

ACQUITY UPLC® System with TUV, PDA, ELS, or FLR Detectors: System Performance Tests

The system performance tests on the ACQUITY UPLC System with TUV, PDA¹, ELS, or FLR detectors include the system precision and gradient performance tests. Performing these tests verify that the ACQUITY UPLC® System is functioning according to its design specifications, producing test results that meet the minimum standards for accuracy and precision.

NOTE: If your ACQUITY UPLC System uses only a mass spectrometer, these tests are not applicable.

NOTE: To be certain you have the latest version of this procedure, please visit the Waters Support Center (<http://www.waters.com>).

Parts Required

Table 1: Parts Required

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

- 100 mL volumetric flask
- 100 mL graduated cylinder
- 1000 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)

Mobile Phase Preparation

Due to the increased sensitivity that both the ACQUITY UPLC 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

1. The term "ACQUITY PDA Detector" refers to two detectors: the ACQUITY UPLC Photodiode Array (PDA) Detector, and ACQUITY UPLC PDA Extended Wavelength (eλ, or eLambda) Detector.

background contamination, low signal-to-noise, and loss of sensitivity. Waters recommends using Fisher Optima HPLC-grade acetonitrile and Baker HPLC-grade water.

All HPLC-grade solvents used with the ACQUITY UPLC System should be properly filtered prior to their use. It is recommended that the solvent be filtered through an appropriate membrane filter using a solvent clarification kit (47mm all-glass filter holder with 1-liter flask) such as Waters p/n WAT200543 or similar, immediately before use. A general-purpose laboratory vacuum pump is also required for use with the all-glass solvent filtration apparatus.

Glassware (e.g. solvent bottles) should not be washed with detergents or washed with other general glassware to prevent contamination. The glassware should 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 2.

Table 2: Mobile Phase and Solvent Concentrations

Mobile Phase and Wash Solvent	Concentration
Mobile Phase A1	10:90 acetonitrile/water (for A1, B2, strong and weak needle-wash, and the plunger-seal wash lines)
Mobile Phase A2	100% acetonitrile (for solvent line B1 and A2)
Mobile Phase B1	100% acetonitrile
Mobile Phase B2	10:90 acetonitrile/water
Weak Wash	10:90 acetonitrile/water
Strong Wash	10:90 acetonitrile/water
Plunger Seal Wash	10:90 acetonitrile/water

NOTE: For the system verification tests, the weak and strong washes will both be 10:90 acetonitrile/water. Other applications may require stronger wash solvents to prevent carryover.

1. Use a 100 mL graduated cylinder to measure 100 mL of filtered HPLC-grade acetonitrile, and then transfer the acetonitrile to a 1 L reservoir bottle.
2. Use a 1000 mL graduated cylinder to measure 900 mL of filtered HPLC-grade water, and then transfer the water to the same 1 L reservoir bottle.
3. Cap the reservoir bottle and mix well.
4. Label the reservoir bottle as 10:90 Acetonitrile:Water.

Sample Preparation

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)

Diluent Preparation

1. Fill an appropriate sized sample vial with 10:90 Acetonitrile/Water.
2. Label the vial as Diluent.

System Precision Test Sample Preparation

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.

Gradient Performance Test Sample Preparation

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.
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System Performance Test Preparation

1. Place the plunger seal wash, strong and weak needle wash, A1, and B2 solvent lines into the mobile phase A1 reservoir.
2. Place the B1 and A2 solvent lines into the mobile phase B1 reservoir.
3. Prime each solvent line of the Binary Solvent Manager for 10 minutes.
4. Prime the seal wash of the Binary Solvent Manager for several minutes.
5. Prime the Sample Manager at least five times.
6. Perform the seal calibration in the Sample Manager.
7. Calibrate the system volume in the Sample Manager.
NOTE: *Additionally, calibrate the system volume whenever you change the mobile phase, wash solvents, sample loop, needle, or any of the syringes.*
8. Place a mobile phase blank (diluent) into the Sample Manager.
9. Place appropriate test sample into the Sample Manager for the system precision test.
10. Place the component mix sample into the Sample Manager for the gradient performance test.

System Flush

CAUTION: *BEFORE CONNECTING THE COLUMN AND IN-LINE FILTER TO THE COLUMN STABILIZER TUBE, FLUSH THE SYSTEM TO REMOVE PARTICULATES. FAILURE TO DO SO COULD CAUSE PREMATURE COLUMN FAILURE.*

1. Flush the system to waste using a 50/50 mixture of mobile phases A1 and B1, at 2 mL/min for 10 minutes.
2. Repeat the flush for solvent lines A2 and B2 using the same conditions.
3. Make ten 10 µL injections of diluent. After each injection perform a 1000 µL strong wash followed by a 1000 µL weak wash.

