ACQUITY UPLC System

Quick Start Guide

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Waters

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Waters contact information

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

Considerations specific to ACQUITY UPLC instruments

High voltage hazard

Warning: To avoid electric shock, do not remove the ACQUITY UPLC instrument's protective panels. The components they cover are not user-serviceable.

Safety advisories

Consult Appendix A in the *Waters ACQUITY UPLC System Operator's Guide* for a comprehensive list of warning and caution advisories.

Operating the ACQUITY UPLC instruments

When operating the ACQUITY UPLC instruments, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
EC REP	Authorized representative of the European Community
CE	Confirms that a manufactured product complies with all applicable European Community directives
ABN 49 065 444 751	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements

Audience and purpose

This guide is intended for personnel who operate ACQUITY UPLC instruments.

Intended use of the ACQUITY UPLC system

Waters designed the ACQUITY UPLC system to isolate, concentrate, separate, detect, and measure individual analytes in solution mixtures for research applications of rapid qualitative analysis, quantitative analysis, and/or micropreparative purification.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards should include the entire range of QC samples, typical specimens, and atypical specimens.

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

ISM classification

ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

EC Authorized Representative

EC REP

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Table of Contents

1 System Overview

This section describes the components and features of the ACQUITY UPLC® system.

Contents

Instruments, components, and data systems

ACQUITY UPLC systems include a binary solvent manager, sample manager, column heater, detectors (tunable ultraviolet, photodiode array, evaporative light scattering, fluorescent, or mass spectrometry), and a specialized ACQUITY UPLC column.

Small-particle chemistries as utilized in UPLC system chromatography generate narrow peaks. To maintain these narrow peaks, extra bandspreading must be controlled by lower detector cell volume, minimized tubing volumes, and specialized fittings. Narrow peak widths sometimes require higher data rates. The TUV, PDA, ELS, and FLR detectors can sample up to 80 data points per second. The SQ and TQ mass spectrometers can sample at fast acquisition speeds suitable for UPLC.

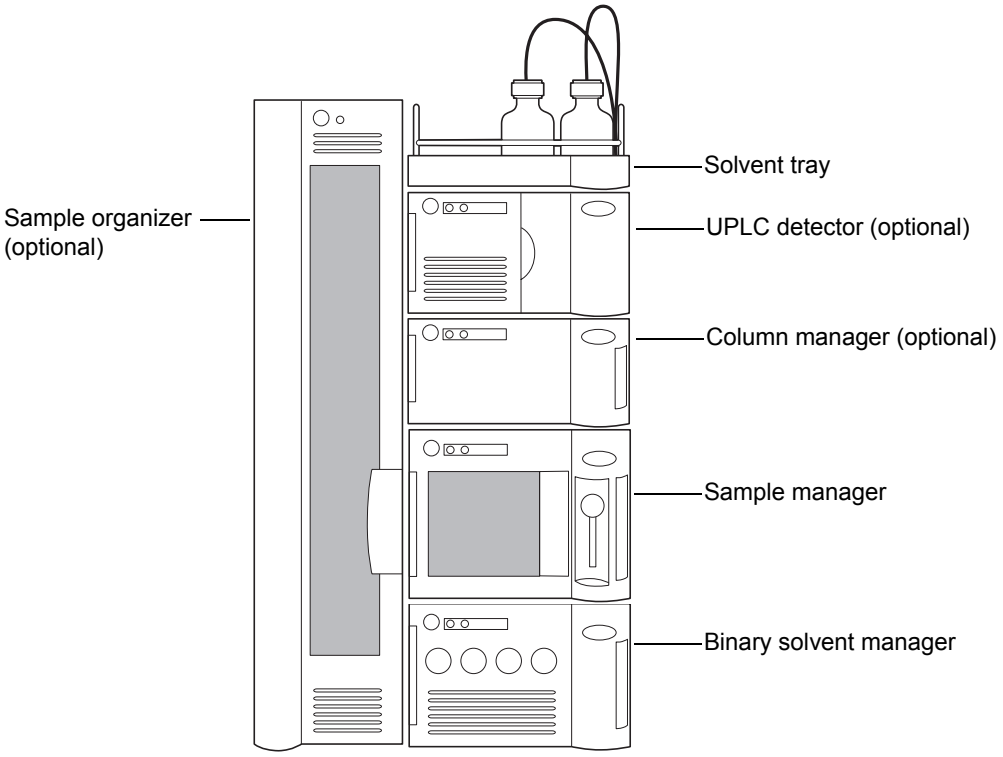
The binary solvent manager and injector can sustain pressures up to 103,421 kPa (1034 bar, 15,000 psi) and can generate high-pressure gradients with minimal gradient delay. The upper limit of the flow rate range is 2 mL/min.

The sample manager can accommodate two plates in a microtiter plate format or 2-mL vials in full-height plate format. An optional sample organizer increases the capacity of the system to as many as 22 microtiter plates (21 in the sample organizer and one in the sample manager), or eight vial racks (seven in the sample organizer and 1 in the sample manager).

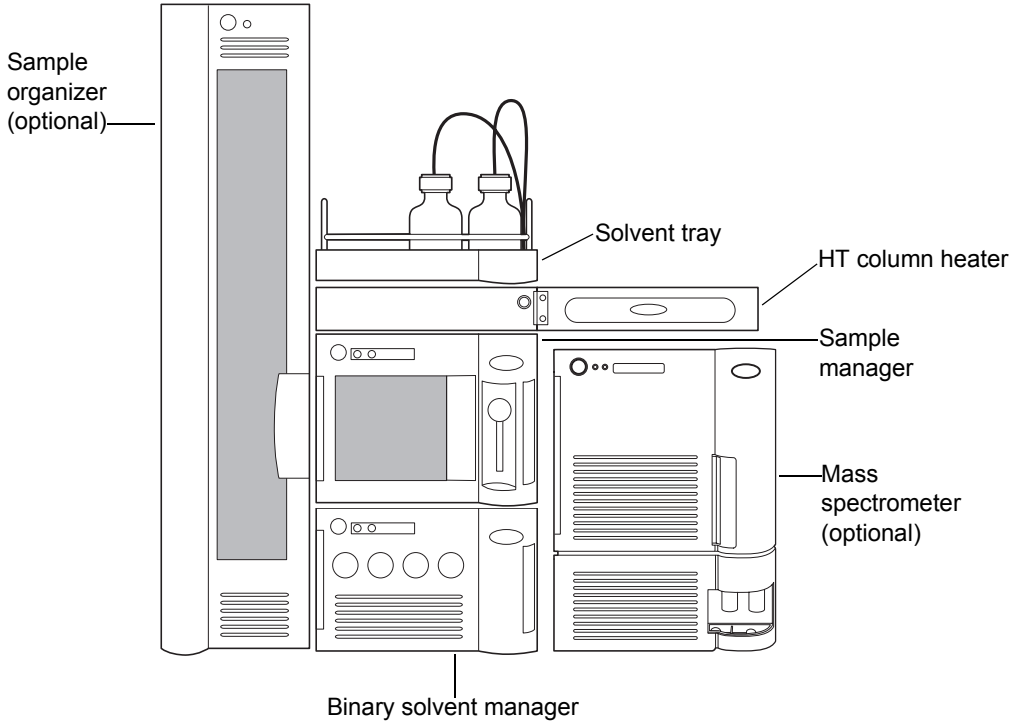
Waters® Empower™ chromatography software, MassLynx™ mass spectrometry software, or certain third-party software controls the ACQUITY UPLC systems.

Examples of Waters ACQUITY UPLC systems

Single detector system with column manager, no mass spectrometer



Single detector system with a mass spectrometer and column heater



UPLC system guidelines

Tip: ACQUITY UPLC system guidelines differ from standard HPLC practices.

When performing fast analyses, note that a peak of interest can be as narrow as 0.5 second. Waters recommends a sampling rate of 25 to 50 points across the peak, which provides good quantitation and peak representation. Sampling rates faster than 20 points per peak yield higher baseline noise and filter time constants must be adjusted accordingly.

The optimal ACQUITY UPLC flow rate differs from that of a typical HPLC column. The table below offers operating guidelines for ACQUITY UPLC columns under both isocratic and gradient conditions. Note that the values provided are approximations and that optimum performance for your molecule or separation can occur at a different flow rate and/or pressure.

Optimal flow rates for molecular weight range

Column size	Molecular weight	Flow rate
2.1 × 50 mm	<500	600 µL/min
2.1 × 50 mm	1000	300 µL/min
2.1 × 50 mm	1500	150 µL/min
2.1 × 50 mm	2000	100 µL/min

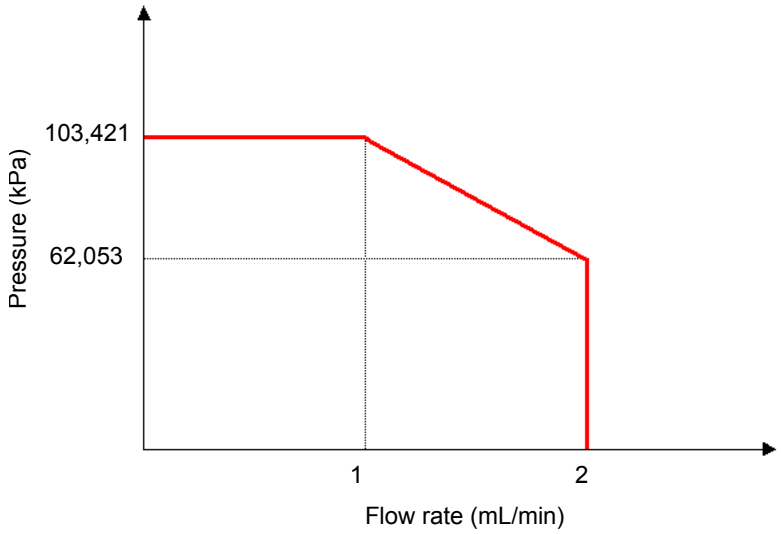
ACQUITY UPLC columns calculator

The ACQUITY UPLC columns calculator estimates the plate count (N) of an isocratic separation or the peak capacity (Pc) of a gradient separation based on your current HPLC conditions. It then offers you a choice of one or more ACQUITY UPLC columns that can provide increased resolving power in the same amount of time or similar resolving power in less time. The chromatographic conditions provided are a starting point and can be further optimized based on your particular requirements. After you install the ACQUITY UPLC software, the ACQUITY UPLC Columns Calculator shortcut appears on your computer desktop.

Binary solvent manager

The binary solvent manager is a high-pressure pump that moves solvent through the system. It provides steady (pulse-free) solvent flow at analytical flow rates. The binary solvent manager delivers solvent at flow rates of 1 mL/min at 103,421 kPa (1034 bar, 15,000 psi) and up to 2 mL/min at reduced pressures to 62,053 kPa (621 bar, 9000 psi). The binary solvent manager can pump two solvents simultaneously.

Pressure flow envelope



How the binary solvent manager works

Each of the binary solvent manager's two independent pump systems, A (on the left-hand side) and B (on the right-hand side), contains two linear-drive actuators (left-hand and right-hand). Each left-hand and right-hand actuator pair comprises a single reciprocating "serial" pump that delivers precise flow of a single solvent. The two pump systems combine their two solvents at a filter/tee mixer. From there, the solvent mixture flows to the sample manager.

The chromatography software controls the two solvents' mixing ratio by varying the flow of pump A relative to that of pump B. A pressure transducer in each pump head relays pressure data to the binary solvent manager, whose firmware measures pump head pressures during the pumping cycle. Thus the binary solvent manager independently pre-compresses the solvents in both the A and B portions to ensure consistent solvent delivery and minimize pump-induced detector baseline disturbances.

Sample manager

The ACQUITY UPLC sample manager injects the samples it draws from microtiter plates or vials onto the chromatographic column. A locating mechanism uses a probe to access sample locations and draw sample from them. In the needle overfill load-ahead mode, the sample manager can perform an injection in approximately 15 seconds. The first injection requires additional overhead time.

The sample manager accepts standard ANSI/SBS footprint plates, 5.03 ±0.02 inch × 3.365 ±0.02 inch, that conform to ANSI standards (maximum height = 2.2 inches, including covers). You can program any combination of these plates and vial holders for automated sample processing. Samples are loaded into the sample manager via the front door or the optional sample organizer, which transfers samples back and forth between the two instruments. The sample manager can maintain samples at any temperature between 4 and 40 ºC (39.2 to 104 ºF) in 25 ºC (77 ºF) or less ambient conditions.

How sample flows

When the default mode, partial loop with needle overfill, is requested, the sample manager needle carriage moves to the specified well location and draws in an air gap. A stainless steel puncture needle pierces the well cover and lowers into the well. The sample needle emerges from within the puncture needle, protrudes into the sample well, and draws in a sample volume equal to the specified injection volume plus 15.0 µL (14.0 µL pre-sample volume and 1.0 µL post-sample volume). The sample needle is then removed from the vial and the sample syringe continues to pull the sample aliquot through the sample needle and through the injection valve until the pre-sample and sample injection volume passes through the injection valve. The valve actuates, switching the sample loop to the load position. The sample is pushed back toward the needle and the sample volume is then pushed into the sample loop. The sample loop moves to the injection position and the sample is carried by the pump to the column.

High temperature column heater

The high temperature (HT) column heater is modular and its footprint is identical to that of the sample manager. The column heater's front compartment can accommodate any Waters column up to 4.6 mm ID and 150 mm long. The column rests in a U-shaped tray that swivels outward to receive the column from either side.

To reduce dispersion associated with dead volume and minimize the length of tubing between system instruments, the column tray swings outward to any position between 0 and 180 degrees. In the 0-degree, "home", position, the column tray is directly above the sample manager and connected to the optical detector (on top of the column heater). In the 180-degree, "away", position, the column heater can be plumbed into a mass spectrometer (located on the system's right-hand side).

The high temperature column heater heats the column compartment to any temperature from 5 ºC (9 ºF) above ambient to 90 ºC (194 ºF). A film element insulated to minimize power consumption and facilitate thermal stability is attached to the tray and produces heat. A passive column stabilizer, inside the tray, reduces sensitivity to ambient temperature swings and minimizes bandspreading.

A receptacle on the column heater's right-hand side receives the column's eCord™ chip. The eCord column chip stores column information that you can access from the ACQUITY UPLC Console.

The column heater drip tray captures any leakage, routing it to the sample manager.

Column manager

The optional column manager can regulate the temperature of up to four columns from 10 to 90 °C (50 to 194 °F). The column manager also offers a bypass channel and automated, programmable switching between columns for methods development. ACQUITY UPLC BEH Technology™ columns are equipped with eCord Information Management Technology, which captures the history of each column to assist in tracking column usage. Reusable high-pressure fittings ease replacement of the columns, when needed.

Column heater/cooler

The optional column heater/cooler can maintain four columns in a series from 10 to 90 °C (50 to 194 °F), but does not have switching valves. One column's eCord connects to the top port to track column usage.

30-cm column heater/cooler

The optional 30-cm column heater/cooler can regulate the temperature of HPLC columns up to 30 cm long, from 4 to 65 °C (39.2 to 149 °F).

Optional sample organizer

The optional sample organizer stores microtiter or vial plates and transfers them to and from the sample manager, automating their processing and increasing throughput.

The sample organizer's storage shelf compartment can hold a selection of ANSI plates. Sample plates are loaded into the organizer through a large, swing-open front door. The shelf compartment is thermally conditioned by sample organizer heater/coolers that, together with the sample manager heater/cooler, control the temperature between 4 and 40 °C (39.2 to 104 °F) in 21 °C (69.8 °F) or less ambient conditions.

Three subassemblies move plates within the sample organizer: the Z-Drive, the sample organizer transfer shuttle (Y-axis), and the sample manager transfer shuttle (X-axis). The Z-Drive moves the Y-axis to the target shelf, where the Y-axis picks the plate. Then the Z-Drive moves the Y-axis to the same elevation as the X-axis. The Y-axis shuttles the plate into the X-axis, which transfers the plate into the sample manager for processing. When the sample manager finishes with the plate, the X-axis pulls it back into the sample organizer. The process is reversed to return the plate to the shelf it came from.

Detectors

The system can be configured with a TUV, PDA, ELS, or FLR detector or a combination of them.

