

ACQUITY UPLC H-Class Series

System Guide

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Waters contact information (continued)

Contacting medium	Information
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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.

System height



Warning: To avoid injury, do not stack modules, including the solvent tray and rails, higher than one meter (39.4 inches) above the bench top.



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



Warning: To avoid crushing your fingers beneath or between modules, use extreme care when installing a module in the system stack.

Safety hazard symbol notice

The symbol indicates a potential hazard. Consult the documentation for important information about the hazard and the appropriate measures to prevent and control the hazard.

Power cord replacement hazard



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

High voltage hazard



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

Bottle placement prohibition



Warning: To avoid injury from electrical shock or fire, and damage to the equipment, follow these guidelines:

- Do not expose the workstation or ancillary equipment to dripping or splashing liquids.
- Do not place objects filled with liquid, such as solvent bottles, on top of the workstation or ancillary equipment.

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 device 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, the protection provided by the equipment may be impaired.

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

The following symbols can be present on the device, system, or packaging.

Symbol	Definition
	Manufacturer
\sim	Date of manufacture
CE	Confirms that a manufactured product complies with all applicable European Community directives
	Australia EMC compliant
CUSTED BIS	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
25	Environmentally friendly use period (China RoHS): indicates the number of years from the date of manufacture until the product, or components within the product, are likely to be discarded or degrade into the environment
ī	Consult instructions for use
\approx	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
	For indoor use only
	No pushing

Symbol	Definition	
10kg max	Indicates the maximum load you can place on that item (for example, 10kg)	
SN	Serial number	
REF	Part number, catalog number	

Audience and purpose

This guide is intended for personnel who operate and maintain the ACQUITY UPLC H-Class Series system. The term "Series" refers to both the latest generation ("PLUS") and previous generations of H-Class systems. It gives an overview of the system's technology and operation.

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 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 CISPR 11 Industrial Scientific and Medical (ISM) instrument requirements.

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

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

EC authorized representative

EC REP

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1 ACQUITY UPLC H-Class Series System

The ACQUITY UPLC H-Class Series 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.

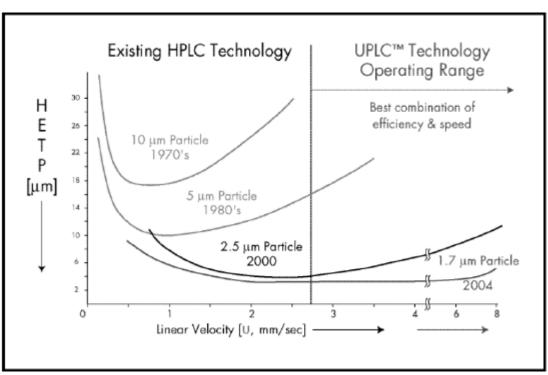
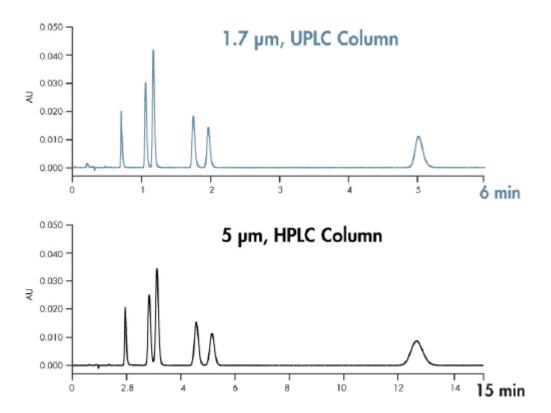


Figure 1–1: Evolution of particle size in liquid chromatography and the impact on separation efficiency

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.





The figure above compares two separations, one using HPLC with a column packed with 5 μ m particles, and the other using UPLC with a column packed with 1.7 μ m 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 × 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 Series system

The ACQUITY UPLC H-Class Series 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 six 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 highpressure 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, organic solvent, or 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.

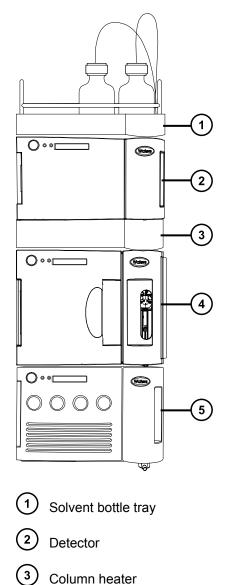
See also:

- Auto•Blend Plus Technology for Ion Exchange, Size Exclusion, and Reversed-phase Chromatography, included on the system documentation media.
- 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.





 $\overline{}$

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 (QSM)

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.

Note: An optional degasser vent line extension exists for applications requiring longer tubing lengths. For more information, contact your local Waters distributor.

1.3.2 Binary solvent manager (BSM)

The binary solvent manager (BSM) delivers solvent compositions for isocratic and binary gradient methods. It is a high-pressure mixing, high-pressure pump. It provides steady (pulse-free) solvent flow at analytical flow rates of 1 mL/min at 103,421 kPa (1034 bar, 15,000 psi) and to 2 mL/min, at reduced pressures, of 82,737 kPa (827 bar, 12,000 psi). Its features include in-line filters upstream of a primary check valve, the Waters Intelligent Intake Valve (*i*²Valve), automated priming functions, and daily system-setup routines.

See also: ACQUITY UPLC Binary Solvent Manager PLUS Overview and Maintenance Guide on your documentation media, or at www.waters.com, for more details.

Note: An optional degasser vent line extension exists for applications requiring longer tubing lengths. For more information, contact your local Waters distributor.

1.3.3 Sample manager-flow through needle (SM-FTN)

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 μ L, 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, autoaddition, and mixing, as well as load-ahead capabilities to reduce inject-to-inject cycle times.

