ACQUITY UPLC H-Class Bio

System Guide

Revision A

Waters

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We seriously consider every customer comment we receive. You can reach us at tech_comm@waters.com.

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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 the ACQUITY UPLC H-Class Bio System

High voltage hazard



Warning:

- To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.
- To avoid nonlethal electric shock when the instrument is in Operate mode, avoid touching the areas marked with the high voltage warning symbol. To touch those areas, first put the instrument in Standby mode.

Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

Operating this ACQUITY UPLC H-Class Bio System

When operating this ACQUITY UPLC® H-Class Bio System, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
	Manufacturer
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use

Audience and purpose

This guide is intended for personnel who install, operate, and maintain ACQUITY UPLC H-Class Bio instruments. It gives an overview of the system's technology and operation.

Intended use of the ACQUITY UPLC H-Class Bio System

The Waters ACQUITY UPLC H-Class Bio System is for research use only and is not intended for use in diagnostic applications.

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.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not an operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

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.

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1 ACQUITY UPLC H-Class Bio System

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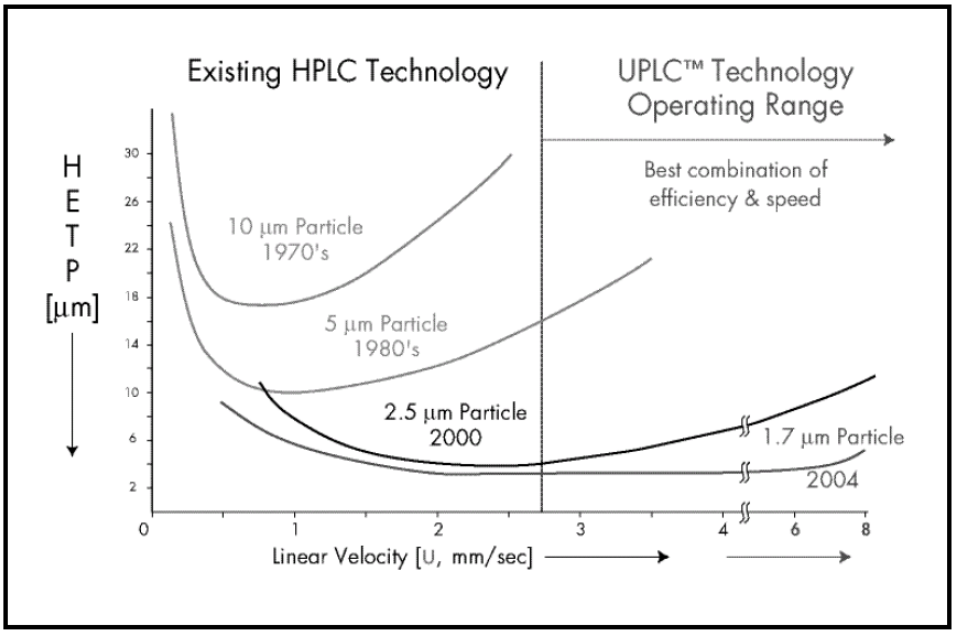
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UltraPerformance liquid chromatography

In 2004, Waters® made significant advances in instrumentation and column design to introduce UPLC® technology to the field of separation science. By employing this technology, Waters' ACQUITY UPLC® systems achieve a marked increase in resolution, speed, and sensitivity in liquid chromatography, when compared to conventional systems.

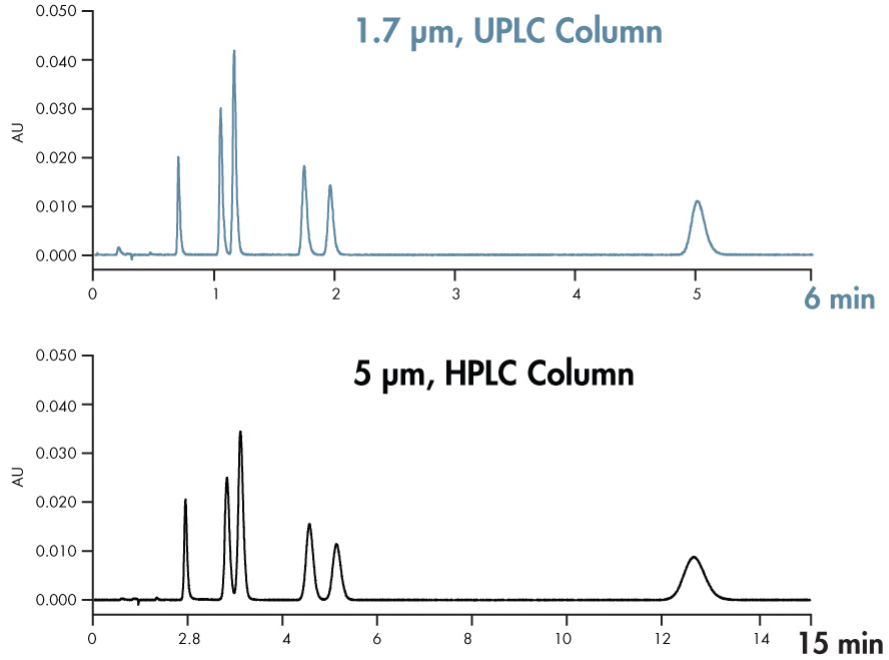
UltraPerformance liquid chromatography uses columns packed with 1.7 µm-diameter, round particles, and operating pressures up to 103,421 kPa (1034 bar, 15,000 psi). The van Deemter equation, an empirical formula that describes the relationship between linear velocity and column efficiency, considers particle size as one of the variables. Thus the equation can be used to characterize theoretical performance across a range of particle sizes.

History of particle size in liquid chromatography:



It is apparent from the figure, above, that using 1.7-µm particles achieves higher efficiency that persists as flow rate increases (lower HETP indicates higher efficiency). When operating in this area of the plot, the peak capacity and the speed of a separation can set limits well beyond those of conventional HPLC technology. Waters has defined this new level of performance as UltraPerformance chromatography.

Comparison of chromatographic separations using 5.0-µm and 1.7-µm particles:



Each separation was performed on a 2.1 × 50 mm column. Chromatographic conditions for the separations were identical, except for the flow rate, which was scaled based on particle size.

Features of the ACQUITY UPLC H-Class Bio System

The ACQUITY UPLC H-Class Bio System is designed for life science and biopharmaceutical investigations. It is optimized for analyzing peptides, proteins, oligonucleotides, and carbohydrates. The core system includes the bioSample Manager - FTN (bioSM-FTN) and the bioQuaternary Solvent Manager (bioQSM). The Auto•Blend Plus™ software feature makes possible enhanced method development capability.

The system's wetted path contains only biocompatible materials that

- do not interact significantly with, adsorb, or modify biomolecules such as proteins.
- do not leach ions into the recommended chromatographic eluents (see Appendix C).
- are not attacked or substantially damaged by mobile phases described in Appendix C, including these:
  - Acidic, halide-containing mobile phases such as HCl (6 mM) and NaCl (up to 1 M) in phosphate buffer (20 to 100 mM).
  - Phosphate buffer at pH from 2 to 12 in the presence of 1M NaCl.

Materials in the wetted path are primarily titanium alloys and MP35N® alloy, which is a nonmagnetic nickel-cobalt-chromium-molybdenum alloy.

The system accommodates several types of column compartment modules. The active preheater in all column compartment modules has wetted surfaces composed of biocompatible materials. Column compartment modules can include the column heater, with active preheating; column manager, with active preheating and column-switching capabilities; auxiliary column manager, with active preheating; 30-cm column heater/cooler; and 30-cm column heater, with active preheating.

The ACQUITY UPLC H-Class Bio system combines the speed and performance of UPLC with the ability to run HPLC separations.

This combination provides many benefits, including these:

- High-pressure, small-particle chromatography allowing faster, higher-resolution analyses, compared with conventional HPLC
- Low solvent consumption (significantly less than conventional HPLC)
- Flexibility in solvent mixing by using a bioQSM
- A bioSM-FTN that facilitates the transfer of methods from HPLC to UPLC.
- bioQSM and bioSM-FTN design enhancements, to minimize dispersion and reduce cycle time

Flow-through-needle injector

The bioSM-FTN in the ACQUITY UPLC H-Class Bio system uses a flow-through-needle mechanism that differs radically from the loop-based

injector used by the ACQUITY UPLC system. The flow-through-needle mechanism aspirates a sample and holds it in the sample needle in preparation for injecting the sample onto the column. The needle serves as part of the injection flow path when the sample is pushed onto the column.

Using the flow-through-needle mechanism, the system operates similarly to most conventional HPLC systems, facilitating the transfer of HPLC methods. The flow-through-needle mechanism also does not require you to learn new injection modes. Moreover, it improves injection accuracy and decreases cycle time for small volume injections. Gradients pass through the needle during injection, ensuring complete sample recovery.

Wash solvent

The wash system uses a single solvent to clean the outside of the sample needle and prime the wash system. The solvent does not enter the injection flow path.

Caution:

- With buffers in the system, do not stop flow for more than 30 min.
- For system shutdown or storage, flush each of the four solvent lines with water for 5 min. Flush the purge line with water for 50 cycles. Flush the needle wash with water for 120 sec. Repeat the same flushes with 20% methanol in water.
- To restart the system, repeat the water flushes before using buffers.
- Inspect the needle and wash port for accumulated salt weekly. Rinse with water, taking care not to bend or otherwise damage the needle.

Restriction: Avoid buffered wash solvents.

Purge solvent

The primary function of the purge solvent is to move sample along the injection pathway. The purge solvent also primes the sample syringe and injection pathway. The solvent's injection onto the column occurs only during auto-dilution, when it is used as the dilution solvent.

Active solvent conditioning

HPLC and UPLC applications benefit from additional pre-column, mobile-phase heating to improve chromatographic separations. The

ACQUITY UPLC H-Class Bio column heater uses an active preheater to condition solvent as it enters the column. The preheater raises the temperature of the incoming mobile phase (and injected sample) to the same set point as that of the column compartment.

Tip: Active preheating is the default configuration for the ACQUITY UPLC H-Class Bio system.

Software enhancements

Auto•Blend Plus

Using Auto•Blend Plus, you create gradients based on the pH and/or salt concentrations of solvents. Solvents intended for use with Auto•Blend Plus are usually installed in solvent reservoirs as follows:

- Solvent A is acidic.
- Solvent B is basic.
- Solvent C is salt buffer.
- Solvent D is aqueous. If the bioQSM has an optional solvent selection valve, you can select one of six solvents (D1 through D6).

You specify or select from a catalog a pKa value or pH calibration curve that is used to calculate the pH. The editable catalog includes known pH solutions, salt solutions, pKa values, and pH calibration curves.