TUV detector

The TUV (tunable ultraviolet) optical detector is a two-channel, ultraviolet/visible (tunable UV/Vis) absorbance detector designed for use in the ACQUITY UPLC system. The detector, controlled by Empower or MassLynx software for both LC/MS and LC applications, operates as an integral part of the system.

The detector offers two flow cell options. The analytical flow cell, with a volume of 500 nanoliters and a pathlength of 10 mm, and the high sensitivity flow cell, with a volume of 2.4 microliters and a 25 mm pathlength, both utilize the Waters patented light-guiding flow cell technology.

The TUV detector operates at wavelengths ranging from 190 to 700 nm.

PDA detector

The PDA (photodiode array) optical detector is an ultraviolet/visible light (UV/Vis) spectrophotometer that operates between 190 and 500 nm.

The detector offers two flow cell options. The analytical flow cell, with a volume of 500 nanoliters and a pathlength of 10 mm, and the high sensitivity flow cell, with a volume 2.4 microliters and a 25 mm pathlength, both utilize the Waters patented light-guiding flow cell technology.

ELS detector

The ACQUITY UPLC ELS detector is an evaporative light scattering detector designed for use in the ACQUITY UPLC system. This detector can be controlled by Empower or MassLynx software.

The detector incorporates a flow-type nebulizer that is optimized for ACQUITY UPLC system performance.

FLR detector

The Waters ACQUITY UPLC FLR detector is a multi-channel, multi-wavelength fluorescence detector designed for use in the ACQUITY UPLC system. Optimized for UltraPerformance LC applications, the FLR detector features a low volume, axially illuminated flow cell (<2 µL), low-noise electronics, and high-intensity Hg-Xe lamp resulting in a design that minimizes stray light while maximizing light throughput, thus enhancing the quality of the fluorescence signal. The detector has an excitation wavelength range of 200 to 890 nm, an emission wavelength range of 210 to 900 nm, support for data rates up to 80 Hz, and offers 3D scanning capability for easier methods development.

Median baseline filter

The median baseline filter is intended to decrease the effects of gradient separations on the chromatographic baseline. The filter is available for the TUV, PDA, and ELS detectors but is most applicable in the absorbance detectors. The median baseline filter enhances the absorbance detector's stability by decreasing its curvature, making the development of integration methods easier.

See also: ACQUITY UPLC Console online Help.

Mass spectrometers

You can configure the system with an SQ, TQ, or other type of mass spectrometer. If your system has a mass spectrometer other than an SQ or TQ, refer to the documentation included with it.

SQ detector

The SQ detector is a single-quadrupole, atmospheric pressure ionization (API) mass spectrometer. Designed for routine ACQUITY UPLC/MS analyses, it can scan at speeds up to 10,000 Da/s.

TQ detector

The TQ detector is a tandem quadrupole, atmospheric pressure ionization (API) mass spectrometer. Designed for routine ACQUITY UPLC/MS/MS analyses in quantitative and qualitative applications, it can operate at fast acquisition speeds compatible with UltraPerformance LC.

Data systems

The system can run under Empower, MassLynx, or certain third-party software control.

Empower software

Empower software provides a graphical, icon-based user interface that acquires, processes, manages, reports, and stores chromatographic data.

The base version of Empower software supports data from TUV, PDA, ELS, and FLR detectors, and single quadrupole mass spectrometers. Popular software options for ACQUITY UPLC system users include System Suitability, Chemical Structures, and Method Validation Manager.

See also: Empower online Help.

MassLynx software

MassLynx is a high-performance mass spectrometry application that acquires, analyzes, manages, and distributes UV and mass spectrometry data. It offers intelligent instrument control and can acquire nominal mass, exact mass, MS/MS, and exact mass MS/MS data.

See also: *MassLynx Getting Started Guide* and MassLynx online Help.

Columns

ACQUITY UPLC columns are packed with 1.7-µm, bridged, ethylsiloxane, hybrid particles that can mechanically endure high-pressure conditions. The column hardware and the matched outlet tubing can withstand up to 103,421 kPa (1034 bar, 15,000 psi). The column dimensions allow optimal MS-compatible flow rates, and matched outlet tubing minimizes the effect of extra-column volume.

Although the system works with any analytical HPLC column, specially designed ACQUITY UPLC columns maximize its high-pressure capabilities.

Compared with traditional HPLC columns, ACQUITY UPLC columns deliver superior resolution and sensitivity in the same run time or equivalent resolution, greater sensitivity, and faster run times.

eCord column chip

ACQUITY UPLC columns include an eCord column chip that tracks the usage history of the column. The eCord column chip interacts with the system software, recording information for up to 50 sample queues run on the column. In regulated environments, the eCord column chip provides documentation of the column used in the validation method.

In addition to the variable column usage data, the eCord column chip also stores fixed column manufacturing data, including

- unique column identification.
- certificate of analysis.
- QC test data.

Once the eCord column chip is attached to the receptacle on the column heater, information is automatically recorded by the system. No user action is required. This information is stored only in the eCord column chip.

FlexCart

The optional FlexCart provides for the ACQUITY UPLC system a mobile platform. It can hold the system instruments as well as the PC and monitor and provides electrical outlets for system instruments and integrated waste management. Used with a mass spectrometer, the cart's adjustable height lets you position the column outlet close to the inlet probe, minimizing system dead volume.

For additional information

Refer to the following documents for further information:

- *ACQUITY UPLC Quick Reference Card* (part number 71508250006)
- *ACQUITY UPLC System Operator's Guide* (part number 71500082502)
- ACQUITY UPLC System Bookshelf Documentation CD (part number 71500082521)
  - *ACQUITY UPLC Photodiode Array Detector Getting Started Guide* (part number 71500108703)
  - *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide* (part number 71500109303)
  - *ACQUITY UPLC Fluorescence Detector Getting Started Guide* (part number 71500142403)
  - *Waters SQ Detector Quick Start Guide* (part number 71500126603)
  - *Waters TQ Detector Quick Start Guide* (part number 71500126803)
  - *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307)
- ACQUITY UPLC Console online Help
- ACQUITY UPLC System release notes

2 Preparing System Hardware

Contents

Powering-on the system

Powering-on the system entails starting the ACQUITY UPLC® system workstation, system instruments, and Empower or MassLynx operating software.

To power on the system

1. Power-on the ACQUITY UPLC system workstation.
2. You must power-on the HT column heater, column manager, or column heater/cooler before any of the other instruments because they contain the internal Ethernet switch that allows the ACQUITY UPLC system workstation to communicate with all the instruments.

Tip: If your system contains an HT column heater, it is automatically powered-on when you power on the sample manager.

To power-on a column manager or column heater/cooler, press the power button on the top, left-hand side of it's front panel. Each system instrument beeps 3 times and runs a series of startup tests.

The power and status LEDs change as follows:

- Each system instrument's power LED shows steady green. The column manager's and column heater/cooler's run LEDs all show red for a few seconds.
- During initialization, each system instrument's power LED shows steady green. The column manager's and column heater/cooler's run LEDs all show flashing green.
- After the instruments are successfully powered-on, each one's power LED shows steady green. The column manager's and column heater/cooler's run LEDs are unlit.

3. Press the power switch on the top, left-hand side of the binary solvent manager's, sample organizer's, and sample manager's door. Each system instrument beeps 3 times and runs a series of startup tests.

   Requirement: If your system has a sample organizer, you must power it on before you power on the sample manager.

   The power and status LEDs change as follows:

   - Each system instrument's power LED shows steady green. The binary solvent manager's flow LED and the sample organizer's and sample manager's run LEDs show red for a few seconds.
   - During initialization, each system instrument's power LED shows steady green. The binary solvent manager's flow LED and the sample organizer's and sample manager's run LEDs show flashing green. Full initialization of the system usually requires about 7 minutes.
   - After the instruments are successfully powered-on, each one's power LED shows steady green. The binary solvent manager's flow LED and the sample organizer's and sample manager's run LEDs are unlit.

4. After the binary solvent manager's, sample organizer's, and sample manager's power LEDs show steady green, press the power switch on the top, left-hand side of the detector(s).

The detector's power and status LEDs change as follows:

- The detector's power LED shows steady green and its lamp LED shows red for a few seconds.
- During initialization, the detector's power LED shows steady green and its lamp LED shows flashing green.
- After the detector is successfully powered-on, its power LED shows steady green. The detector's lamp LED shows steady green, indicating that the lamp is ignited.

Tip: To prevent initialization errors, only power on the detector(s) when the flow cell is wet.

5. Start the Empower or MassLynx operating software. You can monitor the ACQUITY UPLC Console for messages and LED indications.

Monitoring startup tests

These startup tests run when you power-on the ACQUITY UPLC system workstation:

- CPU board
- Memory (RAM and ROM)
- External communication system (Ethernet)
- Clock

If the startup tests indicate a malfunction, consult the ACQUITY UPLC Console online Help.

Monitoring system instrument LEDs

Light emitting diodes on each system instrument indicate the instrument's state of functioning. The LEDs are specific to their instruments, so the significance of their various colors and modes can differ from one instrument to another.

Power LED

The power LED, on the left-hand side of an instrument's front panel, indicates the power-on or power-off status of the instrument. This LED is green when power is on and unlit when power is off.

Tip: To provide adequate ventilation, the sample manager and sample organizer fans are always running, even with the power off. These fans switch off only when the power cable is removed from the back of the instrument.

Status LEDs

Flow LED (binary solvent manager)

The flow LED, on the right-hand side of the power LED on the binary solvent manager's front panel, indicates the flow status. A steady green flow LED indicates that there is a flow through the binary solvent manager.

Run LED (sample manager, column manager, column heater/cooler, and sample organizer)

The run LED, on the right-hand side of the power LED on the sample manager's, column manager's, column heater/cooler's, and sample organizer's front panel, indicates the run status. A steady green run LED indicates that injections are being run.

Lamp LED (detector)

The lamp LED, on the right-hand side of the power LED on the detector's front panel, indicates the lamp status. A steady green lamp LED indicates that the lamp is on.

Status LED indications

LED mode and color	Description
Unlit	• Binary solvent manager, sample manager, column manager, column heater/cooler, and sample organizer – Indicates the instrument is currently idle. • Detector – Indicates the detector lamp is extinguished.

Status LED indications (Continued)

LED mode and color	Description
Steady green	• Binary solvent manager – Indicates solvent is flowing. • Sample manager, column manager, column heater/cooler, and sample organizer – Indicates the sample manager, column manager, column heater/cooler, or sample organizer is operating normally, attempting to complete any outstanding samples or diagnostic function requests. When sample and diagnostic function requests are finished, the LED reverts to the unlit mode. • Detector – Indicates the detector lamp is ignited.
Flashing green	• Binary solvent manager, sample manager, and sample organizer – Indicates the system is waiting for at least one instrument to become operable. Detector lamp warm-up and column temperature equilibration times typically cause such a delay. • Column manager and column heater/cooler – Indicates the system is waiting for the instrument to reach the temperature set point before operating. Column temperature equilibration times typically cause such a delay. The LED also flashes green during initialization or while waiting for initialization. • Detector – Indicates the detector is initializing or calibrating.
Flashing red	Indicates that an error stopped the instrument. Refer to the ACQUITY UPLC Console for information regarding the error.
Steady red	Indicates an instrument failure that prevents further operation. Power-off the instrument, and then power-on. If the LED is still steady red, contact your Waters service representative.

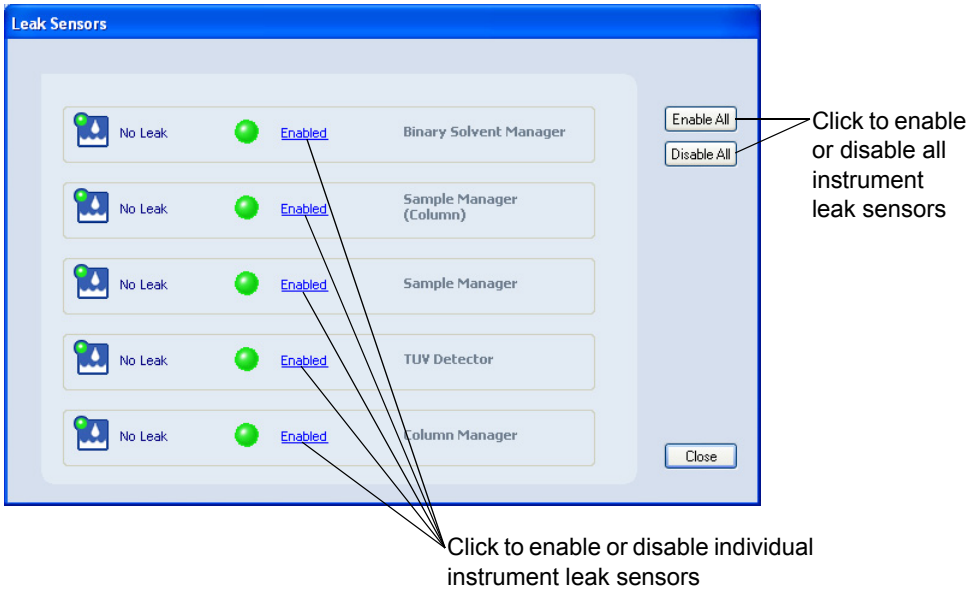
Enabling the leak sensors

Rule: When you power-on the system, the leak sensors default to disabled unless previously enabled.

To enable the leak sensors

1. In the ACQUITY UPLC Console, select Control > Leak Sensors.

Leak Sensors dialog box



2. To enable the leak sensor for an individual instrument, click the status on the left-hand side of the instrument description. Or, to enable all leak sensors, click Enable All.

Preparing the binary solvent manager

For optimal performance of the ACQUITY UPLC system, you must prepare the binary solvent manager for operation.

To prepare the binary solvent manager for operation, you must perform a seal wash prime and then prime the binary solvent manager.

Warning: Observe Good Laboratory Practices when you handle solvents. See the Material Safety Data Sheets for the solvents you use.

Requirement: To maintain the efficiency of the binary solvent manager and to obtain accurate, reproducible chromatograms, use only MS-grade solvents, water, and additives. For details, see the *Waters ACQUITY UPLC System Operator's Guide*.

Caution: To avoid damaging binary solvent manager components, do not use chloroform, methylene chloride, ethyl acetate, or toluene.

Performing a seal wash prime

Prime the seal wash in the binary solvent manager to lubricate the plungers, fill the tubing paths with solvent, and flush away solvent and/or any precipitated salts that have been dragged past the plunger seals from the high-pressure side of the piston chambers.

Prime the plunger seal wash

- after using buffered mobile phase.
- when the binary solvent manager has been inactive for a few hours or longer.
- when the binary solvent manager is dry.

Caution: To avoid damage to the solenoid valve seats and seals in the solvent path, do not use a nonvolatile buffer as the seal wash solvent.

Tip: The seal wash self-primes, but you can use a syringe to hasten the process.

Rule: To prevent contamination, do not recycle seal wash.

Recommendations:

- Seal wash must contain 10% organic solvent. This concentration prevents microbial growth and ensures that the seal wash can solubolize the mobile phase.
- Before priming the plunger seals, ensure the volume of seal wash is adequate for priming.

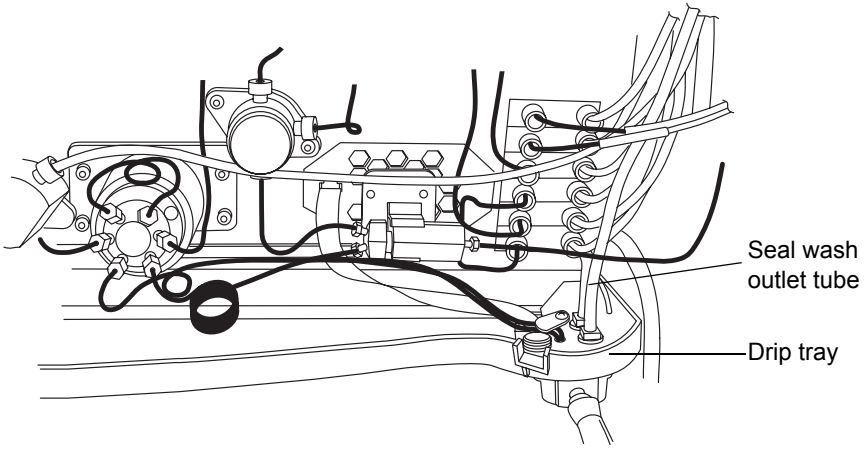
See also: *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD.