Connecting and Equilibrating the Column

1. Attach the outlet of the column stabilizer tube to the in-line filter.
2. Attach the outlet of the in-line filter to the inlet of the ACQUITY UPLC BEH C₁₈ (2.1 x 50 mm, 1.7µm, part number 186002350) column and place it in the column heater.
NOTE: *The direction of the flow is very important. If the column is installed in the wrong direction, the packing material will become disturbed resulting in loss of efficiency and possible damage to the detector flow cell.*
3. 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 optical detector or mass spectrometer.
4. Attach two column support clips to the column.
5. Place the column, in-line filter, and column stabilizer assembly into the column tray of the column heater.
6. Close the column heater door.
7. Attach the eCord to the column heater.
8. Equilibrate the column for at least one hour or until the baseline is stable.

System Precision Test

1. In Empower software, create an Instrument Method with the parameters shown in [Figure 1](#) through [Figure 7](#), as appropriate for your instruments.
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NOTE: The Binary Solvent Manager parameters are the same for the TUV, PDA, or ELSD.

NOTE: Use the same parameters if performing the System Performance Tests using MassLynx software.

System Precision in ACQUITY_UPLC_START_UP as System/Administrator - Instrument ...

File Edit View Help

Acquity UPLC Binary Solvent Manager ACQ-BSM Acquity UPLC Sample Manager ACQ-SM Acquity UPLC PDA Detector ACQ-PDA

Acquity Binary Solvent Manager

General Data Analog Out Events

Solvents:

A1 10/90 ACN/Water ...

B2 10/90 ACN/Water ...

Pressure Limits:

Low: 1000 psi

High: 15000 psi

Seal Wash: 5.0 min

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	initial	0.400	50.0	50.0	initial
2	30.00	0.000	50.0	50.0	11
3					
4					
5					
6					

Comment:

Ready

Figure 1 - System Precision Parameters for the Binary Solvent Manager for the TUV, PDA, or ELSD

10 fast in Acquity FLR Detector as System/Administrator - Instrument Method Editor

File Edit View Help

Acquity UPLC FLR Detector ACQ-FLR Acquity UPLC Binary Solvent Manager ACQ-BSM Acquity UPLC Sample Manager ACQ-SM

Acquity Binary Solvent Manager

General Data Analog Out Events

Solvents:

A1 90%water10%ACN ...

B1 *Acetonitrile ...

Pressure Limits:

Low: 0 psi

High: 15000 psi

Seal Wash: 5.0 min

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	initial	0.400	30.0	70.0	initial
2	30.00	0.000	30.0	70.0	11
3					
4					
5					
6					

Comment:

Ready

Figure 2 - System Precision Parameters for the Binary Solvent Manager for the FLR

NOTE: The Sample Manager parameters are the same for the TUV, PDA, FLR, or ELSD.

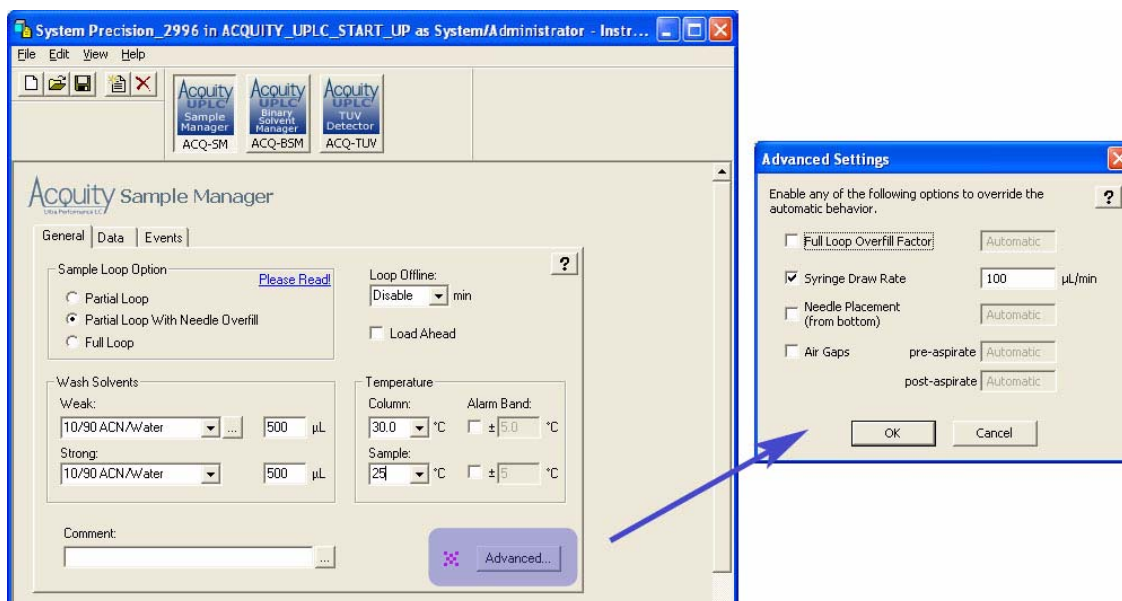


Figure 3 - System Precision Parameters for the Sample Manager

If using the PDA, create an Instrument Method with the following parameters for the system precision test (Figure 4).