Each injection's ratio of pH to salt is constant. You specify the gradient segments' pH concentration, pH curve, salt concentration, salt curve, flow rate, and one of these parameters:

- Time
- Column volume
- Total volume

Quantum Synchronization

Introducing a low-pressure sample into the high-pressure fluid stream during injection causes a pressure pulse that can affect chromatographic results. The Quantum Synchronization feature reduces the effect of this pressure pulse. The bioSM-FTN and bioQSM communicate to automatically coordinate the injection sequence, enabling the bioQSM to provide additional pressure at the

exact moment the bioSM-FTN switches its injector valve to the inject position, to introduce the low-pressure sample.

Gradient Smart Start

Before each sample injection, a bioSM-FTN typically performs wash sequences and aspirates the appropriate sample volume. When these tasks are completed, the bioQSM begins to deliver the gradient to the injection valve. The dwell volume of the system affects the amount of time it takes for this gradient to reach the column and can be a significant component of the overall cycle time.

The Gradient Smart Start feature coordinates pre-injection operations and reduces the effect of the bioQSM's dwell volume on cycle time. The gradient starts before or during the bioSM-FTN's pre-injection functions, resulting in significant time savings.

Wash Plungers

Precipitated material that remains on the bioQSM's pump plungers can damage the high-pressure seals. The Wash Plungers function washes the seals with solvent to remove any precipitate. You can use the Wash Plungers function as needed, or run it as part of the No-Flow Shutdown feature.

No-Flow Shutdown

The No-Flow Shutdown feature runs the Wash Plungers function after the bioQSM remains idle for a specified time interval. This feature prevents depositions of precipitate on the bioQSM plungers while the system is idle.

Automatic Prime

When you enable this function of the bioQSM, the system primes the lines when a new solvent is selected.

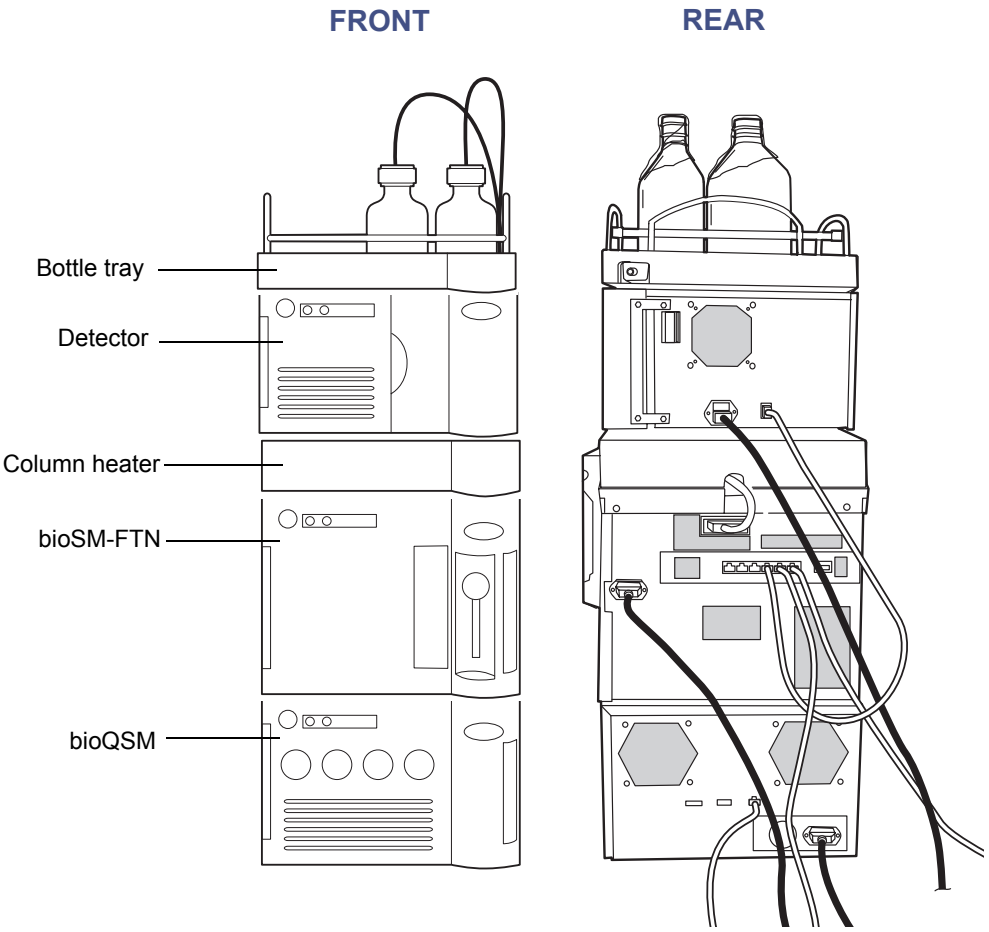
Example: If a first injection uses line D1 and a second injection uses line D2, the solvent manager primes line D2 between the first and second injections.

Flow Ramping

Use this feature to specify the rate at which the bioQSM increases or decreases its flow.

System components

Instrument modules:



The ACQUITY UPLC H-Class Bio system can include a bioQSM fitted with optional, 6-port solvent selection valve, bioSM-FTN, column compartment module, detectors (tunable ultraviolet, photodiode array, or mass spectrometer), and an ACQUITY UPLC column. All materials that contact the sample are biocompatible.

Waters Empower™ chromatography software or MassLynx™ mass spectrometry software controls the ACQUITY UPLC H-Class Bio system.

bioQSM

The bioQSM is a low-pressure mixing, high-pressure pump. It provides steady (pulse-free) solvent flow at analytical flow rates to 1 mL/min at 103,421 kPa (1034 bar, 15,000 psi) and to 2 mL/min, at reduced pressures, to 62,053 kPa (621 bar, 9000 psi). The bioQSM can pump four degassed solvents simultaneously using a gradient proportioning valve (GPV) to dynamically create a specified composition.

bioSM-FTN

The bioSM-FTN uses a direct-injection mechanism to inject samples drawn from plates and vials onto a chromatographic column. Optional extension loops (installed between the sample needle and the injection valve) can increase the injection volume beyond that of the sample needle. The bioSM-FTN can also dilute samples using the auto-dilution option.

Column heater

Column temperature variations can shift peak retention times and alter peak shapes, increasing the difficulty of achieving precise results. The column compartment helps to ensure precise, reproducible separations by controlling the column temperature.

The column compartment heats to any temperature from 20 ºC (or a minimum of 5 ºC above ambient temperature) to 90 ºC. An active preheating device heats the incoming solvent before it enters the column. The column heater can accommodate columns up to 4.6 mm I.D. and up to 150 mm length.

Tip: Active preheating is the default configuration for the ACQUITY UPLC H-Class Bio system.

Local Console Controller (optional)

The ACQUITY UPLC Local Console Controller (LCC) complements chromatography data system (CDS) software, enabling you to control the systems locally. Designed to emulate a simple keypad, the LCC's minimal functionality bars it from operating as a standalone controller. Its installation in a system does not supplant CDS control. Rather, Waters designed the LCC to prepare system modules for operation, define initial conditions, and run system diagnostic tests. These basic functions are rapidly performed, even when a system is remote from the software control and acquisition workstation or LAC/E™[32] module or when network control is unavailable.

FlexCart

The optional FlexCart provides for the ACQUITY UPLC H-Class Bio system a mobile platform. It can hold the system instruments as well as the PC and monitor, and it 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.

Column technology

ACQUITY UPLC columns are packed with 1.7-µm, bridged, ethylsiloxane, hybrid or 1.8-µm high strength silica particles that can mechanically endure high-pressure conditions. The column hardware and the matched outlet tubing can withstand as much as 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 technology

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 as many as 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.

When you attach the eCord column chip to the receptacle on the column compartment, the chip automatically records and stores system information. You need take no further action.

Detectors

The small-particle chemistries used in UPLC system chromatography produce very narrow peaks. The UPLC TUV, PDA, ELS, and FLR detectors and SQ and TQ mass spectrometers collect data at sufficiently fast rates to describe these peaks without affecting the sensitivity or accuracy of the peak measurement. These specially matched detectors employ lower flow cell volume, minimized tubing volumes, and specialized fittings to control bandspreading and maintain these narrow peaks.

Flow cells composed of biocompatible materials such as titanium are available for UPLC TUV and PDA detectors.

For additional information

You can find this additional information about the ACQUITY H-Class Bio system on the system documention CD:

- *Quaternary Solvent Manager Operator's Overview and Maintenance Information*
- *Sample Manager-Flow Through Needle Operator's Overview and Maintenance Information*
- *Column Compartment Operator's Overview and Maintenance Information*
- System specifications

Visit waters.com to find more information and to join the ACQUITY UPLC online community where you can do these things:

- Share information with and ask questions of ACQUITY UPLC experts and scientists
- Access ACQUITY UPLC publications and user experiences from around the globe
- Review exclusive FAQs, tips and tricks, and tutorials
- Explore the latest ACQUITY UPLC applications and information.

2 Optimizing Performance

Follow the advice and guidelines in this chapter to help ensure optimum performance from your ACQUITY system.

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General guidelines

ACQUITY UPLC H-Class Bio system guidelines differ from those governing standard HPLC practices primarily because a chromatography that uses small (less than 2-µm) particles places certain constraints on the system. Chromatography on a UPLC system effects a much smaller-scale, higher-resolution separation than that using HPLC. Moreover, analysis time is shorter for UPLC, and solvent and sample consumption are significantly reduced.

ACQUITY UPLC H-Class Bio chromatography requires optimum performance from the biosample manager (bioSM-FTN) because sample dispersion is more evident when using smaller columns. The reduction in chromatographic run time also makes efficient management of cycle time essential.

When performing fast UPLC analyses, a peak of interest can be less than 0.5 seconds. 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 second yield higher baseline noise, requiring you to adjust filter time constants 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. The values provided are approximations, and 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

Caution:

- With buffers in the system, do not stop flow for more than 30 min.
- For system shutdown or storage, flush each of the four solvent lines with water for 5 min. Flush the purge line with water for 50 cycles. Flush the needle wash with water for 120 sec. Repeat the same flushes with 20% methanol in water.
- To restart the system, repeat the water flushes before using buffers.
- Inspect the needle and wash port for accumulated salt weekly. Rinse with water, taking care not to bend or otherwise damage the needle.

Follow these general recommendations when performing a UPLC analysis:

- Use high quality solvents, buffers and additives (HPLC or MS grade).
- Use high quality water (HPLC- or MS-grade).
- Always use solvent filters on tubing lines in solvent bottles.
- Filter buffers with a 0.2-µm filter membrane.
- Keep concentrated stock solutions, to use when preparing working solutions.
- Do not top off buffers, which can promote microbial growth.
- Do not block the degasser vent line; trim the tubing, if necessary.