Required materials

- 30-mL syringe (startup kit)
- Seal wash solution
- Tubing adapter (startup kit)

To perform a seal wash prime

1. Ensure the seal wash inlet tube is in the solvent reservoir.
2. Remove the seal wash outlet tube from the right-hand side of the drip tray.



3. Push the syringe plunger fully into the syringe barrel.
4. Connect the tubing adapter to the syringe, and then connect the syringe assembly to the outlet tubing from the seal wash system.

5. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
6. Click Control > Prime seal wash, and then click Yes to begin the seal wash priming process.
7. Slowly draw back on the syringe plunger to pull seal wash solvent through the system.
8. When the seal wash solution begins to flow into the syringe without major air bubbles, disconnect the tubing and reinstall it on the fitting on the drip tray.
9. Click Control > Prime seal wash, and then click Yes to stop the priming process.

Priming the binary solvent manager

Priming is used to prepare a new system or binary solvent manager for use, change reservoirs or solvents, and run the system after it has been idle for more than 4 hours. During priming, the vent valve moves to Vent position to both ensure minimal backpressure and direct the flow to waste. The flow rate during priming is 4 mL/min for each pump being primed.

Tip: If you are priming a dry binary solvent manager, using a syringe shortens the time required to complete priming.

Caution: To prevent salts from precipitating in the system, introduce an intermediate solvent, such as water, when changing from buffers to high-organic-content solvents. Be sure to consult the solvent miscibility tables in the *Waters ACQUITY UPLC System Operator's Guide*.

Recommendation: Ensure the solvent reservoirs have enough solvent for adequate priming and the waste container has sufficient capacity for used solvent. The priming flow rate is 4 mL/min for each pump, or 8 mL/min total. For example, priming both solvents for 5 minutes requires approximately 20 mL of each solvent.

Warning: To avoid spills, empty the waste container at regular intervals.

Priming a dry binary solvent manager

To prime a dry binary solvent manager

1. Open the instrument's front door.
2. Locate the appropriate solvent vent line.
3. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
4. In the binary solvent manager information window, click Control > Prime A/B Solvents.
5. In the Prime A/B Solvents dialog box, select solvent A and/or B.
6. In the Time box, specify the number of minutes from 0.1 through 60.0.

   Default: 1.0 minute

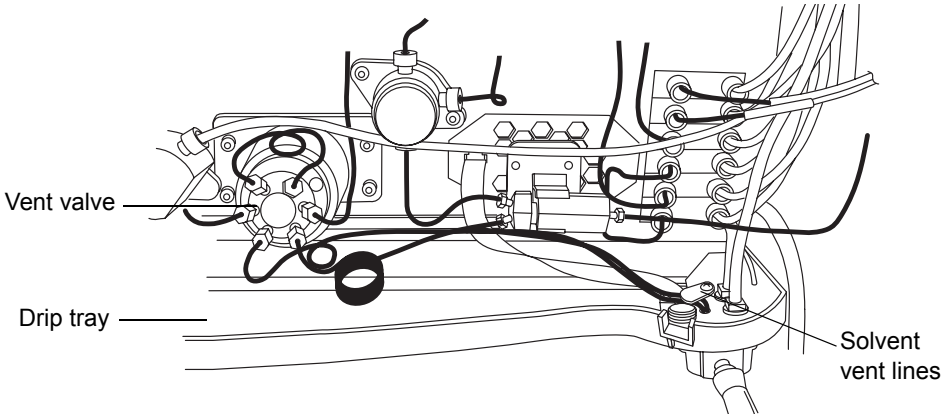
   Recommendations: Prime the binary solvent manager until a steady flow exits the vent tube (typically 7 to 10 minutes).
7. Click Start. When solvent flows out of the vent line without bubbles, the path is primed.
8. Repeat step 3 through step 7 to prime the other solvents.

   Requirement: There must be solvent in the A1, A2, B1, and B2 reservoirs for the degasser to function correctly.

To prime a dry binary solvent manager using a syringe

1. Open the instrument's front door.
2. Locate the appropriate solvent vent line.
   - If you are priming solvent A, follow the stainless steel vent line that is labeled "A-VENT" from port 4 on the vent valve, and lift it out of the drip tray.

- If you are priming solvent B, follow the stainless steel vent line that is labeled “B-VENT” from port 1 on the vent valve, and lift it out of the drip tray.



3. Push the syringe plunger fully into the syringe barrel.
4. Connect the tubing adapter to the syringe.
5. Connect the syringe assembly to the short length of PharMed tubing, and then connect the short length of PharMed tubing to the solvent vent line you located in step 2.
6. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
7. In the binary solvent manager information window, click Control > Prime A/B Solvents.
8. In the Prime A/B Solvents dialog box, select solvent A1.
9. In the Time box, specify the number of minutes from 0.1 through 60.0.

   Default: 1.0 minute

   Recommendation: Prime the binary solvent manager until a steady flow exits the vent tube (typically 3 minutes).
10. Click Start.
11. Slowly draw back on the syringe plunger to pull solvent through the solvent path. When solvent flows out of the vent line without bubbles, the path is primed.

12. Remove the syringe from the vent line, and reconnect the vent line to the drip tray.
13. Repeat step 2 through step 12 for solvent A2, B1, and B2.

    Requirement: The reservoirs and solvent lines for solvents A1, A2, B1, and B2 must not be empty. Otherwise the degasser does not function correctly.

Priming a wetted binary solvent manager

Two functions help prepare the system for operation:

- Refresh system (Sys Prep)
- Start up

The length of time the system has been idle determines which is the better.

Refreshing the system

Use the Refresh (Sys Prep) function after the system has been idle a short period of time (a few hours to overnight) and when you plan to use the same solvents that you used previously.

You can invoke the Sys Prep function from the control panel or by adding it as a line in a sample set.

Recommendations:

- Prime the binary solvent manager for 1 minute if the system has been idle for 4 or more hours and you will use the solvents that are already in the system.
- Prime the binary solvent manager for 4 minutes if you will use new solvents that are of the same composition of what is already in the system.

To refresh the system

1. In the ACQUITY UPLC Console, click Control > Refresh system (Sys Prep).

2. In the Refresh System (Sys Prep) dialog box, review the settings and select a different option, if needed. The system primes your current solvent selections (A1 or A2, B1 or B2).
   - Solvent line A only (default)
   - Solvent line B only
   - Both A and B
3. Click OK.

   Result: The system primes the selected solvents, primes the sample manager with one weak wash prime (using the wash and sample syringes), and ignites the lamp in the detector.

Starting up the system

Use the Start up function to prime the binary solvent manager after changing the mobile phase, after changing the sample needle and/or sample loop, or after the system has been idle a long period of time (overnight or over a weekend). Before you begin this procedure, ensure that the system is correctly configured for use.

Recommendation: Prime the binary solvent manager for 5 minutes if you are changing to solvents whose compositions differ from the compositions of solvents already in the system.

To start up the system

1. In the ACQUITY UPLC Console, click Control > Start up.
2. In the Prime Solvents tab of the System Startup dialog box, review the settings for the A/B Solvents. In the A/B Solvents area, you can select or clear any or all of the solvents: A1, A2, B1, or B2. You can change the length of time to prime solvents A and B by entering a different number in Duration of Prime. All selected solvents are primed for the same duration.

   Allowed values: 0.1 to 60.0 minutes

   Tip: If you want to return settings to their original values on any tab, click Set Defaults.

   Defaults: Solvents A1, A2, B1, and B2 prime for 1.0 minute each.
3. Select or clear priming of the seal wash, strong wash, weak wash, and/or sample syringe.

Default: The seal wash is primed for 1.0 minute, the weak wash once, and the sample syringe once.

4. If necessary, change the number of cycles to prime the syringes by entering a different whole number in the Cycles field.

   Default: 10 cycles for each syringe selected

5. Select the Equilibrate to Method tab to review the settings for the final flow rate, mobile phases, composition, temperatures, and lamp state. Change the values as needed to match your requirements at equilibration.

   Equilibrate to Method tab values

System startup parameters	Default	Allowed values
Method initial flow rate	0.25 mL/min	0.1 to 2.0 mL/min
Composition of A and B (sum must be 100%)	A1, 100 B1, 0%	A1, A2; 0 to 100% B1, B2; 0 to 100%
Column temperature	Off	Off, or 5.0 °C (9 °F) above ambient to 65.0 °C (149 °F)
Sample temperature	Off	Off, or 4.0 to 40.0 °C (39.2 to 104 °F) in 25 °C (77 °F) ambient conditions
Lamp	On	On or off

6. If you changed the sample needle or loop, in the Configuration area, click Change. In the Volume Configuration dialog box, select the new size of loop and/or needle, and then click OK.

7. If you changed the sample needle, click the Optional: Characterize Volume tab, and then select “Characterize seal” and “Characterize needle and loop volumes”.

8. If you changed the sample loop, in the Optional: Characterize Volume tab, select Characterize needle and loop volumes.

9. Click Start.

   Result: The lamp in the optical detector ignites, the ACQUITY UPLC system sets the column sample temperatures, and all priming starts. If

you selected the Characterize seal function after priming finishes, the sample manager finds the position of the needle seal and then logs the results into the database.

Finally, the system establishes the method flow rate, solvent selections, and composition. The default settings for the method initial flow are 100% Solvent A1 at .25 mL/min and 0% B1, the column and sample temperatures are Off, and the detector lamp is ignited.

Preparing the sample manager

Prepare the sample manager for operation after you prepare the binary solvent manager. Preparing the sample manager involves these steps:

- Priming
- Characterizing the seal
- Characterizing the needle and sample loop volumes
- Loading sample plates

Warning: To avoid solvent spills and to maintain proper leak drainage, always close the sample manager fluidics tray before operating the system.

Selecting weak wash and strong wash solvents

For best performance, follow these guidelines when selecting wash solvents. Otherwise performance can be reduced, specifically Area/Height RSD and Linearity. The guidelines do not prohibit all other solvent combinations, however. Other combinations can be run with lower performance expectations or by manipulating default injection parameters.

Use a weak wash solvent based on the sample and mobile phase chemistries of your application, making sure all solutions/buffers are miscible and soluble.

Recommendation: For buffered aqueous, reversed-phase chromatographic conditions and MS applications, use a weak wash solvent of 100% water or 0% to 25% methanol or acetonitrile and a strong wash solvent of 50% to 100% methanol or acetonitrile. High sample concentrations can require other weak wash solvents. If your separation permits, Waters recommends adding a small amount of an organic solvent (~10%) to prevent microbial growth.

See the *Waters ACQUITY UPLC System Operator's Guide* for further information about solvents.

Caution: To avoid damage to the solenoid valve seats and seals in the solvent path, do not use a nonvolatile buffer as the weak wash or strong wash solvent.

Tip: For best performance, the weak wash solvent must be similar or identical to your isocratic or initial gradient solvent conditions, excluding buffers. Do not use salt buffers in wash solvents.

Wash solvent effects

Property	Effect
Organic species	As a general principle, strong and weak solvents must include the same organic species. Note that this is not always be practicable. You can, however, use a 100% organic strong wash solvent.
Solvent composition	The weak wash solvent must reflect as closely as possible the same composition as the initial gradient mobile phase.
pH	Adjust the pH of strong and weak solvents for best peak shape and carryover performance.
Concentration of strong solvent	Strong solvent must be no stronger than the concentration needed to reduce carryover to an acceptable level.
Solubility of sample	The sample must be soluble in both the weak and strong wash solvents. **Caution:** Proteins (in plasma, for example) do not dissolve in solvents whose organic component is greater than 40%.
Sample diluent	The weak wash solvent will contact the sample, so match these as closely as possible. To offset adverse effects on peak shape caused by the matrix's composition, adjust the weak wash composition, especially when using the instrument in partial loop mode.

Wash solvent effects (Continued)

Property	Effect
Wash volume ratio (weak to strong)	Within a method, use a 3:1 ratio, weak wash to strong–sufficient to ensure the weak wash flushes the strong from the needle and sample loop before sampling.
Cycle times	Higher viscosity wash solvents lengthen wash cycles.

Priming the sample manager

The priming process fills the sample needle with solvent, flushes new solvent through the injector lines, and/or purges air from the lines. You prime the sample needle and/or sample syringe to accomplish these tasks:

- Prepare a new sample manager for operation
- Prepare a sample manager for operation after it has been idle for an extended period
- Change the solvent in the syringes
- Remove bubbles from the lines

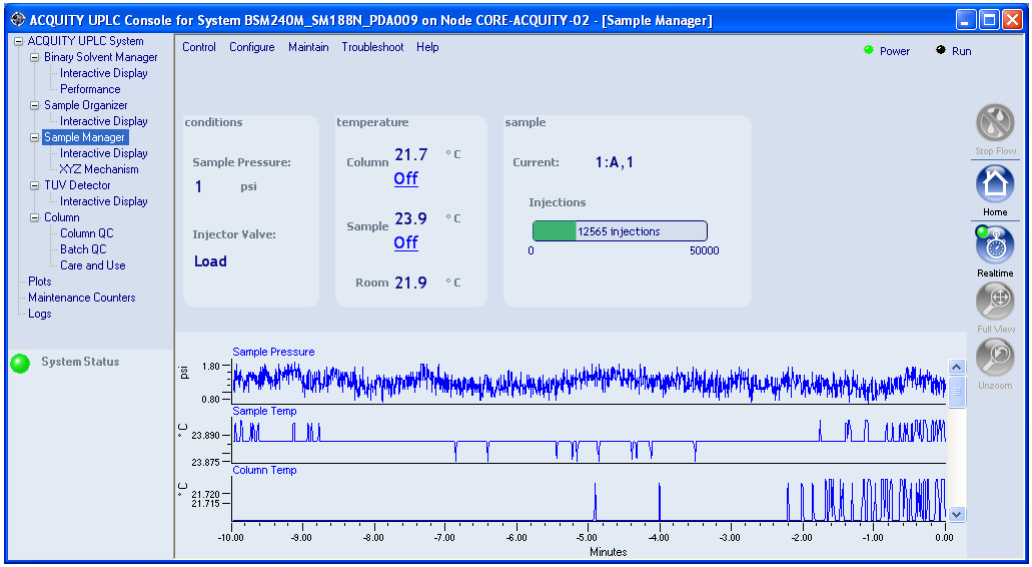
Ensure that the priming solvent is correctly composed and that it is high in quality and miscible with any other solvents used in your system. Use filters in all solvent reservoirs, and ensure the volumes of solvents are sufficient for priming.

Requirement: The sample manager must be primed before you attempt to characterize the seal.

To prime the sample manager

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.

Sample manager information window



2. Click Control > Prime syringes.

   Alternative: Right-click in the Empower or MassLynx sample manager control panel, and then click Prime syringes.

3. In the Prime Syringes dialog box, select Sample syringe and both wash syringes.

   Tip: If you want only to remove air bubbles from the sample syringe, but do not want to prime the wash syringes, select Sample syringe only. However, do not select this option routinely. Priming all components at the same time is good practice.

4. Type the number of primes in the Number of cycles text box.

   Default: 1

   Recommendation: Waters recommends 5 to 7 primes when you are changing solvents.

   Caution: Do not abort the sample manager priming sequence. Doing so can leave strong solvent in the sample needle, which can adversely affect chromatography.

5. Click OK to start priming. When the system status is “Idle,” priming is finished.

   Tip: Each prime takes approximately 2 to 4 minutes.

Washing the sample manager needle

Washing the needle is an optional procedure that flushes strong and/or weak wash solvent through the needle and injection port. Washing the needle removes contaminants from the inside and outside of the needle, the external piercing needle, and the injection port. You can also perform a needle wash to ascertain proper flow through the waste tubing and to confirm that the needle wash system is primed and properly operating.

Rule: Do not use buffered solvents as wash solvents.

Tip: Priming the system washes the sample needle, so whenever you prime the system, you can omit this procedure.

