

ACQUITY UPLC H-Class

System Guide

General information

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Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com
Telephone and fax	From the USA or Canada, phone 800-252-4752, or fax 508-872-1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.


Waters contact information (continued)

Contacting medium	Information
Conventional mail	Waters Corporation Global Support Services 34 Maple Street Milford, MA 01757 USA

Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice (GLP), and consult your organization's standard operating procedures as well as your local requirements for safety.

Safety hazard symbol notice

Documentation needs to be consulted in all cases where the  symbol is used to find out the nature of the potential hazard and any actions which have to be taken.

Power cord replacement hazard



Warning: To avoid electric shock, use the SVT-type power cord in the United States and HAR-type (or better) cord in Europe. The main power cord must be replaced only with one of adequate rating. For information regarding what cord to use in other countries, contact your local Waters distributor.

Hand crush hazard



Warning: To avoid hazards associated with the reciprocating or rotating parts in the source, keep hands clear of the regions marked with yellow and gray labels.

High voltage hazard



Warning: To avoid electric shock, do not remove protective panels from system modules. The components within are not user-serviceable.

Bottle placement prohibition



Warning: To avoid injury from electrical shock or fire, and damage to the equipment, do not place vessels containing liquid atop the workstation or ancillary equipment or otherwise expose those units to dripping or splashing liquids.



Prohibited: Do not place vessels containing liquid—such as solvent bottles—atop the workstation or ancillary equipment or otherwise expose those units to dripping or splashing liquids.

FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the user's authority to operate the equipment. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Electrical power safety notice

Do not position the instrument so that it is difficult to disconnect the power cord.

Equipment misuse notice

If equipment is used in a manner not specified by its manufacturer, protections against personal injury inherent in the equipment's design can be rendered ineffective.












Safety advisories

Consult the "Safety advisories" appendix in this publication for a comprehensive list of warning advisories and notices.

Operating the system

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

Applicable symbols

Symbol	Definition
	Manufacturer
	Date of manufacture
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use
	Alternating current
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with the Waste Electrical and Electronic Equipment Directive (WEEE) 2012/19/EU, contact Waters Corporation for the correct disposal and recycling instructions.
	Serial number
	Part number catalog number

Audience and purpose

This guide is intended for personnel who operate and maintain the ACQUITY UPLC H-Class system.

Intended use

Waters designed this system to perform liquid chromatography separations in these environments:

- Pharmaceutical development and discovery
- Quality assurance and quality control
- Chemical materials
- Environmental
- Food safety

The system is not intended for use in diagnostic applications.

Calibrating

To calibrate LC systems, adopt acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must 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. If sample trays are the same or very similar, vary the location of the QC samples in the trays. 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.

EMC considerations

Canada spectrum management emissions notice

This class A digital product apparatus complies with Canadian ICES-001.

Cet appareil numérique de la classe A est conforme à la norme NMB-001.

ISM classification: ISM group 1 class B

This classification has been assigned in accordance with IEC CISPR 11 Industrial Scientific and Medical (ISM) instrument requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment.

Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

EC authorized representative



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1 ACQUITY UPLC H-Class system

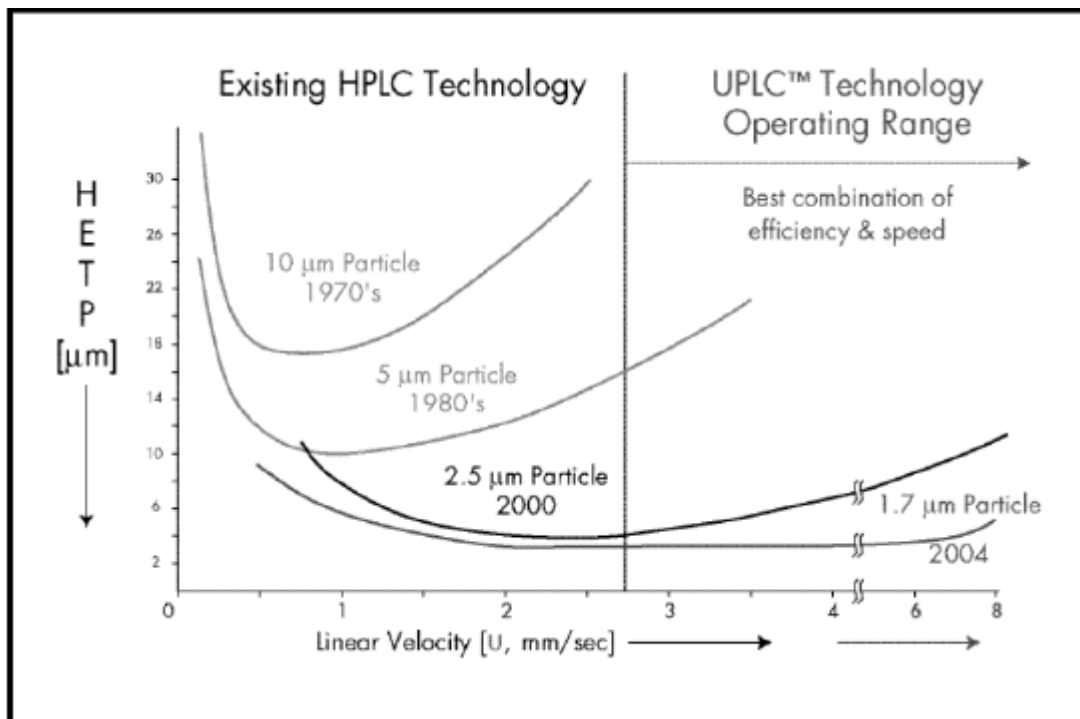
The ACQUITY UPLC H-Class System was designed to support HPLC, UHPLC, and UPLC methods. The low dispersion of the system allows you to maximize chromatographic resolution for the most challenging and complex separations. Software and hardware tools enable simplified transfer of methods and support automated method development.

1.1 UPLC technology

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.

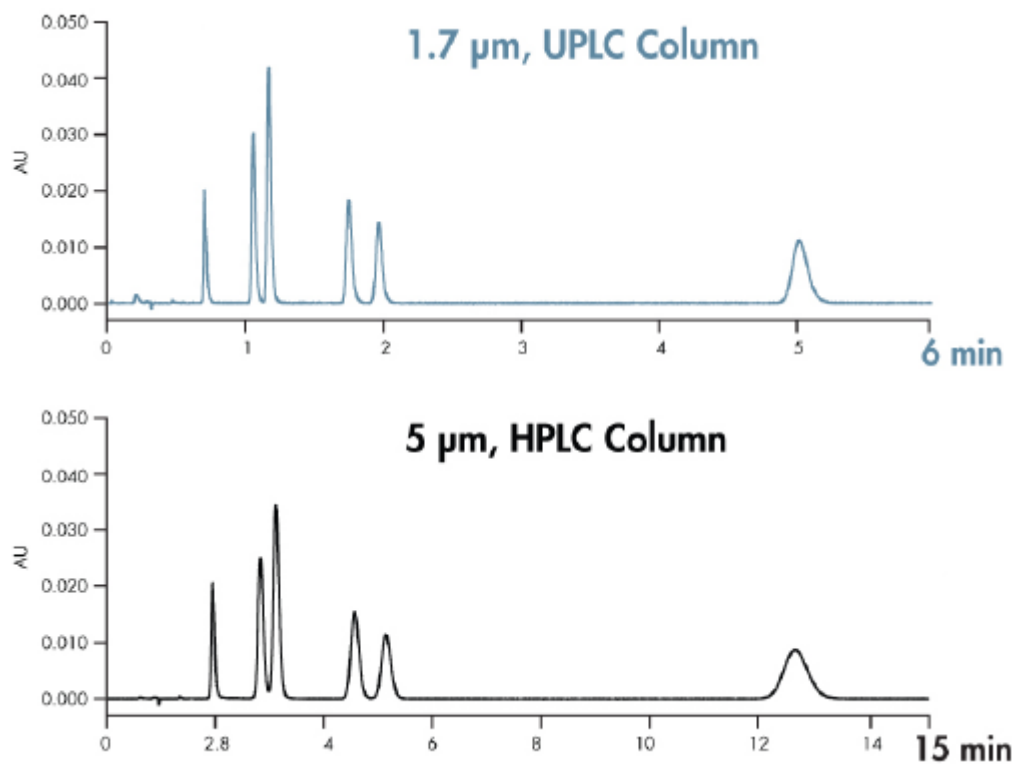
UPLC technology is based on columns packed with 1.7 μm -diameter, spherical particles coupled with low dispersion systems, allowing you to realize the full separation potential of these highly efficient columns.

Figure 1–1: Evolution of particle size in liquid chromatography and the impact on separation efficiency



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.

Figure 1–2: Comparison of chromatographic separations using 5.0- μm and 1.7- μm particles



The figure above compares two separations, one using HPLC with a column packed with 5 μm particles, and the other using UPLC with a column packed with 1.7 μm particles. The improvements in both resolution and the speed of analysis are apparent in the UPLC chromatogram. Each separation was performed on a 2.1 \times 50 mm column. Chromatographic conditions for the separations were identical, except for the flow rate, which was scaled based on particle size.

1.2 Features of the ACQUITY UPLC H-Class system

The ACQUITY UPLC H-Class 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 quaternary solvent manager
- A flow-through-needle sample manager

- Pump and sample manager design enhancements, to minimize dispersion and reduce cycle time
- Flexible column management options to support different column lengths and automated switching of up to 6 columns in independent temperature zones
- An optional sample organizer to expand sample capacity

1.2.1 Software features

1.2.1.1 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 sample manager and solvent manager communicate to automatically coordinate the injection sequence, enabling the solvent manager to provide additional pressure at the exact moment the sample manager switches its injector valve to the inject position, to introduce the low-pressure sample.

1.2.1.2 Gradient Smart Start

Before each sample injection, a sample manager typically performs wash sequences and then aspirates the appropriate sample volume. When these tasks are completed, the solvent manager begins to deliver the gradient to the injection valve. The dwell volume of the system, which affects the amount of time required for this gradient to reach the column, can be a significant component of the overall cycle time.

The Gradient Smart Start feature adjusts when an injection is made relative to when it starts. In this way, when you transfer methods, the feature compensates for differences in dwell volume between chromatographic systems. Moreover, it automatically coordinates all pre-injection operations, minimizing delays that would increase the overall cycle time. In doing so, the feature makes it possible to begin gradient operation before or during the sample manager's pre-injection functions, resulting in significant time savings.

1.2.1.3 Wash Plungers

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

Tip: The Wash Plungers function is not available when the module is operating.

1.2.1.4 No-Flow Shutdown

The No-Flow Shutdown feature runs the Wash Plungers function after the solvent manager remains idle for a specified time interval. This feature prevents deposition of precipitated material on the pump plungers and plunger seals while the system is idle.

1.2.1.5 Automatic Prime

When you enable the solvent manager's Automatic Prime function, the system primes the lines of the optional solvent selection valve when a new line is selected. You can specify the flow rate and duration of the prime for the new solvent line.

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.

1.2.1.6 Flow Ramping

Using the Flow Ramping function, you specify the rate at which the solvent manager increases or decreases flow.

Tip: The default value is set to support rigid, HPLC and UPLC column particles. For pressure sensitive columns (such as gel columns), the flow ramping should be adjusted.

1.2.1.7 Auto•Blend Plus

Auto•Blend Plus technology uses pure solvents and concentrated stocks to blend mobile phase compositions at a specific pH. At the same time, it controls the concentration of salt or organic solvent, to optimize separations. Use the Auto•Blend Plus feature to create and store buffer systems in a solvent catalog that all users of an ACQUITY quaternary solvent manager can share. To prepare and adjust chromatographic mobile phases, you add acid, base, salt or organic solvent, and water to the solvent reservoirs. By doing so you can, for example, optimize protein separations, which are especially sensitive to a buffer's pH and salt concentration. You can also optimize reversed-phase separations that are sensitive to pH and organic-solvent composition.

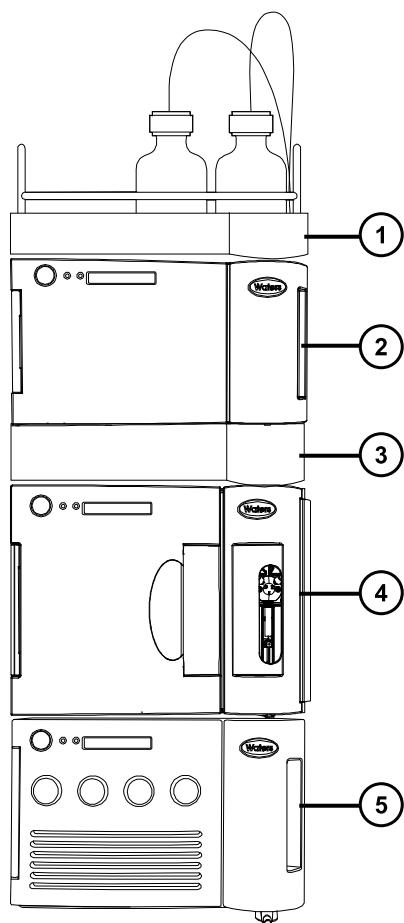
See also:

- *Auto•Blend Plus Technology for Ion Exchange, Size Exclusion, and Reversed-phase Chromatography*
- The Auto•Blend Plus videos on the Support tab on the [Waters Auto•Blend Plus](#) page.

1.3 System components

The following illustration depicts a system stack which includes four core modules and the solvent bottle tray.

Figure 1–3: Example of a system core stack



- ① Solvent bottle tray
- ② Detector
- ③ Column heater
- ④ Sample manager
- ⑤ Solvent manager

The system includes a solvent manager, sample manager, column heater, detector (tunable ultraviolet, photodiode array, evaporative light scattering, fluorescent, conductivity, refractive index, or mass spectrometry), and an ACQUITY UPLC column.

Waters Empower chromatography software, UNIFI, or MassLynx mass spectrometry software controls the system.

1.3.1 Quaternary solvent manager

The quaternary solvent manager (QSM) 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 2.2 mL/min, at reduced pressures, to 53779 kPa (537 bar, 7800 psi). The QSM can pump four degassed solvents simultaneously using a gradient proportioning valve (GPV) to dynamically create a specified composition.

Note: An optional 6-solvent selection valve can be added to the QSM line D for increased solvent flexibility.

1.3.2 Sample manager-flow through needle

The sample manager-flow through needle (SM-FTN) uses a direct-injection mechanism to inject samples drawn from plates and vials onto a chromatographic column. This injection style injects all of the aspirated sample onto the column, without any sample volume overhead. The sample needle is part of the fluidic path and the internal surfaces are flushed by the mobile phase during the separation. The exterior of the needle is washed in the injection port as specified in the method. The standard injection volume range of the SM-FTN is 10 µL, however this can be expanded up to 1 mL through the addition of optional extension loops (installed between the sample needle and the injection valve).

The SM-FTN also features several advanced sample conditioning features such as dilution, auto-addition, and mixing, as well as load-ahead capabilities to reduce inject-to-inject cycle times.

1.3.2.1 Wash solvent

The sample manager needle wash system is used to minimize sample carryover. It uses a single wash solvent and this solvent does not enter the flow path of the system.