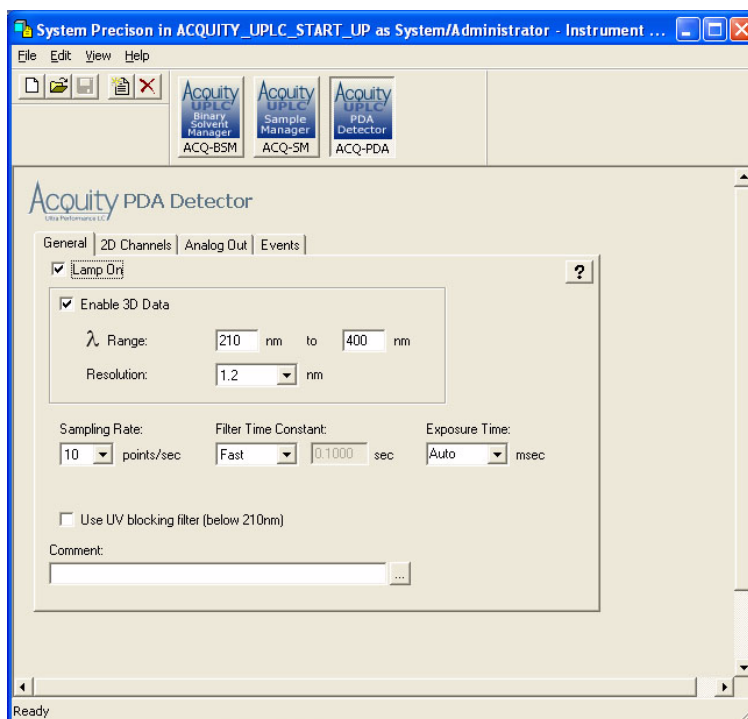


Figure 4 - System Precision Parameters for the PDA

If using the TUV, create an Instrument Method with the following parameters for the system precision test (Figure 5).

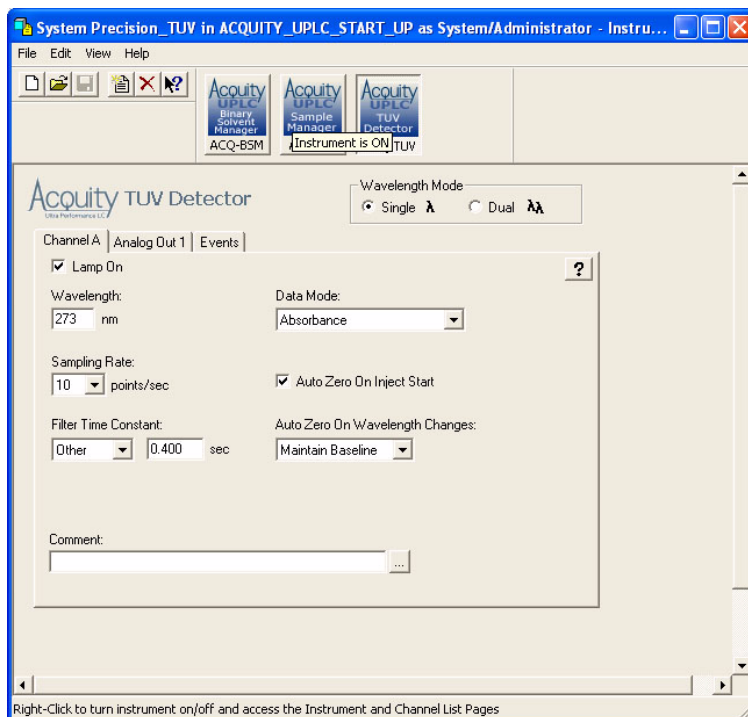


Figure 5 - System Precision Parameters for the TUV

If using the ELSD, create an Instrument Method with the following parameters for the system precision test (Figure 6).