Observing wash solvent recommendations

Waters recommends that you observe these guidelines for washing the needle:

- To ensure that the strong wash solvent is completely removed, the system washes the needle with 500 µL of weak wash solvent after you use strong wash solvent. You can increase, but not decrease, the default value of 500 µL.
- The analytes and sample matrix must be soluble in both weak and strong solvents. Proteins do not dissolve in solvents whose organic component is greater than 40%. Do not use buffers in any wash solvent.

  Example: If the weak wash solvent is 30% acetonitrile and 70% water, the strong wash solvent must contain a greater concentration of acetonitrile in water.
- The weak wash solvent must be the same as the initial eluting solvent, and the strong wash solvent must at least equal the composition of the final eluting solvent.
- Use a weak wash solvent based on the sample and mobile phase chemistries of your application. Make sure all solutions are miscible and soluble. For best results, weak wash solvent must match the initial gradient conditions and mobile phase composition (isocratic). High sample concentrations can require additional weak wash solvents.
- For buffered aqueous, reversed-phase chromatography, use weak wash solvent consisting of 100% water or up to 25% methanol or acetonitrile. For strong wash solvent, use 50% to 100% methanol or acetonitrile.

Before you begin, ensure that the solvents are compatible with your application, that their volumes are sufficient, and that the waste reservoir is large enough to contain the waste solvent.

Needle recommendations

Waters offers needles made from several types of materials to accommodate compounds that may have specific handling requirements or affinities. Users must choose the needle material best suited to their application. For example, the stainless steel needle is recommended when working with samples that are known to be attracted to hydrophobic polymers.

Choose a stainless steel needle or stainless steel/Teflon needle for greater strength and more robust operation. Stainless steel needles are also recommended for use with hexane and tetrahydrofuran; PEEK needles are not recommended for use with either of these solvents.

Choose smaller needles when using partial loop (pressure assist) mode, or when using the smallest loops. However, using a smaller needle will increase cycle times.

To wash the sample manager needle

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree. The sample manager information window appears.
2. Click Control > Wash Needle.

   Alternative: Right-click in the Empower or MassLynx sample manager control panel, and then click Wash Needle.
3. In the Strong Wash box, specify the volume for the strong wash solvent. Or, to omit strong wash solvent, enter 0 in the Strong Wash box, or leave it blank.

   Range: 0.0 through 99,999 µL

   Default: 0.0 µL

   Recommendation: 100 through 500 µL

   Tip: Using both a weak and strong wash solvent increases the wash time and solvent consumption because the system must be fully cleansed of the strong solvent before starting the next injection.

   Caution: To avoid strong wash solvent contacting the sample and contaminating it, use a sufficient quantity of weak wash solvent.

4. In the Weak Wash box, specify the volume for the weak wash solvent.

   Range: 1.0 through 99,999 µL

   Default: 200.0 µL without strong wash or 500 µL with strong wash

   Recommendation: 200 through 500 µL or three times the strong wash volume.

5. Click OK. The needle wash begins.
6. When needle washing is complete, the status returns to Idle.

To stop a needle wash routine before it finishes

From the sample manager information window, click Control > Reset SM.

Alternative: Right-click in the Empower or MassLynx sample manager control panel, and then click Reset SM.

Caution: Do not abort the sample needle wash sequence. Doing so can leave strong solvent in the sample needle, which can adversely affect chromatography.

Characterizing the needle seal

The needle seal characterization procedure finds the position at which the needle obtains a seal within the wash station block. The sample manager must be primed before starting this procedure.

Requirements:

- Perform this procedure before calibrating the needle and sample loop volumes.
- Perform this procedure after you replace and/or adjust these items:
  - Needle
  - any part of the needle assembly
  - Needle (Z) or piercing needle (Zp) flags (home and top-of-plate)
  - Home or top-of-plate sensor
  - Inject port seal
  - Wash station
  - NVRam battery on the CPU2000

To characterize the needle seal

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. In the sample manager information window, click Maintain > Characterize > Needle seal.
3. In the Characterize Needle Seal dialog box, click Start. The calibrate seal operation begins, and the sample manager status displays “Calibrating seal”.
4. When calibration ends, the sample manager status displays “Idle”.
5. Click Results to view the results of the needle seal characterization operation.

Characterizing the needle and sample loop volumes

Whenever you replace the sample loop and/or the sample needle, you must set the system to characterize the volume of the replacement parts. Do this regardless of whether the sizes of the replacement parts are nominally the same as those of the original parts or differ from them. Also perform this procedure when the composition of the weak wash solvent changes, because solvent characteristics such as viscosity, surface tension, and polarity can change. During sample injection, the weak wash solvent precedes and follows the sample in the fluid-carrying lines, so the sample is directly affected by the weak wash solvent.

Characterizing the loop volume compares the loop’s nominal volume (in µL) to its measured volume.

Characterizing the needle volume compares the needle’s nominal volume (in µL) to its measured volume.

Tip: Characterizing the system volume is critical to good sample manager performance.

Requirements:

- Specify the sizes of the sample needle, loop, and syringe in the Volumes dialog box before characterizing the volumes.
- Prime the sample manager and syringes, and characterize the needle seal before characterizing the volumes.

- Create a method (using Empower or MassLynx software) that has the same air gap and sample draw rate that you will be using.
  - If you are running under Empower software control, click File > New Method > Instrument Method in the Project window.
  - If you are running under MassLynx software control, click Inlet Method > Inlet > Autosampler in the Inlet Editor window.

To characterize the needle and sample loop volumes

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. Click Maintain > Characterize > Needle and loop volumes.
3. In the Characterize Needle and Loop Volumes dialog box, click Start.

   Tip: This procedure takes at least 5 minutes.
4. Click Results to view the results of the needle and loop volumes characterization operation.

   Result: If the needle fails the test, suspect it is bent, broken, or blocked. If the sample loop fails the test, suspect that it is blocked or leaking, that it has a loose fitting, or that the draw rate is too high.

Using the extended puncture needle

When using an extended puncture needle, be aware of the following:

- The thumbscrew on the needle mounting bracket is grey plastic or stainless steel instead of red.
- When used with ACQUITY UPLC System Instrument Driver version 1.30 or higher, the extended feature is automatically activated.
- The extended puncture needle is compatible with software released prior to ACQUITY UPLC System Instrument Driver version 1.30, but the extended puncture needle functionality is not active.
- Whenever you calibrate the XYZ mechanism, the extended puncture needle is automatically recognized.

Loading sample plates in the sample manager

The ACQUITY UPLC sample manager holds up to two ANSI/SBS plates that you load through the front door. The left-hand plate is referred to as position 1, the right-hand plate as position 2.

Exception: If the optional sample organizer is installed, you can load only one plate through the sample manager front door. You must load the plate on the right-hand tray. In this case, the right-hand tray becomes the number one position.

Observing vial and plate recommendations

Waters recommends that you observe these guidelines for loading sample vials and plates in the sample manager:

- Use only Waters-certified vials.
- Do not used Waters total recovery vials with the ACQUITY UPLC system default settings. If the needle depth position is suitably offset, total recovery vials can be used successfully with PEEK needles.
- Use only 1860024XX-series vials and cap mats in the ACQUITY UPLC sample manager and sample organizer.
- Always measure other vendor's plates to determine their suitability for use in the ACQUITY UPLC sample manager and sample organizer.
- To avoid warping plates, do not centrifuge them.
- When selecting a new plate supplier, especially for size 384 plates, always compare the plate size to Waters specifications.
- When using PEEK needles:
  - Use pre-slit septa to avoid bending the needle.
  - Use solid septa only when necessary.
  - Use heat-sealed plates instead of cap mats.
  - Do not use re-sealable cap mats, because they can cause alignment errors.

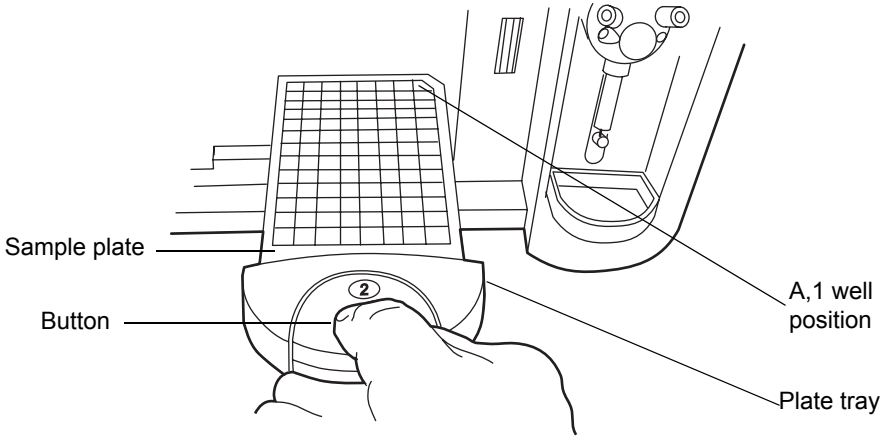
To load a sample plate

1. Open the ACQUITY UPLC sample manager door.
2. Squeeze the tray button while you pull the tray toward you.

3. Load the plate onto the tray so that well position A,1 is at the rear, right-hand corner and the forward edge of the plate is behind the spring inside the front of the carrier.

   Tip: A represents the row number, 1 represents the vial position.

4. Slide the tray into the sample manager until it clicks into place.



Caution: The plates must be positioned correctly to avoid damaging the sample needle.

5. Close the sample compartment door. A mechanism on the door ensures the plates are positioned correctly when the door closes.

Selecting the optimum sample injection mode

The sample manager supports three injection modes – partial loop needle overfill, partial loop, and full loop.

- Partial loop needle overfill mode – Provides superior partial loop accuracy, precision and linearity for a wide range of samples, including strong and weak acids and bases, hydrophilic, and hydrophobic compounds. This mode is the best general purpose mode for partial loop injection.
- Partial loop mode – Use for those situations where analysis time takes precedence over any other concern, where the sample volume is very limited, or where the injection volume is very large.

- Full loop mode – Chosen whenever accuracy and precision are the primary concerns. It is the recommended mode when using 1.0-mm I.D. columns.

Partial loop needle overfill mode

Partial loop overfill mode provides optimum performance when injection volumes are maintained within a range of 10% to 75% measured loop volume. The generally accepted practice for partial loop injection linearity restricts the injection volume selected to ≤ 50% nominal loop volume. However, you can increase the usable loop volume to ≤ 75% measured loop volume by selecting the needle overfill technique. This is based on achieving an injection-to-injection variability of ≤ 1% across the specified volume range. In addition, the correlation between specified injection volume and peak area is $R^2 > 0.999$.

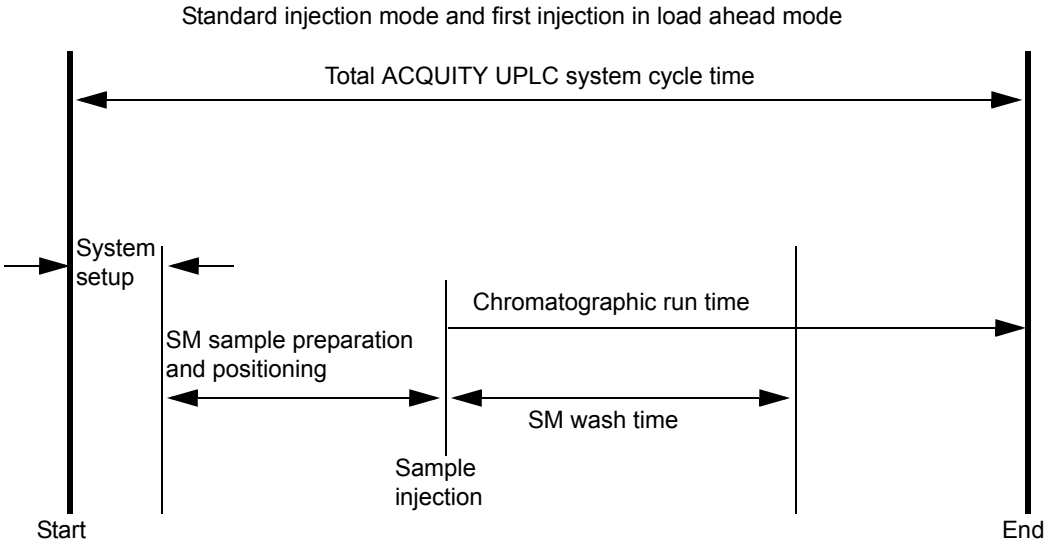
See also: The table titled “Selecting the injection mode and loop volume” on page 2-28.

Load ahead mode

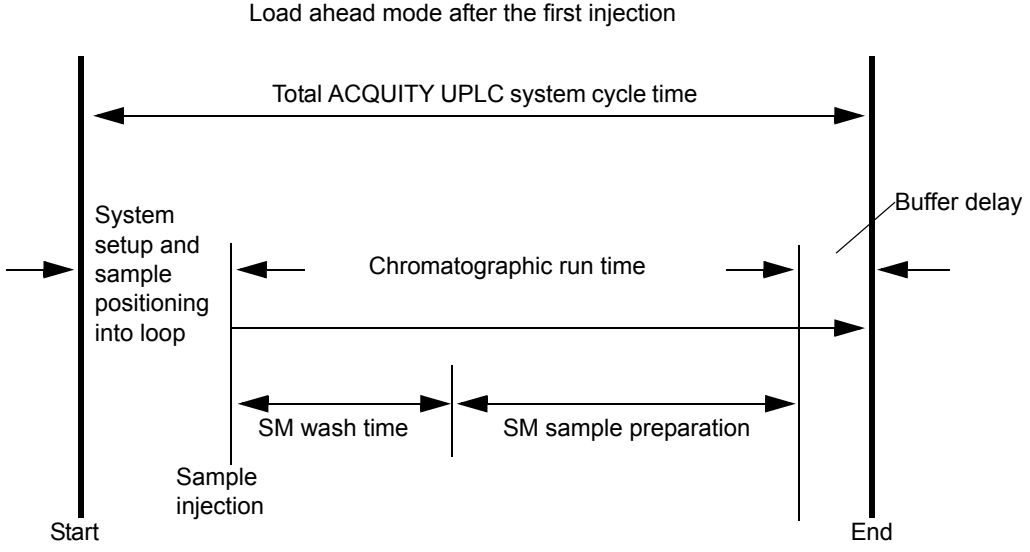
The first injection of a sample set and injection sets with different methods do not utilize load ahead mode. The minimum cycle time is the lesser of either the two run times or sample preparation and wash.

The following figures show a standard ACQUITY UPLC system cycle time and load-ahead cycle time.

Standard ACQUITY UPLC system cycle time definition



ACQUITY UPLC system load-ahead cycle time definition



Tip: The buffer delay is a “wait time” that compensates for variations in the time it takes to load a sample.

The following table provides the minimum and maximum injection volumes for each of the commonly used loops.

Selecting the injection mode and loop volume

Sample injection volume (µL)	Loop volume					
	1 µL	2 µL	5 µL	10 µL	20 µL	50 µL[a]
Partial loop needle overfill mode injection range (µL)	0.1 to .8	0.2 to 1.5	0.5 to 3.8	1.0 to 7.5	2.0 to 15.0	5.0 to 37.0
Partial loop mode injection range (µL)	Not recommended	Not recommended	Not recommended	1.0 to 5.0	2.0 to 10.0	5.0 to 25.0
Full loop mode injection range (µL)	1	2	5	10	20	50

a. The 50-µL loop must be used with a 250-µL sample syringe.

Installing the optional sample manager shade

If your samples are light-sensitive, and you have the optional sample manager foam shade, be sure to install it over the sample compartment window.

For the sample manager foam shade part number, see the Waters Quality Parts Locator on the Waters Web site's Services/Support page.

