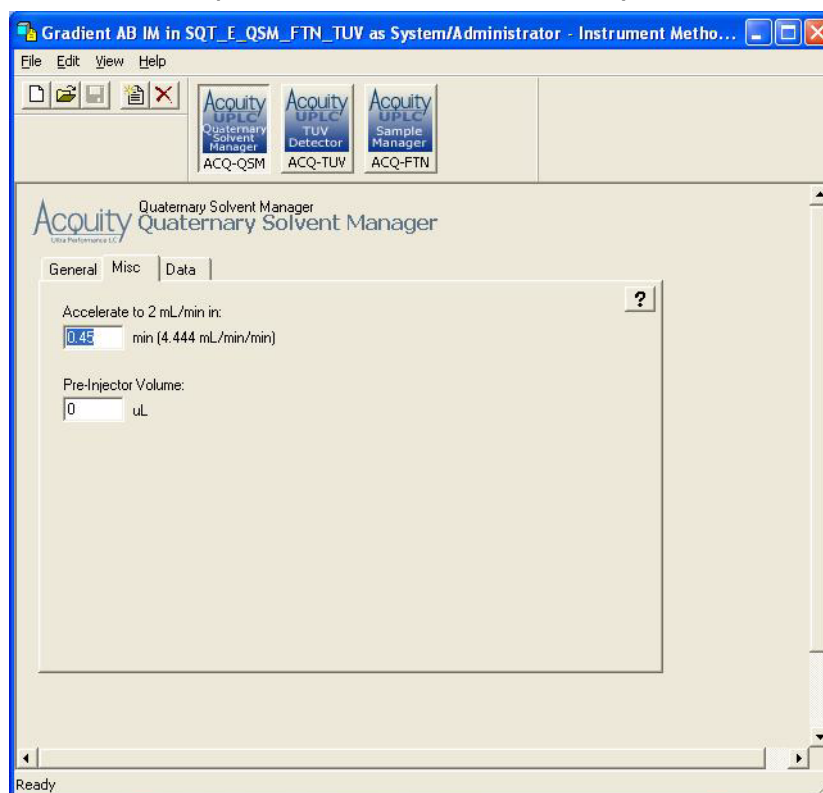
Seal/Wash Period: 5.00 min

Gradient:

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
3	2.50	0.600	5.0	95.0	0.0	0.0	6
4	2.60	0.600	5.0	95.0	0.0	0.0	6
5	3.00	0.600	90.0	10.0	0.0	0.0	6
6	30.00	0.000	90.0	10.0	0.0	0.0	11

Comment:

Figure 35 - Gradient Performance Parameters for the Quaternary Solvent Manager (solvents A and B; rows 3 to 6)



Gradient AB IM in SQT_E_QSM_FTN_TUV as System/Administrator - Instrument Metho...

Acquity Quaternary Solvent Manager

General Misc Data

Accelerate to 2 mL/min in:

0.45 min (4.444 mL/min/min)

Pre-Injector Volume:

0 uL

Figure 36 - Misc Tab - Gradient Performance Parameters for the Quaternary Solvent Manager

NOTE: Use the same Sample Manager parameters for the TUV or the PDA.