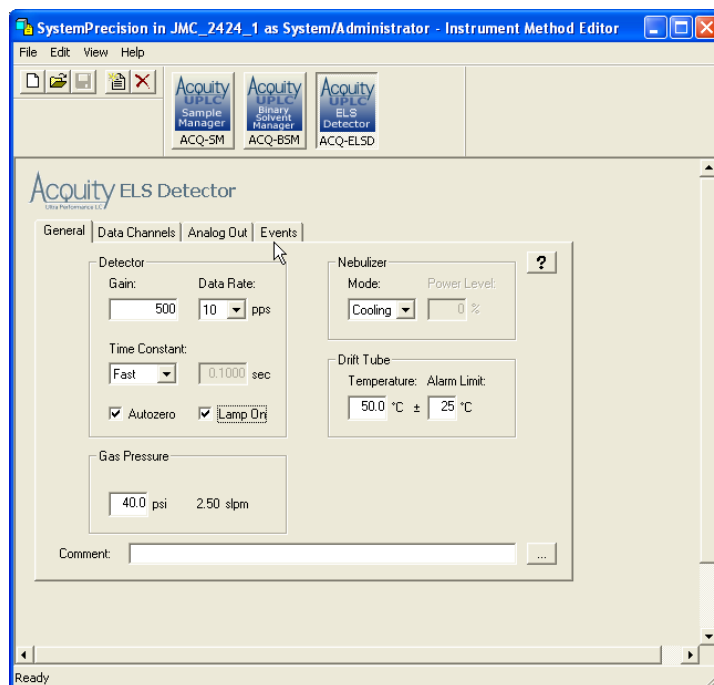


Figure 6 - System Precision Parameters for the ELSD

If using the FLR, create an Instrument Method with the following parameters for the system precision test (Figure 7).

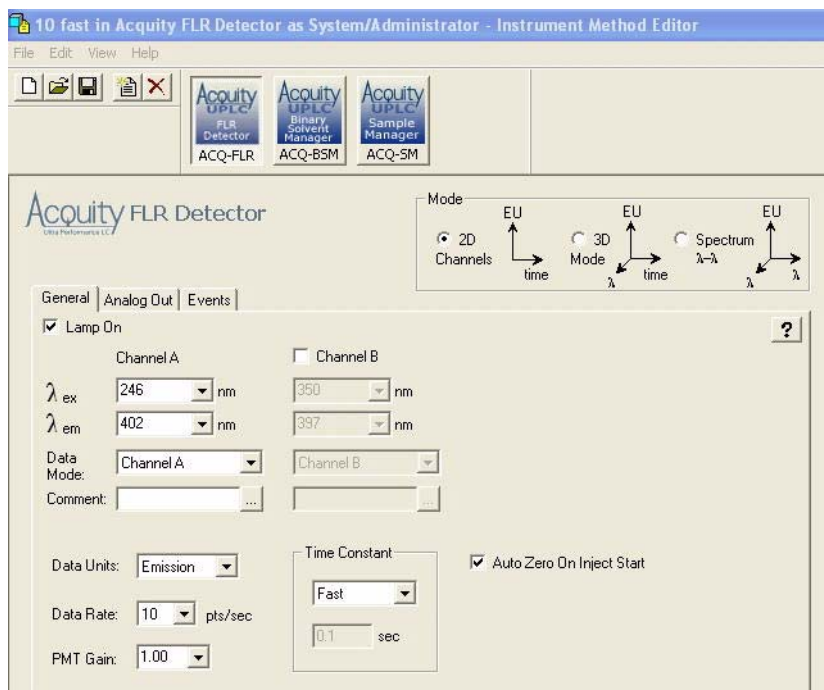


Figure 7 - System Precision Parameters for the FLR

2. Create a Sample Set Method for the system precision test.

NOTE: The Sample Set Method parameters (Inj Vol., # of Injs, Function, Run Time, and Next Inj. Delay) will be the same whether using the TUV or PDA. However, the method set and report methods will vary. Before acquiring, ensure that you choose the appropriate method set.

NOTE: If using the PDA, you will need to derive a channel at 273 nm in the method set.

The sample set parameters are as follows:

- Equilibrate once at initial conditions for a minimum of 30 minutes before making the first blank injection
- Injection volume = 5 μ L (using 10-mm flow cells in PDA and TUV detectors); 1 μ L (using 25-mm flow cells in PDA and TUV detectors); 5 μ L for ELS and FLR detectors

NOTE: If using the 25-mm flow cell and you observe a flat-topped peak, it is likely that a 5- μ L injection (rather than a 1- μ L injection) was made.