1.3.3.1 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.

1.3.3.2 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.

1.3.4 Column heater (CH-A)

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

The CH-A 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 CH-A 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.

1.3.5 Column manager (optional)

The ACQUITY UPLC H-Class Series 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 H 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 CM-AUX) can accommodate as many as four columns (50-mm); a system with a column manager base unit and one CM-AUX module can accommodate as many as four long columns; a system with a column manager base unit and two CM-AUX modules can accommodate as many as six long columns (two columns in the base unit).

1.3.5.1 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.

1.3.6 Column Module Switch Box (optional)

With the optional Waters Column Module Switch Box, you can physically connect a column heater (CH-A) and a 30-cm column module (either a CH-30A or a 30-cm CHC) to the sample manager - flow-through-needle (SM-FTN) and switch the electrical control of the column modules via the SM-FTN console.

The switch box is mounted to the rear of the SM-FTN. The SM-FTN interconnect cable is connected to the sample manager (SM) port of the switch box, the CH-A interconnect cable is connected to the CH-A port, and either the CH-30A or the 30-cm CHC interconnect cable is connected to the 30-cm port.

For complete instructions on installing the Column Module Switch Box, see ACQUITY UPLC Column Heater-Active Overview and Maintenance Guide on your documentation media, or at Waters.com, for more details.

1.3.7 Detection

The 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)
- ACQUITY QDa
- Conductivity detector (2432)

1.3.8 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.

1.3.9 FlexCart (optional)

The optional FlexCart provides a mobile platform for the system. 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 FlexCart is not supported with the ACQUITY QDa detector or any ACQUITY UPLC H-Class Series system with dual detection (split-stack configurations).

1.3.10 Post-Injection Volume Kit (optional)

This optional kit includes a 50-µL extension loop that provides additional post-injection mixing. This additional volume can address peak distortion caused by injections of high organic sample diluents.

For more information on installing this extension loop, see Post-Injection Volume Kit Instructions.

1.3.11 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.

1.3.11.1 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 media, you can find this additional information:

- ACQUITY UPLC Quaternary Solvent Manager PLUS Series Overview and Maintenance Guide
- ACQUITY UPLC Binary Solvent Manager PLUS Overview and Maintenance Guide
- ACQUITY UPLC Sample Manager Flow Through Needle PLUS Series 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 Overview and Maintenance Guide
- 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:

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

ACQUITY UPLC H-Class Series 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.

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

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

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 while gripping the column with the column gripping tool. 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.
- Place the waste container below the system stack to permit gravity flow.
- Ensure that the waste tube does not crimp or bend. A crimp or bend can impede flow to the waste container.
- · Ensure that the exit of the waste tube is not immersed in waste solvent.
- If necessary, shorten the tube so that no portion of it drops below the top of the waste container.

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

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-µm 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% methanol 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.

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- μ L 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 Series system typically exhibits a bandspread (5 σ) of \leq 12 µL, depending on system configuration. A typical HPLC system can exhibit a bandspread between 35 µL and 50 µL. Because of the dispersion differences, a band experiences a threefold increase in dilution, compared with an ACQUITY UPLC H-Class Series 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.

Tips:

- Use additional valve cycle timed events (actuate the valve) if you suspect that sample residue in the valve is causing carryover problems.
- Test your sample in the strong wash solvent to make sure that the strong wash solvent does not cause either the analyte or the matrix to precipitate.
- Do not use the Load Ahead or Loop Offline options when you are troubleshooting carryover problems, as the shorter cycle times will prevent effective troubleshooting.

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 that different techniques apply to re-tightening fittings versus installing them for the first time.

2.5.1 Installation recommendations for fittings

Three types of fitting assemblies are used within the system: PEEK (polymer-based), SST (stainless steel), and MP35N (alloy-based). 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 biologically hazardous materials, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Recommendations:

- To prevent band spreading, ensure that the tubing is fully bottomed in its connection 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.

Required tools and materials

- · Chemical-resistant, powder-free gloves
- · Protective eyewear
- 1/2-inch open-end wrench
- 1/4-inch open-end wrench for tightening or loosening stainless steel (gold-plated) fittings with two-piece ferrules
- Column gripping tool for holding the column while tightening or loosening the dual-threaded fitting
- · Permanent marker

2.5.1.1 Assembling new fittings

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



Warning: To avoid eye injury, use eye protection when performing this procedure.



Notice: To prevent contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

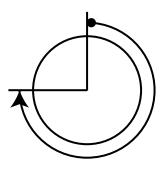
Required tools and materials

- Chemical-resistant, powder-free gloves
- · Protective eyewear
- 1/2-inch open-end wrench
- 1/4-inch open-end wrench for tightening or loosening stainless steel (gold-plated) fittings with two-piece ferrules
- Column gripping tool for holding the column while tightening or loosening the dual-threaded fitting
- Permanent marker

To assemble the new fittings:

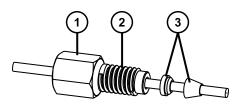
- 1. Insert the end of a tube into the hexagonal end of the compression screw.
- 2. Insert the tube into the larger end of the ferrule.
- 3. Insert the tube into the connection port.
- 4. Rotate the compression screw, clockwise, into the connection port until the screw is fingertight.
- 5. Using a permanent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

First-use tightening:



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

First use





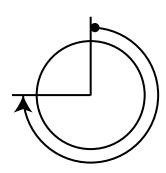


3 2-piece stainless steel ferrule

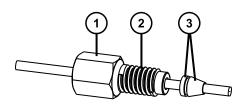
Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench. For detailed instructions about assembling new fittings, see Assembling new fittings.