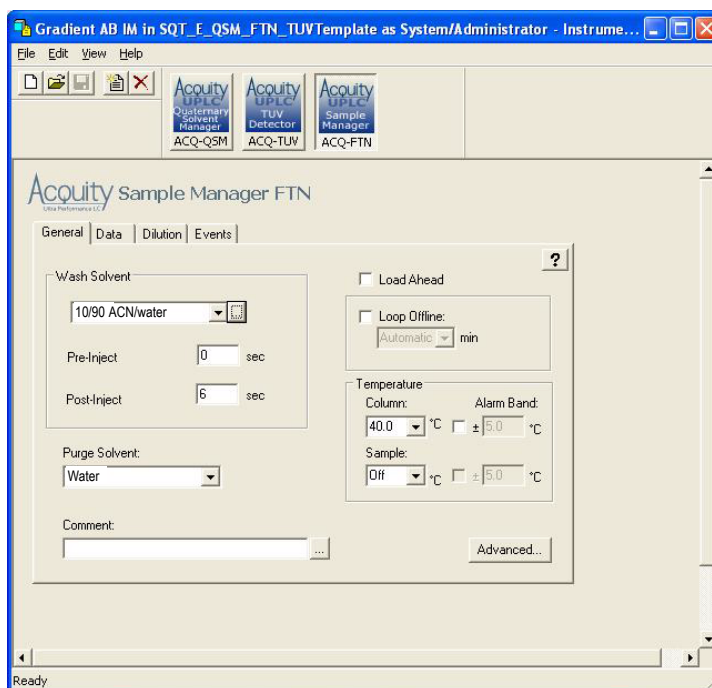


Figure 37 - Gradient Performance Parameters for the Sample Manager

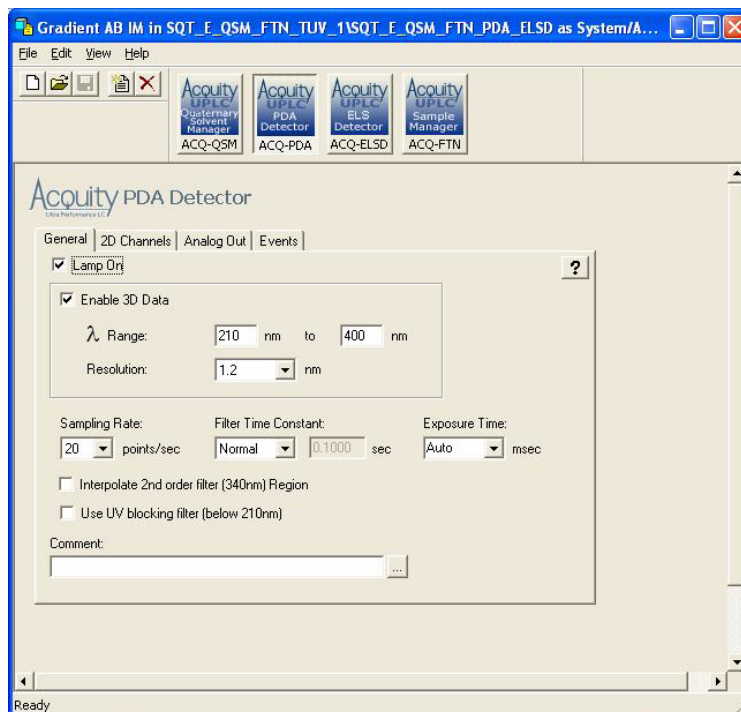


Figure 38 - Gradient Performance Parameters for the PDA

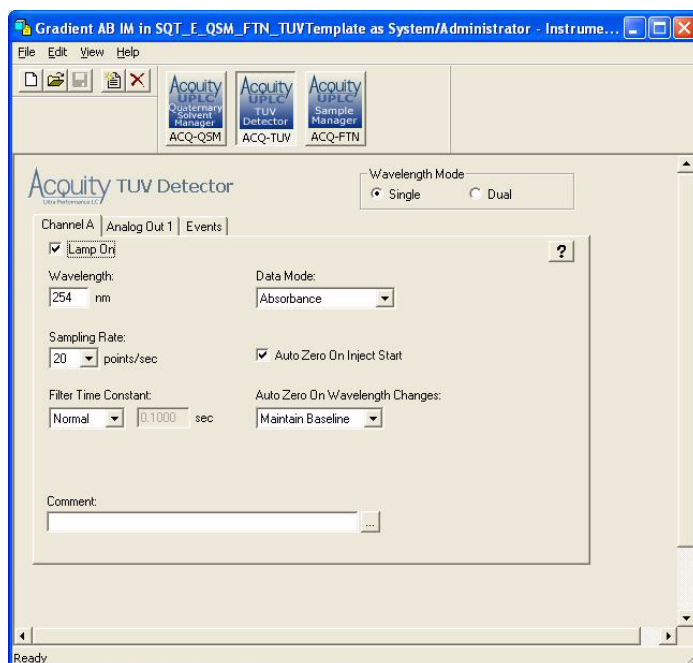


Figure 39 - Gradient Performance Parameters for the TUV

2. Open the appropriate sample set method for the gradient test and confirm the following settings are in place:
 - A 6-minute condition column step that uses initial conditions
 - Three sample injections
 - Injection volume = 5 μ L (using 10-mm or 25-mm flow cells)
 - Run time = 4.0 minutes
3. Run the sample set.
4. When the run finishes, process the samples, then analyze the results and view the reports.