Notice:

- Do not leave buffers stored in the system.
- Flush all flow paths, including the needle wash, with plenty of non-buffered solvent before shutting down the system.
- For extended shutdown periods (longer than 24 hours), use 10% to 20% methanol in water.
- When using a buffered wash solvent, prime it for a minimum of 30 sec.
- Use of buffers can cause salt build-up on the needle and wash port, which can require periodic cleaning.

Restriction: Avoid buffered wash solvents.

1.3.2.2 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.

Notes:

- Purge solvent must be miscible with the needle wash solvent.
- Waters recommends a purge solvent of 90/10 Water/Methanol whenever possible.

1.3.3 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 °C (or a minimum of 5 °C above ambient temperature) to 90 °C. 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.

Tips:

- Active preheating is the default configuration for the system.
- An optional, passive, column stabilizer is available for existing chromatographic methods not suitable for active preheating.

1.3.4 Column manager (optional)

The ACQUITY UPLC H-Class column manager is an option for helping to ensure precise, reproducible separations. The column manager can regulate the temperature of columns from 4 to 90 °C. Its troughs can accommodate columns of up to 4.6-mm I.D. and up to 150-mm length, depending on the configuration. Each of the two column troughs can condition one 15-cm column (with filter) or two 50-mm columns (without filters).

The column manager offers a waste channel as well as a bypass channel and automated, programmable switching between columns, for methods development.

You can configure the column manager to work with as many as two column manager auxiliary modules, in addition to the base unit. The column manager auxiliary modules are controlled by the column manager and operate in the same temperature range. Each of the two column troughs can accommodate one column of up to 150 mm in length with a pre-column filter, or two columns up to 500 mm in length without pre-column filters.

Note: A system configured with a column manager base unit (and no additional heater/cooler modules) can accommodate as many as four columns (50-mm); a system with a column manager base unit and one column heater/cooler module can accommodate as many as four columns; a

system with a column manager base unit and two column heater/cooler modules can accommodate as many as six columns (two columns in the base unit).

1.3.4.1 Active solvent conditioning

HPLC and UPLC applications benefit from pre-column, mobile-phase heating to improve chromatographic separations. The column heaters in the system use 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 system. When using the CH-A, an optional, passive, column stabilizer is available for existing chromatographic methods not suitable for active preheating.

1.3.5 Sample organizer (optional)

Requirement: Verify that the sample organizer you use with your system is compatible with sample managers that have a rotary tray.

The sample organizer stores multiple microtiter or vial plates and transfers them to and from the sample manager. This automates processing and increases throughput.

The sample organizer's storage shelf compartment can hold a selection of ANSI plates, which you load into the organizer through a large, swing-open front door. Heaters and coolers thermally condition the shelf compartment and together with the sample manager's heater/cooler, maintain the temperature at a set point determined by the user.

1.3.6 Detection

The optical detectors compatible with your system detect and quantify concentrations of sample analyte. The system accommodates these detectors:

- Photodiode array (PDA)
- Photodiode array eλ (PDA eλ)
- Photodiode array with TaperSlit (PDA-TS)
- Tunable ultraviolet (TUV)
- Evaporative light scattering (ELS)
- Fluorescence (FLR)
- Refractive index (RI)
- Conductivity detector (2432)

1.3.7 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/E³² module or when network control is unavailable.

1.3.8 FlexCart (optional)

The optional FlexCart provides for the 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.

Note: The ACQUITY Flex Cart is not supported with the ACQUITY QDa detector or any ACQUITY UPLC H-Class System with dual detection (split stack configurations).

1.3.9 Column technology

The ACQUITY UPLC columns are packed with the following: 1.7- μm , bridged, ethylsiloxane, hybrid; 1.8- μm high strength silica particles, or 1.6- μm solid-core 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 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.

1.3.9.1 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. The eCord column chip provides documentation of the column used for each chromatographic run and records the following information:

- The name of the sample set (or sample list) run on the column.
- Number of injections onto the column.
- Number of samples injected onto the column.
- The highest pressure that the column has experienced (and the date).
- The highest temperature the column has experienced (and the date).

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 column's eCord fob to the receptacle on the column compartment, the chip automatically records and stores system information. You need take no further action.

1.4 For additional information

On the system documentation CD, you can find this additional information:

- *ACQUITY UPLC Quaternary Solvent Manager Overview and Maintenance Guide*
- *ACQUITY UPLC Sample Manager - Flow Through Needle Overview and Maintenance Guide*
- *ACQUITY UPLC Column Heater-Active Overview and Maintenance Guide*
- *ACQUITY UPLC Column Manager - Active and Column Manager - Auxiliary Overview and Maintenance Guide*
- *ACQUITY UPLC 30-cm Column Heater-Active Overview and Maintenance Guide*
- *ACQUITY UPLC 30-cm Column Heater/Cooler Overview and Maintenance Guide*
- *ACQUITY UPLC Sample Organizer Operator's Overview and Maintenance Information*
- *ACQUITY UPLC Photodiode Array and e λ Photodiode Array Detector Operator's Overview and Maintenance Guide*
- *ACQUITY Photodiode Array Detector with TaperSlit Overview and Maintenance Guide*
- *ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide*
- *ACQUITY UPLC Fluorescence Detector Getting Started Guide*
- *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide*
- *ACQUITY Refractive Index Detector Overview and Maintenance Guide*
- *ACQUITY QDa Detector Overview and Maintenance Guide*

- *2432 Conductivity (Cond) Detector Overview and Maintenance Guide*
- *Auto•Blend Plus Technology for Ion Exchange, Size Exclusion, and Reversed-phase Chromatography*

Visit www.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 Performance optimization

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

2.1 General guidelines

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

ACQUITY UPLC H-Class chromatography requires optimum performance from the sample manager 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 in width. Waters recommends a sampling rate that will generate between 25 and 50 points across the narrowest integrated peak in the separation in order to ensure repeatable quantification and while maximizing sensitivity. Based on the van Deemter equation, the optimal linear velocity for 1.7 μ m columns will be higher than that on a 5 μ m column. The table below offers optimal flow rate conditions 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.

Table 2–1: Optimal flow rates for molecular weight range

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

2.1.1 Follow these general recommendations when performing a UPLC analysis

Select appropriate solutions

- Use high-quality solvents, buffers, and additives (HPLC or MS grade).
- Keep concentrated stock solutions, to use when preparing working solutions.
- 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.
- Ensure that gradients include an organic component (0.1%, for example) to start, to provide more consistent and predictable.

Set up the system properly

- 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.
- Always use solvent filters on tubing lines in solvent bottles.
- Use the **Load Ahead** option when you desire a shorter cycle time.

Prime properly

- Prime solvent lines during system start-up.
- Keep the seal wash line and all solvent lines primed.

Manage waste properly

- 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 solvent manager overview and maintenance guide for details on how to route the tubing.)
- Monitor the waste level, to ensure that it is never too high.

Use care when using buffers

- Do not use buffers in the wash solvent line.
- Do not top off buffers, which can promote microbial growth.
- Filter buffers with a 0.2- μ m filter membrane.
- Do not leave buffers stored in the system.

- 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 with purge solvent for a minimum of 10 cycles.
- 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.

Follow proper shutdown procedures

- Flush all flow paths, including the needle wash, with plenty of non-buffered solvent before shutting down the system. For extended shutdown periods (longer than 24 hours), use 10% to 20% methanol in water.
- Do not use the **Load Ahead** or **Loop Offline** options when you are troubleshooting carryover problems.

2.2 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 system typically exhibits a bandsread between 10 and 12 μL, depending on system configuration. An Alliance HPLC system can exhibit a bandsread between 35 μL and 50 μL. Because of the dispersion differences, a band on an Alliance system experiences a threefold increase in dilution, compared with an ACQUITY UPLC H-Class system.

As a result, 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.

2.3 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.

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 re-equilibrate 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, and whatever time savings you gain can be lost in terms of 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.

2.4 Cycle time (between injections)

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

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

The Loop Offline option on the sample manager 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.

2.5 Preventing leaks

Preventing leaks during an analysis ensures adequate flow pressure in the system and the integrity of the sample.

Leaks can occur at any tubing connection, gasket, or seal but are most common at tubing connections. Low pressure leaks (on the intake side of the solvent manager's pump) cause solvent loss and air introduction during the intake cycle. Leaks at high pressure fittings (downstream of the "intelligent" intake valves) 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 re-tightening fittings versus installing them for the first time.

2.5.1 Installation recommendations for fittings

Two types of fittings are used within the system: PEEK (polymer-based) fittings and tubing and SST (stainless steel) tubing and fittings. When connecting tubing, heed the following recommendations for installing and tightening fittings.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biohazards, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Recommendations:

- To prevent band spreading, ensure that the tubing is fully bottomed in the fitting port before tightening the compression screw.
- For easier accessibility, use long compression screws to attach tubes to the injector and vent valve.
- Perform the solvent manager leak test whenever you replace or loosen fittings during maintenance (see the console online Help).
- Whenever you loosen fittings during maintenance, examine for cracks, stripped threads, and deformations.
- Do not reuse stainless steel fittings more than six times.

2.5.1.1 Assembling new fittings

For metallic (SST or MP35N) fitting assemblies with ferrules not previously assembled or set to tubing, you must mark the compression screw and fitting body, and ensure that the two marks line up when you tighten them.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required tools and materials

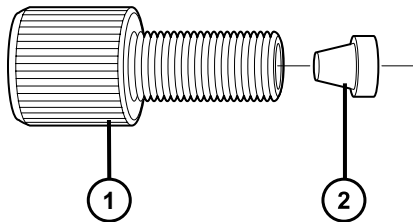
- 1/4-inch open-end wrench – For tightening or loosening stainless steel (gold-plated) fittings with 2-piece ferrule.
- Permanent marker
- Chemical-resistant, powder-free gloves

To assemble the new fittings:

1. Insert the end of a tube into the hexagonal end of the compression screw.
2. Insert the tube into the larger end of the ferrule.
3. Insert the tube into the fitting body.
4. Rotate the compression screw, clockwise, into the fitting body until the screw is finger-tight.
5. Using a permanent marker, mark the compression screw at the 12 o'clock position.
6. Mark the fitting body at the 9 o'clock position.
7. Ensure that the tubing makes contact with the bottom of the fitting body, and use the 1/4-inch open-end wrench to rotate the compression screw clockwise 3/4-turn until the two marks line up.

2.5.1.2 1/4-28 flangeless fitting with ferrule

First use or re-installed



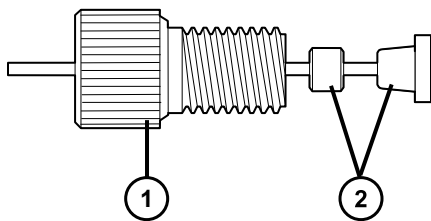
① Compression screw

② Ferrule

Tighten the fitting finger-tight.

2.5.1.3 1/4-28 flangeless fitting with 2-piece ferrule

First use or re-installed

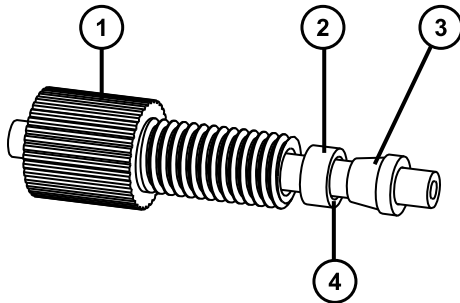


- ① Compression screw
- ② 2-piece ferrule

Tighten the fitting finger-tight.

2.5.1.4 Long 1/4-28 fitting with flangeless ferrule and stainless steel lock ring installed on 1/8-inch OD tubing

First use or re-installed

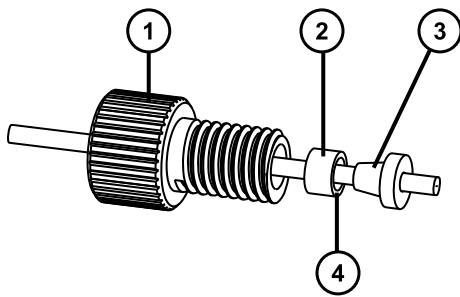


- ① Compression screw
- ② Lock ring
- ③ Ferrule
- ④ End of lock ring with larger inside diameter (ID)

Tighten the fitting finger-tight.

2.5.1.5 Short 1/4-28 fitting with flangeless ferrule and stainless steel lock ring installed on 1/16-inch OD tubing

First use or re-installed

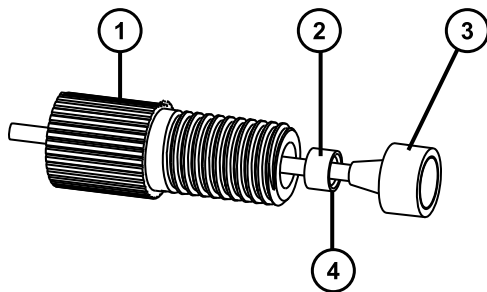


- ① Compression screw
- ② Lock ring
- ③ Ferrule
- ④ End of lock ring with larger inside diameter (ID)

Tighten the fitting finger-tight.

2.5.1.6 5/16-24 fitting with filter and stainless steel lock ring

First use or re-installed

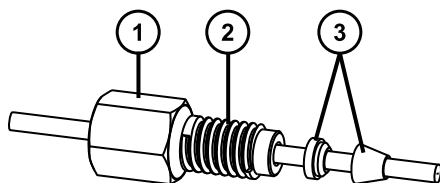


- ① Compression screw
- ② Lock ring
- ③ Ferrule and filter
- ④ End of lock ring with larger inside diameter (ID)

Tighten the fitting finger-tight.

2.5.1.7 Stainless steel (gold-plated) fitting with long flats and 2-piece stainless-steel ferrule

First use

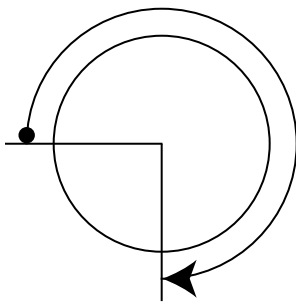


- ① Long flats
- ② Compression screw
- ③ 2-piece stainless-steel ferrule

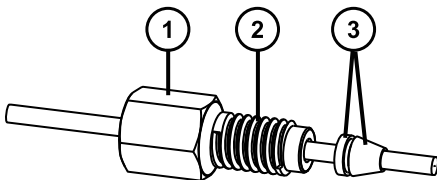
Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench. For detailed instructions about assembling new fittings, see [Assembling new fittings](#).

Tip: To prevent band spreading, ensure that the tubing is fully bottomed in the fitting before tightening the compression screw.