- Do not submerge the waste or degasser vent lines in liquid. (See the *ACQUITY UPLC H-Class Quaternary Solvent Manager Operator's Overview and Maintenance Information* for details on how to route the tubing.)
- Keep all solvent lines primed.
- Flush buffers from the system, with aqueous solvent, if you keep the system idle for extended periods (longer than 24 hours). Use 10 to 20% organic solvent in water as a "storage" solvent. Prime the bioSM-FTN with purge solvent for a minimum of 10 cycles.
- Use of buffers in needle wash solvents can cause salt build-up, which can require periodic cleaning. Prime the wash solvent for a minimum of 30 sec and the purge solvent for a minimum of 10 cycles.
- Keep the seal wash line primed.
- Running continuously with salt concentrations higher than 1M can result in a need to change pump seals more frequently than the scheduled PM. To help increase seal life and prevent salt crystal buildup on the pump seals, flush the pump, high salt line, and reservoir periodically. Salt concentration, flow rate, and other factors can affect the frequency of flush procedures. Some applications can require weekly flushing.
- Prime solvent lines during system start-up.
- Monitor the waste level, to ensure that it is never too high.
- Start gradients that include an organic component (0.1%, for example) to provide more consistent and predictable gradient formation than when you start with 0% organic.
- Use the Load Ahead option when you desire a shorter cycle time.
- Do not use the Load Ahead or Loop Offline options when you are troubleshooting carryover problems.
- When installing or removing a column, always hold the active preheater's reusable compression fitting in place. Rotate the column or optional in-line filter to install or remove it.

ACQUITY UPLC columns calculator

The ACQUITY UPLC columns calculator is a software tool that helps you transfer methods from an HPLC system to a UPLC system, or from a UPLC

system to an HPLC system. The calculator differentiates between systems with binary and quaternary pumps.

When you input parameter values from a current separation, choose a target column for which the resolving power (L/dp) is similar. (L/dp values are automatically calculated and displayed). Specify the dwell volumes for the current and target systems (the calculator recommends chromatographic conditions for the target system). You can further optimize these conditions based on your particular requirements.

See also: The ACQUITY UPLC columns calculator documentation and the ACQUITY Console online Help for additional details and methodology.

Tip: You can install the calculator from the ACQUITY UPLC system driver CD or the Method Assistance Kit CD. An icon for the ACQUITY UPLC Columns Calculator appears on your computer's desktop after installation.

Dispersion

UPLC systems and autosamplers exhibit low dispersion—a fixed, instrument characteristic measured by the extent of peak broadening that occurs because of the system design.

Small particle chromatography uses small, high-efficiency columns. A typical 2.1 × 50 mm UPLC column has an approximate 174-µL volume, compared with 2.5 mL for a typical 4.6 × 150 mm HPLC column. The smaller column and particle size require a system whose low dispersion reduces dilution and band broadening, thus maintaining the peak shape, height, and sensitivity produced by the high efficiency column.

An ACQUITY UPLC H-Class Bio system typically exhibits a bandspread of 20 µL (value depends on system configuration). UPLC peak concentrations are higher than HPLC concentrations. Because solubility effects are more apparent in low dispersion, high pressure systems, it is important to adjust column load appropriately.

Carryover

You observe carryover in chromatographic systems when a previously injected analyte appears as a peak in the chromatogram of subsequent samples. Carryover tends to occur when a small amount of analyte remains in the system after a sample is injected. You can measure carryover by observing

analyte peaks that appear when you run a blank sample immediately after an analytical sample.

See also: *ACQUITY UPLC H-Class System Specifications* for carryover on the ACQUITY UPLC H-Class Bio system.

A common cause of carryover is inadequate washing of the system. Choosing an appropriate wash solvent can minimize carryover for a particular analysis. The wash solvent must be strong enough to dissolve any remaining sample, and the wash duration must be long enough to remove the residue from the system.

Method conditions also affect carryover. Too short a hold-time at the final conditions of a gradient, especially if the gradient is steep, can fail to remove all analytes from the system. It is important to completely flush the system and reequilibrate the column before proceeding to a subsequent analysis. Use caution when choosing the load-ahead and loop-offline options. Initiating these options before the highly organic part of the gradient reaches the needle can leave sample residue in the system. The time savings you gain can lead to inadequate system cleaning.

The hydrophobicity and solubility of samples, as well as cleanliness during sample preparation, are additional factors to consider when trying to minimize carryover, as is contamination from sample preparation tools.

Precision and accuracy

The injection volume precision using the ACQUITY UPLC H-Class Bio system is less than 1% RSD for injection volumes from 0.2 to 10.0 µL. The system's injection volume accuracy is 100 ± 2%. (See *ACQUITY UPLC H-Class System Specifications* for details).

Cycle time (between injections)

The short run time of a UPLC separation requires efficient use of the time between analyses.

The bioSM-FTN has a load-ahead option that can help decrease cycle time. This option instructs the bioSM-FTN to aspirate the next sample while the current sample is running.

The Loop Offline option on the bioSM-FTN reduces the impact of delay volume on cycle time by taking the needle and extension loop offline before the

gradient reaches the injection valve and after the sample transfers to the injection port.

Setting an appropriate syringe draw rate can also help reduce cycle time. By default, the system uses feedback information from a pressure transducer to optimize the syringe draw rate for maximum throughput and performance.

Preventing leaks

Preventing leaks ensures that the system maintains adequate pressure and sample integrity throughout the analysis.

Leaks can potentially occur at any tubing connection, gasket, or seal but are most common at tubing connections. Low pressure leaks (on the intake side of the bioQSM's pump) cause solvent loss and air introduction during the intake cycle. Leaks at high pressure fittings (downstream of the *i*^{2}*Valve*™) can leak solvent but do not introduce air.

To prevent leaks, follow Waters' recommendations for the proper tightening of system fittings. Note specifically that different techniques apply to retightening fittings versus installing them.

Sample preparation

UPLC analysis places some additional restrictions on sample preparation.

Particulates

The small column frit size (0.2 µm) can become blocked more easily than larger HPLC column frits (2.0 µm). As a result, particle-free mobile phase solvents and sample solutions are essential for UPLC analysis. See "General guidelines" on page 2-1 for recommendations on choosing and handling solvents.

Matching sample diluents

When you use the auto-dilution option on the bioSM-FTN, the purge solvent serves as the sample diluent. Ensure that your sample solution is soluble and miscible in your chosen purge solvent.

3 Preparing the System

Contents:

Topic	Page
Preparing system hardware	3-1
Configuring chromatography data software	3-7
ACQUITY control panels	3-7
Starting the ACQUITY UPLC Console	3-13

Preparing system hardware

Powering-on the system

Powering-on the system entails starting the ACQUITY UPLC H-Class Bio system workstation, system instruments, and chromatography software. Each device or instrument beeps three times and runs a series of startup tests.

Tip: If your system includes a column compartment module, it is automatically powered-on when you power-on the bioSM-FTN.

To power on the system:

1. Power-on the bioQSM and the bioSM-FTN by pressing the power switch on the top, left-hand side of each device's door.

   See also: "Status LEDs" on page 3-2 and "Power LED" on page 3-2 for information on how to interpret LED modes for device or instrument flow status and whether the units are powered-on.

2. After power LEDs on the bioQSM and on the bioSM-FTN show steady green, press the power switch on the top, left-hand side of the detector (or detectors).

   Tip: To prevent initialization errors, only power-on the detector (or detectors) when the flow cell is wetted.

3. Start the chromatography software.

   Tip: 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 H-Class Bio system's workstation:

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

If the startup tests indicate a malfunction, consult the console's online Help.

Monitoring system module LEDs

Light emitting diodes on each system module 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 a device or instrument's front panel, indicates the power-on or power-off status of the instrument. This LED is green when power is applied to the unit and unlit when power is not applied.

Tip: To provide adequate ventilation, the bioSM-FTN fans run continuously, even when the power switch is in the "off" position. These fans switch off only when you disconnect the power cable from the back of the instrument.

Status LEDs

Flow LED (bioQSM)

The flow LED, on the right-hand side of the power LED on the bioQSM's front panel, indicates the flow status. A steady-green flow LED indicates flow through the quaternary solvent manager.

Run LED (bioSM-FTN)

The run LED, on the right-hand side of the power LED on the bioSM-FTN'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 ignited.

Status LED indications:

LED mode and color	Description
Unlit	• bioQSM and bioSM-FTN – Indicates the device is currently idle. • Detector – Indicates the detector lamp is extinguished.
Steady green	• bioQSM – Indicates solvent is flowing. • bioSM-FTN – Indicates the bioSM-FTN 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	• bioQSM and bioSM-FTN – Indicates that the device is initializing. • Detector – Indicates the detector is initializing or calibrating.
Flashing red	Indicates that an error stopped the instrument or device. Refer to the console for information regarding the error.
Steady red	Indicates an instrument or device failure that prevents further operation. Power-off the unit, and then power-on. If the LED is still steady red, contact your Waters service representative.

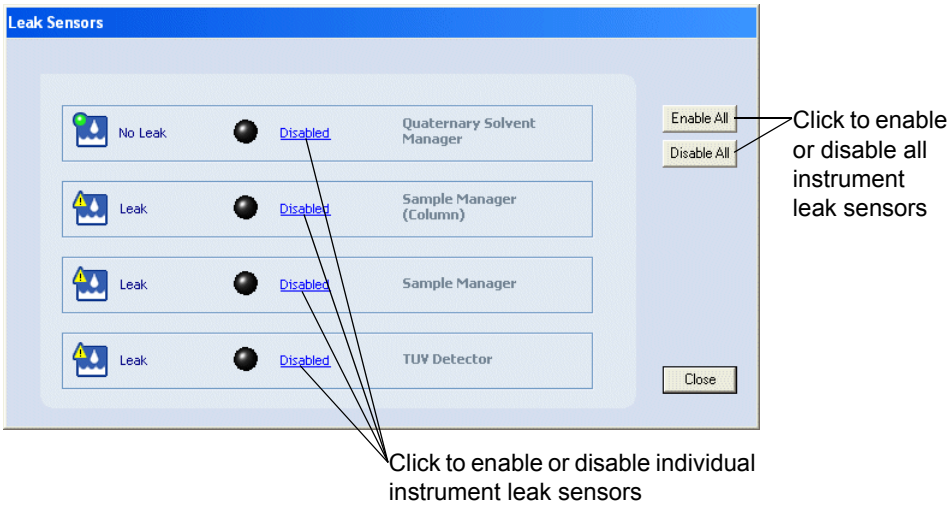
Enabling the leak sensors

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

To enable the leak sensors:

1. In the 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.

   Tip: To enable all leak sensors, click Enable All.

Starting up the system

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

Recommendation: Prime the bioQSM for a minimum of five 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 console, click Control > Start up system.
2. In the Prime Solvents tab of the System Startup dialog box, review the settings for the A/B/C/D Solvents (mobile phase).

   Tips:

   - In the A/B/C/D Solvents area, you can select or clear any or all of the solvents: A, B, C, D.
   - You can change the duration of priming for solvents A through D by entering a different value in the Duration of Prime field. All selected solvents are primed for the same duration.
   - If you want to return settings to their original values on any tab, click Set Defaults.