Gradient performance test results

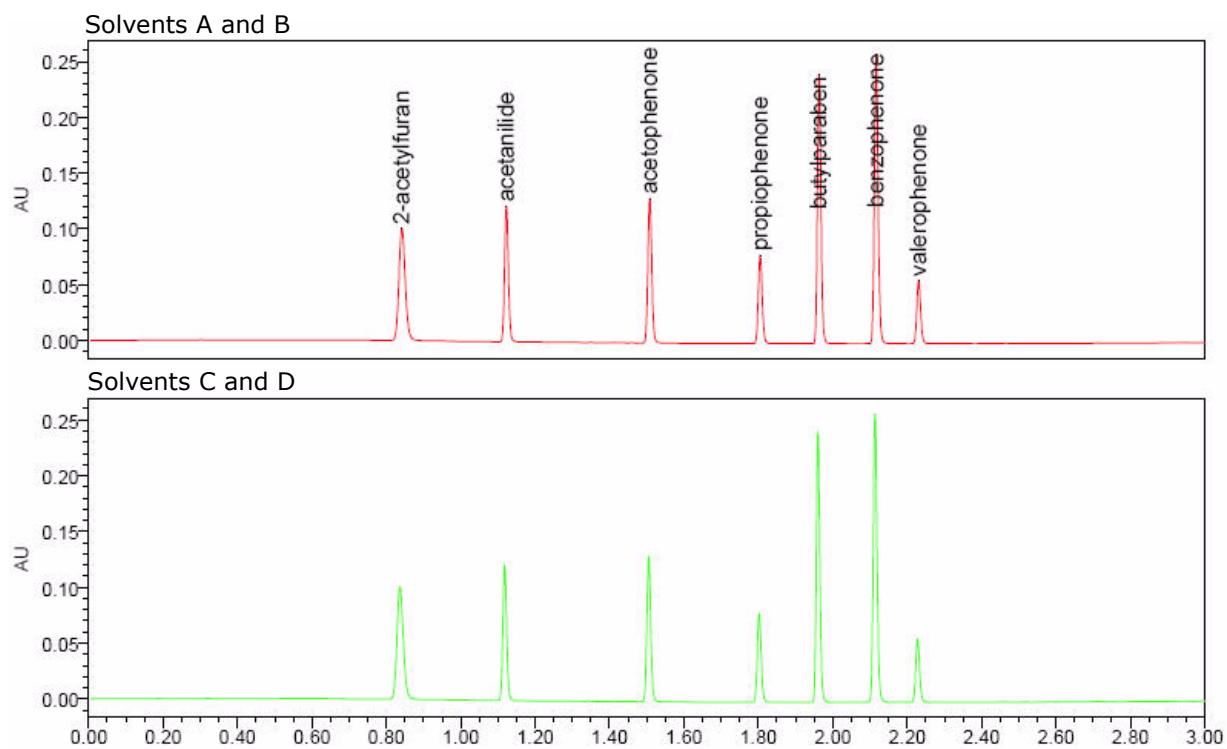


Figure 40 - Gradient Performance Chromatograms for AB and CD

NOTE: If using the 25-mm flow cell, the peak areas will be approximately 4x larger.

1. Verify that the peaks are symmetrical, integrated, and identified correctly.
2. The standard deviation for the peak retention over three replicate injections must be ≤ 1.5 seconds.
3. Repeat the gradient performance tests that start on [page 31](#) for mobile phases C and D.
4. Record the results in the appropriate table in the *Waters ACQUITY UPLC H-Class System with TUV, PDA, ELS, or FLR Detectors Installation Checklist*.