First use tightening



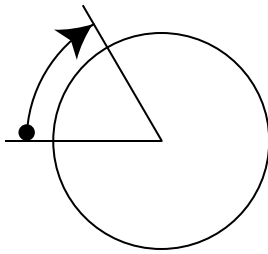
Re-installed



- ① Long flats
- ② Compression screw
- ③ 2-piece stainless-steel ferrule

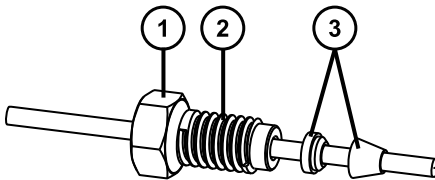
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

Re-installed tightening



2.5.1.8 Stainless steel (gold-plated) fitting with short flats and 2-piece stainless-steel ferrule

First use

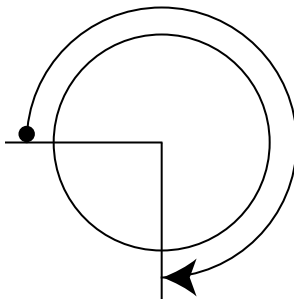


- ① Short flats
- ② Compression screw
- ③ 2-piece stainless steel ferrule

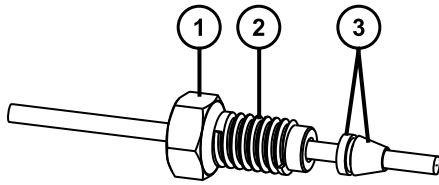
Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench. For detailed instructions about assembling new fittings, see [Assembling new fittings](#).

Tip: To prevent band spreading, ensure that the tubing is fully bottomed in the fitting before tightening the compression screw.

First use tightening



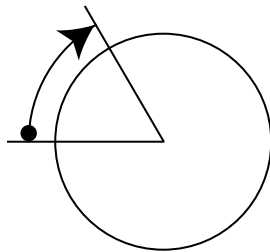
Re-installed



- ① Short flats
- ② Compression screw
- ③ 2-piece stainless steel ferrule

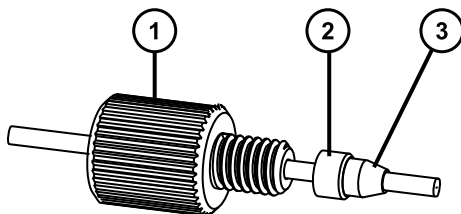
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

Re-installed tightening



2.5.1.9 PEEK fitting with PEEK ferrule and stainless steel lock ring

First use or re-installed

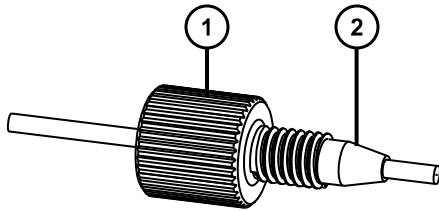


- ① Compression screw
- ② Lock ring
- ③ Ferrule

Tighten the fitting finger-tight.

2.5.1.10 One-piece PEEK fitting

First use or re-installed



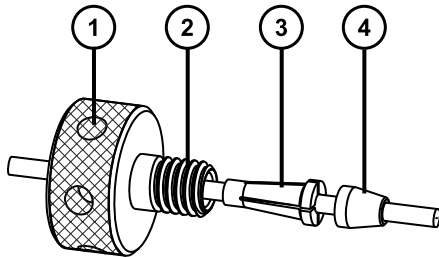
① Compression screw

② Ferrule

Tighten the fitting finger-tight.

2.5.1.11 Gold-plated compression screw with collet

First use



① Hole for inserting the collet and compression-screw multi-tool

② Compression screw

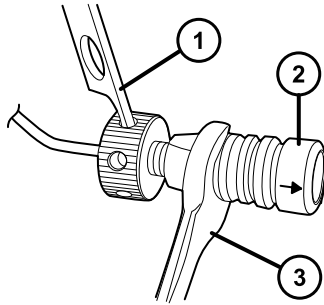
③ Collet

④ PEEK ferrule

To tighten this fitting for first use:

1. Finger-tighten the fitting.
2. Using the collet and compression-screw multi-tool and a 5/16-inch open-end wrench, tighten the fitting as much as an additional 1/6-turn.

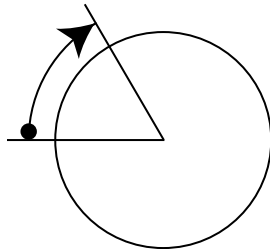
Placement of collet and compression-screw multi-tool and 5/16-inch open-end wrench



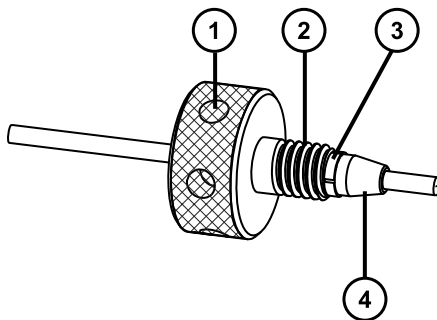
- ① Collet and compression-screw multi-tool
- ② Column or column in-line filter
- ③ 5/16-inch open-end wrench

Tip: To prevent band spreading, ensure that the tubing bottoms in its fitting before you tighten the compression screw.

First use tightening



Re-installed

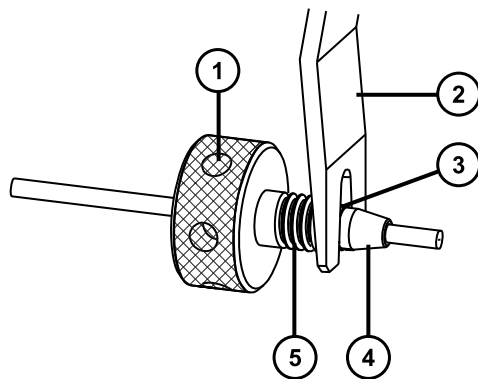


- ① Hole for collet and compression-screw multi-tool
- ② Compression screw
- ③ Collet
- ④ PEEK ferrule

To tighten a re-installed fitting:

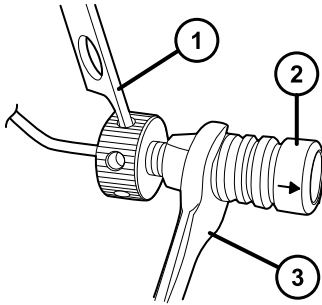
1. Insert the tool between the collet and compression screw and pry the collet until it loosens from the inside of the fitting.

Re-installed



- ① Hole for collet and compression-screw multi-tool
 - ② Collet and compression-screw multi-tool
 - ③ Collet
 - ④ PEEK ferrule
 - ⑤ Compression screw
2. Tighten the fitting finger-tight.
 3. Using the collet and compression-screw multi-tool and a 5/16-inch open-end wrench, tighten the fitting as much as an additional 1/6-turn.
 4. If the connection leaks, tighten the fitting an additional 1/8-turn.

Placement of collet and compression-screw multi-tool and 5/16-inch open-end wrench

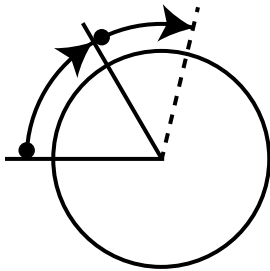


- ① Collet and compression-screw multi-tool
- ② Column or column in-line filter
- ③ 5/16-inch open-end wrench

Tip: When reinstalling the fitting,

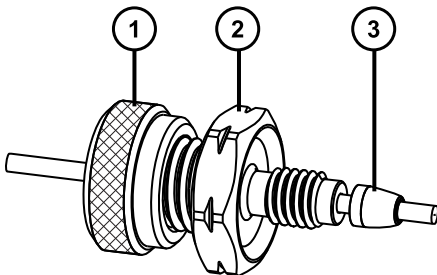
- examine the collet and ferrule for damage, and replace them, if necessary;
- always loosen the collet before re-connecting the reusable finger-tight fitting.

Re-installed tightening



2.5.1.12 Dual-threaded, finger-tight fitting with loose ferrule

First use or re-installed



- ① Locking cap nut
- ② Gold-plated compression screw
- ③ Ferrule

To tighten the fitting:

1. Loosen the cap nut from the gold compression screw.
2. Slide the gold compression screw with the ferrule into the inlet of the column (or in-line filter).
3. Finger-tighten the gold compression screw into the inlet of the column (or in-line filter).
4. Engage the column (or in-line filter), and tighten the column (or in-line filter) onto the gold compression screw.
5. Tighten the cap nut onto the gold compression screw.

Important: When re-installing this fitting, examine the ferrule for any damage, and replace it, if necessary.

2.6 Developing methods

See also: For information about method development and validation, consult the *Auto•Blend Plus Technology for Ion Exchange, Size Exclusion, and Reversed-phase Chromatography* documentation.

For the greatest flexibility in method development, Waters recommends configuring the system with the column manager and optional Auxillary column managers as well as installing the optional 6-solvent select valve in the QSM. Using the standard Auto•Blend Plus technology will automate the preparation of any pH specific mobile phase from pure solvents for easier method development.

2.7 Sample preparation

The use of UPLC columns may result in additional considerations when it comes to sample preparation.

2.7.1 Particulates

Waters recommends filtering all samples with particulates through a 0.2 µm sample filter or installing a column pre-filter. 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](#) for recommendations on choosing and handling solvents.

2.7.2 Matching sample diluents

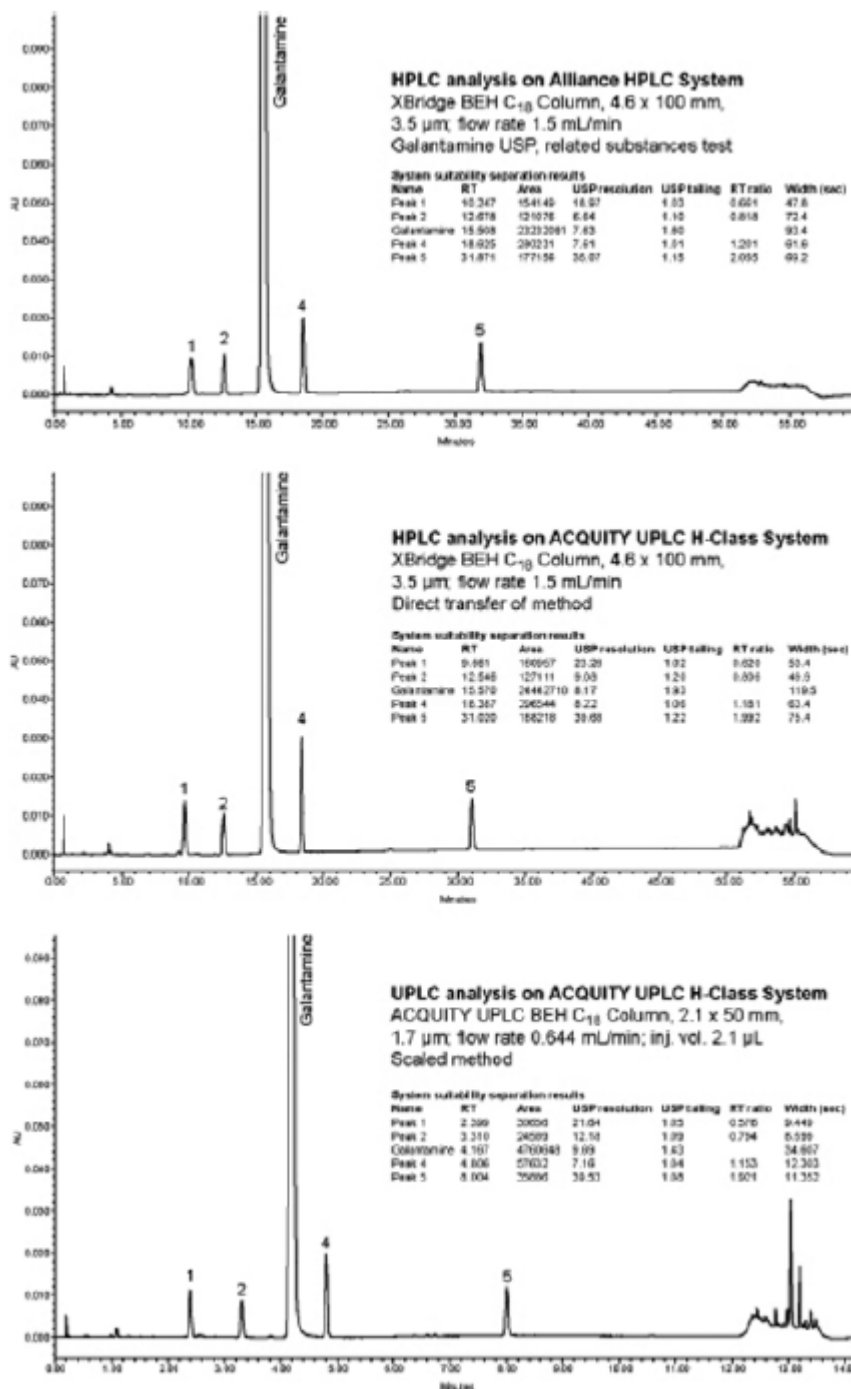
When you use the sample manager's auto-dilution option, the purge solvent serves as the sample diluent. Ensure that your sample solution is soluble and miscible in your chosen purge solvent.

2.8 Transferring methods

Transferring an LC method from one system to another can sometimes be necessary. For such transfers, the object is to maintain a separation's performance or enhance it by reducing runtime or improving selectivity and/or resolution. The ACQUITY UPLC H-Class system, which Waters designed specifically to facilitate method transfer, are the ideal tools to achieve that goal with quaternary solvent capability, low pressure mixing, and flow through needle design. The optional column manager facilitates switching between target columns while exhibiting low bandspread and maintaining the same temperature profile as the standard column heater.

The following example of a method transfer shows the related substances test for galantamine, an alkaloid used to treat Alzheimer's disease. The USP method (monograph: USP32-NF27 supplement: no. 2, page 4245) is demonstrated first using an HPLC system. The method is then transferred to the system fitted with an HPLC column, whereby selectivity and resolution are maintained. Using the ACQUITY UPLC columns calculator, it is then scaled to UPLC and optimized for the shortest analysis time at equal peak capacity. The run time decreased by 46 minutes.

Figure 2-1: Method transfer from HPLC to UPLC



When transferring methods from one system to another, you must define and characterize the original method well. Doing so includes noting information about column dimensions, dwell volumes, configurations, injection volumes, analyte molecular weights, and gradient profiles. You must calculate various measurements of both systems, using the same method, to ensure a successful transfer. Using the Waters ACQUITY UPLC Columns Calculator ensures the best results for transferring the LC method from HPLC to UPLC or UPLC to HPLC.

2.8.1 Columns calculator

The columns calculator enables you to scale a method by calculating operating parameters that give equivalent chromatographic performance. It can quickly define methods to test further in the laboratory.

See also: The ACQUITY console online Help for additional details.

2.8.2 Transferring from HPLC to UPLC

Follow these guidelines to preserve a chromatographic profile when transferring from one system to another:

- Consider the difference in dwell volume between the two systems.
- Pre-injector volume, specified in the instrument method, enables the gradient to start before the injection is triggered. Use a pre-injection volume to maintain a constant dwell volume to column volume ratio on both systems.

$$\text{Pre-injector volume} = \frac{[\text{System 1 dwell volume (mL)} - \text{System 2 dwell volume (mL)}] \times \text{Column 2 volume (mL)}}{\text{Column 1 volume (mL)}}$$

- For a target system, with a smaller volume, use an isocratic hold to account for the dwell volume differences.
- Active preheating is the default configuration for the ACQUITY UPLC H-Class system. An optional, passive, column stabilizer is available for existing chromatographic methods not suitable for active preheating.
- Select the column with the most similar selectivity using the Interactive Waters Reversed Phase Column Selectivity Chart, which you can download from the Waters Web site or by double-clicking the shortcut icon on the desktop. The Waters columns are highlighted (larger white dots).
- For the initial evaluation, keep conditions as consistent as possible. You can optimize the separation later.