   Default: All solvents are primed for 2.0 minutes each. (Range: 0.1 to 60.0 minutes.)

   Recommendation: Prime for 3 minutes or for 7 minutes after changing solvents.

   Caution:

   - With buffers in the system, do not stop flow for more than 30 min.
   - For system shutdown or storage, flush each of the four solvent lines with water for 5 min. Flush the purge line with water for 50 cycles. Flush the needle wash with water for 120 sec. Repeat the same flushes with 20% methanol in water.
   - To restart the system, repeat the water flushes before using buffers.
   - Inspect the needle and wash port for accumulated salt weekly. Rinse with water, taking care not to bend or otherwise damage the needle.

3. Select or clear priming of the seal wash, wash solvent, and purge solvent. If necessary, change the duration specified to prime the seal wash and wash solvent and the number of cycles specified to prime the purge solvent.

   Default: The seal wash is primed for 2.0 minutes, the wash solvent for 15 seconds, and the purge solvent for 5 cycles.

4. Select the Equilibrate to Method tab, and review the settings for the flow rate, mobile phases, composition, temperatures, and lamp state at equilibration.

Equilibrate to Method tab values:

System startup parameters	Default	Allowed values
Method initial flow rate	0.500 mL/min	0.1 to 2.0 mL/min
Composition of A, B, C, and D (sum must be 100%)	A: 100% B,C,D: 0%	A: 0 to 100% B: 0 to 100% C: 0 to 100% D: 0 to 100%
Column temperature	Off	Depends on type of column compartment module
Sample temperature	Off	Off, or 4.0 to 40.0 °C
Lamp	On	On or off **Note:** For light guiding flow cells, do not power-on, operate, or ignite the lamp of the detector when there is no flow through the cell, or when the cell is dry.

5. Click Start.

Result: The lamp in the optical detector ignites, the system sets the column and sample temperatures, and all priming starts. After priming, the sample manager characterizes the needle and seal, if selected, and then logs the results of the characterizations into the database. Finally, the system establishes the method flow rate, solvent selections, and composition.

Configuring chromatography data software

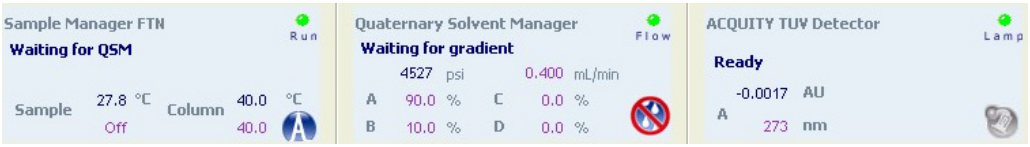
Configure the chromatography data system software for use with ACQUITY:

- Start the chromatography data system software, and log in.
- Select system instruments, and name the system (see Empower or MassLynx Help for details).
- Open the ACQUITY Console and control panels.

ACQUITY control panels

You can monitor control panels for the bioQSM, bioSM-FTN, and detector from your chromatography data system.

Control panels:



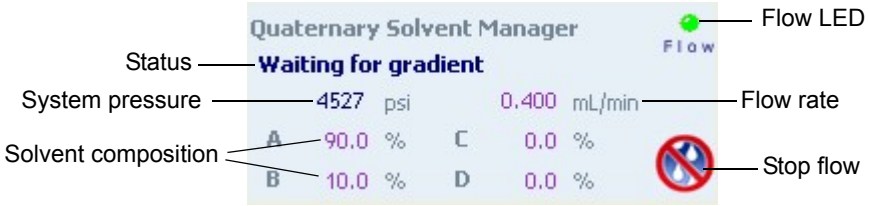
Where Empower software controls the system, the control panels appear at the bottom of the Run Samples window. Where MassLynx software controls the system, the control panels appear on the Additional Status tab of the Inlet Editor window.

bioQSM control panel

The bioQSM 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 the underlined value. You cannot edit quaternary solvent manager parameters while the system is running samples.

bioQSM control panel:



bioQSM control panel items:

Control panel item	Description
Flow LED	Displays the actual flow LED on the front panel of the bioQSM unless communications with the bioQSM are lost.
Status	Displays the status of the current operation.
System Pressure	Displays system pressure in kPa, bar, or psi. You can customize pressure units via the console.
Flow Rate	Displays the flow rate of solvent through all lines of the bioQSM, from 0.000 to 2.000 mL/min under normal operation and 0.000 to 4.000 mL/min when priming.
Solvent Composition	Displays the percentage of solvent to be drawn from the solvent lines (A through D). Composition values range from 0.0 to 100.0%.
(Stop Flow)	Immediately stops all flow from the bioQSM.

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

Additional functions in the bioQSM control panel:

Control panel function	Description
Start up system	Brings the system to operational conditions after an extended idle period or when switching to different solvents. See "Starting up the system" in the *Quaternary Solvent Manager Operator's Overview and Maintenance Information*.
Prime solvents	Displays the Prime Solvents dialog box. See "Priming the quaternary solvent manager" in the *Quaternary Solvent Manager Operator's Overview and Maintenance Information*.
Prime seal wash	Starts priming the seal wash. See "Priming the seal wash system" in the *Quaternary Solvent Manager Operator's Overview and Maintenance Information*.
Wash plungers	Initiates the plunger wash sequence, which fills and then slowly empties the primary and accumulator chambers (with the current solvent composition) while performing a high speed/volume seal wash. This action helps to prevent the build-up of precipitates on the pump plungers, which can damage the high pressure seals.
Launch ACQUITY UPLC Console	Launches the console.
Reset QSM	Resets the bioQSM after an error condition.
Help	Displays online Help for the console.

bioSM-FTN control panel

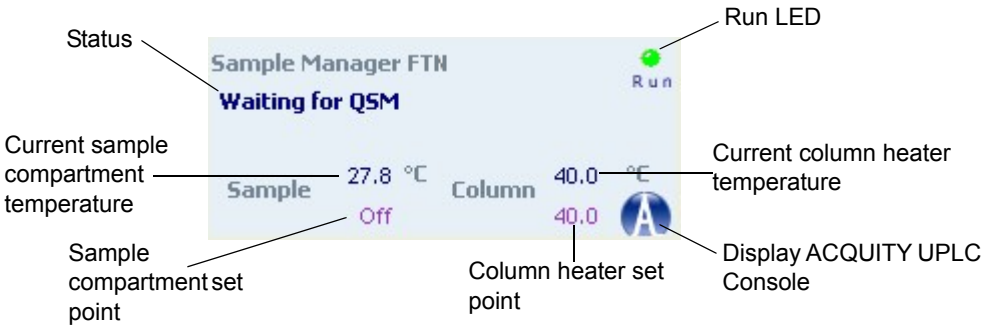
The bioSM-FTN control panel displays current temperatures and set points for the sample compartment and column compartment module. You can edit

these values when the system is idle by clicking the underlined value. You cannot edit sample manager set points while the system is running samples.

Tips:

- To keep the sample compartment at a constant temperature, open its door only when necessary.
- The bioSM-FTN's fans stop circulating air whenever the sample compartment door is open.

bioSM-FTN control panel:



bioSM-FTN control panel items:

Control panel item	Description
Run LED	Displays the actual run LED on the front panel, unless communications 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, even when active temperature control is disabled.
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".
Current Column Heater Temperature	Displays the current column heater temperature to 0.1 °C resolution, even when active temperature control is disabled.

bioSM-FTN control panel items: (Continued)

Control panel item	Description
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 additional functions by right-clicking anywhere in the bioSM-FTN control panel.

Additional functions in the bioSM-FTN control panel:

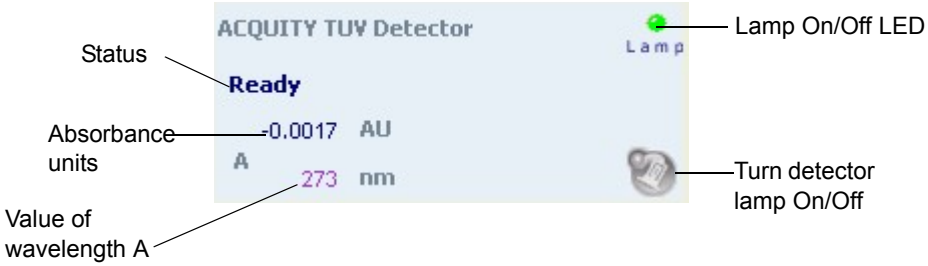
Control panel function	Description
Prime	Displays the Prime dialog box. See “Priming the SM-FTN” in the *Sample Manager - Flow Through Needle Operator’s Overview and Maintenance Information*.
Wash needle	Displays the Wash Needle dialog box. See “Washing the SM-FTN needle” in the *Sample Manager - Flow Through Needle Operator’s Overview and Maintenance Information*.
Reset SM	Resets the sample manager following an error condition.
Help	Displays online Help for the console.

TUV detector control panel

The TUV detector’s control panel displays absorbance units and wavelength values, which you can edit when the system is idle by clicking the underlined value. Nevertheless, you cannot edit detector parameters when the system is running samples.

Control panels for other detectors function similarly. If your system includes a PDA detector, see the *ACQUITY UPLC Photodiode Array Detector Getting Started Guide*.

TUV detector control panel:



The following table describes the controls and indicators in the TUV detector's 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 additional functions described in the table, below, 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 online Help for the console.

Starting the ACQUITY UPLC Console

The ACQUITY UPLC Console is a software application that provides a convenient way to configure settings, monitor performance, run diagnostic tests, and maintain the system and its modules. It replaces the keypads and small screen displays traditionally found on the fronts of system instruments. The ACQUITY UPLC Console functions independently of data applications and does not recognize or control them.

From the ACQUITY UPLC Console's interface, you can quickly navigate to visual representations of each module and its components. You can also navigate to interactive diagrams, which show interconnections and provide diagnostic tools for troubleshooting problems.

To start the ACQUITY UPLC Console from Empower software:

In the Run samples window, click Display console  in the bioSM-FTN control panel.

ACQUITY UPLC Console window:



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 .

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

Contents:

Topic	Page
Warning symbols	A-2
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-6
Electrical and handling symbols	A-11

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of sharp-point puncture injury.)



Warning: (Risk of hand crush injury.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



Warning: (Risk of exposure to a toxic substance.)



Warning: (Risk of personal exposure to laser radiation.)



Warning: (Risk of exposure to biological agents that can pose a serious health threat.)



Warning: (Risk of tipping.)



Warning: (Risk of explosion.)



Warning: (Risk of eye injury.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



Warning: Pressurized nonmetallic, or polymer, tubing can burst. Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



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

This warning applies to certain instruments when they are in Operate mode.



Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.



Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.



Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.



Caution: To avoid damage, do not use abrasives or solvents to clean the instrument's case.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the equipment guidelines in this section.

Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意：未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意：未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

주의: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告：当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

警告：使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

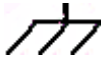
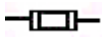
경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告：ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

Electrical and handling symbols

Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

B External Connections

This section describes the ACQUITY UPLC® H-Class Bio system's external connections.

Tip: A Waters Technical Service representative unpacks and installs your ACQUITY UPLC H-Class Bio instruments.

Warning: To avoid back injuries, do not attempt to lift the instruments without assistance.

Caution:

- Contact Waters Technical Service before moving the ACQUITY UPLC H-Class Bio instruments.
- If you must transport an instrument or remove it from service, contact Waters Technical Service for recommended cleaning, flushing, and packaging procedures.

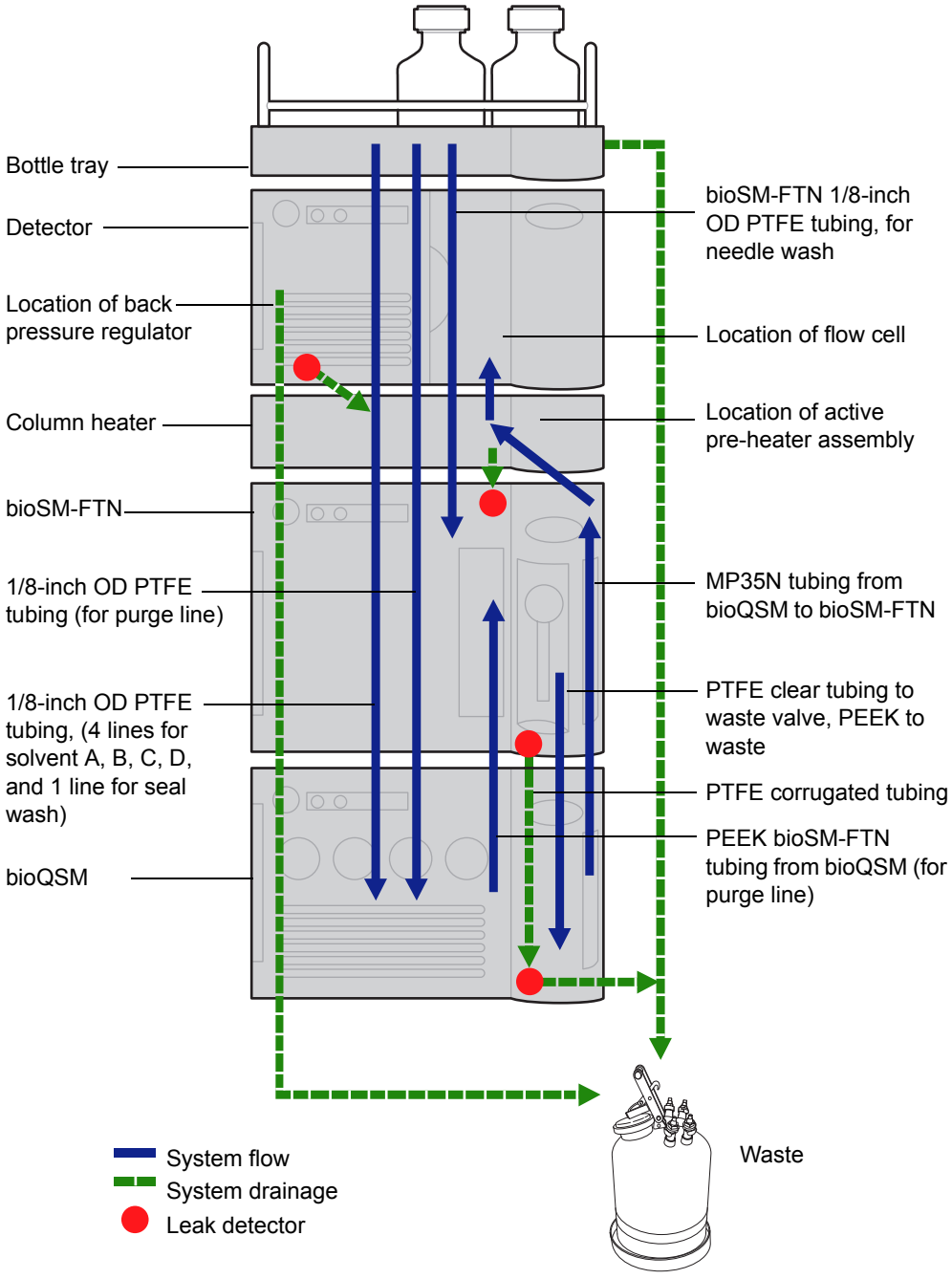
Contents:

Topic	Page
System tubing connections	B-1
Instrument external wiring connections	B-3
Flow path connections for column managers	B-5
Signal connections	B-7
Connecting to the electricity source	B-11

System tubing connections

The system's external tubing connections for solvent flow and drainage are shown below.

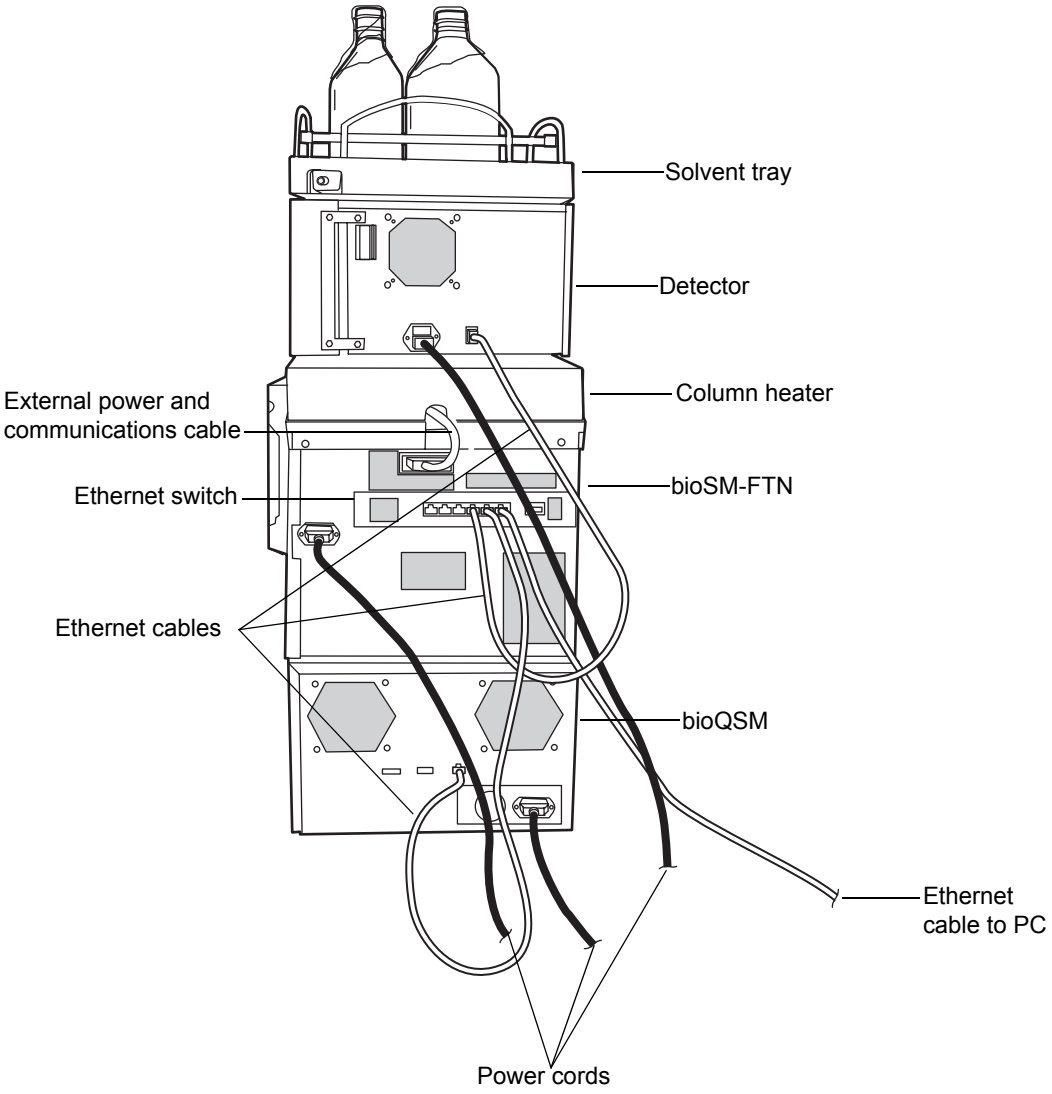
Solvent flow and drainage:



Instrument external wiring connections

ACQUITY UPLC H-Class Bio instrument external wiring connections

The rear panel connections for ACQUITY UPLC H-Class Bio instruments are shown below.



Ethernet connections

The bioSM-FTN incorporates an internal Ethernet switch that accommodates the PC (workstation) and up to six ACQUITY UPLC H-Class Bio modules. Connect the shielded Ethernet cables from each module to the electronic connections on the rear panel of the bioSM-FTN. The bioSM-FTN is connected internally to the Ethernet switch.

If the system includes an optional column manager (CM-A), connect up to two optional auxiliary column managers (CM-Aux) to the CM-A.

Column heater connection

The bioSM-FTN powers and communicates with the column heater. The external communication cable must be connected to the rear of the column heater and the bioSM-FTN.

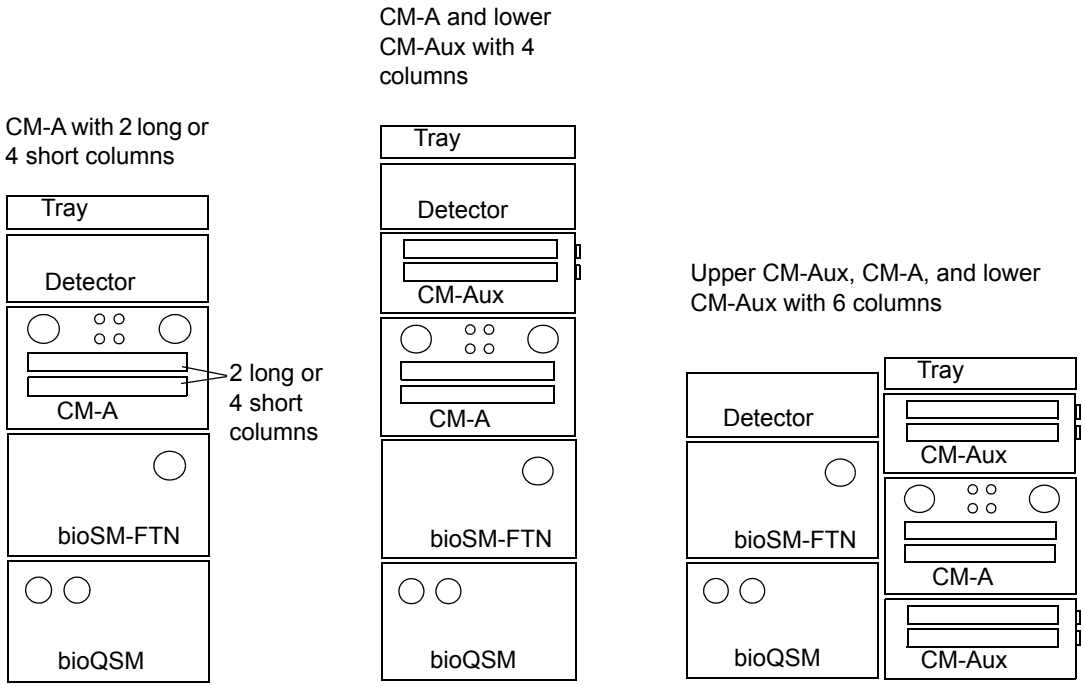
To make column heater connections:

Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To interrupt power to an instrument, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds thereafter before you disconnect an assembly.