Required material

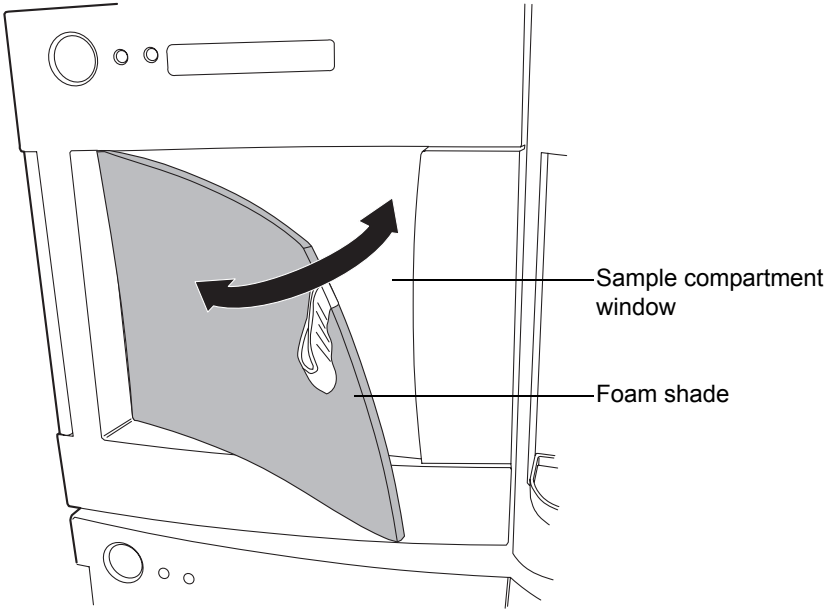
Sample manager foam shade

To install the sample manager foam shade

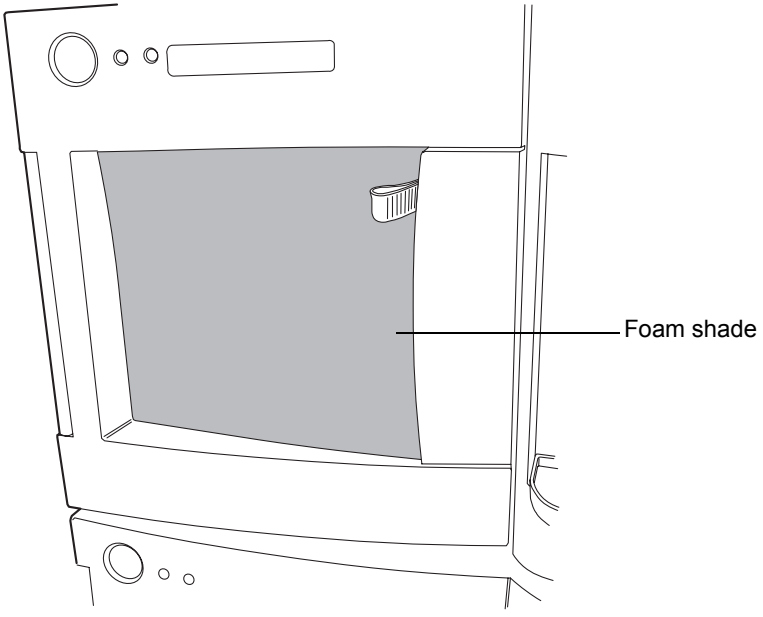
1. Turn off the sample manager compartment lights.

   Tip: To turn the sample manager compartment lights off, right-click in the sample manager control panel and then select Turn lights on/off.

2. Insert the foam shade over the outside of the sample compartment window.



3. Press the foam shade into place over the window.



Preparing the column manager

To prepare the column manager, ensure that each column's eCord is connected to the correct port.

See also: For more information on checking the eCord status, consult the ACQUITY Console online Help.

Preparing the sample organizer

If your system includes a sample organizer, prepare it for operation according to the procedures in this section.

Initiating communications

To initiate communications between the sample manager and sample organizer

1. Open the sample manager door, load a plate onto the right-hand tray, and then close the sample manager door.

   Tip: When the system has both a sample manager and a sample organizer, the right-hand tray of the sample manager becomes position 1, and the left-hand tray is not available. The bottom shelf in the sample organizer becomes position 2.

   Exception: If the system does not include a sample organizer, the left-hand tray in the sample manager is designated position 1 and the right-hand tray is position 2.

2. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
3. In the sample manager information window, click Configure > Sample Organizer.
4. In the Sample Organizer Configuration dialog box, select the sample organizer from the list of serial numbers, and then click OK.
5. The sample organizer automatically detects which shelves contain plates and illuminates their corresponding LEDs.

Loading sample plates

The sample organizer holds up to 21 standard ANSI/SBS footprint plates that you load through the front door. However, the actual number of plates you can load depends on their height. When the system has both a sample manager and sample organizer, the right-hand shelf in the sample manager is referred to as position 1 and the bottom shelf in the sample organizer as position 2.

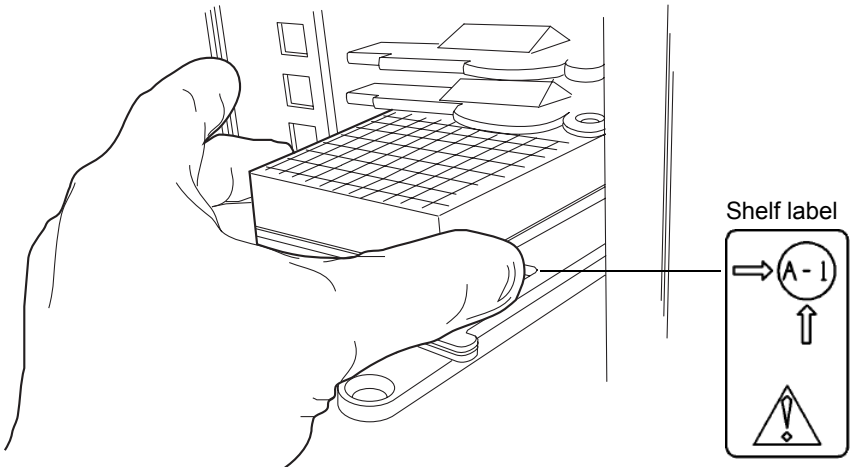
To load sample plates

1. Open the sample organizer door.

   Tip: To keep the sample compartment from freezing, open its door only when necessary. (Opening the door admits humid air into the sample compartment, which causes condensation and freezing.)

2. Pull the shelf toward you.

3. Load the plate onto the shelf so that position A,1 is at the rear, right-hand corner and the forward edge of the plate is behind the stop at the front, left-hand corner.



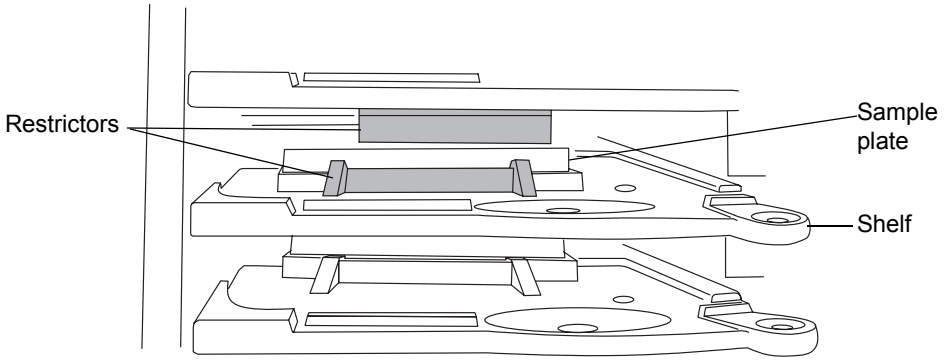
Caution: To prevent spillage, use Waters-approved cap mats, sealing caps, or heat seal film on the samples. Consult the current ACQUITY UPLC system release notes for a list of approved sample covers.

4. Ensure that the plate does not extend beyond the plate stop at the rear of the shelf.

Caution: To ensure the transfer shuttle moves freely and without damaging the sample organizer, you must be able to slide a shelf/plate/vial combination in or out without interfering with the shelves or plates directly above and below it.

5. Slide the shelf into the sample organizer until it stops.

Shelf/plate combination and restrictors



6. Repeat step 2 through step 5 for the remaining plates.

Caution: To avoid jarring the plates from their shelves, do not slam the sample organizer door closed.

7. Close the sample organizer door. A mechanism on the door ensures the shelves are positioned correctly when the door closes.
8. Click Configure > Scan and store shelf layout to update and save the new shelf configuration.

To remove and replace the same plate on the same shelf

1. Open the sample organizer door, and then remove plates that have finished processing.
2. Pull a shelf toward you, and then insert a plate of the same type and size on the shelf.

   Caution: To prevent spillage, use Waters-approved cap mats, sealing caps, or heat seal film on the samples. Consult the current ACQUITY UPLC system release notes for a list of approved sample covers.

3. Load a plate onto a shelf so that position A,1 is at the rear, right-hand corner and the forward edge of the plate is behind the stop at the front, left-hand corner. Ensure that the plate does not extend beyond the plate stop at the back of the shelf.

   Caution: To ensure the transfer shuttle moves freely and without damaging the sample organizer, you must be able to slide a shelf/plate/vial combination in or out without interfering with the shelves or plates directly above and below it.

4. Slide the shelf into the sample organizer until it stops.
5. Repeat step 3 and step 4 until all plates and holders are placed correctly on the shelves.

   Caution: To avoid jarring the plates from their shelves, do not slam the sample organizer door closed.

6. Close the sample organizer door. A mechanism on the door ensures the shelves are positioned correctly when the door closes.
7. Click Verify. The sample organizer automatically scans the plates and shelves, senses which shelves contain plates, compares them to the saved layout, verifies that they match, and illuminates the corresponding LEDs inside the sample organizer door.
8. Configure the plates and shelves in the Empower or MassLynx data application.

   Rule: You can load plates and shelves either before or after configuring them in the data application, but you must configure them before running samples.

To rearrange the shelves for a different plate configuration

1. Open the sample organizer door, and then remove plates that have finished processing.
2. Add, move, or remove shelves from the sample organizer so that the shelf configuration suits the plates you intend to run.

   Tip: Standard microtiter plates need one slot. Intermediate plates need two slots, so allow empty slots above the plate or holder. Deep-well plates and all vial holders need three slots, so allow two empty slots above the plate or holder.

   Caution: To prevent spillage, use Waters-approved cap mats, sealing caps, or heat seal film on the samples. Consult the current ACQUITY UPLC system release notes for a list of approved sample covers.

3. Load a plate onto a shelf so that position A,1 is at the rear, right-hand corner and the forward edge of the plate is behind the stop, at the front, left-hand corner. Ensure that the plate does not extend beyond the plate stop at the back of the shelf.

   Caution: To ensure the transfer shuttle moves freely and without damaging the sample organizer, you must be able to slide a shelf/plate/vial combination in or out without interfering with the shelves or plates directly above and below it.

4. Slide the shelf into the sample organizer until it stops.
5. Repeat step 3 and step 4 until all plates and holders are placed correctly on shelves.

   Caution: To avoid jarring the plates from their shelves, do not slam the sample organizer door closed.

6. Close the sample organizer door. A mechanism on the door ensures the shelves are positioned correctly when the door closes.
7. Click Configure > Scan and store shelf layout.

   The sample organizer initializes and scans the shelves. When it detects a new shelf, it illuminates the LED to the left-hand side of the shelf, inside the sample organizer door.

Tip: In the ACQUITY UPLC Console, a thin, gray bar appears for each empty shelf. After the plate or shelf is configured using Empower or MassLynx software, plate identification appears in a thicker bar.

8. Configure the plates and shelves in the Empower or MassLynx data application.

   Rule: You can load plates and shelves either before or after configuring them in the data application, but you must configure them before running samples.

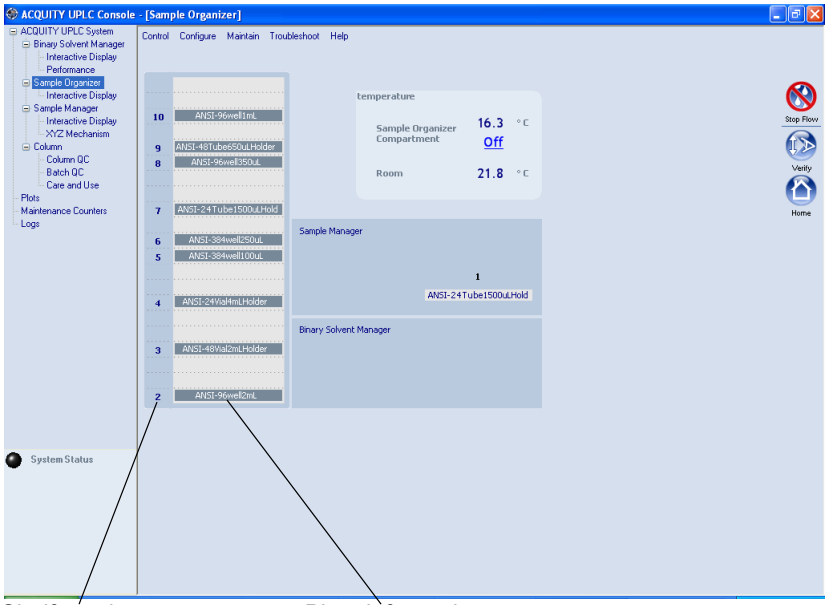
Displaying sample plate information

To display sample plate information

1. In the ACQUITY UPLC Console, select Sample Organizer from the system tree.
2. In the sample organizer information window, click Configure > Scan and store shelf layout to update and save the configuration of plates on shelves.

   The number designations of shelves that contain sample plates appear beside information about the plates.

Sample organizer information window



Tips:

- The shelf and plate information appear only after method setup.
- Move the pointer over a shelf to display the number of samples remaining to be run from that shelf.

Starting the TUV detector

If your system includes a TUV detector, prepare it for operation by following the procedures in this section.

If your system includes a PDA detector, see the *ACQUITY UPLC Photodiode Array Detector Getting Started Guide* for information on preparing it.

If your system includes an ELS detector, see the *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide* for information on preparing it.

If your system includes an FLR detector, see the *ACQUITY UPLC Fluorescence Detector Getting Started Guide* for information on preparing it.

If your system includes a mass spectrometer, see the documentation that accompanies the instrument for information on preparing it.

Starting the TUV detector

Caution: Use only thoroughly degassed HPLC-grade solvents. Gas in the mobile phase can form bubbles in the flow cell and cause the detector to fail the startup diagnostic tests.

Tip: To prevent errors on startup, be sure the flow cell contains degassed, transparent solvent (acetonitrile or water) and the detector door is closed firmly.

To start the TUV Detector

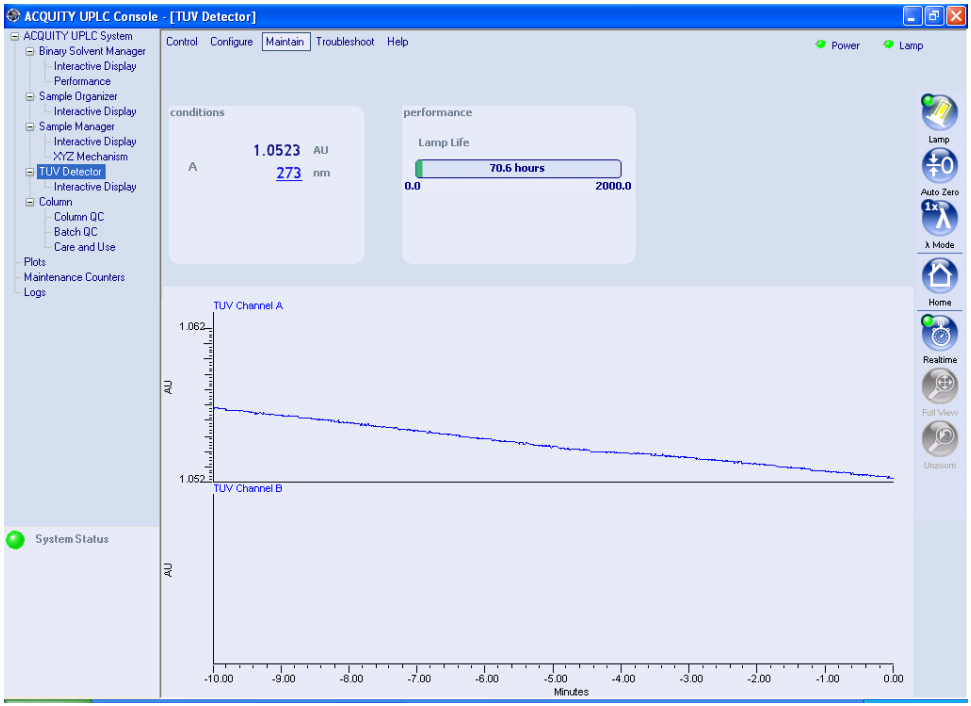
1. Ensure the detector flow cell is filled with degassed, transparent solvent (acetonitrile or water) and free of air bubbles.
2. Ensure the detector door is closed firmly.
3. Press the power switch on the door to power-on the detector. The detector beeps 3 times and runs a series of startup tests while the lamp LED blinks. The power LED shows steady green.