- # of Injections = 6
- Run time = 2.0 minutes

3. Perform the analysis.

System Precision Test Results

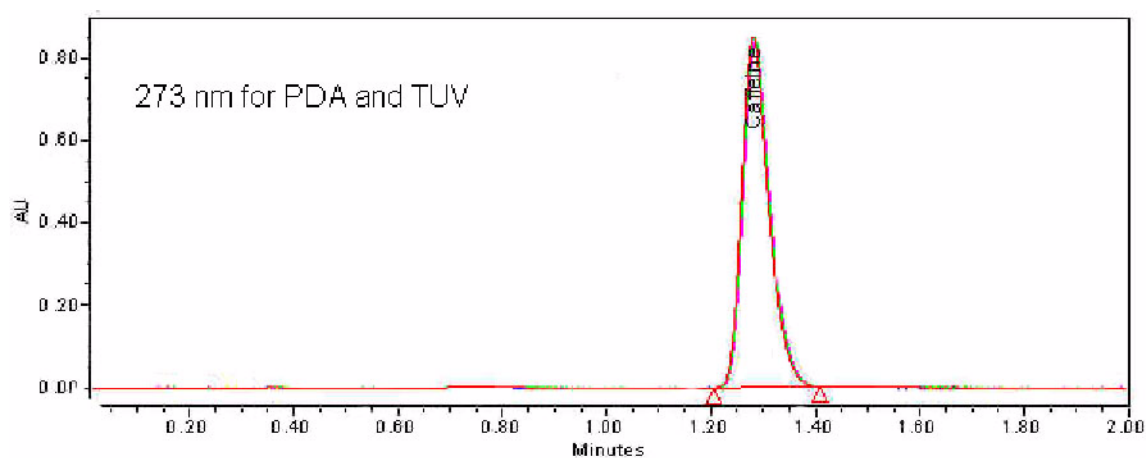


Figure 8 - System Precision Chromatogram for TUV and PDA (10-mm flow cell)