See also: *Transferring methods* in the ACQUITY Console online Help.

2.8.3 Transferring from UPLC to HPLC

Follow these guidelines to preserve the integrity of a chromatographic separation.

- Match the ratio of column length to particle size (L/dp), the measure of resolving power.
- Maintain the number of gradient column volumes for each step of the gradient, to preserve its separation power.
- Calculate appropriate gradient hold volumes at initial gradient conditions when going from a larger system volume to a smaller one.

$$\text{Gradient hold volume} = \frac{[\text{System 1 dwell volume (mL)} - \text{System 2 dwell volume (mL)}] \times \text{Column 2 volume (mL)}}{\text{Column 1 volume (mL)} \times \text{Column 2 flow rate (mL/min)}}$$

After you input the required information, the calculator displays the target method conditions. The calculator automatically displays the L/dp of the existing method column and the target column.

Because the dwell volumes of the ACQUITY UPLC H-Class system are far smaller than those of a conventional HPLC system, often a gradient hold is required.

Identical chemistries are available in UPLC and HPLC columns simplifying their selection and allowing for a simple method transfer from the ACQUITY UPLC H-Class system to standard analytical HPLC.

Finally, note that the chromatographic conditions provided by the calculator serve as a starting point. You can further optimize these conditions based on the requirements for the separation.

See also: *Transferring methods* in the ACQUITY Console online Help.

3 System preparation

Before proceeding, ensure that all of the procedures that explain how to prepare the system modules for operation were performed as specified in the modules' overview and maintenance guides.

3.1 Powering-on the system

To power-on the system, you must power-on the system workstation, system modules, and the chromatography software. When powered-on, each module beeps three times and performs a series of startup tests. After all modules complete their startup tests, you open the system software and prime the system.

Tip: When you power-on a new system for the first time, its leak sensors default to disabled status. Subsequently, they retain their last specified setting. To enable or disable leak sensors, see the console online Help.

To power on the system:

1. Power-on the system's workstation.

Result: The following start-up tests run: CPU board, memory (RAM and ROM), external communication system (Ethernet), and clock. If the start-up tests indicate a malfunction, consult the console online Help.

Important: If the system includes a column heater, it is automatically powered-on when you power-on the sample manager.

2. Power-on the sample manager and then the solvent manager, by pressing the power switch on the top, left-hand side of each device's door.

Note: The system's communications occur at an internal Ethernet switch in the sample manager. This module must be powered-on for any other system modules to communicate with the data system.

3. After power LEDs on the solvent manager and sample manager glow steady green, power-on each detector by pressing the power switch on the detector's top, left-hand side.

Tip: Power-on detectors only when the flow cell is wetted, to prevent initialization errors.

See also: [Monitoring module LEDs](#)

4. Launch the chromatography data system software, and open the system.

Requirement: If this is the first time you are using this system, you must define a new system. For instructions, see the online Help.

5. Open the control panels and console.

See also: [Monitoring from control panels](#) and [Opening the console](#)

6. Prime the system.

See also: [Priming the system](#)

3.2 Opening the console

You can perform the following tasks in the console:

- Monitor system performance
- Specify settings for certain module parameters
- Run diagnostic tests
- View an interactive diagram of the module components

See also: The console online Help for additional information on how to perform these tasks.

3.2.1 To open the console from Empower software

1. From the Empower navigation bar, select **Run Samples** and then **Control Panel**.

Result: A control panel for each device in the system appears.

2. In the sample manager control panel, click **Display console** .

Alternative: Right-click the control panel for any module, and select **Launch Console** from the menu that appears.

3.2.2 To open the console from MassLynx software

1. From the MassLynx window, click **Inlet Method**.
2. Click the **Additional Status** tab.

Result: A control panel for each device in the system appears.

3. In the sample manager control panel, click **Display console** .

Alternative: Right-click the control panel for any module, and select **Launch Console** from the menu that appears.

3.2.3 To open the console from UNIFI software

1. From the UNIFI Portal, click the **My Work** tab.
2. From the **My Work** tab, select **Instrument Systems**, and then double-click on the device that you want to monitor.

Alternative: Launch the **System Console** from the **System Control Panel** menu.

Result: A control panel for the selected device appears.

3.3 Priming the system

Requirement: You must prime the system after starting up the system, as well as after changing the mobile phase, after changing the sample needle, and after the system has been idle for four hours or more.

Recommendation: If you are introducing new solvents, prime them at 10 mL/min for 7 minutes. Alternatively, prime the solvents at 10 mL/min for 3 minutes. Ensure that sufficient quantities of solvent are available for priming.

Tip: In the console, you can select the Startup System feature to prime all solvents and to specify the solvent composition, flow rate, column and sample temperatures, and needle characterization. For details, see the console online Help.

3.4 Monitoring module LEDs

The LEDs on each module indicate its operational status. Note, however, that the significance of an LED's color differs from one module to another.

3.4.1 Power LED

The power LED indicates the power-on or power-off status. Two LEDs appear on each device or instrument, typically located on the left-hand side of the front panel or door. The one on the left is the power LED, which glows green when power is applied to the device and unlit when power is not applied.

Note: To provide adequate ventilation, the sample manager's 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 ac wall outlet or rear panel.

3.4.2 Status LEDs

3.4.2.1 Run LED

Run status is indicated by an LED on the sample manager's front panel. The run LED is on the right-hand side of the power LED. If the run LED is a steady-green color, injections or a diagnostic tests are in progress.

Table 3–1: Run LED descriptions

Run LED mode and color	Indication
Unlit	The sample manager is idle.
Steady green	The sample manager is operating normally, completing any outstanding samples or diagnostic function requests.
Flashing green	The sample manager is initializing.
Flashing red	An error stopped the sample manager. Refer to the console log for information about the error. Alternative: Firmware upload is in progress.
Steady red	A failure is preventing operation. Cycle power to the sample manager. If the LED remains red, report the problem to Waters Technical Support. Alternative: Firmware upload is complete.

3.4.2.2 Flow LED

Flow status is indicated by an LED on the solvent manager's front panel. The flow LED is on the right-hand side of the power LED. If the flow LED is a steady-green color, solvent is flowing through the solvent manager as programmed.

Table 3–2: Flow LED descriptions

Flow LED mode and color	Indication
Unlit	The solvent manager is idle.
Steady green	The solvent manager is operating normally, flow is moving through the system as programmed.
Flashing green	The solvent manager is initializing.
Flashing red	An error stopped the solvent manager. Refer to the console log for information about the error. Alternative: Firmware upload is in progress.

Table 3–2: Flow LED descriptions (continued)

Flow LED mode and color	Indication
Steady red	A failure is preventing operation. If, after you cycle power to the solvent manager, the LED remains red, report the problem to Waters Technical Service. Alternative: Firmware upload is complete.

3.4.2.3 Detector LED

An LED on the detector's front panel indicates the run status of the lamp or detector. For detectors equipped with a lamp, the LED is a steady-green color when the lamp is ignited. For detectors that are not equipped with a lamp, the LED is a steady-green color when the detector is operating normally.

Table 3–3: Detector LED descriptions

Detector LED color	Description
Unlit	If the detector is equipped with a lamp, the lamp is extinguished. If the detector is not equipped with a lamp, the detector is idle.
Steady green	If the detector is equipped with a lamp, the lamp is ignited. If the detector is not equipped with a lamp, the detector is operating normally.
Flashing green	The detector is initializing or calibrating.
Flashing red	An error stopped the detector's operation. Refer to the console log for information about the error.
Steady red	A failure is preventing the detector from operating. If, after you cycle power to the detector, the LED remains red, report the problem to Waters Technical Service.

3.5 Monitoring from control panels

You can monitor the sample manager, solvent manager, column module, and detector from control panels, which you access via the chromatography data system.

- When Empower software controls the system, the control panels appear at the bottom of the Run Samples window.
- When MassLynx software controls the system, the control panels appear on the Additional Status tab of the Inlet Editor window.
- When UNIFI software controls the system, the control panels appear in the right-hand utility pane of the main window whenever a system is selected for the System Console or data analysis activities.

You can update a parameter set point directly from a control panel, providing sample analysis is not running. When the parameter value is underscored and appears in blue, sample analysis is not running, and you can click the parameter value and specify a new value in the dialog box that appears.

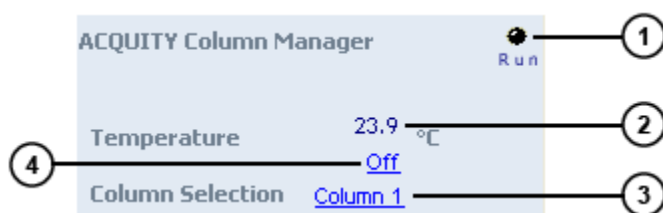
3.5.1 Column Manager control panel

The column manager's control panel displays the current column temperature and set point of the column manager (CM-A) and column manger auxiliary (CM-AUX). Other compatible column modules are controlled via the sample manager control panel.

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

You can edit the set point when the system is idle by clicking on the underlined value. You cannot edit temperature set point and column selection while the system is running samples.

Figure 3–1: Column Manager control panel



- ① Run LED – Mirrors the run status LED on the column manager's front panel, unless communications are interrupted.
- ② Current temperature – Displays the current column compartment temperature.
- ③ Column currently in use – Displays the column that is currently in use.
- ④ Temperature set point – Displays the column compartment set point. When active temperature control is disabled, this field displays "Off".

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

Table 3–4: Additional functions in the column manager's control panel

Control panel function	Description
Reset module	Resets the column manager after an error condition.
Help	Displays the console online Help.

3.5.2 Sample manager control panel

The control panel of the sample manager-flow through needle (SM-FTN) displays the current temperatures of the sample compartment and column heater, their set points, and the selected column.

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.

Figure 3–2: SM-FTN control panel



- ① Run LED – Mirrors the run status LED on the sample manager's front panel, unless communications are interrupted.
- ② Current sample compartment temperature – Displays the current temperature of the sample compartment.
- ③ Display console icon – When clicked, launches the console software.
- ④ Column compartment temperature set point – Displays the temperature set point for the column compartment.
- ⑤ Sample compartment temperature set point – Displays the temperature set point for the sample compartment.
- ⑥ Current column compartment temperature – Displays the current temperature in the column compartment.
- ⑦ Status – Displays the status of the current operation.

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

Table 3–5: Additional functions in the sample manager control panel

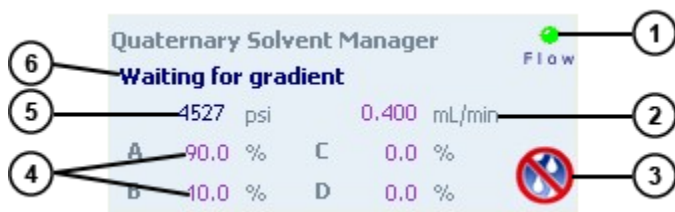
Control panel function	Description
Prime	Displays the Prime dialog box.
Wash needle	Displays the Wash Needle dialog box.
Launch Console	Launches the console software.
Reset SM	Resets the sample manager following an error condition.
Help	Displays the console online Help.

3.5.3 Solvent manager control panel

The control panel of the quaternary solvent manager (QSM) displays system pressure, total flow rate, and solvent composition.

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

Figure 3–3: QSM control panel



- ① Flow LED – Mirrors the flow status LED on the solvent manager's front panel, unless communications are interrupted.
- ② Flow rate – Displays the flow rate of solvent through all lines of the solvent manager.
- ③ Stop flow – When clicked, immediately stops all flow from the solvent manager.
- ④ Solvent composition – Displays the percentage of solvent to be drawn from solvent lines A through D. Composition values range from 0.0 to 100.0%.
- ⑤ System pressure – Displays system pressure, in kPa, bar, or psi. You can specify pressure units in the console software.
- ⑥ Status – Displays the status of the current operation.

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

Table 3–6: Additional functions in solvent manager the 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 also: Console online Help
Prime solvents	Displays the Prime Solvents dialog box.
Prime seal wash	Starts or stops priming the seal wash.
Wash plungers	Initiates the plunger wash sequence.
Launch console	Launches the console software.
Reset QSM	Resets the QSM following an error condition.
Help	Displays the console online Help.

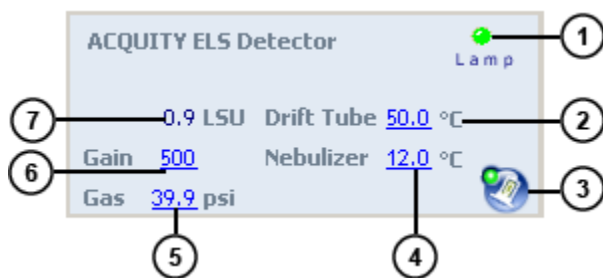
3.5.4 ELS control panel

The evaporative light scattering (ELS) detector's control panel displays light scattering units, photomultiplier tube gain factor, gas pressure, nebulizer temperature, and drift tube temperature.

If Empower software controls the system, the detector's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the detector's control panel appears at the bottom of the Inlet Editor window.

You can edit detector parameters when the system is idle by clicking the underlined value. You cannot edit these values while the system is running samples.

Figure 3–4: ELS detector control panel



- ① Lamp LED – Mirrors the lamp status LED on the front panel of the detector, unless communications with the detector are lost.
- ② Current drift tube temperature – Displays the current drift tube temperature.

- ③ Lamp icon – When clicked, ignites or extinguishes the lamp. If the icon is green, the lamp is ignited. If the icon is red, the lamp is extinguished.
- ④ Current nebulizer temperature – Displays the current nebulizer temperature.
- ⑤ Current nebulizer gas pressure – Displays the current nebulizer gas pressure.
- ⑥ Photomultiplier tube gain factor – Displays the current photomultiplier-tube gain factor.
- ⑦ Current sample energy – Displays the sample signal, in light scattering units.

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

Table 3–7: Additional functions in the ELS detector control panel

Control panel function	Description
Autozero	Resets the detector offsets.
Reset module	Resets the detector after an error condition.
Help	Displays the console online Help.

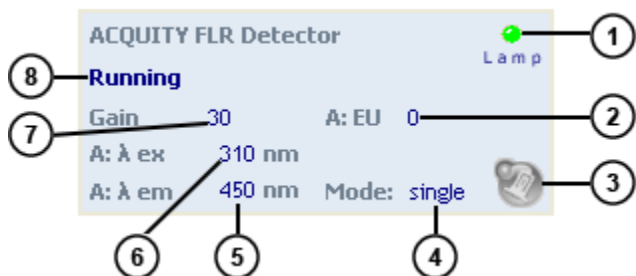
3.5.5 FLR control panel

The fluorescence (FLR) detector's control panel displays emission or energy units, the excitation and emission wavelengths, and the photomultiplier tube gain factor.

If Empower software controls the system, the detector's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the detector's control panel appears at the bottom of the Inlet Editor window.

You can edit detector parameters when the system is idle by clicking the underlined value. You cannot edit these values while the system is running samples.