Tip: To prevent band spreading, ensure that the tubing is fully bottomed in the connection port before you tighten the compression screw.

First use tightening



Reinstalled



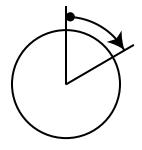
1 Long flats

(2) Compression screw



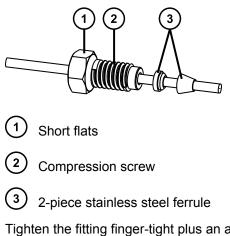
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

Reinstalled tightening



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

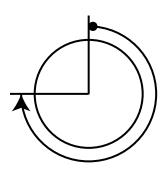
First use



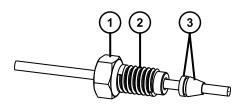
Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench. For detailed instructions about assembling new fittings, see Assembling new fittings.

Tip: To prevent band spreading, ensure that the tubing is fully bottomed in the connection port before you tighten the compression screw.

First use tightening



Reinstalled





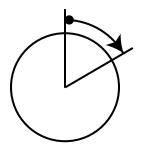


Compression screw

(3) 2-piece stainless steel ferrule

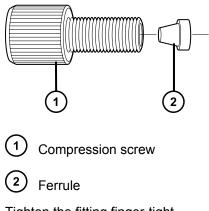
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

Reinstalled tightening



2.5.1.4 1/4-28 flangeless fitting with ferrule

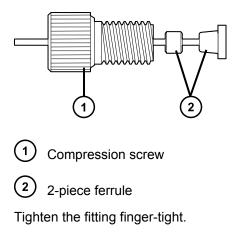
First use or re-installed



Tighten the fitting finger-tight.

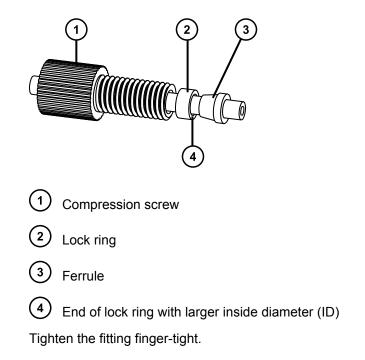
2.5.1.5 1/4-28 flangeless fitting with 2-piece ferrule

First use or re-installed



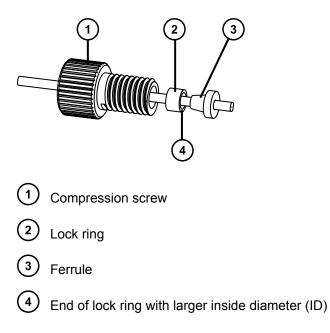
2.5.1.6 Long 1/4-28 fitting with flangeless ferrule and stainless steel lock ring installed on 1/8-inch OD tubing

First use or re-installed



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

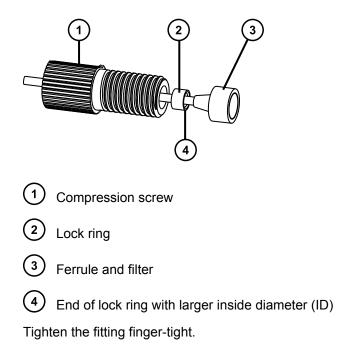
First use or re-installed



February 13, 2019, 715005702 Rev. B Page 35 Tighten the fitting finger-tight.

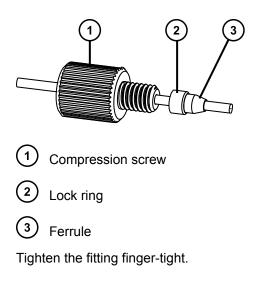
2.5.1.8 5/16-24 fitting with filter and stainless steel lock ring

First use or re-installed



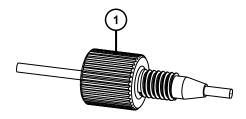
2.5.1.9 PEEK fitting with PEEK ferrule and stainless steel lock ring

First use or re-installed



2.5.1.10 One-piece PEEK fitting

Figure 2–1: First use or reinstalled



(1) Compression screw

Tighten the fitting finger-tight.

Tips:

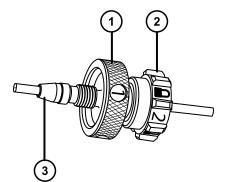
- You can also use the column gripping tool when tightening this fitting.
- To prevent band spreading, ensure that the tubing is fully bottomed in the connection port before tightening the fitting.

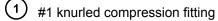
2.5.1.11 Dual-threaded fitting with locking cap nut

Required tools and materials

• Column gripping tool

Figure 2–2: APH outlet fitting first use or reinstalled





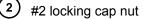
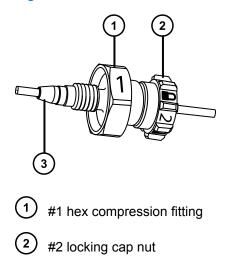


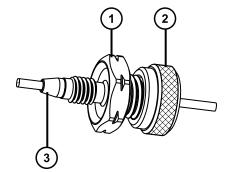


Figure 2–3: Column-stabilizer outlet fitting first use or reinstalled



3 Back-locking PEEK ferrule

Figure 2–4: Legacy fitting first use or reinstalled



1 Hex compression fitting

2 Locking cap nut

3 Back-locking or standard PEEK ferrule

To tighten the fitting:

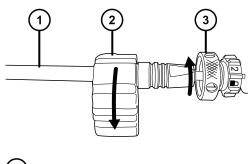
- 1. Loosen the cap nut from the compression fitting.
- 2. Slide the compression fitting together with the ferrule into the inlet of the column (or the inline filter).

Important: To prevent band spreading, ensure that the tubing bottoms in the connection port before you tighten the compression fitting.