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

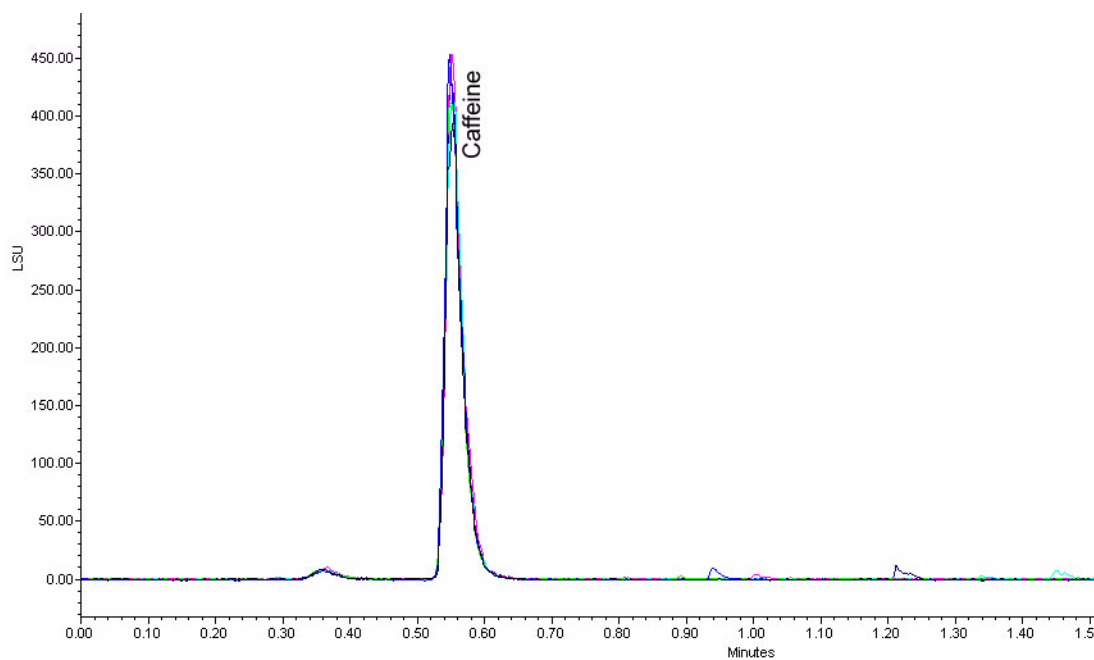


Figure 9 - System Precision Chromatogram for ELSD

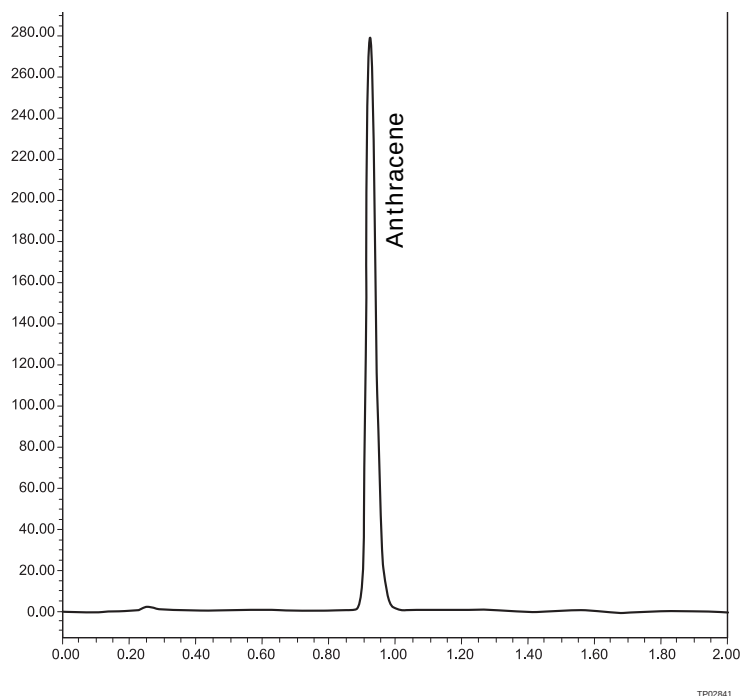


Figure 10 - System Precision Chromatogram for FLR

1. Verify that the peak is symmetrical, integrated, and identified correctly.
2. Calculate the standard deviation for the peak retention times for caffeine and anthracene over six replicate injections. The standard deviation should be ≤ 1.0 second for caffeine and ≤ 2.8 seconds for anthracene. To use the Windows® scientific calculator to calculate standard deviation:
 - 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.
3. Calculate the %RSD for the peak areas over six replicate injections. The %RSD should be $\leq 0.5\%$ (TUV and PDA), $\leq 5.0\%$ (ELSD), and $\leq 3.0\%$ (FLR).
 Use the following formula to calculate the %RSD: $\%RSD = (Standard\ Deviation \times 100) / Mean$
4. Calculate the %RSD for the peak heights over six replicate injections. The %RSD should be $\leq 0.9\%$ (TUV and PDA), $\leq 5.0\%$ (ELSD), and $\leq 4.5\%$ (FLR).
5. Report the results in the appropriate tables in the *Waters ACQUITY UPLC System with TUV, PDA, ELS, or FLR Detectors Installation Checklist*.

Gradient Performance Test

1. Create an Instrument Method with the parameters shown in [Figure 11](#) through [Figure 14](#).

NOTE: Use the same parameters if performing the System Performance Tests with Empower or MassLynx.