Figure 3–5: FLR detector control panel



- ① Lamp LED – Mirrors the lamp status LED on the front panel of the detector, unless communications with the detector are lost.
- ② Emission units or energy units – Displays the emission units or energy units.
- ③ Lamp icon – When clicked, ignites or extinguishes the lamp. If the icon is green, the lamp is ignited. If the icon is red, the lamp is extinguished.
- ④ Operating mode – Displays the current operating mode of the detector: single channel, multichannel, spectrum scanning, or 3D.
- ⑤ Em λ – Displays the emission wavelength.
- ⑥ Ex λ – Displays the excitation wavelength.
- ⑦ Photomultiplier tube gain factor – Displays the current photomultiplier-tube gain factor.
- ⑧ Status – Displays the status of the current operation.

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

Table 3–8: Additional functions in the FLR detector control panel

Control panel function	Description
Autozero	Resets the detector offsets.
Reset module	Resets the detector after an error condition.
Help	Displays the console online Help.

3.5.6 PDA control panel

Note: The PDA, PDA e λ , and the PDA-TS have the same control panel.

The photodiode array (PDA) detector's control panel displays the detector status.

If Empower software controls the system, the detector's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the detector's control panel appears at the bottom of the Inlet Editor window.

You can edit detector parameters when the system is idle by clicking the underlined value. You cannot edit these values while the system is running samples.

Figure 3–6: PDA detector control panel



- ① Lamp LED – Mirrors the lamp status LED on the front panel of the detector, unless communications with the detector are interrupted.
- ② Lamp icon – When clicked, ignites or extinguishes the lamp. If the icon is green, the lamp is ignited. If the icon is red, the lamp is extinguished.
- ③ Shutter position – Displays the current position of the detector shutter: open, closed, erbium, or UV-blocking.
- ④ Status – Displays the status of the current operation.

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

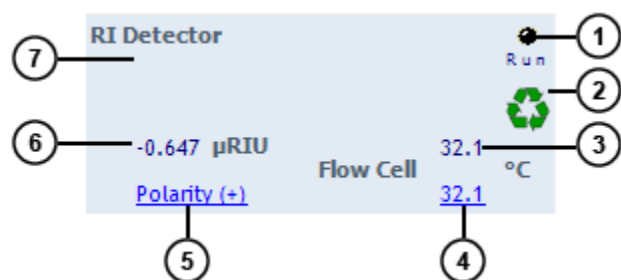
Table 3–9: Additional functions in the PDA detector control panel

Control panel function	Description
Autozero	Resets the detector offsets.
Reset module	Resets the detector after an error condition.
Help	Displays console online Help.

3.5.7 RI control panel

The refractive Index detector's control panel displays signal measurement, peak polarity, and the temperatures of the flow cell and external column manager.

Figure 3–7: RI detector control panel



- ① Run status LED – Mirrors the run status LED on the front panel of the detector, unless communications with the detector are interrupted.
- ② Recycle indicator – When clicked, the recycle valve changes positions to avoid wasting solvent when equilibrating the detector.
- ③ Current flow cell temperature – Displays the current flow cell temperature.
- ④ Flow cell temperature set point – Displays the set point for the flow cell temperature.
- ⑤ Peak polarity – Displays the polarity of the output signal. If the polarity is negative, the chromatogram is inverted.
- ⑥ Signal measurement – Displays the signal, in µRIU.
- ⑦ Status – Displays the status of the current operation.

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

Table 3–10: Additional functions in the RI detector control panel

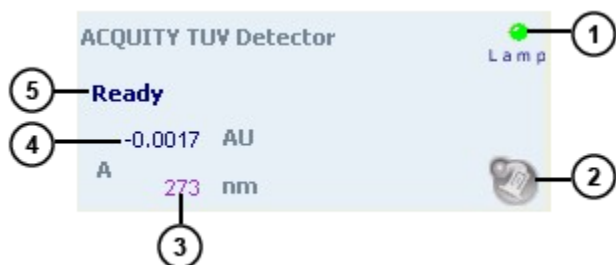
Control panel function	Description
Autozero	Resets the detector's offsets.
Reset module	Resets the detector after an error condition.
Help	Displays the console online Help.

3.5.8 TUV control panel

The tunable ultraviolet (TUV) detector's control panel displays absorbance units and wavelength values. When the detector is running in dual mode, the values for both wavelength A and B appear.

You can edit detector parameters when the system is idle by clicking the underlined value. You cannot edit these values while the system is running samples.

Figure 3–8: TUV detector control panel



- ① Lamp LED – Mirrors the lamp status LED on the front panel of the detector, unless communications with the detector are interrupted.
- ② Lamp ignition – When clicked, ignites or extinguishes the lamp. If the icon is green, the lamp is ignited. If the icon is red, the lamp is extinguished.
- ③ Value of wavelength A – Displays the value of wavelength A, in nm. If the detector is in dual wavelength mode, the value of wavelength B also appears.
- ④ AU – Displays the absorbance units of wavelength A. If the detector is in dual wavelength mode, the absorbance units of wavelength B also appears.
- ⑤ Status – Displays the status of the current operation.

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

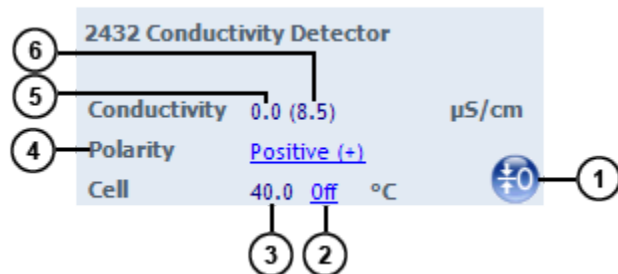
Table 3–11: Additional functions in the TUV detector control panel

Control panel function	Description
Autozero	Resets the absorbance value to 0.
Reset module	Resets the detector after an error condition.
Help	Displays console online Help.

3.5.9 2432 conductivity control panel

The 2432 conductivity detector's control panel displays the conductivity, peak polarity, and the cell temperature.

Figure 3–9: 2432 conductivity detector control panel



- ① Autozero button – Eliminates the eluent's contribution to conductivity.
- ② Cell temperature set point – Displays the set point for the flow cell temperature.
- ③ Current cell temperature – Displays the current flow cell temperature.
- ④ Peak polarity – Displays the polarity of the output signal. If the polarity is negative, the chromatogram is inverted.
- ⑤ Relative conductivity – The autozeroed conductivity.
- ⑥ Absolute conductivity – The conductivity reading including the eluent's contribution.

3.6 Starting up the system

Use the Start up system function to prime the solvent manager 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.



Notice:

- Do not leave buffers stored in the system.
- Flush all flow paths, including the needle wash, with plenty of non-buffered solvent before shutting down the system.
- For extended shutdown periods (longer than 24 hours), use 10% to 20% methanol in water.
- When using a buffered wash solvent, prime it for a minimum of 30 sec.
- Use of buffers can cause salt build-up on the needle and wash port, which can require periodic cleaning.

To start up the system:

1. From the system view of the console, click **Control > Start up system**.

Alternative: Right-click in the control panel and click **Start up system**.

2. On the **Prime Solvents** tab, click the solvent manager sub-tab and if necessary, change the value in the **Duration of Prime** field.

Notes:

- If you are priming with solvents that differ significantly from the current solvents, prime the solvent manager for minimum of 5 minutes.
 - If you are using a concentrated acid and a concentrated base, it is recommended that the lines are not primed in succession. Instead, to reduce the risk of salt formation, ensure the pump is flushed or primed with water prior to priming the second buffer.
 - If you want to return settings to their original values on any tab, click **Set Defaults**.
3. On the **Prime Solvents** tab, click the sample manager sub-tab and if necessary, change the settings for the wash and purge solvent.

Table 3–12: Sample manager priming parameter values

Parameter	Wash solvent	Purge solvent
Priming range	1 to 600 seconds	1 to 100 cycles Note: Each cycle takes approximately 0.5 minutes.
Default priming	15 seconds	5 cycles
Recommended priming: dry inlet tube	180 seconds	100-µL syringe: 60 cycles 250-µL syringe: 24 cycles 500-µL syringe: 12 cycles
Recommended priming: changing solvents	180 seconds	100-µL syringe: 50 cycles 250-µL syringe: 20 cycles 500-µL syringe: 10 cycles

4. On the **Equilibrate to Method** tab, click each module sub-tab and if necessary, change the settings for the flow rate, solvent composition, temperature, and lamp state to match your requirements at equilibration.

Table 3–13: Equilibrate to Method table values

System startup parameters	Default	Allowed values
Method initial flow rate	0.500 mL/min	0.1 to 2.0 mL/min

Table 3–13: Equilibrate to Method table values (continued)

System startup parameters	Default	Allowed values
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 Note: Column selection for the Column Manager will default to Column 1	Depends on type of column compartment
Sample temperature	On	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.

Note: Change the settings on the **Optional Characterize** tab only if the needle has been replaced.

5. Click **Start**.

Result:

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

4 System maintenance

Perform the maintenance activities described in this chapter to ensure an optimally operating system.

4.1 Contacting Waters Technical Service

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800-252-4752). From elsewhere, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. The Waters Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Visit www.waters.com.

When you contact Waters, be prepared to provide this information:

- Error message (if any)
- Nature of the symptom
- Serial number of the system module and its firmware version, if applicable
- Flow rate
- Operating pressure
- Solvent(s)
- Detector settings (sensitivity and wavelength)
- Type and serial number of column(s)
- Sample type and diluent
- Chromatography data software version and serial number
- System workstation model and operating system version

Note: For an explanation about how to report shipping damages and submit claims, see the document *Waters Licenses, Warranties, and Support Services*.

4.1.1 Viewing module information

Each system module bears a serial number that facilitates service and support. Serial numbers also provide a way to create single log entries for each module so that you can review the usage history of a particular unit.

Be prepared to provide the serial numbers of the modules in your system when you contact Waters customer support.

To view module information:

1. In the console, select a module from the system tree.
2. Click **Configure > View module information**.
The Module Information dialog box displays this information:
 - Serial number
 - Firmware version
 - Firmware checksum
 - Component software version

Alternatives:

- In the main window, point to the visual representation of the system module that you want information about.
- Obtain the serial number from the printed labels on the module's rear panel or inside the sample compartment door.

4.2 Maintenance procedures and frequency

Consult the individual module's overview and maintenance guide on the documentation CD for routine maintenance procedures and frequency.

4.3 Spare parts

To ensure that your system operates as designed, use only Waters Quality Parts. Visit www.waters.com/wqp for information about Waters Quality Parts, including how to order them.

4.4 Troubleshooting with Connections INSIGHT

Connections INSIGHT is an intelligent device management (IDM) Web service that enables Waters to provide proactive service and support for a system. To use Connections INSIGHT, you must install its service agent software on a MassLynx or Empower workstation. In a client/server system, you must also install the service agent on the computer that controls the system. The service agent software automatically and securely captures and sends information about the support needs of a system directly to Waters. If you encounter a performance problem when using the console software, manually submit a Connections INSIGHT request to Waters Customer Support. Alternatively, use **Remote Desktop**, a real-time collaboration option that

controls the two-way connection with the system by enabling the Connections INSIGHT iAssist service level. These sources, available on the Waters' Web site, provide more information about Connections INSIGHT and Connections INSIGHT iAssist:

- *Connections INSIGHT Quick Start Guide*
- *Connections INSIGHT User's Guide*

4.4.1 To submit a Connections INSIGHT request:

1. Select **Troubleshoot > Submit Connections INSIGHT request**.
2. In the Connections INSIGHT Request dialog box, type your name, telephone number, e-mail address, and a description of the problem.
3. Click **Submit**, and allow approximately 5 minutes to save the service profile.

Result: A ZIP file containing your Connections INSIGHT profile is forwarded to Waters customer support for review.

Note: Saving a service profile or plot file from the instrument console can require as much as 150 MB of file space.

4.5 Configuring maintenance warnings

Maintenance counters, if available for a particular component, provide information about real-time usage that can help you determine when to schedule routine maintenance for specific components. You can specify usage thresholds and maintenance warnings that alert you when a component reaches a specified threshold. Thus you can minimize unexpected failures and unscheduled downtime during important work. For information explaining how to specify maintenance warnings, consult the Waters console Help.

5 External connections

See also: For information explaining how to connect chromatographic tubing, see [Installation recommendations for fittings](#).

Note: A Waters Technical Service representative unpacks and installs the system components.



Warning: To avoid spinal and muscular injury, do not attempt to lift a system module without assistance.

If you must transport a system component, or remove it from service, request recommended cleaning, flushing, and packaging procedures from Waters Technical Service.

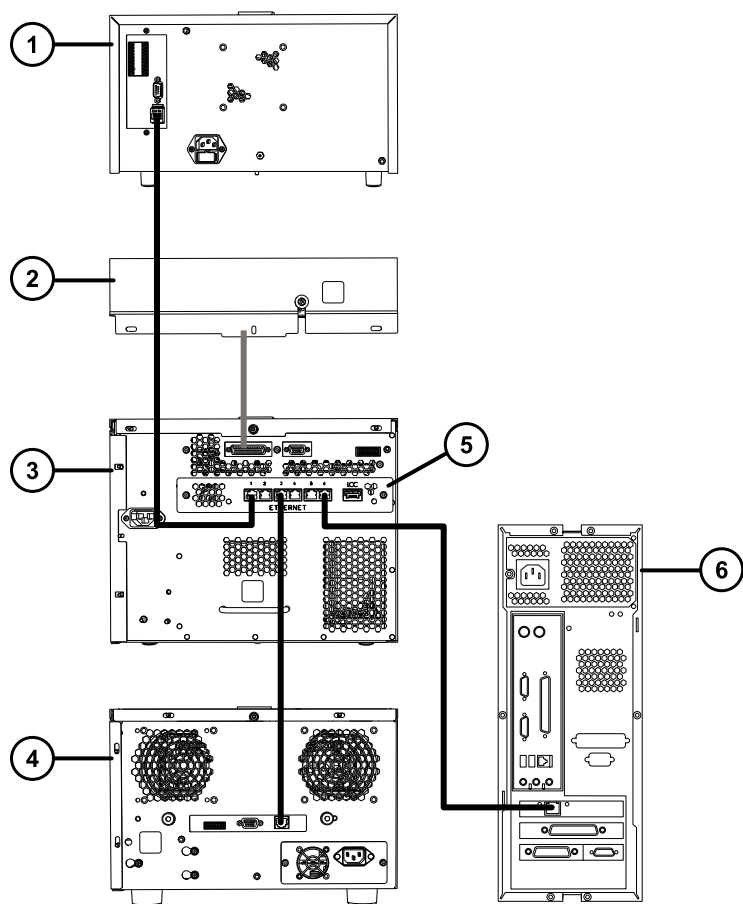
5.1 Ethernet connections

The sample manager incorporates an internal Ethernet switch that accommodates the PC (workstation) and as many as six system modules. Connect the shielded Ethernet cables from each module to the electronic connections on the rear panel of the sample manager. The sample manager is connected internally to the Ethernet switch.

Alternative: Some column modules connect to the sample manager using an interconnect (D-sub) cable assembly.