   Tip: Initialization usually requires approximately 2 minutes, and lamp warm-up requires approximately 3 minutes.
4. When the lamp LED shows steady green, start the Empower or MassLynx software. You can monitor the ACQUITY UPLC Console for messages and LED indications. For best results, allow at least 30 minutes to equilibrate the detector and stabilize the baseline.

   Tips:

   - The absorbance value appears in the ACQUITY UPLC Console and also in Empower's Run Samples window or MassLynx's Inlet Editor window. If the detector is in dual wavelength mode, two absorbance values appear.
   - Absorbance values have a resolution of 0.0001 AU.
   - When the lamp is extinguished, "Lamp Off" appears in the software instead of absorbance values.
5. Configure the detector according to the instructions in the Empower or MassLynx online Help, or as provided in "Configuring Empower software" on page 3-1 and "Configuring MassLynx software" on page 3-13.

TUV detector information window



Recording sample and reference energies

After you install the detector or perform maintenance tasks, like changing the lamp or flow cell, complete the procedures in this section to verify that the detector optics and electronics work properly.

To record sample and reference energies

1. Ensure that the detector is connected to the workstation.
2. Flush the system tubing with filtered, degassed HPLC-grade acetonitrile.

   Caution: The maximum allowable pressure drop across the flow cell is 6895 kPa (69 bar, 1000 psi). If the solvent is viscous (methanol-water, for example), decrease the maximum flow rate to prevent breaking the cell.

3. Pump mobile phase for 15 minutes or more at 0.3 mL/min.

4. Ensure the detector cell is filled with solvent and free of air bubbles.
5. When both LEDs show steady green, initialization is complete.
6. Start the Empower or MassLynx software.
7. Launch the ACQUITY UPLC Console from the sample manager control panel.

   For additional information, see "Starting the ACQUITY UPLC Console from Empower software" on page 3-12 and "Starting the ACQUITY UPLC Console from MassLynx software" on page 3-14.
8. Select the TUV detector view in the ACQUITY UPLC Console.
9. Set the wavelength to 230 nm.
10. In the Console, select TUV Detector > Interactive Display from the system tree.
11. Record the sample and reference energies at 230 nm.

Conditioning the column

Conditioning the column involves running a solvent gradient through it without injecting samples or running the Events table. The run time for conditioning the column must equal the gradient table run time.

Caution: To prevent damage to the detector flow cell, ensure that the waste solvent does not flow through the detector during this procedure. After installing a new column, flush solvent through it and out to waste before connecting the column to the detector (for example, 10 column volumes).

To condition the column

1. Remove the column inlet line from the detector, and place the end in a small waste container.
2. If Empower software controls the system, proceed as follows:
   a. In the Samples table, add a row to the method.
   b. Select Equilibrate/Condition Column (Isocratic or Gradient) as the function in the new row.
   c. Run the separation method.

3. If MassLynx software controls the system, proceed as follows:

    a. Open the Sample Set window, and select an inlet method that includes the chromatographic conditions you want to use.

    b. Run the sample set line. The system runs the separation method.

See also: For more information about column conditioning, consult the Empower or MassLynx online Help.

Shutting down the system

Caution: Buffers left in the detector can precipitate and damage instrument components.

Tips:

- If you are using Empower software to control the system, set system shutdown parameters in the Instrument Method. Consult the Empower online Help for more information.
- If you are using MassLynx software to control your system, set system shutdown parameters in the Shutdown Editor. Consult the MassLynx online Help for more information.

Shutting down for less than 24 hours

To shut down the system for less than 24 hours

1. Continue to pump the initial mobile phase mixture through the column. Doing so maintains the column equilibrium necessary for good retention time reproducibility.
2. To lengthen lamp life, extinguish the detector lamp by clicking  (Lamp Off) in the detector control panel.

    Tip: If you are running under MassLynx control, ensure that Auto-Shutdown for your shutdown method is deactivated.
3. If a few hours will pass before the next injection, slow the flow rate in the interim to a few tenths of a mL/min to conserve solvent.

    Tip: Ensure that the shutdown method is deactivated.
4. Keep the detector operating and the column heater at operating temperature during this period.

Shutting down for more than 24 hours

To shut down the system for more than 24 hours

1. To lengthen lamp life, extinguish the detector lamp by clicking  (Lamp Off) in the detector control panel.
2. Remove buffer salts and additives by flushing with water.
3. Flush the column and flow cell with 100% pure organic solvent.

   See also: Waters *ACQUITY UPLC BEH Column Care and Use Instructions* or *ACQUITY UPLC HSS Column Care and Use Instructions*.

   Warning: Risk of electric shock. The power switch on each system instrument controls the basic operational state of that instrument. Nevertheless, some instrument circuits remain live after the instrument is switched off. To completely interrupt power to a system instrument, set the power switch to Off, and then unplug the instrument's power cord from the AC outlet.

4. Power-off the system.

   Alternative: If you prefer to leave the system powered-on, turn off the column heater or reduce the column heater temperature to 40 °C (104 °F).

   Caution: Before using any system or instruments that have been shut down, under the recommended conditions, ensure that the new mobile phase is miscible with the recommended storage solvents: water/methanol or water/acetonitrile. If the mobile phase and solvents for the new analysis are not directly miscible with the recommended storage solvents, ensure that an intermediate solvent, one that is miscible with both the storage solvents and those for the new analysis, is used to flush the storage solvents from the system and all of its components.

5. Cap the flow cell inlet and outlet ports.

Running HPLC methods on an ACQUITY UPLC system

For more information on transferring methods, consult the ACQUITY Console online Help.

The Waters ACQUITY UPLC Columns Calculator (provided with your ACQUITY UPLC software and also available on the Waters Web site) automates the scaling calculations required to convert isocratic or gradient HPLC methods to UPLC methods.

System considerations

Traditional HPLC methods run on an ACQUITY UPLC system can differ in retention time—and in some cases peak order—from the same methods run on traditional HPLC equipment. The differences are primarily due to lower system volume and reduced bandspreading in the ACQUITY UPLC system, which operates with a maximum injection volume of 50 µL and a maximum flow rate of 2 mL/min. You can minimize discrepancies between HPLC and UPLC methods by compensating for the differences in system volume.

The ACQUITY UPLC system uses a loop-based autosampler. The amount of sample loaded on the column for a given injection volume, and the subsequent sample recovery, can be different from when you use a traditional HPLC injector (direct-inject style). So an injection volume of 10 µL on a UPLC system can yield different area counts from an equivalent volume injected on an HPLC system. Full loop injections yield better recoveries (lower area-count losses) than those made using partial loop injection modes. If an appropriate sample loop is available (one with a characterized loop volume that matches your desired injection volume), Waters recommends using full loop injections when initially transferring an HPLC method to an ACQUITY UPLC system. If necessary, you can adjust injection volumes or change injection modes after the initial method transfer.

For information on available sample loop sizes, see the Waters Quality Parts Locator on the Waters Web site's Services/Support page.

For more information on ACQUITY UPLC injection modes, consult the ACQUITY Console online Help.

Requirements for using an HPLC column on an ACQUITY UPLC system

Observe the following requirements when fitting an HPLC column on an ACQUITY UPLC system:

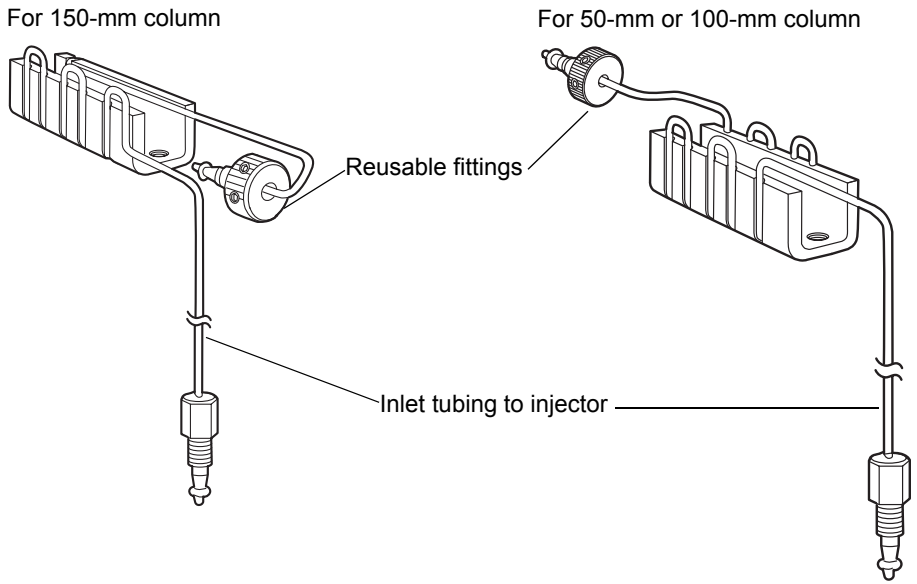
- When running columns without an eCord, verify that the console software is configured to ignore the absence of the eCord.

  For information on how to enable/disable the eCord column chip requirement, consult the ACQUITY Console online Help.

- Install the column stabilizer. For 150-mm columns, use the optional 150-mm column stabilizer.

  For detailed instructions on how to install the column stabilizer, see the *Waters ACQUITY UPLC System Operator's Guide*.

Column stabilizer assembly (two styles)



- Do not use the ACQUITY UPLC column in-line filter unit with an HPLC column.
- Set the high pressure limit of the binary solvent manager to the maximum pressure recommended for the HPLC column.

Recommendation: To reduce variability from temperature fluctuations, use a column oven with a thermostat. For columns longer than 150 mm, use the

ACQUITY UPLC system 30-cm column heater/cooler or another appropriate external oven.

Tip: If you observe a discrepancy between the selectivity and resolution of the HPLC and UPLC methods, ensure that you properly compensated for system volume differences. If the discrepancy persists, adjust the temperature of the column heater by +/-2 °C and assess the result.

Choosing fittings

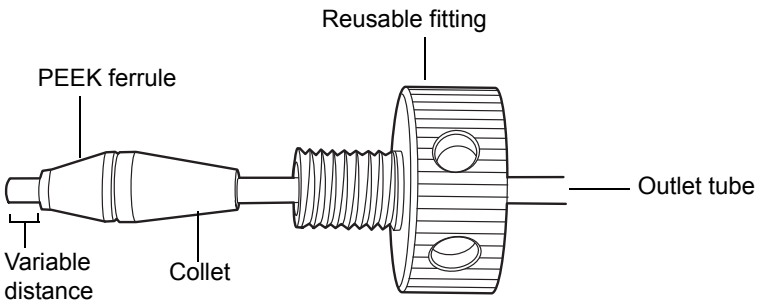
Column inlet

When connecting the column stabilizer to an HPLC column, choose among the following types of fittings:

- Use the Waters-supplied, reusable, UPLC fitting.
- Replace the three-piece Waters fitting with a one-piece, reusable fitting suitable for your pressure and temperature requirements.
- Use a stainless-steel (swage) fitting recommended by the HPLC column manufacturer.

Waters supplies a finger-tight, reusable fitting for connecting the column stabilizer to the column inlet. The fitting assembly consists of a ferrule (PEEK), collet, and compression screw. Because the position of the ferrule is adjustable, you can use this fitting assembly with any style of column end-fitting.

Column stabilizer reusable fitting



Note: Older reusable fittings do not have holes in the side of them.

For more information on replacing fittings and installing columns, see the *Waters ACQUITY UPLC System Operator's Guide*.

If you prefer the convenience of a single-piece fitting, you can replace the three-piece Waters fitting with a single-piece fitting that meets your temperature and pressure requirements.

Caution: To avoid leaks and connection failures, do not exceed the temperature or pressure limitations of your fittings.

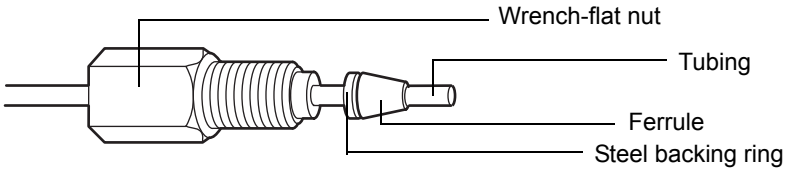
You can also use stainless-steel fittings recommended by your HPLC column's manufacturer. For stainless-steel fittings, the ferrule position remains fixed (swaged) once it is set. The distance from the ferrule to the end of the tubing when connected to a UPLC column is approximately 3 mm. Your HPLC column can require a different distance. Ensure that the distance between the ferrule and the end of the tubing is appropriate for the end-fitting of the column you are using. Band broadening and leaks can occur if the fittings are not properly seated.

Column outlet

If your system has a Waters-supplied, wrench-flat, column outlet fitting that has been used for a UPLC column connection, replace the swage fitting with a reusable fitting of your choice, or a swage fitting recommended by the HPLC column manufacturer.

For all fittings, ensure that the distance between the ferrule and the end of the tubing is appropriate for the end-fitting of the HPLC column you are using.

Wrench-flat column outlet fitting



For information on tubing and fittings for post-column tubing connections, see the *Waters ACQUITY UPLC System Operator's Guide* and the appropriate detector user documentation.

3 Configuring System Software

Contents

Configuring Empower software

Perform these tasks to configure Empower software:

- Start the software and log in
- Select system instruments
- Name the system

Starting Empower software and logging in

To start Empower software and log in

1. Select Start > Programs (for Windows XP, All Programs) > Empower > Empower Login.

   Alternative: Start the Empower software through the Empower desktop shortcut.

2. In the Empower Login dialog box, type your user name and password.
3. Click OK.

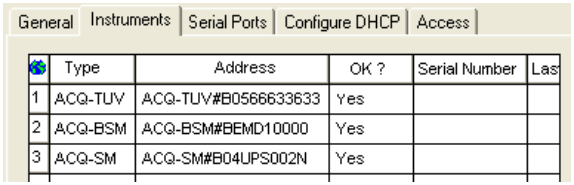
Selecting system instruments

To select system instruments

1. In the Empower Pro window, click Configure System.
2. In the Configuration Manager window, click Acquisition Servers, right-click the node name, and then select Properties.

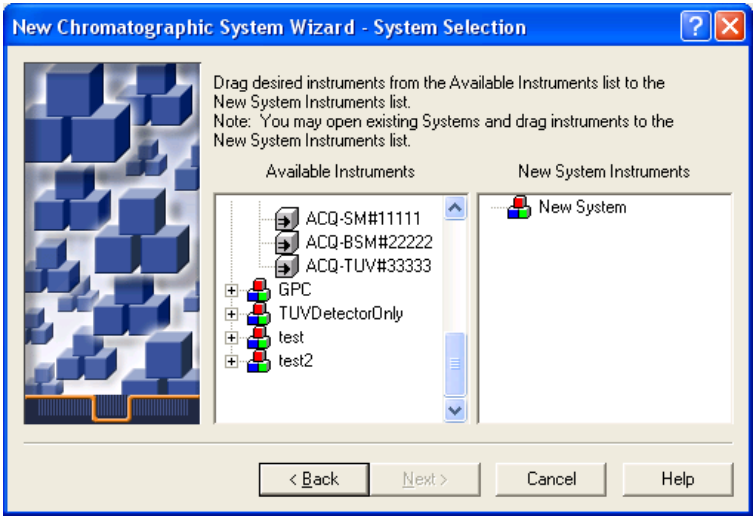
   Tip: If you are using Empower 1154 software to control the system, the node name is referred to as the acquisition server name.
3. In the Acquisition Server dialog box, click the Instruments tab. The system instruments that are successfully communicating with your system are shown with a Yes in the "OK?" column.