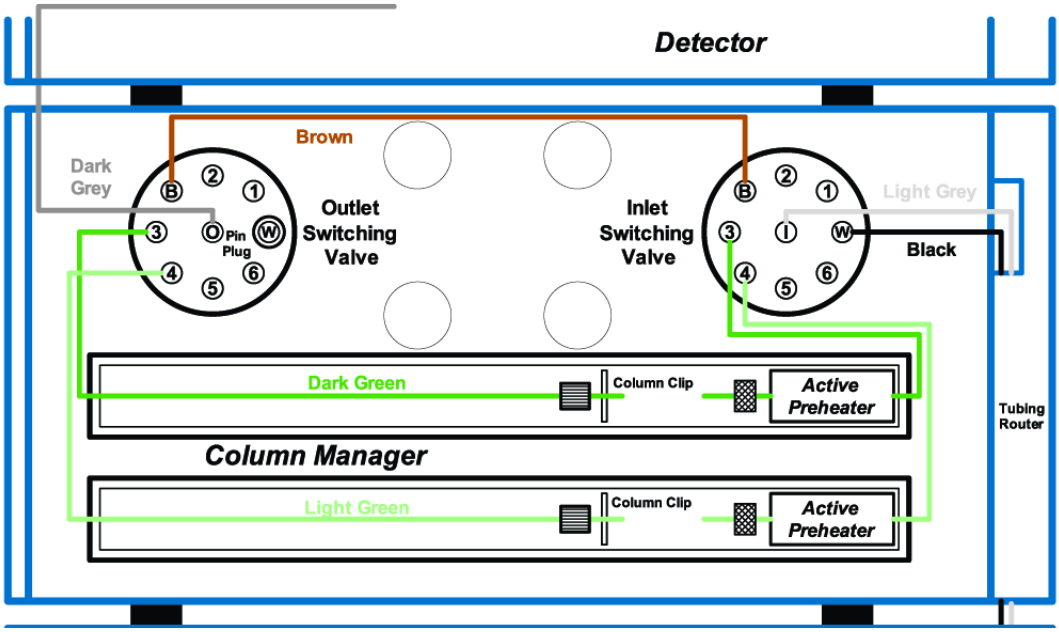
1. Make sure the bioSM-FTN and the column heater are powered-off.
2. Connect the external communication cable to the High Density (HD) port on the rear of the column heater.
3. Connect the other end of the external communication cable to the QSPI port on the rear of the bioSM-FTN.

Flow path connections for column managers

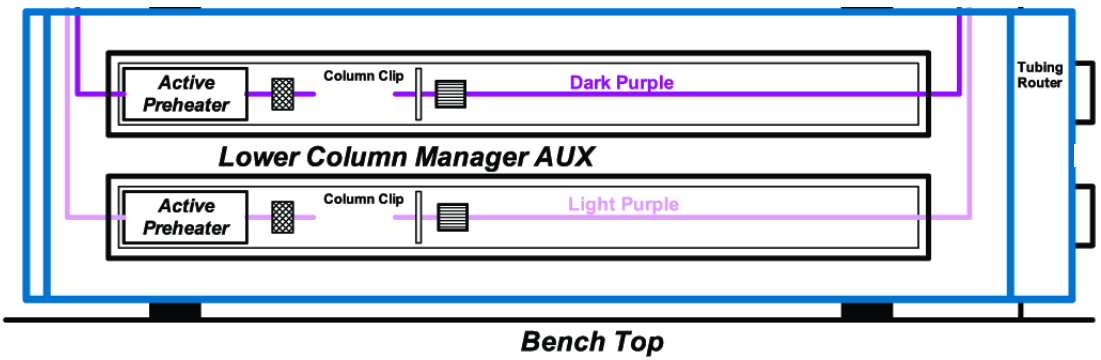
Column compartment modules in three system configurations:



Column manager with active preheaters on right-hand side:



Auxiliary column manager with active preheaters on left-hand side:



Signal connections

Making signal connections

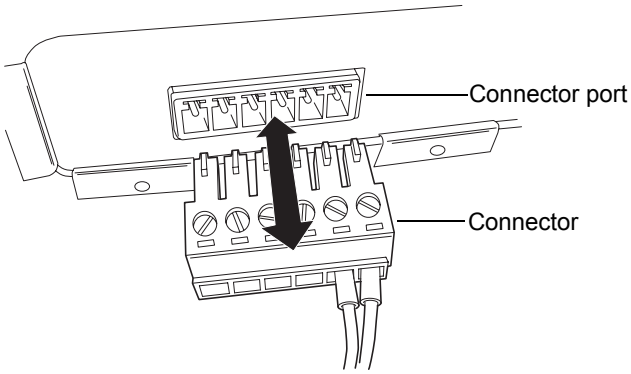
Refer to the signal connection location shown on the silk-screened label affixed to the rear panel of each instrument.

Required materials

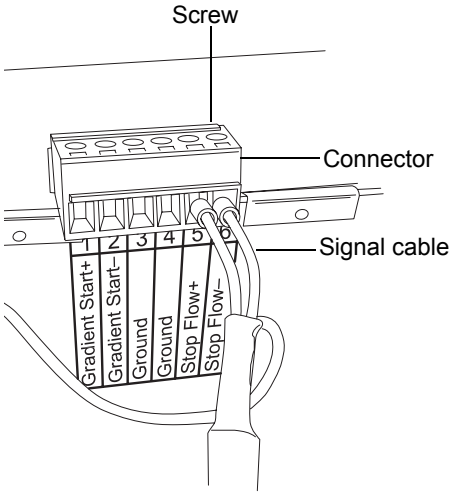
- 9/32-inch nut driver
- Flat-blade screwdriver
- Connector
- Signal cable

To make signal connections:

1. Insert the connector into the connector port on the back of the instrument.

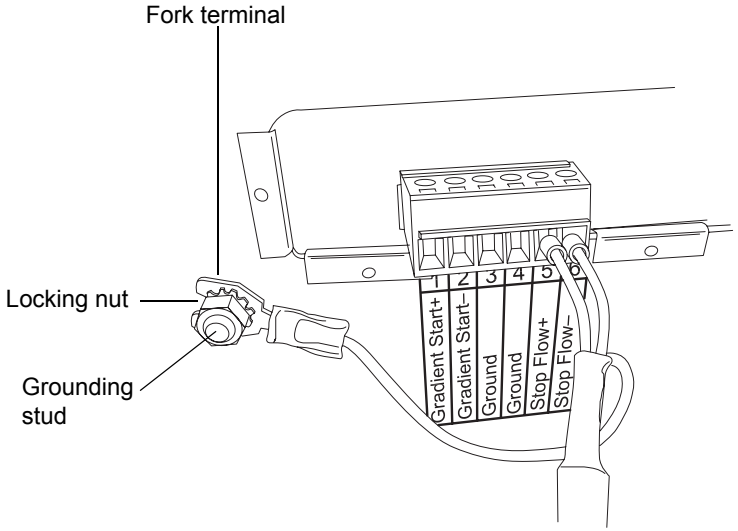


2. Using the flat-blade screwdriver, attach the positive and negative leads of the signal cable to the connector.



3. Fit the grounding cable's fork terminal on the rear-panel grounding stud, and secure it with the locking nut.

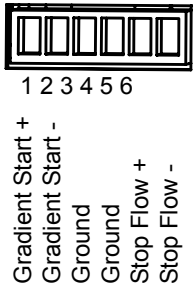
   Tip: Use the 9/32-inch nut driver to tighten the locking nut until the fork terminal does not move.



bioQSM I/O signal connectors

The rear panel of the bioQSM includes a removable connector that holds the screw terminals for I/O signal cables. This connector is keyed so that it can be inserted only one way.

bioQSM I/O signal connections:



For electrical specifications, see the *ACQUITY UPLC H-Class System Specifications*.

bioQSM event-in connections:

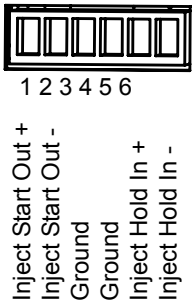
Signal connection	Description
Gradient Start	Initiates the pumps to begin gradient operation by either contact closure input or 0-volt input.
Stop Flow	Allows you to stop the flow from the quaternary solvent manager when it receives a contact closure input or 0-volt input (an error condition or hardware failure on another instrument, for example).

bioSM-FTN I/O signal connectors

The rear panel of the bioSM-FTN includes a removable connector that holds the screw terminals for I/O signal cables. This connector is keyed so that it can receive a signal cable inserted only one way.

Requirement: You must configure a contact closure output connection (Inject Start Out) from the bioSM-FTN, to trigger a mass spectrometer, an ACQUITY 2996 PDA detector, or an ACQUITY ELS detector running under MassLynx software control to start.

bioSM-FTN I/O signal connectors:



For electrical specifications, see the *ACQUITY UPLC H-Class System Specifications*.

bioSM-FTN event-out/event-in connections:

Signal connections	Description
Inject Start	Indicates (with a contact closure output) that an injection has started.
Inject Hold	Delays the next injection when the bioSM-FTN receives a contact closure input (from another system instrument, for example).

TUV detector signal connectors

If your system includes a TUV detector, see the *ACQUITY UPLC Tunable Ultraviolet Detector Getting Started Guide* for information on signal connectors.

PDA detector signal connectors

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

ELS detector signal connectors

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

FLR detector signal connectors

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

Connecting to the electricity source

Each system instrument requires a separate, grounded power source. The ground connection in all power outlets must be common and physically close to the system.



Warning: Avoid electrical shock:

- Use power cord SVT-type in the United States and HAR-type or better in Europe. For other countries' requirements, contact your local Waters distributor.
- Power-off and unplug each system instrument before performing any maintenance operation on the instrument.
- Connect each system instrument to a common ground.