5.2 External cable connections

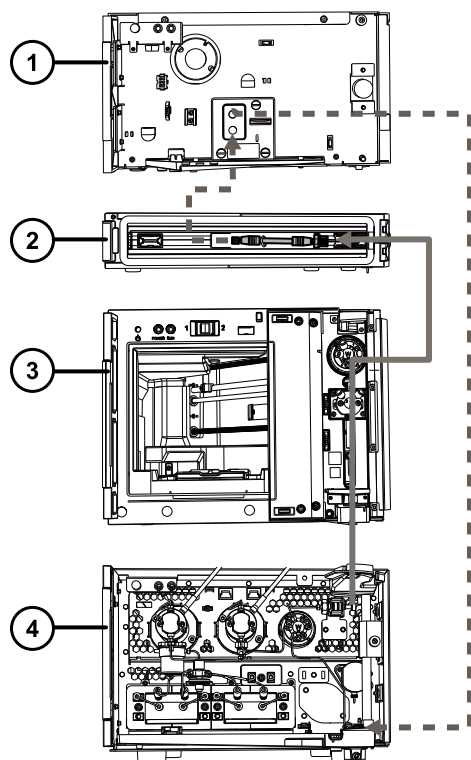
Figure 5–1: System rear-panel cable connections



- ① Detector
 - ② Column heater
 - ③ Sample manager (SM-FTN)
 - ④ Solvent manager (QSM)
 - ⑤ Ethernet switch
 - ⑥ Workstation
- Interconnect (D-sub) cables
- Ethernet cables

5.3 Plumbing connections

Figure 5–2: System plumbing connections



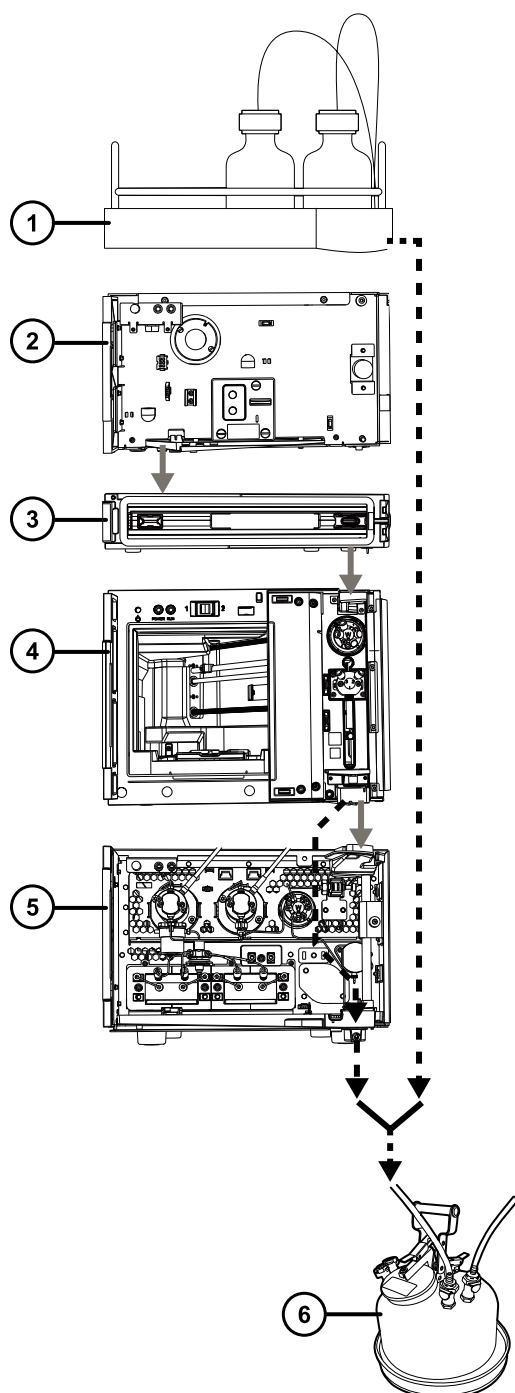
- ① Detector
- ② Column heater
- ③ Sample manager (SM-FTN)
- ④ Solvent manager (QSM)

← Stainless steel tubing

← - - - PEEK tubing

5.4 Waste-tubing connections

Figure 5–3: System waste-tubing connections



① Solvent bottle tray

② Detector

- ③ Column heater
- ④ Sample manager (SM-FTN)
- ⑤ Solvent manager (QSM)
- ⑥ Waste container
- ← Leak path
- ← - - - Required waste lines

5.5 Electricity source

Most modules require a separate, grounded, power source. The ground connection in the power outlet must be common and physically close to the module.



Warning: To avoid electric shock, do not remove protective panels from system modules. The components within are not user-serviceable.



Notice: To avoid damaging the electronic components of the sample manager and the column heater or column heater/cooler, always power-off the sample manager and column heater/cooler before connecting or disconnecting the interconnect cable.

5.5.1 Connecting to a wall electricity source



Warning: To avoid electrical shock, observe these precautions:

- Use SVT-type power cord in the United States and HAR-type power cord, or better, in Europe. For requirements elsewhere, contact your local Waters distributor.
- Inspect the power cord for damage, and replace it, if necessary.
- Power-off and unplug each module before performing any maintenance operation on it.
- Connect each module to a common ground.

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

To connect to a wall electricity source:

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

5.5.2 Connecting to a cart's electricity source

If your system includes the optional FlexCart or micro cart, follow this procedure to connect each module to a power source.



Warning: To avoid electrical shock, observe these precautions:

- Use SVT-type power cord in the United States and HAR-type power cord, or better, in Europe. For requirements elsewhere, contact your local Waters distributor.
- Inspect the power cord for damage, and replace it, if necessary.
- Power-off and unplug each module before performing any maintenance operation on it.
- Connect each module to a common ground.

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

To connect to a cart's electricity source:

1. Connect the female end of the cart's electrical cables (included in the startup kit) to the receptacle on the rear panel of each system module.
2. Connect the hooded, male end of the cart's electrical cables to the power strips on its back.
3. Connect each power strip's cable to a wall outlet operating on its own circuit.

5.6 Connecting signal cables

The rear panel of the module includes a removable connector that holds the screw terminals for the I/O signal cables. The connector is keyed so that it can be inserted only one way. Refer to the cable-connection label affixed to the rear panel of the module.

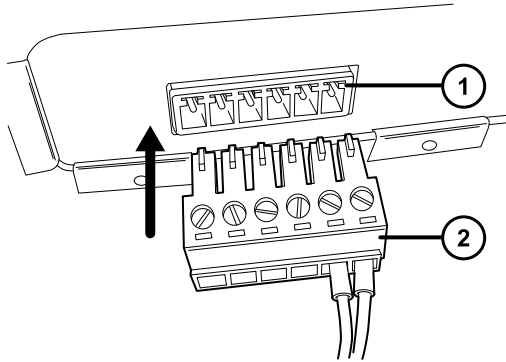
Required tools and materials

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

To connect the cables:

1. Insert the connector into the connector port on the module's rear panel.

Figure 5-4: Inserting connector into connector port

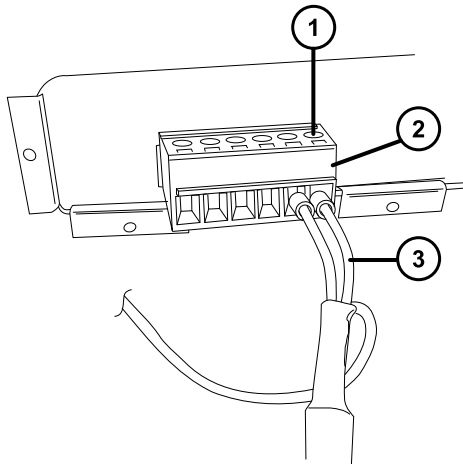


① Connector port

② Connector

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

Figure 5-5: Positive and negative lead connections



① Screw

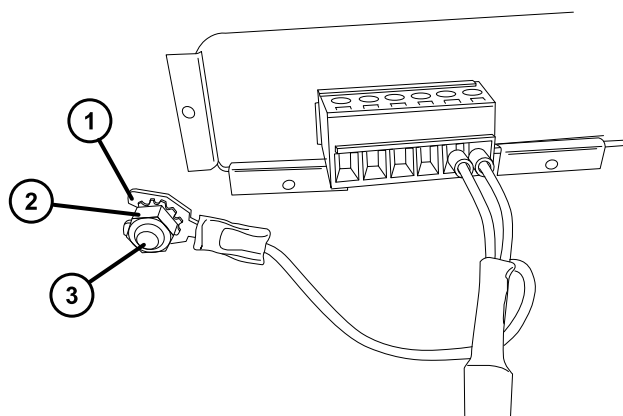
② Connector

③ Signal cable

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

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

Figure 5–6: Grounding cable fork terminal on grounding stud



- ① Fork terminal
- ② Locking nut
- ③ Grounding stud

5.7 Connecting to a column module

The following column modules are compatible with the ACQUITY UPLC H-Class system:

- Column heater
- Column heater 30 cm
- Column heater/cooler
- Column manager
- Column manager auxiliary

The sample manager powers and communicates with the column module. The external communication cable must be connected to the rear of the column module and the sample manager.

To connect the column module:



Notice: To avoid damaging the electronic components of the sample manager and the column heater or column heater/cooler, always power-off the sample manager and column heater/cooler before connecting or disconnecting the interconnect cable.

1. Make sure the sample manager and the column module are powered-off.
2. Connect the interconnect cable to the High Density (HD) port on the rear of the column module.
3. Connect the other end of the interconnect cable to the QSPI port on the rear of the sample manager.

A Safety advisories

Waters instruments and devices display hazard symbols that alert you to the hidden dangers associated with a product's operation and maintenance. The symbols also appear in product manuals where they accompany statements describing the hazards and advising how to avoid them. This appendix presents the safety symbols and statements that apply to all of Waters' product offerings.

A.1 Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appear in a manual's narrative sections or procedures, an accompanying statement identifies the applicable 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 injury caused by moving machinery.)



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.)

A.1.1 Specific warnings

A.1.1.1 Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.



Warning: To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- 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 compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

A.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process material containing biohazards, which are substances that contain biological agents capable of producing harmful effects in humans.



Warning: To avoid infection with potentially infectious, human-sourced products, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the biohazard safety representative for your organization regarding the proper use and handling of infectious substances.

A.1.1.3 Biohazard and chemical hazard warning

This warning applies to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.



Warning: To avoid personal contamination with biohazards, toxic materials, or corrosive materials, you must understand the hazards associated with their handling.

Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the safety representative for your organization regarding its protocols for handling such materials.

A.2 Notices

Notice advisories appear where an instrument or device can be subject to use or misuse that can damage it or compromise a sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.



Notice: To avoid damaging the instrument's case, do not clean it with abrasives or solvents.

A.3 Bottles Prohibited symbol

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.



Prohibited: To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

A.4 Required protection

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization's standard operating procedures.



Requirement: Use eye protection when performing this procedure.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

A.5 Warnings that apply to all Waters instruments and devices

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



Warning: 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.



Avertissement : 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.



Warnung: 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.



Advertencia: 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.



警告： 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。



警告： 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。



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



警告: 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.



Avertissement : 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.



Warnung: 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.



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

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnerle 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 tetrahydrofuran (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。
- 不要使用已經被壓癰或嚴重彎曲管線。
- 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 디메틸설폭시드(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。

This warning applies to Waters instruments fitted with nonmetallic tubing. This warning applies to instruments operated with flammable solvents.



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.



Avertissement : 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.



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



Avvertenza: 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削弱。



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削弱。



경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호수단이 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。

A.6 Warnings that address the replacing of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

Finding fuse types and ratings when that information appears on the instrument or device:



Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.



Avertissement : pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boîte à fusible de l'instrument.



Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.



Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.



Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.



警告： 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。



警告： 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。



경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.



警告: 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Finding fuse types and ratings when that information does not appear on the instrument or device:



Warning: To protect against fire, replace fuses with those of the type and rating indicated in the "Replacing fuses" section of the Maintenance Procedures chapter.



Avertissement : pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués dans la rubrique "Remplacement des fusibles" du chapitre traitant des procédures de maintenance.



Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.



Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".



Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características indicados en la sección "Sustituir fusibles".



警告： 為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。



警告： 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。



경고: 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.



警告: 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

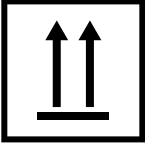



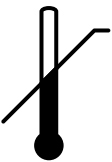

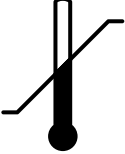
A.7 Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.

Symbol	Description
	Electrical power on
○	Electrical power off
⏻	Standby
≡	Direct current
~	Alternating current
3 ~	Alternating current (3 phase)
⏏	Safety ground
⏏	Frame, or chassis, terminal
⏏	Fuse
⏏	Functional ground
⏏	Input
⏏	Output

A.8 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation

B Solvent considerations



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

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

- ACQUITY UPLC H-Class system modules
- ACQUITY UPLC PDA detector
- ACQUITY UPLC PDA eλ detector
- ACQUITY UPLC PDA-TS detector
- ACQUITY UPLC TUV detector
- ACQUITY UPLC ELS detector
- ACQUITY UPLC FLR detector
- 2432 Conductivity detector

B.1 Solvent recommendations

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

Notes:

- When implementing passive check valves on ACQUITY systems, Waters recommends that you use MS Grade solvents.
- You can use some normal-phase solvents on the system if you make the appropriate modifications. See [Other solvents](#) and [Hexane/THF Compatibility Kit](#).

This section lists solvents recommended for the 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.

B.2 Recommended solvents

- HPLC-grade acetonitrile
- Acetonitrile/water mixtures
- Isopropyl alcohol
- Methanol
- Methanol/water mixtures
- HPLC-grade water

B.3 Preventing contamination

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

B.3.1 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.

B.3.2 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: Heed the recommendations of the filter's manufacturer or vendor to ensure that the filter is appropriate for the solvents used.

B.3.3 Solvent preparation

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

Recommendation: Store mobile phases in borosilicate glass reservoirs type 1, class A² or type 3.3³. Use high-quality, brown-tinted glassware to inhibit microbial growth. Use aluminum foil or Waters caps to cover the reservoirs.

B.3.4 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- μ m membrane filter.

! **Notice:** 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.

B.4 Buffered solvents

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 wet priming using at least five system volumes of HPLC-grade distilled or deionized water. When using a buffer, choose good quality reagents, filtering them through a 0.2- μ m membrane filter.

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

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

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

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

B.5 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

Note: 1 - 4% aqueous solutions of Hexafluoroisopropanol (HFIP) for oligonucleotide applications.

Note: HFIP should never be used in wash solvents.

Note: For additional information, see [System recommendations](#).

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.

B.6 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.

B.7 Additives/modifiers

- 0.1% ethylene diaminetetraacetic acid (EDTA)
- 0.1% heptafluorobutyric acid
- 0.1% triethyl amine (TEA)
- 0.1% trifluoroacetic 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

B.8 Sample diluents

- Acetonitrile
- Acetonitrile/water mixtures
- Chloroform
- Dimethylformamide (DMF)
- Dimethyl sulfoxide (DMSO)
- Isooctane
- Isopropanol

- Methanol
- Methanol/water mixtures
- Methylene chloride
- Water

Recommendation: Do not use buffers as needle wash.