- 3. Finger-tighten the compression fitting into the inlet of the column (or the in-line filter).
- 4. Place the column gripping tool onto the column.

5. While holding the column with the column gripping tool, tighten the column onto the compression fitting.





- 1 Compression fitting
- 2) Column gripping tool
- 3 Column

Tip: If you are operating the system at, or close to, its maximum system operating pressure limit and the fitting is a hex, use the 1/2-inch open-end wrench to tighten the compression fitting an additional 1/8- to 1/6-turn.

6. Tighten the cap nut onto the compression fitting.

Tip: When reinstalling the fitting:

- Examine the PEEK ferrule for damage and replace it if necessary. For instructions on replacing the PEEK ferrule, see Replacing the ferrule on the column-inlet fitting.
- Always loosen the locking cap nut before reconnecting the fitting.

2.5.1.12 Replacing the ferrule on the column-inlet fitting

Replace the column-inlet ferrule on the APH assembly fitting if the column is leaking or the ferrule looks damaged.



Warning: To prevent burn injuries, set the column temperature to Off, and then allow the column compartment and its components to cool for 60 minutes before touching them. Monitor the column compartment's internal temperature to ensure that all components are cool.



Warning: To avoid personal contamination with biologically hazardous, toxic, and corrosive materials, wear chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

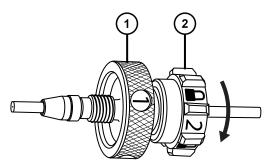
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Replacement ferrule

To replace the ferrule on the column fitting:

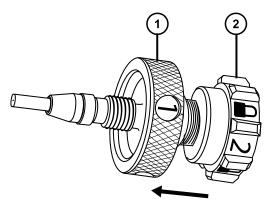
1. Unscrew the #2 locking cap nut from the #1 knurled compression fitting.

Figure 2–6: Unscrewing cap nut from fitting



- 1) #1 knurled compression fitting
- 2) #2 locking cap nut
- 2. Slide the #1 knurled compression fitting off the tubing.

Figure 2–7: Sliding fitting off tubing

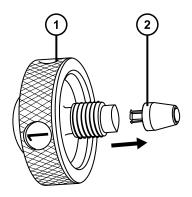


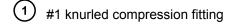
- 1) #1 knurled compression fitting
- 2 Tubing

Note: If the assembly contains a captive ferrule, the ferrule remains locked in the fitting.

3. If the assembly contains a captive ferrule, remove the ferrule from the fitting.

Figure 2–8: Removing ferrule from fitting

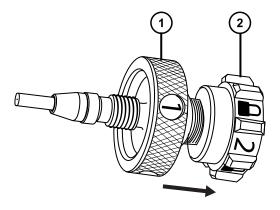




2 Ferrule (captive ferrule shown)

- 4. Discard the used ferrule.
- 5. Install the new ferrule on the fitting.
- 6. Slide the #1 knurled compression fitting and ferrule onto the tubing.

Figure 2–9: Sliding fitting and ferrule onto tubing



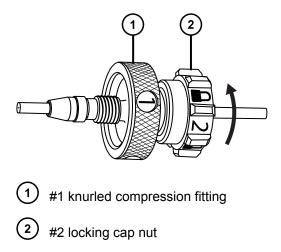


2 #1 knurled compression fitting

Note: If it is a captive ferrule, the ferrule locks into the fitting.

7. Screw the #2 locking cap nut onto the #1 knurled compression fitting.

Figure 2–10: Screwing cap nut onto fitting



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, included on the system documentation media.

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

2.7.1 Particulates

Waters recommends filtering all samples with particulates through a 0.2 μ m sample filter or installing a column pre-filter. The small column frit size (0.2 μ m) can become blocked more easily than larger HPLC column frits (2.0 μ m). 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

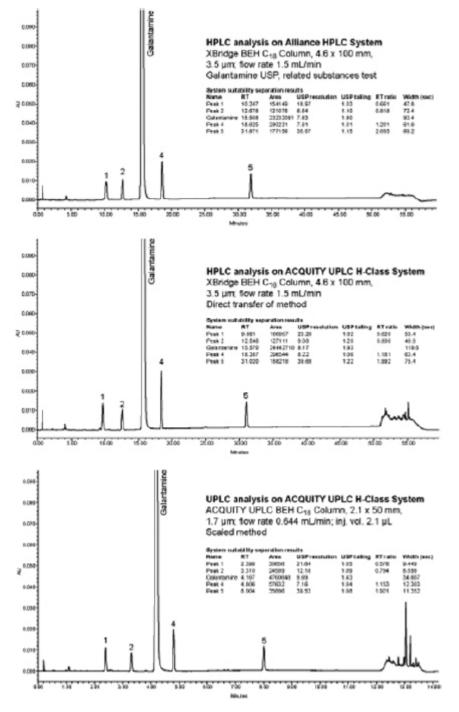
When you use the sample manager's auto-dilution option, the purge solvent serves as the sample diluent. Ensure that your sample solution is soluble and miscible in your chosen purge solvent.

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 Series system, which Waters designed specifically to facilitate method transfer, is the ideal tool 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 an ACQUITY UPLC H-Class Series system fitted with an HPLC column, whereby selectivity and resolution are maintained. Using the ACQUITY UPLC columns calculator, it is then scaled to use a UPLC column and optimized for the shortest analysis time at equal peak capacity. The run time decreased by 46 minutes.





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.

Pre-injector volume = [System 1 dwell volume (mL) - System 2 dwell volume (mL)] × Column 2 volume (mL) 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 Series 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 Waters.com, or by doubleclicking 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.