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

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

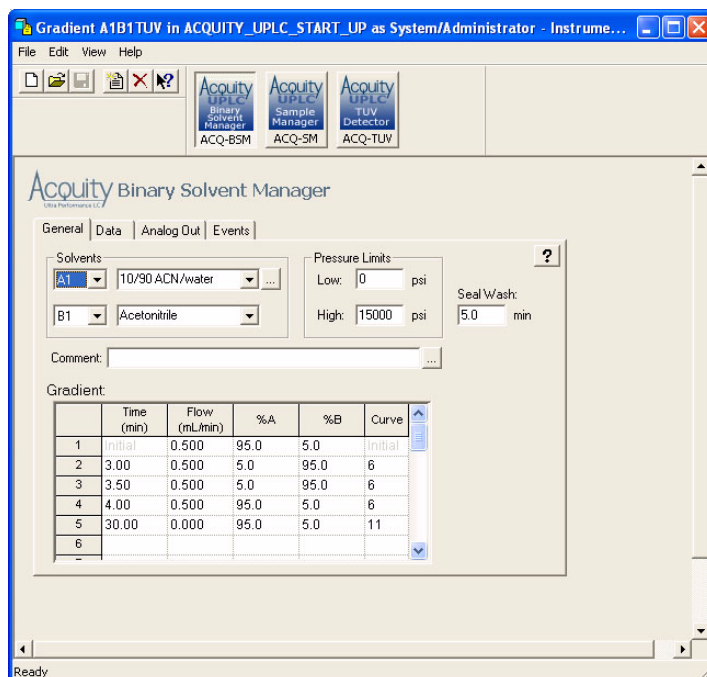


Figure 11 - Gradient Performance Parameters for the Binary Solvent Manager

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

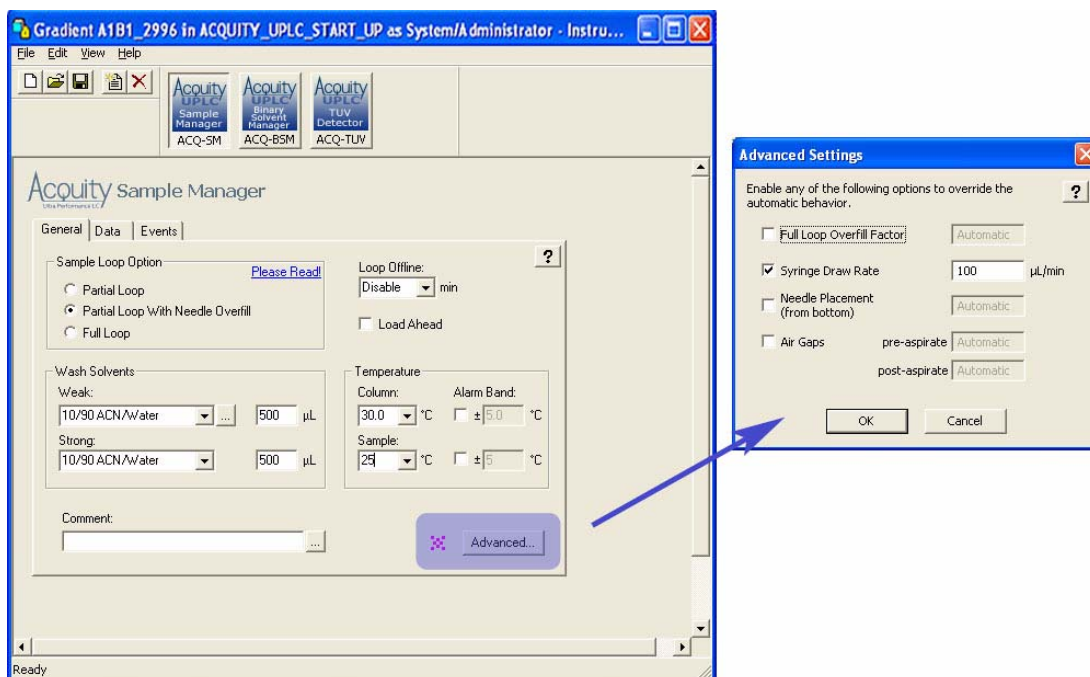


Figure 12 - Gradient Performance Parameters for the Sample Manager

If using the PDA, create an Instrument Method using the parameters for the gradient performance test shown in Figure 13.

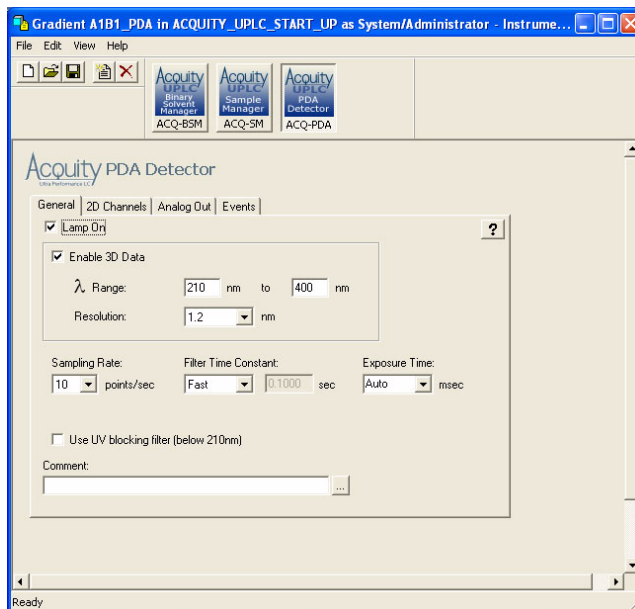


Figure 13 - Gradient Performance Parameters for the PDA

If using the TUV, create an Instrument Method using the parameters for the gradient performance test shown in [Figure 14](#).

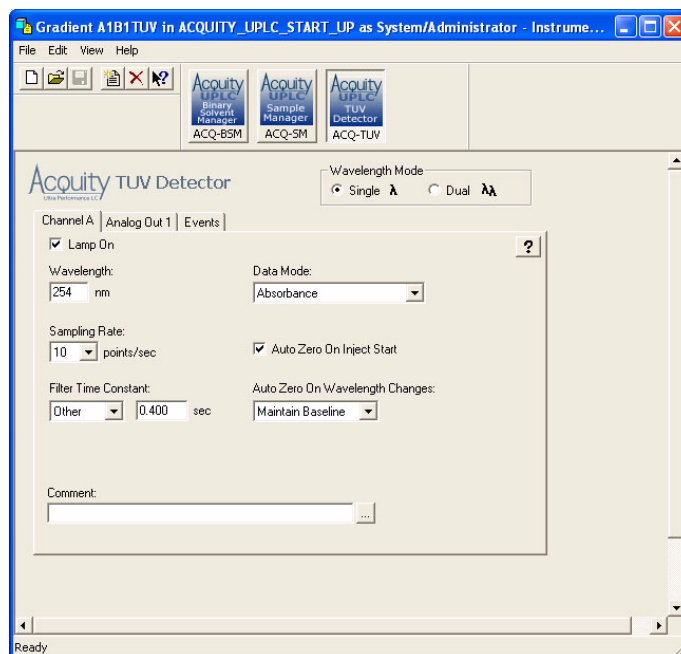


Figure 14 - Gradient Performance Parameters for the TUV

2. Create a Sample Set Method for the gradient performance test.

NOTE: The Sample Set Method parameters (Inj Vol., # of Injs, Function, Run Time, and Next Inj. Delay) will be the same whether using the TUV or PDA. However, the method set and report methods will vary. Before acquiring, ensure that you choose the appropriate method set.