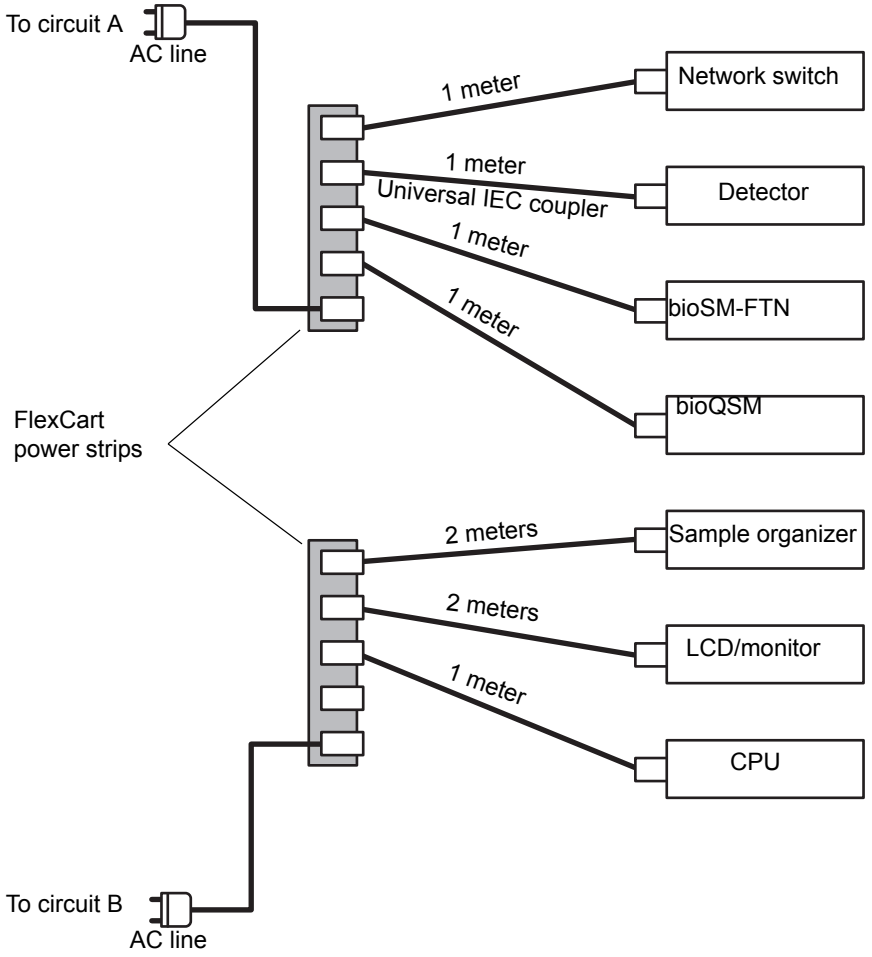
To connect to the electricity source:

Recommendation: Use a line conditioner and uninterruptible power supply (UPS) for optimum, long-term, input voltage stability.

1. Connect the female end of the power cord to the receptacle on the rear panel of each instrument.
2. Connect the male end of the power cord to a suitable wall outlet.

   Alternative: If your system includes the optional FlexCart, connect the female end of the Flexcart's electrical cables (included in the startup kit) to the receptacle on the rear panel of the each instrument. Connect the hooded, male end of the Flexcart's electrical cables to the power strips on the back of the cart. Finally, connect each power strip's cable to a wall outlet operating on its own circuit.

FlexCart power connections:



C Solvent Considerations

Warning: To avoid chemical hazards, always observe Good Laboratory Practices when operating your system, handling solvents, or changing tubing. See the Material Safety Data Sheets for the solvents you use.

The information in this appendix applies only to the following instruments:

- ACQUITY UPLC® H-Class Bio system modules
- ACQUITY UPLC PDA detector
- ACQUITY UPLC PDA eλ detector
- ACQUITY UPLC TUV detector

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Introduction

Preventing contamination

For information on preventing contamination, refer to *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the Waters Web site. Visit www.waters.com.

Clean solvents

Clean solvents ensure reproducible results and permit you to operate with minimal instrument maintenance.

Dirty solvents can cause baseline noise and drift, and they can clog solvent reservoir filters, inlet filters, and capillary lines.

Solvent quality

Use MS-grade solvents for the best possible results; the minimum requirement is HPLC-grade. Filter solvents through an appropriate membrane filter.

Recommendation: Ensure your solvent choices are consistent with the recommendations of the membrane filter manufacturer or supplier.

Solvent preparation

Proper solvent preparation, primarily filtration, can prevent many pumping problems.

Recommendation: Use brown-tinted glassware to inhibit microbial growth.

Water

Use water only from a high-quality water purification system. If the water system does not deliver filtered water, filter the water through a 0.2-µm membrane filter.

Caution: Using 100% water can cause microbial growth. Waters recommends changing 100% water solutions daily. Adding a small amount of an organic solvent (~10%) prevents microbial growth.

Using buffers

Adjust the pH of aqueous buffers. Filter them to remove insoluble material, and then blend them with appropriate organic modifiers. After you use a buffer, flush it from the pump by running a wet-prime with at least five system volumes of HPLC-grade distilled or deionized water.

For shutdowns of more than a day, flush the pump with a 20% methanol/water solution to prevent microbial growth.

Caution: Some buffers can be incompatible with mass spectrometers. Consult the documentation that accompanies your instrument for compatible buffers.

Tip: To avoid salt precipitation, nonvolatile buffer concentrations must not exceed 100 mM.

Buffered solvents

When using a buffer, choose good quality reagents, filtering them through a 0.2-µm membrane filter.

Recommendation: To discourage microbial growth, replace 100% mobile aqueous phase daily.

See also: For information on preventing contamination, refer to *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the Waters Web site. Visit www.waters.com.

Solvent recommendations

The ACQUITY UPLC H-Class Bio system was designed for reversed-phase chromatography and ACQUITY UPLC BEH column chemistries. Waters evaluated the system's reliability using traditional reversed-phase solvents.

This section lists solvents recommended for the ACQUITY UPLC H-Class Bio system. Contact Waters Customer Service to determine whether you can use solvents that do not appear in the list without adversely affecting instrument or system performance.

General solvent guidelines

Always observe the following general solvent guidelines:

- Use high-quality, brown-tinted glassware to inhibit microbial growth.
- Filter solvents. Small particles can permanently block a system's capillary lines. Filtering solvents also improves check valve performance.

Recommended solvents

- Acetonitrile
- Acetonitrile/water mixtures
- Isopropanol
- Methanol
- Methanol/water mixtures
- Water

Other solvents

You can use the following solvents. Note, however, that these solvents can shorten instrument life. If you routinely use the solvents on this list, Waters recommends you install the Hexane/THF Compatibility Kit.

- Tetrahydrofuran (THF)
- Hexane
- Acetone
- Ethyl acetate
- Hexafluoroisopropanol (HFIP)

  Notes:

  – 1-4% aqueous solutions of HFIP for oligonucleotide applications.

  – HFIP must never be used in wash solvents.

For additional information, see page C-6.

Consider solvent polarity when you change typical reversed-phase solvents. Flush the system with a solvent of intermediate polarity, like isopropanol, before introducing nonpolar solvents like THF or hexane.

Hexane/THF Compatibility Kit

The ACQUITY UPLC System Hexane/THF Compatibility Kit (contact Waters for part number) can be installed in ACQUITY UPLC systems with closed waste management. It is designed for users that need to run their systems with hexane or THF at high concentrations and high pressure and is recommended for many ELS detector-based applications where THF is used in the mobile phase, at high concentrations.

Additives/modifiers

- 0.1% ethylene diaminetetraacetic acid (EDTA)
- 0.1% heptafluorobutyric acid
- 0.1% triethyl amine (TEA)
- 0.1% trifluoracetic acid (TFA)
- 0.2% formic acid
- 0.3% acetic acid
- 10 mM ammonium bicarbonate
- 10 mM phosphate buffer
- 50 mM ammonium acetate
- 50 mM ammonium hydroxide

Sample diluents

- Acetonitrile
- Acetonitrile/water mixtures
- Chloroform
- Dimethylformamide (DMF)
- Dimethyl sulfoxide (DMSO)
- Isooctane
- Isopropanol
- Methanol
- Methanol/water mixtures
- Methylene chloride
- Water

Recommendation: Avoid buffers as needle wash.

Cleaning agents

Recommendation: See the cleaning procedures in *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the Waters Web site. Visit www.waters.com.

- Phosphoric acid (≤30%)
- Sodium hydroxide (≤1M)
- Formic acid (≤10%)

Solvents to avoid

Avoid these solvents:

- Toluene, methyl chloride, trichlorobenzene
- Solvents that contain halogens: fluorine, bromine, or iodine.
- Strong acids. (Use them only in weak concentration, <5%, unless as cleaning agents. Avoid using acids as mobile phases when their pH <1.0.)
- Peroxidizable compounds such as UV-grade ethers, non-stabilized THF, dioxane, and diisopropylether. (If you must use peroxidizable compounds, be sure to filter them through dry aluminium oxide to adsorb formed peroxides.)
- Solutions that contain strong concentrations of complexing agents like ethylene diaminetetraacetic acid (EDTA).

ACQUITY UPLC H-Class Bio system recommendations

Contact Waters for recommended system cleaning and flushing procedures.

Flush buffers from the system, with aqueous solvent, if you keep the system idle for extended periods (longer than 24 hours). Use 10 to 20% organic solvent in water as a “storage” solvent. Prime the sample manager-flow through needle with wash solvent for a minimum of 30 sec and purge solvent for a minimum of 10 cycles.

See also: *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the Waters Web site. Visit www.waters.com.



Warning: Explosion hazard: Peroxide contaminants in THF can spontaneously and destructively explode when you partially or completely evaporate the THF.



Warning: Health hazard: Hexane is a neurotoxin, and THF can irritate eyes, skin and mucous membranes and cause harmful neurologic effects. If you use either or both of these volatile solvents, locate your ACQUITY UPLC H-Class Bio system inside a fume hood or walk-in chamber to minimize exposure to harmful solvent vapors.

- THF, hexane, ethyl acetate, and acetone can be used as the mobile-phase in ACQUITY UPLC H-Class Bio systems. However, as with many nonaqueous solvents, they can shorten system and instrument life compared with equipment running typical reversed-phase solvents. If you routinely use THF, hexane, ethyl acetate, or acetone, Waters recommends you install the Hexane/THF Compatibility Kit.
- When using unstabilized THF, ensure that your solvent is freshly prepared. Previously opened bottles contain peroxide contaminants, which cause baseline drift.
- Chloroform, methylene chloride, halogenated solvents, and toluene are generally not recommended for use in ACQUITY UPLC H-Class Bio systems. Nevertheless, you can use these solvents in weak dilutions (<10%) as additives, sample diluents, or modifiers.
- Contact your Waters sales representative or local technical support organization to determine whether a specific method is suitable to use with the ACQUITY UPLC H-Class Bio system instruments and components.
- When using THF or hexane, install stainless steel tubing, and minimize the use of PEEK™ components.