Gradient hold volume = [System 1 dwell volume (mL) - System 2 dwell volume (mL)] × Column 2 volume (mL) Column 1 volume (mL) × 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 Series 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 Series 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.

2.9 Solvent recommendations

For more information on recommended solvents for your system, refer to ACQUITY UPLC H-Class, H-Class Bio, and I-Class Series Systems Solvent Considerations on your documentation media, or visit www.waters.com.

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, if it has leak sensors, they default to disabled status. Subsequently, they retain their last specified setting. To enable or disable leak sensors, see "Enabling the leak sensors".

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, contact your local Waters representative.

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.

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

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, poweron 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 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**.

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 it, as well as after changing the mobile phase, after changing the sample needle, and after the system is idle for four hours or more.

Recommendation: If you are introducing new solvents, prime them at 4 mL/min for seven minutes. Alternatively, prime the solvents at 4 mL/min for three 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 your next system start-up. For details, see the console online Help.

3.4 Monitoring module LEDs

The LEDs on each module indicate its operational status. Note 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.

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.			

Table 3–1: Run LED descriptions

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.

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

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. Alternative: Firmware upload is in progress.
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.
	Alternative: Firmware upload is in progress.

3.5 Enabling the leak sensors

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

To enable the leak sensors:

- 1. From the system view of the ACQUITY UPLC Console, select **Control > Leak Sensors**.
- 2. To enable the leak sensor for an individual module, click the status on the left-hand side of the module's description.

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

3.6 Monitoring from control panels

You can monitor the system module 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.

3.6.1 Quaternary 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 3–1: QSM control panel



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) **Pressure Delta –** The difference between the maximum and minimum pressures observed during the previous minute of operation.
- (5) Solvent composition Displays the percentage of solvent to be drawn from solvent lines A through D. If you have the optional solvent selection valve, values for solvent lines D1 through D6 will appear here. Composition values range from 0.0 to 100.0%.
- 6 System pressure Displays system pressure, in kPa, bar, or psi. You can specify pressure units in the console software.

(7) Status – Displays the status of the current operation.

You can access these additional functions by right-clicking anywhere in the solvent manager 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 Solvent dialog box and allows for manual changeover or refreshing of solvent. Solvents are automatically shunted to waste.
Prime seal wash	Starts priming the seal wash which lubricates the plungers, fills the tubing paths with solvent and flushes away solvent and or any precipitated salts that have been dragged past the plunger seals from the high-pressure side of the piston chambers.
Wash plungers	Initiates the plunger-wash sequence, which fills and then slowly empties the primary and accumulator chambers (with the current solvent composition) while performing a high speed/volume seal wash. This action helps prevent precipitate buildup on the pump plungers. Such a buildup can damage the high pressure seals.
Launch console	Launches the console software.
Reset QSM	Resets the QSM following an error condition.
Help	Displays the console online Help.

Table 3-4: Additional functions in the QSM control panel

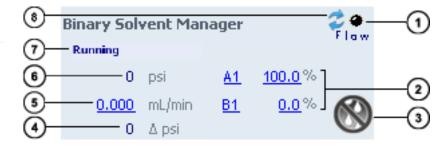
Note: If you are in a degasser pump down state, a separate degasser pump down status icon appears on your control panel to provide you with system status feedback.

3.6.2 Binary solvent manager control panel

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

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

Figure 3–2: BSM control panel



The following table describes the items in the binary solvent manager control panel:

- (1) **Flow LED** Displays the actual flow LED on the front panel of the binary solvent manager, unless communications with the binary solvent manager are lost.
- (2) **Solvent composition** Displays the percentage of solvent to be drawn from the solvent lines (A and B). Composition values range from 0.0 to 100.0%.
- 3 **Stop flow** Immediately stops all flow from the binary solvent manager.
- 4 **Pressure Delta -** The difference between the maximum and minimum pressures observed during the previous minute of operation.
- 5 **Flow rate** Displays the total flow rate of the binary solvent manager, from 0.000 to 2.000 mL/min under normal operation. The flow rate range is 0.000 to 4.000 mL/min when priming a single solvent and 0.000 to 8.000 mL/min when priming both solvents.
- 6 System pressure Displays system pressure in kPa, bar, or psi.

Tip: To change how your system pressure is displayed, from the system tree in the ACQUITY UPLC Console, select **Binary Solvent Manager** and click **Configure > System preferences**.

Status - Displays the status of the current operation.

Begasser health check running - Displays on power-up and when you manually run the degasser health check. Waters does not recommend data acquisition until the degasser health check passes. When it is recommended that the degasser health check should be run, the icon is displayed.

You can access these additional functions by right-clicking anywhere in the binary solvent manager 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.		
Prime solvents	Displays the Prime solvents dialog box.		
Prime seal wash	Starts priming the seal wash.		
Launch Console	Launches the ACQUITY UPLC Console.		
Reset BSM	Resets the binary solvent manager after an error condition.		
Help	p Displays the ACQUITY UPLC Console online Help.		

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

3.6.3 ISM control panel

The control panel of the ISM displays flow status, system pressure, and total flow rate.

Rule: You can edit these settings when the system is idle by clicking on the underlined value. You cannot edit ISM settings while the system is running samples.

Figure 3–3: ISM control panel



- (1) **Flow LED** Displays on the front panel of the solvent manager the status of the flow state, unless communications are lost.
- Flow rate Displays the flow rate of solvent through all lines of the ISM, from 0.000 to 2.000 mL/min, under normal operation, and 0.000 to 4.000 mL/min, when priming.
- 3 Stop flow When clicked, immediately stops all flow from the solvent manager.
- Currently selected solvent line Displays the currently selected solvent line (S1 through S6). If the optional solvent selection valve is not installed, the display will be blank.
- 5 **System pressure** Displays system pressure, in kPa, bar, or psi. You can customize pressure units via the console.
- 6 **Status** Displays the status of the current operation. (In this representation of the control panel, the status is blank.)
- Degasser health check running Displays on power-up and when you manually run the degasser health check. Waters does not recommend data acquisition until the degasser health check passes. When it is recommended that the degasser health check should be run, the icon is displayed.