B.9 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 ($\leq 30\%$)
- Sodium hydroxide ($\leq 1M$)
- Formic acid ($\leq 10\%$)

B.10 Solvents to avoid

Avoid these solvents:

- Solvents that contain halogens: fluorine, bromine, or iodine.
- Strong acids. (Use 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).

B.11 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 UltraPerformance LC/MS and HPLC/MS Systems* (part number 715001307) on the Waters Web site. Visit www.waters.com.



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



Warning: Hexane is a neurotoxin, and THF can irritate eyes, skin, and mucous membranes and cause harmful neurological effects. If you use either or both of these volatile solvents, locate your 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 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 systems. Nevertheless, you can use these solvents in weak dilutions (<5%) 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 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 systems.

B.12 Quaternary solvent manager recommendations

- The 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.

B.13 Sample manager - flow through needle 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.



Notice:

- Do not leave buffers stored in the system.
- Flush all flow paths, including the needle wash, with plenty of non-buffered solvent before shutting down the system.
- For extended shutdown periods (longer than 24 hours), use 10% to 20% methanol in water.
- When using a buffered wash solvent, prime it for a minimum of 30 sec.
- Use of buffers can cause salt build-up on the needle and wash port, which can require periodic cleaning.

B.14 Common solvent properties

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

Table B-1: 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
<i>n</i> -butyl acetate	7.8 at 20 °C	126.11	22
<i>n</i> -butyl alcohol	4.4 at 20 °C	117.5	37
<i>n</i> -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
<i>o</i> -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

Table B–1: Properties of common solvents (continued)

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
<i>N, N</i> -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
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	182.6	164
Methanol	97 at 20 °C	64.7	11
Methyl <i>t</i> -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
<i>N</i> -Methylpyrrolidone	0.33 at 25 °C	202.0	86
Pentane	420 at 20 °C	36.07	-49
<i>n</i> -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	
<i>o</i> -xylene	6 at 20 °C	144.41	17

B.14.1 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.

Note: λ cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.

Table B–2: Solvent miscibility

Polarity index	Solvent	Viscosity cP, 20 °C (at 1 atm)	Boiling point °C (at 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	Dimaethylformamide	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	—	—

B.14.1.1 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 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.

B.14.2 Solvent stabilizers

Certain solvents degrade, or become unstable, over time. Highly unstable solvents represent a potential explosion hazard. Solvent stabilizers are added to slow or stop solvent degradation.

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.

B.14.3 Solvent viscosity

Generally, viscosity is not a consideration when you use a single solvent or under low pressure. In gradient chromatography, however, the viscosity changes that occur as the solvents are mixed in varying proportion can effect pressure changes during the run.

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

B.14.4 Wavelength selection

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

- Common solvents
- Common mixed mobile phases

B.14.4.1 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.

Table B–3: 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
Tetrahydrofuran (THF)	230

B.14.4.2 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.

Note: λ cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.

Table B–4: Wavelength cutoffs for different mobile phases

Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230
Ammonium acetate, 10 mM	205
Ammonium bicarbonate, 10 mM	190

Table B–4: Wavelength cutoffs for different mobile phases (continued)

Mobile phase	UV cutoff (nm)
Polyoxyethylene (35) lauryl ether (BRIJ 35), 0.1%	190
3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (CHAPS) 0.1%	215
Diammonium phosphate, 50 mM	205
(Ethylenedinitrilo) tetraacetic acid disodium salt (disodium EDTA), 1 mM	190
4-(2-hydroxyethyl)-1-piperazineet hanesulfonic acid (HEPES), 10 mM, pH 7.6	225
Hydrochloric acid, 0.1%	190
Morpholinoethanesulfonic acid (MES), 10 mM, pH 6.0	215
Potassium phosphate, monobasic, 10 mM	190
Potassium phosphate, dibasic, 10 mM	190
Sodium acetate, 10 mM	205
Sodium chloride, 1 M	207
Sodium citrate, 10 mM	225
Sodium dodecyl sulfate	190
Sodium formate, 10 mM	200
Triethylamine, 1%	235
Trifluoroacetic acid, 0.1%	190
TRIS HCl, 20 mM, pH 7.0	202
TRIS HCl, 20 mM, pH 8.0	212
Triton X-100, 0.1%	240
Waters PIC Reagent A, 1 vial/liter	200
Waters PIC Reagent B-6, 1 vial/liter	225
Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Waters PIC Reagent D-4, 1 vial/liter	190

B.14.4.3 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 caused only by 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. Wavelength, pH, and concentration of the mobile phase affects its absorbance. Examples of several mobile phases are given in the table below, where the absorbances are based on a 10-mm path length.

Table B–5: Mobile phase absorbance measured against air or water

	Absorbance (AU) 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
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

Table B–5: Mobile phase absorbance measured against air or water (continued)

	Absorbance (AU) at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
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	—	—	—	—	—	—
Ethylenedinitrilotetraacetic 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	—	—
Morpholinoethanesulfonic 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 ₂ PO ₄), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K ₂ HPO ₄), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—
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	—	—	—	—	—	—	—	—

Table B–5: Mobile phase absorbance measured against air or water (continued)

	Absorbance (AU) at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
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
BRI J 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
Sodiumdodecyl 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

C Specifications

The reproducibility of the specifications presented in this document depends on the conditions in individual laboratories. Contact the Waters Technical Service organization for additional information about specifications.

C.1 System specifications

See also: For individual module physical, environmental, and input/out specifications consult the module's overview and maintenance guide.

Item	Specification
Dwell volume, system	<400 μ L with 100- μ L mixer
Integrated leak management	Drip trays direct all leaks to the front of the instrument and then into waste line.
Leak detection	Leak sensors, installed in drip trays.
Quantum synchronization	Injection synchronization between pump and sample manager enhances retention time reproducibility.
Settable flow rate range	<ul style="list-style-type: none">• 0.010 to 2.000 mL/min, in 0.001-mL increments (firmware version 1.5x and earlier)• 0.010 to 2.200 mL/min, in 0.001-mL increments (firmware version 1.60)• 0.010 to 2.200 mL/min, in 0.001-mL increments (firmware version 1.65 and later)
Maximum operating pressure	<ul style="list-style-type: none">• 103,421 kPa (1034 bar, 15,000 psi) up to 1.0 mL/min; 62,053 kPa (621 bar, 9000 psi) up to 2.0 mL/min (firmware version 1.5x and earlier)• 103,421 kPa (1034 bar, 15,000 psi) up to 1.0 mL/min; 53,779 kPa (538 bar, 7800 psi) up to 2.2 mL/min (firmware version 1.6x and later)
pH range	2 to 12

Item	Specification
Cycle time	<p><30 s inject-to-inject</p> <p>System cycle time (or overhead) is equal to the chromatographic run time subtracted from the injection-to-injection time.</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • System: ACQUITY UPLC H-Class quaternary solvent manager (QSM), ACQUITY UPLC H-Class sample manager with flow through needle (SM-FTN), ACQUITY UPLC H-Class column heater with active pre-heater (CH-A), and ACQUITY UPLC TUV detector • Isocratic chromatography • Flow rate: ≥ 0.4 mL/min • Injection volume: 1 mL • Sample manager parameters: Default aspiration speeds and wash times • Load ahead mode: enabled • Loop offline: 0.2 min • Run time: 2.0 min
Gradient mixers	<ul style="list-style-type: none"> • Standard: stainless steel, 100-mL mixer/filter • Optional: stainless steel, 250-mL mixer/filter
Plunger wash feature	Wash pump plungers using seal-wash solvent, can be primed manually or run automatically.
No-flow shutdown feature	Automatically runs the wash plungers function after a user-specified period of idle time.
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through instrument control software.
Auto•Blend Plus	Automated, on-line pH, ionic strength, and organic modifier blending from pure solvents.

C.1.1 Instrument control specifications


Item	Specification
Informatics compatibility	Empower software, MassLynx software, UNIFI, or standalone, through ACQUITY UPLC Console software
Communications	Ethernet interfacing via RJ45 connection to host PC
Event inputs/outputs	Contact closure and/or TTL inputs/outputs

Item	Specification
Connections INSIGHT	Provides real-time monitoring and automatic notification of instrument performance and diagnostic information
Local control	ACQUITY UPLC Local Console Controller (LCC)

C.1.2 Environmental specifications

Attribute	Specification
Acoustic noise, system	<65 dBA
Ambient operating temperature	4.0 to 40.0 °C
Ambient operating humidity	20% to 80%, non-condensing

C.1.3 Electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC
Power requirements	100 to 240 VAC
Line frequency	50 to 60 Hz

Attribute	Specification
Power consumption	QSM: 360 VA SM-FTN: 400 VA Column manager with active pre-heater (CM-A): 400 VA Sample organizer: 540 VA

- a. **Protection Class I** – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. **Overvoltage Category II** – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. **Pollution Degree 2** – A measure of pollution on electrical circuits that can produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.
- d. **Moisture Protection** – Normal (IPXO) – IPXO means that no Ingress Protection against any type of dripping or sprayed water exists. The “X” is a placeholder that identifies protection against dust, if applicable.

C.1.4 Physical Specifications

This table provides the physical specifications for a system that includes the Quaternary Solvent Manager, Sample Manager FTN, Column Heater, and Solvents Tray.

Attribute	Specification
Width	34.3 cm (13.5 in.)
Height	71.1 cm (28.0 in.)
Depth	71.2 cm (28.0 in.)
Weight	61.6 kg (135.5 lbs) ^a

- a. Actual system weight will vary depending upon the solvent and number of solvent bottles in the solvent tray.

C.2 Performance specifications

The following tables list the performance specifications for the system modules.

C.2.1 QSM and bioQSM performance specifications

Attribute	Specification
Number of solvents	One to four (A, B, C, and D), in any combination. Optional 6-position solvent selection valve enables solvent selections D1 through D6 on line D, in addition to A, B, and C (a total of nine solvents to select from).
Solvent degassing	Integrated vacuum degassing, four chambers. One additional chamber for the sample manager purge solvent.
Gradient formation	Low-pressure mixing, quaternary gradient
Gradient profiles	11 gradient curves, including linear, step (2), concave (4), and convex (4)
Primary check valve	Intelligent Intake Valve (<i>i²Valve</i>), standard Passive check valve, optional
Flow accuracy	±1.0% of set flow at 0.500 to 2.000mL/min using 100% solvent A (with <i>i²Valve</i>). Back pressure 4137 to 6895 kPa (41 to 69 bar, 600 to 1000 psi), with degassed water.
Flow precision	0.075% RSD or ±0.020 min SD, whichever is greater, based on six replicates (<i>i²Valve</i>). Test conditions: <ul style="list-style-type: none"> • Mobile phase: 60:40 water/methanol mixed via Auto•Blend Plus technology • Flow rate: 0.5 mL/min • Sample mix: alkylphenone mix (5.0 µL injection volume) • Column: ACQUITY UPLC BEH C₁₈, 1.7 µm, 2.1 × 50 mm • Column temperature: 35 °C ±0.3 °C • Detector: UV, 254 nm wavelength
Composition ripple (baseline noise)	<1.0 mAU (<0.1 mAU with optional 250-µL mixer), with <i>i²Valve</i> Test conditions: <ul style="list-style-type: none"> • Mobile phase: A: water + 0.1%, trifluoroacetic acid; B: acetonitrile + 0.1% trifluoroacetic acid • Flow rate: 0.5 mL/min • Gradient conditions: 1.0 to 33% B in 10 min; time average window, 10 s. Noise range 4.00 to 6.00 min • Column: ACQUITY UPLC BEH C₁₈, 1.7 µm, 2.1 × 50 mm • Detector: ACQUITY TUV, 214 nm wavelength, 10 Hz sampling rate

Attribute	Specification
Composition accuracy	<p>±0.5% absolute (full scale) from 5 to 90% from 0.5 to 2.0 mL/min, with <i>i</i>²Valve</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Mobile phase: degassed 90:10 acetonitrile/water; 90:10 acetonitrile/water with caffeine at 12 mg/L concentration • Back pressure: 13,790 kPa (138 bar, 2000 psi) • Gradient conditions: Step gradient method • Detector: UV, 273 nm wavelength
Composition precision	<p><0.15% RSD or ±0.04 min SD, whichever is greater, based on six replicate injections, with <i>i</i>²Valve</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Mobile phase: 60:40 water/methanol mixed via Auto•Blend Plus technology • Flow rate: 0.5 mL/min • Sample mix: alkylphenone mix (5.0 µL injection volume) • Column: ACQUITY UPLC BEH C₁₈, 1.7 µm, 2.1 × 50 mm • Column temperature: 35 °C ±0.3 °C • Detector: UV, 254 nm wavelength
Compressibility compensation	Automatic and continuous
Priming	Wet priming can run at flow rates up to 4 mL/min
Pump seal wash	<p>Equipped with a wash system, to flush the rear of the high pressure seal and the plunger.</p> <p>QSM: The default interval between seal wash pump activations is 5.0 min.</p> <p>bioQSM: The default interval between seal wash pump activations is 0.1 min (6 s).</p>
Flow ramping	<p>Range: 0.01 to 30.00 min to reach 2.0 mL/min</p> <p>Default: 0.45 min, to reach 2.0 mL/min at 4.44 mL/min</p>
Vent valve	Used for priming the pump and automated leak testing. When the column manager switches columns, the vent valve switches to the vent position, to reduce system pressure.
Solvent lines	Set of factory-installed inlet tubing assemblies. Each assembly includes a 10-µm reservoir filter.
Composition range	0.0 to 100.0% settable in 0.1% increments.