Instruments tab



4. Ensure that a binary solvent manager (ACQ-BSM), sample manager (ACQ-SM), and detector (ACQ-TUV, ACQ-PDA, or ACQ-ELS) appear in the instrument list and are successfully communicating with your system, and then click OK.
5. Right-click Systems, and then select New > Chromatographic System.
6. In the System Type area of the New Chromatographic System Wizard dialog box, select Create New System, and then click Next.

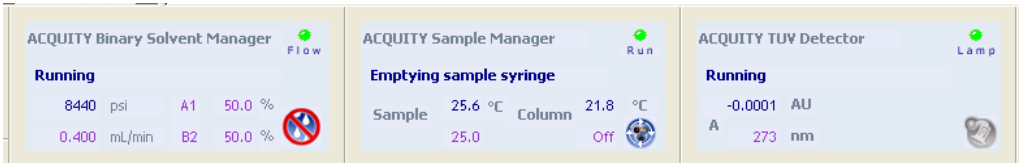
System Selection dialog box



7. In the System Selection dialog box, drag the name of the instrument(s) you want to include in the new system from the Available Instruments pane to the New System Instruments pane. Click Next.
8. When the Access Control dialog box appears, click Next.
9. In the Name Selection dialog box, specify a name for your system. Enter comments, if any, and then click Finish. A confirmation dialog box appears.
10. Click Projects, right-click a project, and then select Open.
11. In the Project window, click Run Samples  .

    Alternative: Access the Run Samples window via the Empower QuickStart menu.
12. On the Run Samples window, you can monitor control panels for the binary solvent manager, sample manager, detector, and optional column manager.

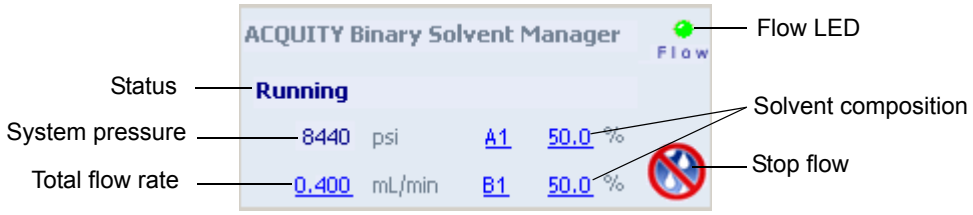
Control panels



About the binary solvent manager control panel

If Empower software controls the system, the binary solvent manager's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the binary solvent manager's control panel appears on the Additional Status tab of the Inlet Editor window.

Binary solvent manager control panel



The binary solvent manager control panel displays flow status, system pressure, total flow rate, and solvent composition parameters.

Rule: You can edit these parameters when the system is idle by clicking on the underlined value. You cannot edit binary solvent manager parameters while the system is running samples.

The following table lists the items in the binary solvent manager control panel.

Binary solvent manager control panel items

Control panel item	Description
Flow LED	Displays the actual flow LED on the front panel of the binary solvent manager unless communications with the binary solvent manager are lost.
Status	Displays the status of the current operation.
System Pressure	Displays the binary solvent manager pressure, in kPa, bar, or psi. Pressure units can be customized through the ACQUITY UPLC Console.
Total Flow Rate	Displays the total flow rate of the binary solvent manager. Total flow rate values range from 0.000 to 2.000 mL/min under normal operation and 0.000 to 8.000 mL/min when priming.
Solvent Composition	Displays the percentage of solvent (1 and 2) to be drawn from the pumps (A and B). Composition values range from 0.0 to 100.0%.
 (Stop Flow)	Immediately stops all flow from the binary solvent manager.

You can access these additional functions by right-clicking anywhere in the binary solvent manager control panel:

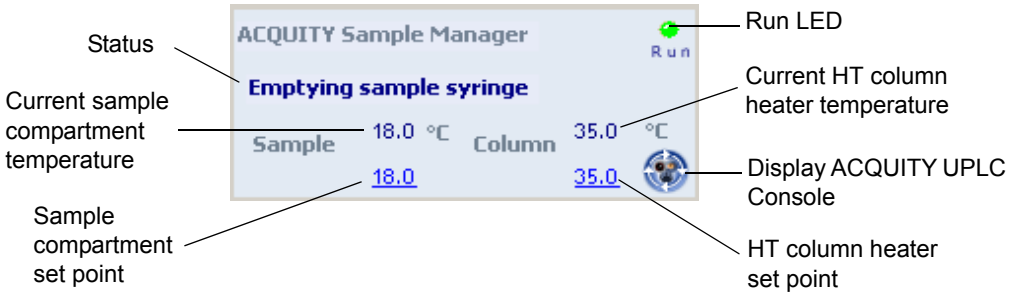
Additional functions in the binary solvent manager control panel

Control panel function	Description
Refresh system (Sys Prep)	Refreshes the fluid-carrying lines according to the current method conditions. See "Refreshing the system" on page 2-12.
Startup	Brings the system to operational conditions after an extended idle period or when switching to different solvents. See "Starting up the system" on page 2-13.
Prime A/B solvents	Displays the Prime A/B Solvents dialog box. See "Priming the binary solvent manager" on page 2-9.
Prime seal wash	Displays the Prime Seal Wash dialog box. See "Performing a seal wash prime" on page 2-7.
Reset BSM	Resets the binary solvent manager after an error condition.
Help	Displays the ACQUITY UPLC Console online Help.

About the sample manager control panel

If Empower software controls the system, the sample manager's control panel appears at the bottom of the Run Samples window. If Masslynx software controls the system, the sample manager's control panel appears on the Additional Status tab of the Inlet Editor window.

Sample manager control panel



The sample manager control panel displays current sample compartment and HT column heater temperatures and set points. You can edit these values when the system is idle by clicking on the underlined value. You cannot edit sample manager set points while the system is running samples.

Tip: To keep the sample compartment from freezing, open its door only when necessary. (Opening the door admits humid air into the sample compartment, which causes condensation and freezing.)

The following table lists the items in the sample manager control panel.

Sample manager control panel items

Control panel item	Description
Run LED	Displays the actual run LED on the front panel of the sample manager unless communications with the sample manager are lost.
Status	Displays the status of the current operation.
Current Sample Compartment Temperature	Displays the current sample compartment temperature to 0.1 °C resolution. When active temperature control is disabled, this field displays “Off”.
Sample Compartment Set Point	Displays the current sample compartment set point to 0.1 °C resolution. When active temperature control is disabled, this field displays “Off”.

Sample manager control panel items (Continued)

Control panel item	Description
Current Column Heater Temperature	Displays the current column heater temperature to 0.1 °C resolution, even when active temperature control is disabled.
Column Heater Set Point	Displays the current column heater set point to 0.1 °C resolution. When active temperature control is disabled, this field displays “Off”.
 (Display Console)	Displays the ACQUITY UPLC Console.

You can access these additional functions by right-clicking anywhere in the sample manager control panel:

Additional functions in the sample manager control panel

Control panel function	Description
Run Sys Prep	Primes the sample manager with one weak wash prime (wash and sample syringes). See “Refreshing the system” on page 2-12.
Prime syringes	Displays the Prime Syringes dialog box. See “Priming the sample manager” on page 2-17.
Wash needle	Displays the Wash Needle dialog box. See “Washing the sample manager needle” on page 2-19.
Turn lights on/off	Turns the sample manager compartment and optional sample organizer lights on or off.

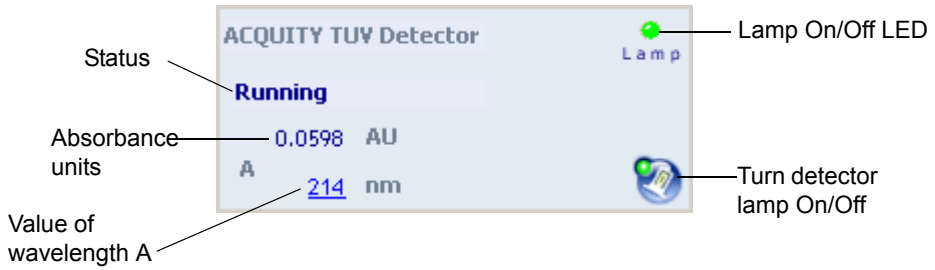
Additional functions in the sample manager control panel (Continued)

Control panel function	Description
Reset SM	Resets the sample manager after an error condition.
Help	Displays the ACQUITY UPLC Console online Help.

About the TUV detector control panel

If Empower software controls the system, the TUV detector's control panel appears at the bottom of the Run Samples window. If Masslynx software controls the system, the detector's control panel appears on the Additional Status tab of the Inlet Editor window.

TUV detector control panel



The TUV detector control panel displays absorbance units and wavelength values. You can edit these parameters when the system is idle by clicking on the underlined value. You cannot edit detector parameters while the system is running samples.

If your system includes a PDA detector, see the *ACQUITY UPLC Photodiode Array Detector Getting Started Guide* for information on the control panel.

If your system includes an ELS detector, see the *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide* for information on the control panel.

If your system includes an FLR detector, see the *ACQUITY UPLC Fluorescence Detector Getting Started Guide* for information on the control panel.

The following table lists the items in the TUV detector control panel.

TUV detector control panel items

Control panel item	Description
Lamp On/Off LED	Displays the actual lamp on/off LED on the front panel of the detector unless communications with the detector are lost.
Status	Displays the status of the current operation.
AU	Displays the absorbance units.
nm	Displays the value of wavelength A, in nm. If the detector is in dual wavelength mode, the value of wavelength B also appears.
 (Lamp On)	Ignites the detector lamp.
 (Lamp Off)	Extinguishes the detector lamp.

You can access these additional functions by right-clicking anywhere in the detector control panel:

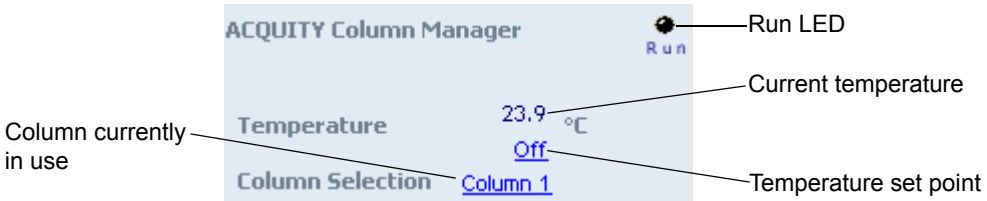
Additional functions in the detector control panel

Control panel function	Description
Autozero	Resets the absorbance value to 0
Reset TUV	Resets the detector, when present, after an error condition
Help	Displays the ACQUITY UPLC Console online Help

About the column manager control panel

If Empower software controls the system, the column manager's control panel appears at the bottom of the Run Samples window. If Masslynx software controls the system, the column manager's control panel appears on the Additional Status tab of the Inlet Editor window.

Column manager control panel



The column manager control panel displays the current column temperature and set point. You can edit the set point when the system is idle by clicking on the underlined value. You cannot edit any underlined values (temperature set point and column selection) while the system is running samples.

The following table lists the items in the column manager control panel.

Column manager control panel items

Control panel item	Description
Run LED	Displays the actual run LED on the front panel of the column manager unless communications with the column manager are lost.
Temperature	Displays the current column compartment temperature and set point to 0.1 °C resolution. When active temperature control is disabled, this field displays "Off".
Column	Displays the column that is currently in use.

You can access these additional functions by right-clicking anywhere in the column manager control panel:

Additional functions in the column manager control panel

Control panel function	Description
Reset CM	Resets the column manager, when present, after an error condition
Help	Displays the ACQUITY UPLC Console online Help

Starting the ACQUITY UPLC Console from Empower software

To start the ACQUITY UPLC Console from Empower software

In the Run samples window, click Display console  in the Sample Manager control panel.

ACQUITY UPLC Console window

Configuring MassLynx software

Perform these tasks to configure MassLynx software:

- Start the application
- Select system instruments

To start MassLynx software

1. Select Start > Programs > MassLynx > MassLynx.

   Alternative: Use the MassLynx desktop shortcut.

   If MassLynx Security is not enabled, MassLynx software starts, and the MassLynx window appears. If MassLynx Security is enabled, the MassLynx Login dialog box appears.

2. Type your user name and password, and select your domain.
3. Click OK. The MassLynx window appears.

To select system instruments

1. In the MassLynx window, click Inlet Method.
2. In the Inlet Method window, select Instrument Configuration from the Tools menu of the Inlet Method window.
3. In the Inlet Configuration window, click Configure and then click Next.
4. In the Select Pump dialog box, select Waters ACQUITY as the pumping device, and then click Next.
5. Select Waters ACQUITY as the autosampler, and then click Next.
6. Select Waters ACQUITY TUV, Waters ACQUITY PDA, or Waters ACQUITY ELS as the detection device, and then click Next.
7. Click Next.
8. Click Finish.
9. Click Finish, and then click OK.
10. In the Instrument Control Option Pack dialog box, ensure that “Install new instrument software or upgrade existing installation(s)” is selected, and then click Next.

11. Select ACQUITY Binary Solvent Manager, ACQUITY Sample Manager, ACQUITY Column Manager, and ACQUITY TUV Detector (or ACQUITY PDA Detector and/or ACQUITY ELS Detector), and then click Next. A progress bar appears at the bottom of the dialog box.
12. When the instrument control option pack installation is finished, the Results screen of the Instrument Control Option Pack dialog box appears.
13. Click Finish. The Inlet Method window appears.

Starting the ACQUITY UPLC Console from MassLynx software

To start the ACQUITY UPLC Console from MassLynx software

1. In the MassLynx window, click Inlet Method.
2. In the Inlet Method window, click the ACQUITY Additional Status tab.
3. Click Display console  .

4 Verifying System Operation

This chapter explains how to run a gradient performance test on a system with a TUV detector to verify that your system is operating properly. The sample you use to verify the system is included in the system startup kit.

To ensure you have the latest version of this procedure, visit http://www.waters.com and click Waters Division > Services & Support.

Contents

Before you begin this procedure, your system must be set up and configured as described in the *Waters ACQUITY UPLC System Operator's Guide*.

Restrictions:

- This gradient performance test is not applicable to ACQUITY UPLC® systems that have a mass spectrometer.
- There is no gradient performance test for ACQUITY UPLC systems that have an ELS detector.

Preparing the system

Preparation is the same whether the system is controlled by the Empower or MassLynx data system.

Requirements: Because of the increased sensitivity of the ACQUITY UPLC system and detectors

- All solvents, including water and additives, must be of the highest chemical purity (MS-grade). Failure to use MS-grade solvents results in high background concentration, low signal-to-noise ratios, and loss of sensitivity.
- All MS-grade solvents used with the ACQUITY UPLC system must be properly filtered prior to their use. Waters recommends that the solvent be filtered through an appropriate .22-µm or smaller membrane filter using a solvent clarification kit (47-mm all-glass filter holder with 1-L flask) immediately before use. A general-purpose laboratory vacuum pump is also required for use with the all-glass solvent filtration apparatus.
- Glassware (such as solvent bottles) must not be washed with detergents or with other general glassware to prevent contamination. The glassware must be rinsed with the high-purity solvents to be used.

  See also: *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD.

For the system verification test, the mobile phases and wash solvents must be mixed as follows:

- 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

To prepare the system for verification

Warning: To avoid chemical exposure risk, always observe Good Laboratory Practices when you use this equipment and when you work with solvents and test solutions. Know the chemical and physical properties of the solvents and test solutions you use. See the Material Safety Data Sheet for each solvent and test solution in use.

1. Prepare a 10:90 acetonitrile/water mobile phase:
   a. Measure 100 mL of filtered acetonitrile into a 100-mL graduated cylinder.
   b. Carefully transfer the acetonitrile to a 1-L reservoir bottle.
   c. Measure 900 mL of filtered HPLC-grade water into a 1000-mL graduated cylinder.
   d. Carefully transfer the water to the same 1-L reservoir bottle.
   e. Cap the reservoir bottle and mix well.
   f. Label the reservoir bottle as 10:90 acetonitrile/water.
   g. Submerge lines A1, B2, plunger seal wash, strong needle wash, and weak needle wash in the reservoir bottle containing the 10:90 acetonitrile/water.
   h. Place the reservoir bottle in the solvent tray.
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 as acetonitrile.
   c. Submerge lines A2 and B1 in the acetonitrile reservoir bottle.

d. Place the reservoir bottle in the solvent tray.