You can access these additional functions by right-clicking anywhere in the ISM 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.				
Prime solvent	Displays the Prime Solvent dialog box and allows for manual changeover or refreshing of solvent. Solvents are automatically shunted to waste.				
Prime seal wash	Starts priming the seal wash which lubricates the plungers, fills the tubing paths with solvent and flushes away solvent and or any precipitated salts that have been dragged past the plunger seals from the high-pressure side of the piston chambers.				
Wash plungers	Initiates the plunger-wash sequence, which fills and then slowly empties the primary and accumulator chambers (with the current solvent composition) while performing a high speed/volume seal wash. This action helps prevent precipitate buildup on the pump plungers. Such a buildup can damage the high pressure seals.				
Launch console	Launches the console.				
Reset ISM	Resets the ISM after an error condition.				

Table 3–6: Additional functions in the ISM control panel

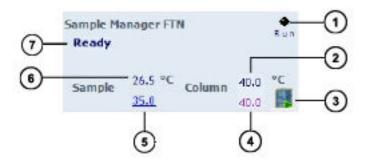
Control panel function	Description
Help	Displays the online Help for the console software.

3.6.4 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 3–4: 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 column compartment temperature Displays the current temperature of the column compartment.
- 3 **Display console** When clicked, launches the console software.
- 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 sample compartment temperature Displays the current temperature in the sample 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.

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.

Table 3–7: Additional functions in the sample manager control panel

3.6.5 Column manager control panel

The column manager's control panel displays the current temperature and set point of the selected column. 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 3–5: Column manager control panel

Column Manager		e Run	
			(2)
Column Selection	<u>Column 1</u>		~
Temperature	30.0 °C		3
· comp croccar c	30.0		

The following table describes the items in the column manager's control panel:

Run LED - Mirrors the run status LED on the column manager's front panel, unless communications are interrupted.



Column currently in use - Displays the column that is currently in use.

3 Current temperature - Displays the current column compartment temperature.

(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 3–8:	Additional	functions	in the	column	manager's	control panel
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Control panel function	Description	
Reset CM	Resets the column manager after an error condition.	
Help	Displays the console online Help.	

3.6.6 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 3–6: ELS detector control panel



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 temperatures - Displays both the set point temperature and current drift tube temperatures.

3) Current nebulizer temperatures - Displays the current nebulizer temperature.

(4) **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.

(5) Current nebulizer gas pressure - Displays the current nebulizer set gas pressure from 20 to 60 psi.

6 Photomultiplier tube gain factors - Displays the gain setting, programmable from 0 to 1000.

(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 3–9: Additional functions in the ELS detector control panel

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

3.6.7 FLR control panel

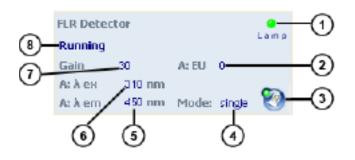
2)

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 3–7: FLR detector control panel



Lamp LED - Mirrors the lamp status LED on the front panel of the detector, unless communications with the detector are lost.

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 3–10: Additional functions in the FLR detector control panel

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

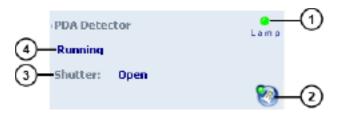
3.6.8 PDA control panel

Note: The PDA, PDA $e\lambda$, and 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.

Figure 3–8: 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.

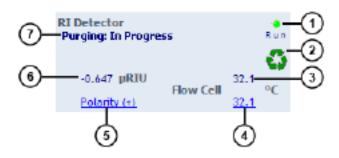
Control panel function	Description
Auto zero	Resets the detector offsets.
Reset PDA	Resets the detector after an error condition.
Help	Displays console online Help.

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

3.6.9 RI control panel

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

Figure 3–9: 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 μ RIU.

(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 3–12: Additional functions in the RI detector control panel

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

3.6.10 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 3–10: TUV detector control panel



The following table describes the items in the TUV's 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 gray, the lamp is off. If the icon is red, the lamp is in an error state.

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 3–13: Additional functions in the TUV detector control panel

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

3.6.11 ACQUITY QDa

The ACQUITY QDa mass detector characterizes LC-separated analytes, determining their mass by means of a photomultiplier detection system and chromatography data software. The instrument acquires data in one of these operating modes:

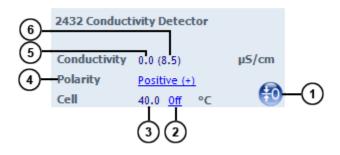
- Scanning, where the instrument scans mass-to-charge (*m*/*z*) ratios to produce a mass spectrum.
- Selected ion recording (SIR), where the instrument records the signal intensity at a static *m*/*z* ratio.

For more information, see the ACQUITY QDa Detector Overview and Maintenance Guide on your documentation media, or at www.waters.com.

3.6.12 2432 conductivity control panel

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

Figure 3–11: 2432 conductivity detector control panel



- 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.

3.7 Starting up the system

Use the Start up system function to prime the solvent manager after the system has been idle a long period of time (overnight, for example). 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.