C.2.2 SM-FTN and bioSM-FTN performance specifications

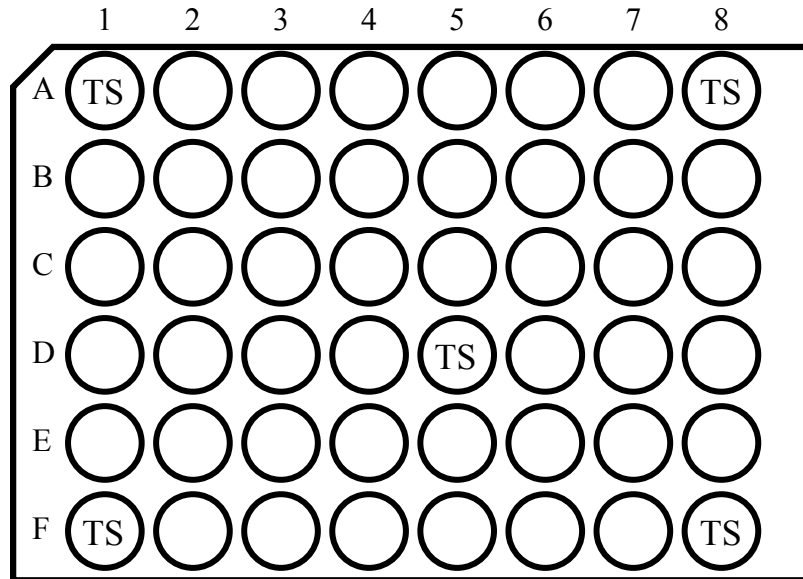
Item	Specification
Injection volume range	<ul style="list-style-type: none"> • With the standard loop fitted, 0.1 to 10.0 μL, in 0.1 μL increments. • With one of the optional extension loops fitted, as much as the volume of the extension loop (50, 100, 250, or 1000 μL).
Accuracy (aspiration)	$\pm 0.2 \mu\text{L}$, measured by fluid weight removed from vial by means of 10- μL injections, averaged over 20 injections, using a 100- μL syringe.
Linearity	<p>>0.999 (standard needle)</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Chromatography: isocratic • Mobile phase: 10:90 acetonitrile/water • Flow rate: 0.6 mL/min • Needle volume: 1 to 70% • Sample mix: caffeine 0.03 mg/mL (0.2 to 10.0 μL, with 15 μL needle installed, no extension loop) • Column: ACQUITY UPLC BEH C_{18}, 1.7 μm, 2.1 \times 50 mm • Detector: UV, 273-nm wavelength • Column temperature: 40 $^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$
Precision	<p><1% area RSD, 0.2 to 1.9 μL (0.25 to 0.50 mg/mL caffeine), <0.5% area RSD, 2.0 to 10.0 μL (0.03 mg/mL caffeine)</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Chromatography: isocratic • Replicates: 6 • Mobile phase: 10:90 acetonitrile/water • Flow rate: 0.6 mL/min • Column: ACQUITY UPLC BEH C_{18}, 1.7 μm, 2.1 \times 50 mm • Detector: UV, 273-nm wavelength • Column temperature: 40 $^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$

Item	Specification
Number of sample plates	<p>Any two of the following Waters-certified plates:</p> <ul style="list-style-type: none"> • 96 and 384 microtiter plates • 48-position, 2.00-mL vial plates • 48-position, 0.65-mL micro-centrifuge tube plates • 24-position, 1.50-mL micro-centrifuge tube plates <p>For more information, see the <i>Waters Sample Vials and Accessories Brochure</i>, part number 720001818 or visit the plate selector and vial selector on the Waters website.</p>
Maximum sample capacity	<p>768 in two, 384-well Waters-certified plates, or 96 in 2-mL vial holders. Four additional positions for dilution functions.</p> <p>For more information, see the <i>Waters Sample Vials and Accessories Brochure</i>, part number 720001818 or visit the plate selector and vial selector on the Waters website.</p>
Sample compartment temperature range	<p>Between 4 and 40 °C, settable in increments of 0.1 °C; maintains 19 °C below ambient with a tolerance range of between -2.0 and +4.0 °C</p> <ul style="list-style-type: none"> • At a set point of 4 °C with ambient temperature <23 °C and humidity <80%, maintains a sample temperature of 2 to 8 °C. • At ambient temperatures >23°C and humidity <80%, maintains an average sample temperature of 18 °C below ambient, ±3.0 °C.
Minimum sample compartment temperature specifications	<p>See the graph that follows this table for the achievable sample compartment temperature and expected variation at various sample temperatures.</p> <p>The graph shows achievable sample compartment temperature and expected variation at various sample temperatures.</p>
Recommended locations for temperature sensors	<p>See the diagram that follows this table for the recommended locations for temperature sensors on the sample tray when validating specifications.</p>
Temperature accuracy	±0.5 °C at sensor
Temperature stability	±1.0 °C (at the sensor with sample compartment door closed)
Injection needle wash	Integral, active, and programmable
Minimum sample required	3 µL, residual, using total recovery 2-mL vials (zero offset)

Item	Specification
Sample carryover - UV	<p data-bbox="537 275 808 302"><0.004% caffeine (UV)</p> <p data-bbox="537 310 724 338">Test conditions:</p> <ul data-bbox="537 363 1427 1146" style="list-style-type: none"> <li data-bbox="537 363 850 390">• Solvent A: 100% water <li data-bbox="537 415 911 443">• Solvent B: 100% acetonitrile <li data-bbox="537 468 1008 495">• Weak wash: 10:90 acetonitrile/water <li data-bbox="537 520 1019 548">• Strong wash: 10:90 acetonitrile/water <li data-bbox="537 573 1252 600">• Column: ACQUITY UPLC BEH C₁₈ 1.7 mm, 2.1 × 50 mm <li data-bbox="537 625 1101 653">• Mobile phase: 90% solvent A:10% solvent B <li data-bbox="537 678 837 705">• Flow rate: 0.6 mL/min <li data-bbox="537 730 1427 842">• Sample: caffeine, at 0.16 mg/mL (standard) and 4 mg/mL (challenge) in 10:90 acetonitrile/water, compared with blanks of 10:90 acetonitrile/water <li data-bbox="537 867 841 894">• Injection volume: 5 µL <li data-bbox="537 919 906 947">• Column temperature: 40 °C <li data-bbox="537 972 1325 1041">• Detection: UV at 273 nm, sampling rate = 20 points/s, filter time constant = normal (0.2 s) <li data-bbox="537 1066 764 1094">• Run time: 2 min <li data-bbox="537 1119 1122 1146">• Data system: Empower or MassLynx software <p data-bbox="537 1171 1427 1270">Basis of calculation: Any peak in the blanks following the challenge sample are compared with the known (0.005%) standard. Carryover peak areas below the standard area are within specification.</p>

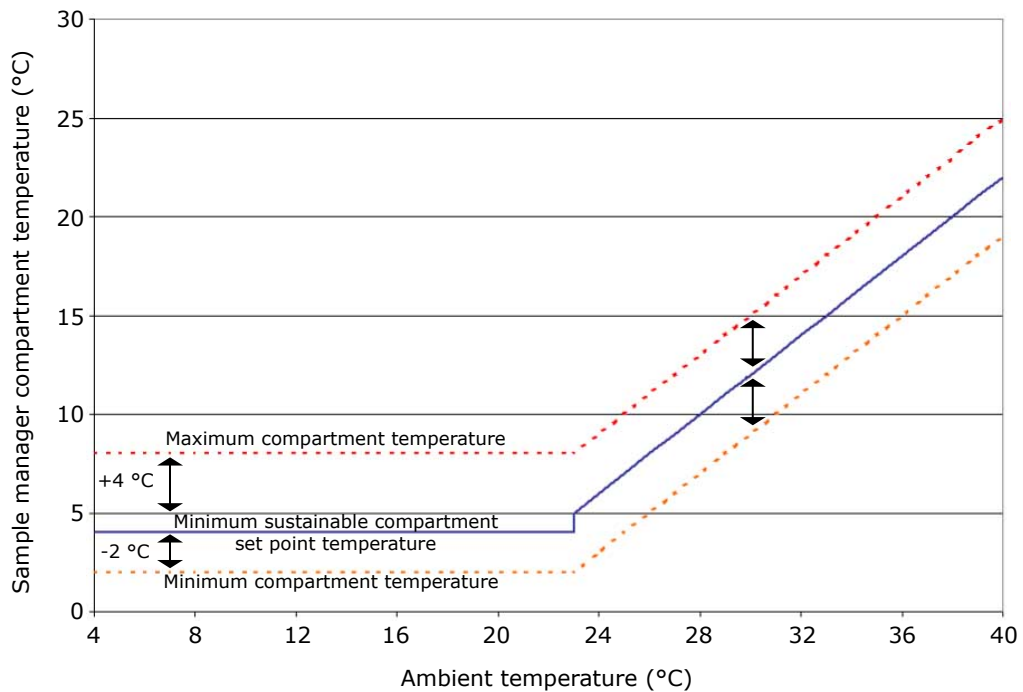
Item	Specification
Sample carryover - MS	<p data-bbox="537 275 938 302"><0.005% sulphadimethoxine (MS)</p> <p data-bbox="537 310 724 338">Test conditions:</p> <ul data-bbox="537 365 1393 1213" style="list-style-type: none"> <li data-bbox="537 365 1029 392">• Solvent A: water with 0.1% formic acid <li data-bbox="537 415 1094 443">• Solvent B: acetonitrile with 0.1% formic acid <li data-bbox="537 466 992 493">• Weak wash: 5:95 acetonitrile/water <li data-bbox="537 516 1019 543">• Strong wash: 50:50 acetonitrile/water <li data-bbox="537 567 1101 594">• Mobile phase: 80% solvent A:20% solvent B <li data-bbox="537 617 834 644">• Flow rate: 0.3 mL/min <li data-bbox="537 667 1393 779">• Sample: sulphadimethoxine at 5 pg/μL (standard) and 1 μg/μL (challenge) in 10:90 acetonitrile/water +0.1% formic acid, compared with blanks of 10:90 acetonitrile/water +0.1% formic acid <li data-bbox="537 802 834 829">• Injection volume: 5 μL <li data-bbox="537 852 1247 879">• Column: ACQUITY UPLC BEH C₁₈ 1.7 μm, 2.1 × 50 mm <li data-bbox="537 903 899 930">• Column temperature: 40 °C <li data-bbox="537 953 899 980">• Sample temperature: 10 °C <li data-bbox="537 1003 1393 1031">• Detection: MS SIR at 311.3 Da, 0.5 s dwell or MRM at 156.0 to 310.0 <li data-bbox="537 1054 753 1081">• Ion mode: ES+ <li data-bbox="537 1104 769 1131">• Run time: 5 min <li data-bbox="537 1155 1122 1182">• Data system: Empower or MassLynx software <p data-bbox="537 1226 1403 1337">Basis of calculation: Any peaks in the blanks following the challenge sample are compared with the known (0.005%) standard. Carryover peak areas <80% of the standard area are within specification.</p>

Figure C-1: Recommended temperature sensor locations



TS Temperature sensor

Figure C-2: Minimum sample compartment temperature specifications



C.2.3 CH-A performance specifications

Item	Specification
Settable temperature range	20.0 to 90.0 °C, in 0.1 °C increments
Controllable temperature range	(Ambient +5 °C) to 90.0 °C
Temperature accuracy ^a	Tested to ± 0.5 °C between 20 and 50 °C (± 1.0 °C for remaining range) Test qualification: The temperature measured by a NIST-traceable probe located next to the measurement sensor must fall within this specification.
Temperature stability ^a	± 0.3 °C at the sensor
Solvent conditioning	Active pre-heating as standard
Leak control	Compartment drip tray with leak sensor installed in sample manager upper drip tray or under compartment (for extended configuration only). Single exit drain manages leaks to waste.
Door open to heater cutoff delay	1-minute maximum
Column tracking	eCord technology column information management tracks and archives column usage history

a. Operating with active pre-heating via the APH assembly

C.2.4 CH-30A performance specifications

Item	Specification
Settable temperature range	20.0 to 90.0 °C, in 0.1 °C increments
Controllable temperature range	(Ambient + 5 °C) to 90.0 °C
Temperature accuracy ^a	Tested to ± 0.5 °C between 20 and 50 °C (± 1.0 °C for remaining range) Test qualification: The temperature measured by a NIST-traceable probe located next to the measurement sensor must fall within this specification.
Temperature stability ^a	± 0.3 °C at the sensor
Solvent conditioning	Active pre-heating

Item	Specification
Leak control	Compartment drip tray with leak sensor installed under compartment. Single exit drain manages leaks to waste.
Door open to heater cutoff delay	1-minute maximum
Column tracking	eCord technology column information management tracks and archives column usage history.

a. Operating with active pre-heating via the APH assembly

C.2.5 30-cm CHC performance specifications

Item	Specification
Settable temperature range	4.0 to 65.0 °C, in 0.1 °C increments
Controllable temperature range	(Ambient – 15 °C) to 65.0 °C
Temperature accuracy	Tested to ± 0.8 °C between 20 and 50 °C (± 1.0 °C for remaining range) Test qualification: The temperature measured by a NIST-traceable probe located next to the measurement sensor must fall within this specification.
Leak control	Compartment drip tray with additional condensate drain. Single exit drain manages leaks to waste.
Door open to heater cutoff delay	1-minute maximum

C.2.6 CM-A and CM-Aux performance specifications

Item	Specification
Settable temperature range	4.0 to 90.0 °C, in 0.1 °C increments
Controllable temperature range	(Ambient -25 °C) to 90.0 °C

Item	Specification
Temperature accuracy ^a	<p>Tested to ± 0.5 °C</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Door closed • No column installed • No flow • Measurement taken with traceable, external temperature measurement device • Measurement taken after 1 hour of thermal equilibration at set point • Measurement taken at column compartment sensor location • Tested at 35 °C, 55 °C, and 90 °C
Time to return to steady-state temperature on door open/close	<p>12 minutes maximum</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • No column installed • No flow • Measurement taken with internal temperature sensor • Measurement taken after 1 hour of thermal equilibration at set point • Door is opened for 30 seconds • Tested at 35 °C, 55 °C, and 85 °C
Temperature precision	<p>Tested to ± 0.1 °C</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Door closed • No column installed • No flow • Measurement taken with traceable, external temperature measurement device • Measurement taken at column compartment sensor location • Temperature is ramped from ambient to 90 °C • Measurement taken after 1 hour of thermal equilibration • Temperature is returned to ambient • Test is repeated four additional cycles

Item	Specification
Temperature stability	<p>Tested to ± 0.3 °C</p> <p>Test conditions:</p> <ul style="list-style-type: none"> • Door closed • No column installed • No flow • Measurement taken with traceable, external temperature measurement device • Measurement taken for 1 hour after thermal equilibration at set point • Measurement taken at column compartment sensor location • Tested at 35 °C, 60 °C, and 90 °C
Ambient temperature stability	Within 2.0 °C/60 minute maximum
Pre-heater temperature (not user-settable)	Defined by set point of the column zone
Solvent conditioning	Active pre-heating
Leak control	Compartment drip tray with leak sensor installed under compartment. Single exit drain manages leaks to waste.
Column tracking	eCord technology column information management tracks and archives column usage history

a. Operating with active pre-heating via the APH assembly

C.2.7 Sample organizer performance specifications

Item	Specification
Sample plate compatibility	Consult the sample manager's overview and maintenance guide or system consumables catalog for a list of approved plates, vials, sealing caps, and sample covers for use with your sample manager and sample organizer.
Sample plate capacity	<p>Maximum of 19 plates, as high as 15.5 mm</p> <p>Maximum of 9 plates, as high as 40.0 mm</p> <p>Maximum of 6 plates, as high as 53.0 mm</p>
Minimum sample plate height	13 mm

Item	Specification
Maximum sample plate height (includes vials, caps, and cap mats)	53 mm
Sample compartment temperature range	<p>Between 4 and 40 °C, in increments of 0.1 °C, with a tolerance range of between -2 and +4 °C</p> <ul style="list-style-type: none"> At a setpoint of 4 °C with ambient temperature <23 °C and humidity <80%, maintains a sample temperature of 2 to 8 °C. At ambient temperatures >23 °C and/or humidity >80%, the sample manager and sample organizer can maintain an average sample temperature of 18 °C below ambient, ±3.0 °C.
Temperature accuracy	No more than ±1.0 °C in temperature between a traceable external temperature measurement device and an instrument temperature measurement device.
Temperature stability	±1.0 °C (at the sensor, with the sample compartment door closed)
Pneumatic system operating pressure range	414 to 758 kPa (4 to 8 bar, 60 to 110 psi)
Optional external pneumatic source pressure range	517 to 689 kPa (5 to 7 bar, 75 to 100 psi)