Caution: Never change directly between immiscible eluents or between buffered solutions and organic eluents. Immiscible eluents form emulsions in the flow path. Buffered solutions and organic eluents in combination can result in salt precipitation in the gradient proportioning valves, pump heads, check valves, or other parts of the system. Confirm that all fluids in the system are miscible with acetonitrile. If you need additional information about priming your system, see "Priming the binary solvent manager" on page 2-9.

3. Install the ACQUITY UPLC column in the column heater, close the column tray, and replace the column heater's front cover. If you need more information about installing the column, see the *Waters ACQUITY UPLC System Operator's Guide*.
4. Access the ACQUITY UPLC Console and perform these tasks:
   a. Prime each solvent line of the binary solvent manager for 10 minutes. See "Priming a wetted binary solvent manager" on page 2-12.
   b. Prime the seal wash of the binary solvent manager for several minutes. See "Priming a wetted binary solvent manager" on page 2-12.
   c. Prime the sample manager at least 5 times. See "Priming the sample manager" on page 2-17.
   d. Perform the seal characterization procedure in the sample manager. See "Characterizing the needle seal" on page 2-21.
   e. Calibrate the needle and loop volume in the sample manager.

   Alternative: Use the Refresh (Sys Prep) function to prime the binary solvent manager. See "Refreshing the system" on page 2-12.
5. Prepare the sample as listed on the sample instructions, using 10:90 acetonitrile/water.
6. Place the sample in the vial plate, noting the vial position, and put the plate in position 2 of the sample manager.

Creating the test methods

The gradient performance test method parameters are the same whether Empower or MassLynx software controls the system. Follow the steps below to create the methods, setting the parameter values to match those pictured in the screen representations.

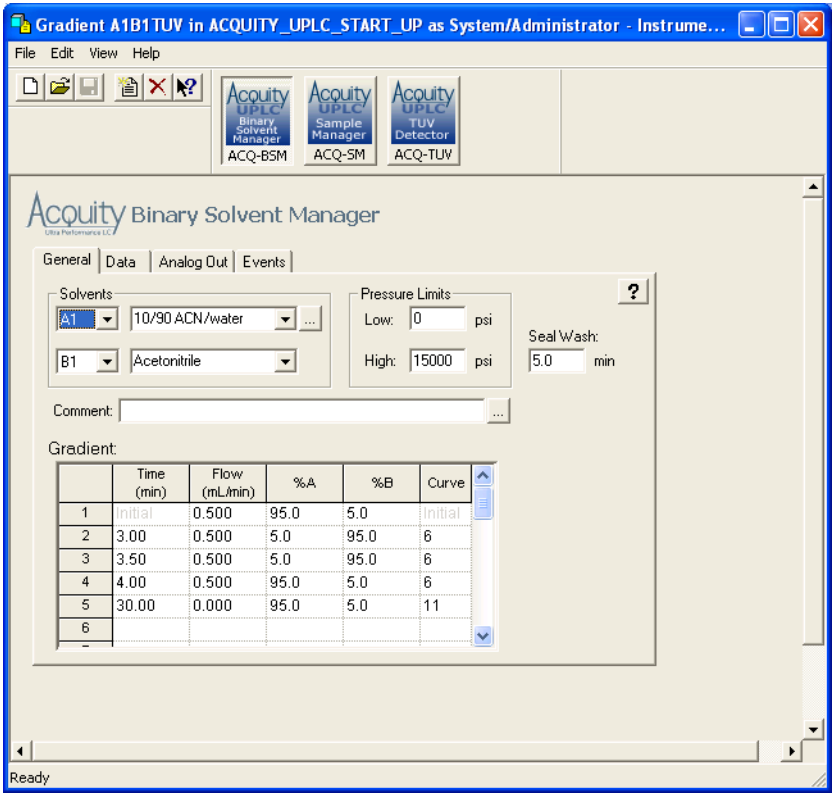
Tip: Click ? on the tabs to display online Help.

Creating the instrument method

To create the instrument method

1. Create an instrument method with the binary solvent manager parameters shown in the following screen representation.

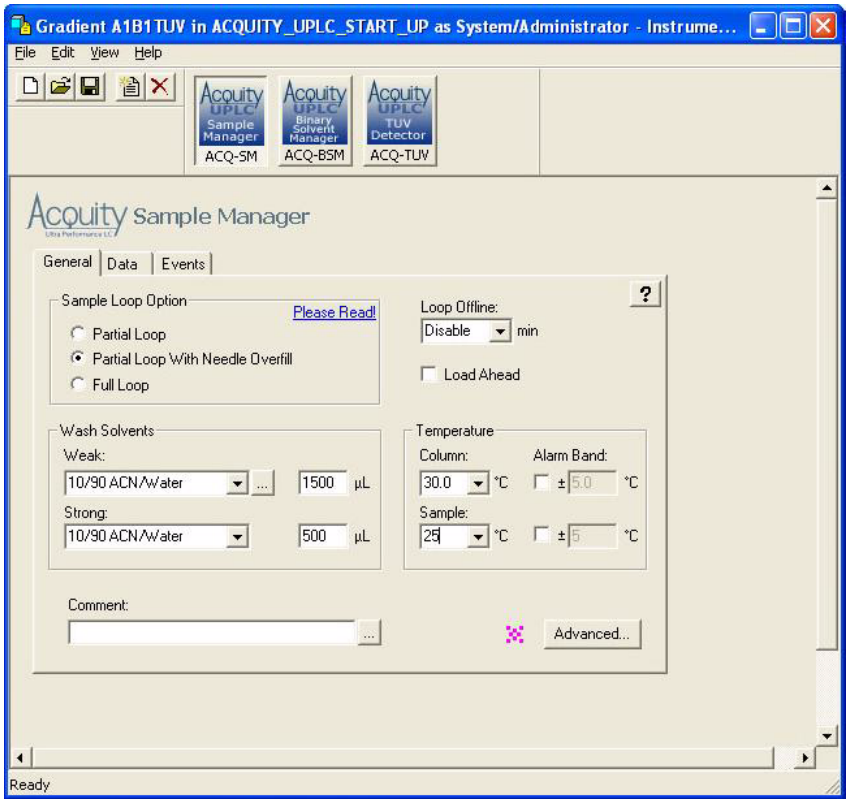
Binary solvent manager instrument parameters



Tip: The binary solvent manager parameters are the same regardless of whether your system includes the TUV or PDA detector.

2. Set instrument method parameters for the sample manager as shown in the following screen representation.

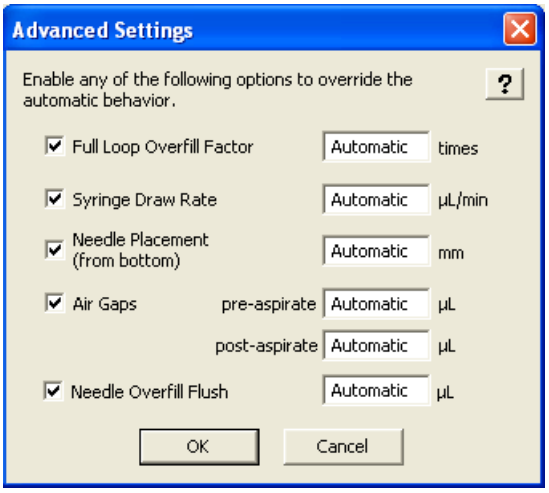
Sample manager instrument parameters



Tip: The sample manager parameters are the same regardless of whether your system includes the TUV or PDA detector.

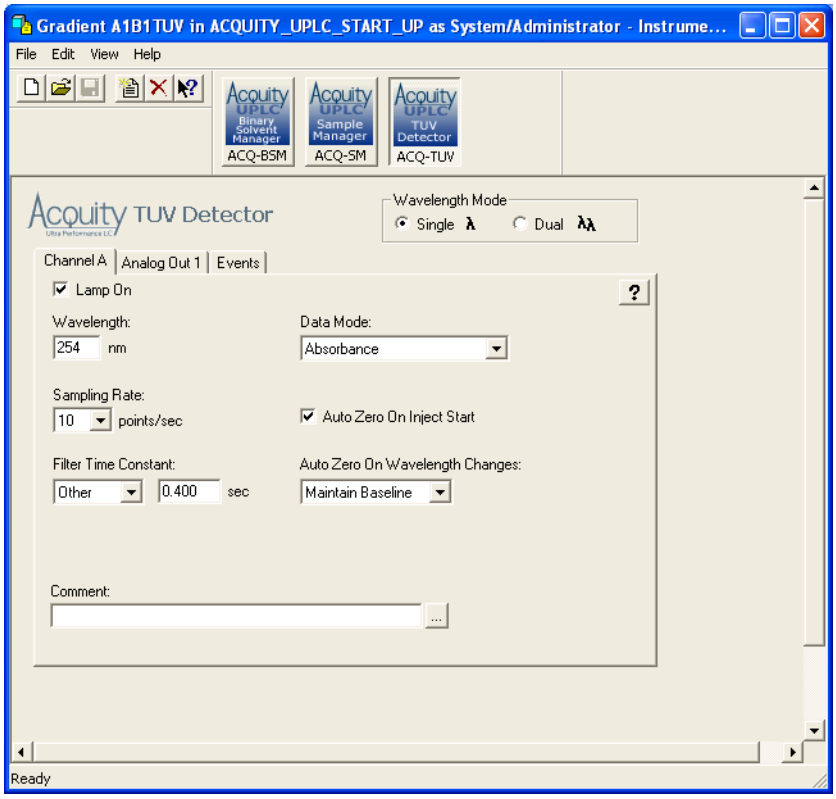
3. On the General tab, click Advanced and then set the parameters shown in the following screen representation.

Sample manager instrument advanced settings



Tip: The sample manager parameters are the same regardless of whether your system includes the TUV or PDA detector.

TUV detector instrument parameters



4. If your system includes the TUV detector, set instrument method parameters as shown in the following screen representation.

See also:

- For information on default values, consult the ACQUITY Console online Help.
- For more information on detector parameters, consult the ACQUITY UPLC Console online Help, Empower online Help, or MassLynx online Help.
- If your system includes a PDA detector, see the *ACQUITY UPLC Photodiode Array Detector Getting Started Guide* for information on verifying it.
- If your system includes an ELS detector, see the *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide* for information on performing a run.

- If your system includes an FLR detector, see the *ACQUITY UPLC Fluorescence Detector Getting Started Guide* for information on verifying it.
- If your system includes an SQ detector, see the *SQ Detector Operator's Guide* for information on verifying it.
- If your system includes a TQ detector, see the *TQ Detector Operator's Guide* for information on verifying it.

5. Save the instrument method.

Creating the sample set method

If Empower software controls the system, you must create a sample set method. The sample set method parameters (Inj Vol., # of Injections, Function, Run Time, and Next Injection Delay) are the same whether your system includes the TUV or PDA detector. However, the method set and report methods vary. Before acquiring data, ensure that you chose the appropriate method set.

For more information on creating a sample set method, consult the Empower online Help.

To create the sample set method

1. Set these sample set method parameters:
   - Condition column once (run time = 6.0 minutes)
   - Injection volume = 5 µL (10-mm or 25-mm flow cell or ELSD)
   - # of Injections = 3
   - If Empower software controls the system, run time = 4.0 minutes (with Next Injection Delay = 2.5 minutes). If MassLynx software controls the system, total run time = 6.5 minutes
2. Save the sample set method.

Performing the gradient performance test

Restriction: If your ACQUITY UPLC system uses only a mass spectrometer, the gradient performance test is not applicable.

When the system is prepared and the test methods are created, you are ready to perform the gradient performance test. The steps for running the test vary slightly, depending on whether your system uses Empower or MassLynx software, but the desired results are the same.

To perform the test

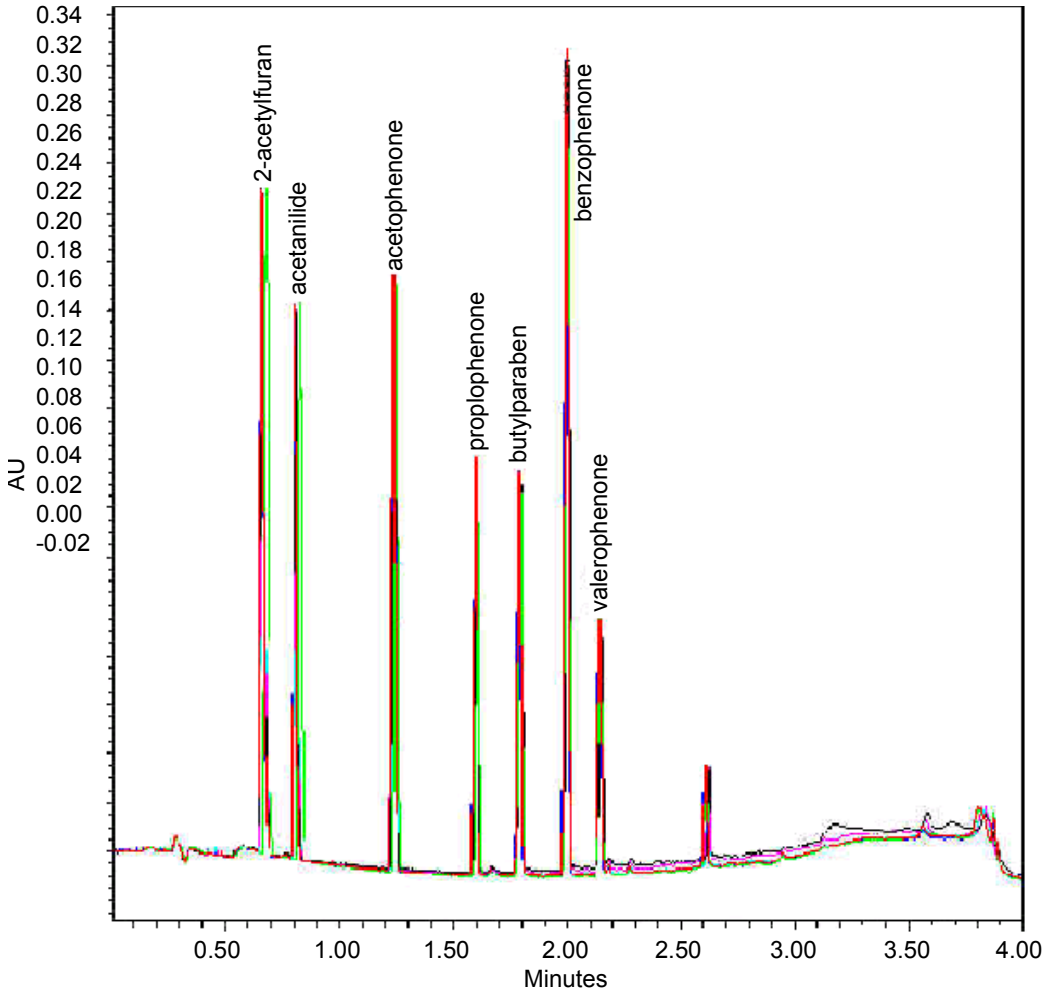
1. Start the run:
   - If the system is controlled by Empower software, open the project in Run Samples, select the gradient performance test sample set, and then select Run and Report.
   - If the system is controlled by MassLynx software, access the MassLynx main page, and select Start from the Run menu.
2. When the sample set is complete, enter the appropriate results in the table, below.

Retention time reproducibility (three replicates)

Peak	Peak retention time mean value	Std dev	Acceptable std dev
2-acetylfuran			≤1.5 secs
acetanilide			≤1.5 secs
acetophenone			≤1.5 secs
propiophenone			≤1.5 secs
butylparaben			≤1.5 secs
benzophenone			≤1.5 secs
valerophenone			≤1.5 secs

3. Review the gradient performance report. The gradient performance test result is "passing" when these conditions are realized:
   - The peaks are symmetrical, integrated, and identified correctly. (Compare the chromatogram on the report to the sample chromatogram, below.)
   - The peak retention times show a standard deviation of ≤ 2.0 s. (Consult the table you completed.)

Sample gradient performance test chromatogram



Note that this is a representative chromatogram. The results from your system can vary slightly.