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

Table 3–14: Sample manager priming parameter values

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 3–15: 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
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%

Table 3–15:	Equilibrate	to Method	table values	(continued)
	- quint ato			(001101000)

System startup parameters	Default	Allowed values
Column temperature	Off Note: Column selection for the Column Manager defaults 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 system begins to prime.
- 2. The system establishes the method flow rate, column and sample temperatures, and ignites the lamp.
- 3. After priming, the sample manager characterizes the needle and seal, if selected, and then logs the results of the characterizations into the database.

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 website includes phone numbers and email addresses for Waters locations worldwide. Visit www.waters.com.

When you contact Waters, be prepared to provide the following 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 or solvents
- Detector settings (sensitivity and wavelength)
- · Type and serial number of column or columns
- · Sample type and diluent
- · 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* on the Waters website (www.waters.com).

Note: For troubleshooting information, visit support.waters.com.

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.
- 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 module door.

4.2 Maintenance procedures and frequency

Consult the individual module's overview and maintenance guide on the documentation media 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 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

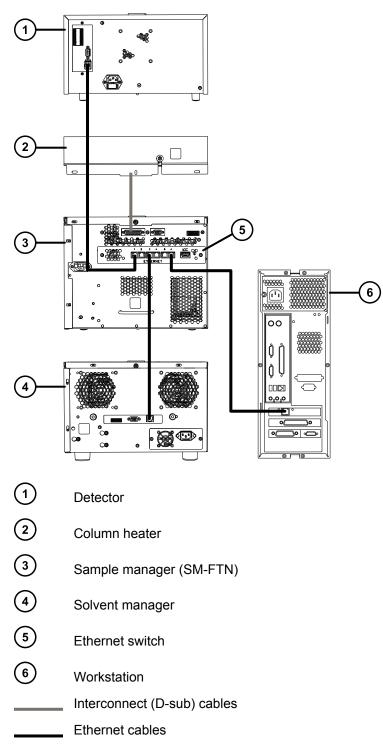
The sample manager incorporates an internal Ethernet switch that accommodates the PC (workstation) and as many as six system modules. Connect the shielded Ethernet cables from each module to the electronic connections on the rear panel of the sample manager.

Note: The sample manager is connected internally to the Ethernet switch.

Tip: Use a Waters switch box if you are running multiple stacks of modules.

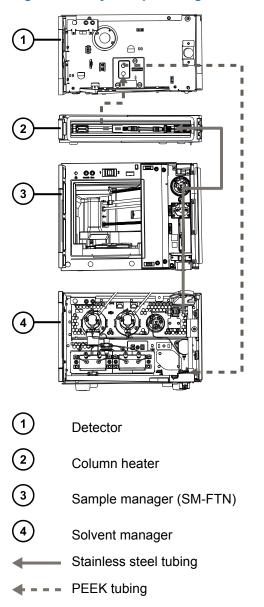
5.2 External cable connections





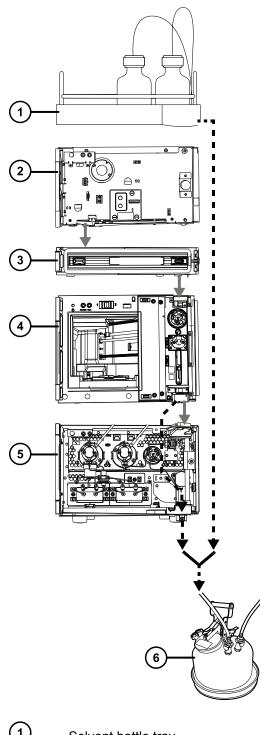
5.3 Plumbing connections

Figure 5–2: System plumbing connections



5.4 Waste-tubing connections





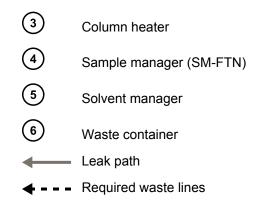


Solvent bottle tray

Detector

2

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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 the device. 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 electric shock, observe these precautions:

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

Note: Some column modules, such as the column heater (CH-A) and the column heater 30-cm (CH-30A), receive their power from the sample manager via the interconnect cable.

Recommendation: Use a line conditioner and uninterruptible power supply (UPS) for optimum, long-term, input-voltage stability. Contact Waters to ensure the correct selection and size.

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 grounded wall outlet.

5.5.2 Connecting to a cart's electricity source

If your system includes an optional cart, follow this procedure to connect each module to a power source.



Warning: To avoid electric shock, observe these precautions:

- Use SVT-type power cords in the United States and HAR-type power cords, or better, in Europe. For requirements elsewhere, contact your local Waters distributor.
- Inspect the power cords for damage and replace them 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 start-up 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.

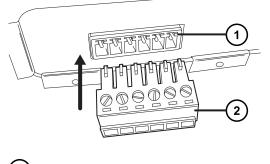
Required tools and materials

- 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

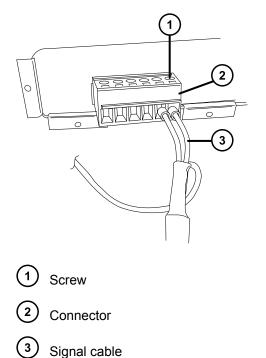




2. Using the flat-blade screwdriver, attach the positive and negative leads of the signal cable to the connector.

Tip: Refer to the cable-connection label affixed to the rear panel of the module.

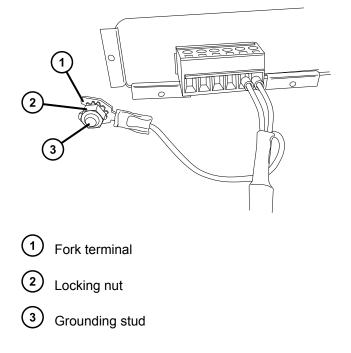
Figure 5–5: Positive and negative lead connections



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



5.7 Connecting to a column module

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

- Column heater (CH-A)
- Column heater 30 cm (CH-30A)
- Column heater/cooler (30 cm CHC)
- Column manager (CM-A)
- Column manager auxiliary (CM-Aux)

The sample manager powers and communicates with the column module (CH-A, CH-30A, and the CM-Aux). The external communication cable must be connected to the rear of the column module and the sample manager.