- Aqueous solvents must not remain in a shut-down system because they serve as a substrate for microbial colonies. Microbes can clog system filters and capillary lines. To prevent their proliferation, add a small amount (~10%) of an organic solvent such as acetonitrile or methanol.
- Methanesulfonic acid is not recommended for use in ACQUITY UPLC H-Class Bio systems.

bioQSM recommendations

- The plunger seal wash system must never run dry, particularly during separations that use a polar mobile phase.
- Isopropyl alcohol or mixtures of methanol and water, like 20% methanol/water, are effective seal wash solvents for THF solvent mixtures.
- For reversed-phase applications, use aqueous seal wash solutions with a weak organic component (for example 1:9 methanol/water).
- Do not use 100% organic seal wash solutions.

bioSM-FTN recommendations

- Do not use concentrations of THF or hexane greater than 10% as purge solvent.
- Typical organic sample diluents such as dimethylsulfoxide (DMSO) and dimethylformamide (DMF) are supported.

Caution:

- With buffers in the system, do not stop flow for more than 30 min.
- For system shutdown or storage, flush each of the four solvent lines with water for 5 min. Flush the purge line with water for 50 cycles. Flush the needle wash with water for 120 sec. Repeat the same flushes with 20% methanol in water.
- To restart the system, repeat the water flushes before using buffers.
- Inspect the needle and wash port for accumulated salt weekly. Rinse with water, taking care not to bend or otherwise damage the needle.

Detector recommendations

To transport a flow cell while temperatures are below 5 °C, fill it with alcohol.

Common solvent properties

The following table lists the properties for some common chromatographic solvents.

Properties of common solvents:

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Acetone	184.5 at 20 °C	56.29	-20
Acetonitrile	88.8 at 25 °C	81.6	6
n-Butyl acetate	7.8 at 20 °C	126.11	22
n-Butyl alcohol	4.4 at 20 °C	117.5	37
n-Butyl chloride	80.1 at 20 °C	78.44	-9
Chlorobenzene	8.8 at 20 °C	131.69	28
Chloroform	158.4 at 20 °C	61.15	
Cyclohexane	77.5 at 20 °C	80.72	-20
Cyclopentane	400 at 20 °C	49.26	-7
o-Dichlorobenzene	1.2 at 20 °C	180.48	66
Dichloromethane	350 at 20 °C	39.75	
Dimethyl acetamide	1.3 at 25 °C	166.1	70
N,N-Dimethylformamide	2.7 at 20 °C	153.0	58
Dimethyl sulfoxide	0.6 at 25 °C	189.0	88
1,4-Dioxane	29 at 20 °C	101.32	12
Ethyl acetate	73 at 20 °C	77.11	-4
Ethyl alcohol	43.9 at 20 °C	78.32	15
Ethyl ether	442 at 20°C	34.55	-45
Ethylene dichloride	83.35 at 20 °C	83.48	13
Heptane	35.5 at 20 °C	98.43	-4
Hexane	124 at 20 °C	68.7	-22

Properties of common solvents: (Continued)

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Iso-octane	41 at 20 °C	99.24	-12
Isobutyl alcohol	8.8 at 20 °C	107.7	28
Isopropyl alcohol	32.4 at 20 °C	82.26	12
Isopropyl myristate	<1 at 20 °C	192.6	164
Methanol	97 at 20 °C	64.7	11
Methyl t-butyl ether	240 at 20 °C	55.2	-28
Methyl ethyl ketone	74 at 20 °C	79.64	-9
Methyl isobutyl ketone	16 at 20 °C	117.4	18
N-Methylpyrrolidone	0.33 at 25 °C	202.0	86
Pentane	420 at 20 °C	36.07	-49
n-Propyl alcohol	15 at 20 °C	97.2	23
Propylene carbonate		241.7	135
Pyridine	18 at 25 °C	115.25	20
Tetrahydrofuran	142 at 20 °C	66.0	-14
Toluene	28.5 at 20 °C	110.62	4
1,2,4-Trichlorobenzene	1 at 20 °C	213.5	106
Triethylamine	57 at 25 °C	89.5	-9
Trifluoroacetic acid	97.5 at 20 °C	71.8	-3
Water	17.54 at 20 °C	100.0	
o-Xylene	6 at 20 °C	144.41	17

Solvent miscibility

Before you change solvents, refer to the table below to determine solvent miscibility. Be aware of these effects:

- Changes involving two miscible solvents can be made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water) require an intermediate solvent like n-propanol.
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- Buffers dissolved in water can precipitate when mixed with organic solvents.

When you switch from a strong buffer to an organic solvent, thoroughly flush the system with distilled water before you add the organic solvent.

Solvent miscibility:

Polarity index	Solvent	Viscosity cP, 20 °C (@1 atm)	Boiling point °C (@1 atm)	Miscibility number (M)	λ Cutoff (nm)
0.0	N-Hexane	0.313	68.7	29	—
1.8	Triethylamine	0.38	89.5	26	—
4.2	Tetrahydrofuran (THF)	0.55	66.0	17	220
4.3	1-Propanol	2.30	97.2	15	210
4.3	2-Propanol	2.35	117.7	15	—
5.2	Ethanol	1.20	78.3	14	210
5.4	Acetone	0.32	56.3	15, 17	330
5.5	Benzyl alcohol	5.80	205.5	13	—
5.7	Methoxyethanol	1.72	124.6	13	—
6.2	Acetonitrile	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	—
6.4	Dimethylformamide	0.90	153.0	12	—
6.5	Dimethylsulfoxide	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
9.0	Water	1.00	100.0	—	—

Using miscibility numbers (M-numbers)

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent.

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger M-number value.

- If the difference between the two M-numbers is 15 or less, the two liquids are miscible, in all proportions, at 15°C.
- A difference of 16 indicates a critical solution temperature from 25 to 75 °C, with 50 °C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible, or their critical solution temperature is above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

Solvent stabilizers

Do not leave solvents containing stabilizers, like THF with butylated hydroxytoluene (BHT), to dry in the system's flow path. A dry flow path, including the detector flow cell, becomes contaminated with residual stabilizer, and a substantial cleaning effort is needed to restore the flow path to its initial condition.

Solvent viscosity

Generally, viscosity is not a consideration when you operate with a single solvent or under low pressure. However, with gradient chromatography, the viscosity changes that occur as the solvents are mixed in different proportions can effect pressure changes during the run. For example, a 1:1 water/methanol mixture produces twice the pressure of either water or methanol alone.

If you do not know the extent to which pressure changes affect the analysis, monitor the pressure during the run.

Wavelength selection

The tables in this section provide UV cutoff values for these items:

- Common solvents
- Common mixed mobile phases

UV cutoffs for common solvents

The table below shows the UV cutoff (the wavelength at which the absorbance of the solvent equals 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise because of solvent absorbance.

UV cutoff wavelengths for common chromatographic solvents:

Solvent	UV cutoff (nm)
Acetone	330
Acetonitrile	190
Diethyl amine	275
Ethanol	210
Isopropanol	205
Isopropyl ether	220
Methanol	205
n-Propanol	210

UV cutoff wavelengths for common chromatographic solvents: (Continued)

Solvent	UV cutoff (nm)
Tetrahydrofuran (THF)	230

Mixed mobile phases

The following table provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's law, because absorbance is proportional to concentration.

Wavelength cutoffs for different mobile phases:

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
Polyoxyethylene (35) lauryl ether (BRIJ 35), 0.1%	190	Sodium formate, 10 mM	200
3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate) (CHAPS) 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190
(Ethylenedinitrilo) tetraacetic acid disodium salt (disodium EDTA), 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 10 mM, pH 7.6	225	Triton™ X-100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC® Reagent A, 1 vial/liter	200
Morpholinoethanesulfonic acid (MES), 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225

Wavelength cutoffs for different mobile phases: (Continued)

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Potassium phosphate: monobasic, 10 mM dibasic, 10 mM	 190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

Mobile phase absorbance

This section lists the absorbances at several wavelengths for frequently used mobile phases. Choose the mobile phase carefully to reduce baseline noise.

The best mobile phase for your application is one that is transparent at the chosen detection wavelengths. With such a mobile phase, ensure that any absorbance is due only to the sample. Absorbance by the mobile phase also reduces the linear dynamic range of the detector by the amount of absorbance the autozero function cancels, or "autozeroes", out. Wavelength, pH, and concentration of the mobile phase affect its absorbance. Examples of several mobile phases are given in the table below.

The absorbances in the table below are based on a 10-mm pathlength.

Mobile phase absorbance measured against air or water:

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Solvents										
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<0.01	—	—	—	—
Methanol (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	—
Methanol (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	—	—
Isopropanol	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02

Mobile phase absorbance measured against air or water: (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Unstablized tetrahydrofuran (THF, fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstablized tetrahydrofuran (THF, old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45
Acids and bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	—
Hydrochloric acid, 0.1%	0.11	0.02	<0.01	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—
Trifluoroacetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Buffers and salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	—	—	—
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	—	—	—	—	—	—

Mobile phase absorbance measured against air or water: (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Ethylene-dinitrilo-tetraacetic acid disodium salt (disodium EDTA), 1 mM	0.11	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	—	—
Morpholino-ethanesul-fonic acid (MES), 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	—	—	—
Potassium phosphate, monobasic (KH_2PO_4), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K_2HPO_4), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—

Mobile phase absorbance measured against air or water: (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	—	—	—
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	—	—	—	—	—
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	—	—	—
Sodium phosphate, 100 mM, pH 6.8	1.99	0.75	0.19	0.06	0.02	0.01	0.01	0.01	0.01	<0.01
Tris HCl, 20 mM, pH 7.0	1.40	0.77	0.28	0.10	0.04	<0.01	—	—	—	—
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	—	—	—	—
Waters PIC® reagents										
PIC A, 1 vial/L	0.67	0.29	0.13	0.05	0.03	0.02	0.02	0.02	0.02	<0.01
PIC B6, 1 vial/L	2.46	2.50	2.42	2.25	1.83	0.63	0.07	<0.01	—	—
PIC B6, low UV, 1 vial/L	0.01	<0.01	—	—	—	—	—	—	—	—
PIC D4, 1 vial/L	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01

Mobile phase absorbance measured against air or water: (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Detergents										
BRIJ 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	—	—	—
3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (CHAPS), 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
Sodium dodecyl sulfate (SDS), 0.1%	0.02	0.01	<0.01	—	—	—	—	—	—	—
4-octylphenol polyethoxylate (Triton™ X-100), 0.1%	2.48	2.50	2.43	2.42	2.37	2.37	0.50	0.25	0.67	1.42
Polyoxyethylene sorbitan monolaurate (Tween™ 20), 0.1%	0.21	0.14	0.11	0.10	0.09	0.06	0.05	0.04	0.04	0.03

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