NOTE: If using the PDA, you must derive a PDA MaxPlot in the method set.

The sample set parameters are as follows:

- Condition Column once (Run time = 6.0 minutes)
- Injection volume = 5 μ L (10-mm or 25-mm flow cell, or ELSD)
- # of Injections = 3
- Run time = 4.0 minutes (w/ Next Inj. Delay = 2.5 minutes). If using MassLynx the total run time will equal 6.5 minutes.

3. Perform the analysis.

Gradient Performance Test Results

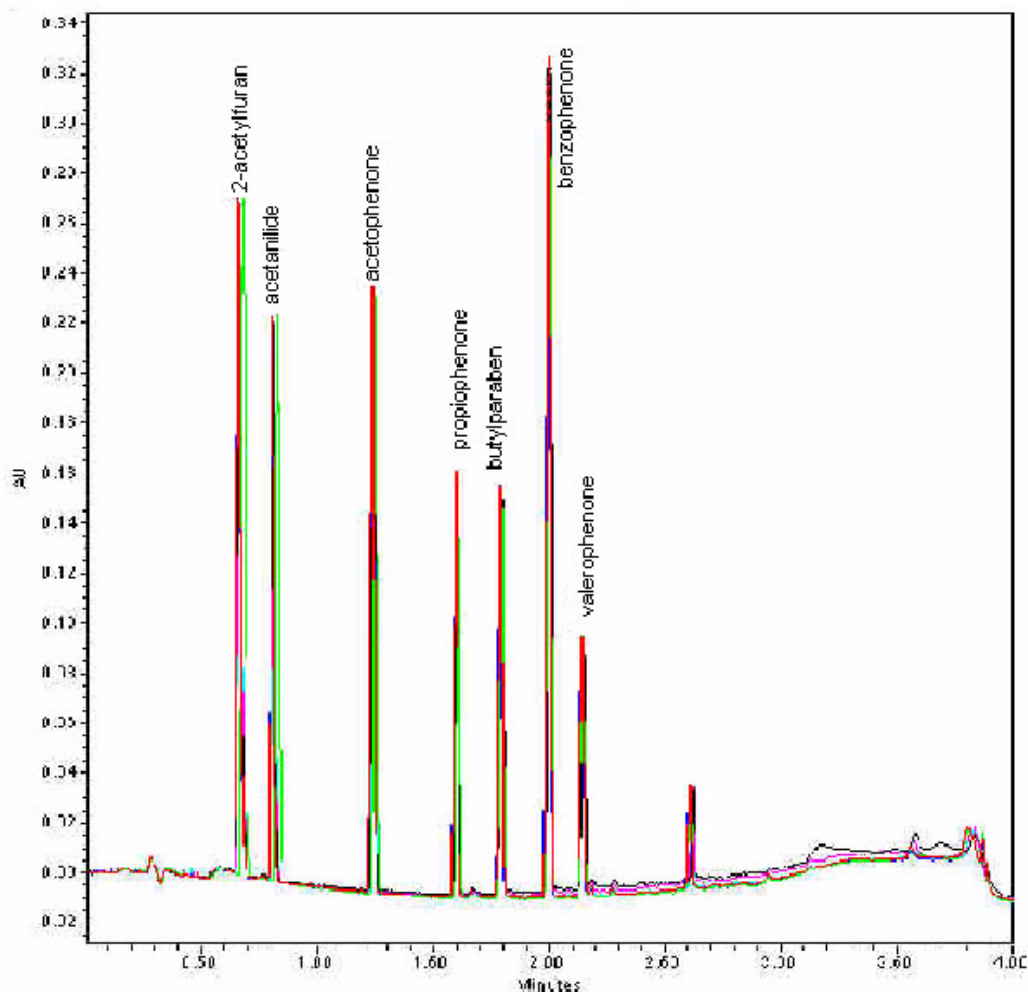


Figure 15 - Gradient Performance Chromatogram

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. Calculate the standard deviation for the peak retention times over three replicate injections. The standard deviation should be ≤ 1.5 minutes.
3. Report the results in the appropriate table in the *Waters ACQUITY UPLC System with TUV, PDA, ELS, or FLR Detectors Installation Checklist*.