Note: The CM-A and 30-cm CHC both require their own separate power supplies to operate.

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. Ensure that 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 PSPI port on the rear of the sample manager.

Notes:

- The CM-A connects to the sample manager via an Ethernet connection.
- The CM-Aux connects to the CM-A via an SDL cable.

A Post-Injection Volume Kit instructions



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



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

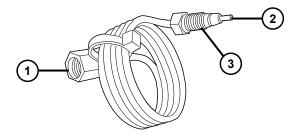
Required tools and materials

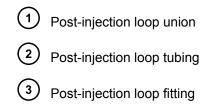
- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- · Post-injection loop kit

To install or replace a post-injection loop:

- 1. Power-off the sample manager.
- 2. Open the fluidics compartment door.
- 3. Fully insert the post-injection loop tubing into port 6 of the injection valve, and then thread the fitting into the port.
- 4. Holding the extension post-injection loop tubing against the bottom of the port, fingertighten the post-injection loop fitting.
- 5. Use the 1/4-inch open-end wrench to tighten the post-injection loop fitting an additional 1/6turn for existing fittings, or 3/4-turn for a new fitting.

Figure A-1: Post-injection loop





- 6. Screw the active preheater (APH) fitting into the post-injection loop union, and then use the 1/4-inch open-end wrench to tighten the fitting 3/4-turn beyond finger-tight for a new fitting, or 1/6-turn beyond finger-tight for existing fittings.
- 7. Close the fluidics compartment door.
- 8. Power-on the sample manager.

B Safety advisories

Waters products display safety symbols that identify hazards associated with the product's operation and maintenance. The symbols also appear in product manuals with statements that describe the hazards and advise how to avoid them. This appendix presents all safety symbols and statements that apply to Waters' product offerings. The symbols and statements can apply to a specific product, or apply to other products within the same system.

B.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 appears 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.)



Warning: (Risk of high-pressure gas release.)

B.1.1 Specific warnings

B.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.

B.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process biologically hazardous materials. Biologically hazardous materials are substances that contain biological agents capable of producing harmful effects in humans.



Warning: To avoid infection from blood-borne pathogens, 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).



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.

B.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 biologically hazardous, toxic, 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.

B.2 Notices

Notice advisories appear where an instrument, device, or component 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 case of the instrument or device, do not clean it with abrasives or solvents.

B.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.

B.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.

B.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。



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。



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



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

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



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



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 질 산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.



警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

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

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



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



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



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

B.6 Warnings that address the replacement 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 boite 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: 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。



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。



경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타 입 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: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。



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。



경고: 화재의 위험을 막으려면 유지관리 절차 단원의 "퓨즈 교체" 절에 설명된 것과 동 일한 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。

B.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
\bigcirc	Electrical power off
\bigcirc	Standby
	Direct current
\sim	Alternating current
3~	Alternating current (three phase)
	Safety ground
<i></i>	Frame or chassis terminal connection
\blacksquare	Fuse
<u> </u>	Functional ground
\rightarrow	Input
()→	Output
	Indicates that the device or assembly is susceptible to damage from electrostatic discharge (ESD)

B.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!
Y	Fragile!
X	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation

B.9 Stacking system modules with interlocking features

This procedure applies to system modules equipped with interlocking features.



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



Warning: To avoid crushing your fingers beneath or between modules, use extreme care when installing a module in the system stack.

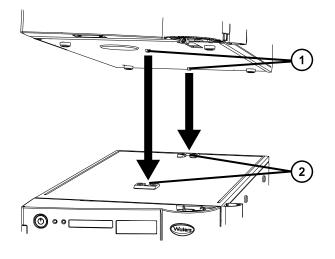


Warning: To avoid injury, do not stack modules, including the solvent tray and rails, higher than one meter (39.4 inches) above the bench top.

To stack the modules:

1. Place the rear feet of the module that you are adding atop the previously added module in the system stack, and slide it backward until its rear alignment pin rests in the rear alignment slot on the previously added module.

Figure B-1: Aligning pins with slots



- 1 Alignment pins
- 2 Alignment slots
- 2. Lower the front of the module that you are adding so that its front alignment pin rests in the front alignment slot on the previously added module.
- 3. Repeat steps 1 and 2 for the remaining system modules.

B.10 Stacking system modules without interlocking features

This procedure applies to system modules that are not equipped with interlocking features. Most modules will, however, have interlocking features.



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

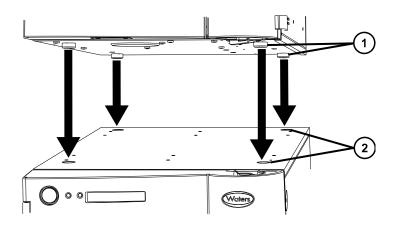


Warning: To avoid crushing your fingers beneath or between modules, use extreme care when installing a module in the system stack.

To stack the modules:

1. Align the front and rear feet of the module that you are adding with the corresponding indents in the top of the chassis of the previously added module in the system stack.

Figure B-2: Aligning feet with indents



1) Feet on underside of module being stacked

2 Indents on top side of previously added module

2. Carefully lower the module so that the feet rest in the indents.

Important: To maintain the integrity of the system stack and integrated waste system, ensure that the feet of the upper module rest in the indents of the lower module.

3. Repeat steps 1 and 2 for the remaining system modules.