

ACQUITY UPLC System

Operator's Guide

71500082502 / Revision F

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Copyright © Waters Corporation 2014
All rights reserved

Copyright notice

© 2014 WATERS CORPORATION. PRINTED IN THE UNITED STATES OF AMERICA AND IN IRELAND. ALL RIGHTS RESERVED. THIS DOCUMENT OR PARTS THEREOF MAY NOT BE REPRODUCED IN ANY FORM WITHOUT THE WRITTEN PERMISSION OF THE PUBLISHER.

The information in this document is subject to change without notice and should not be construed as a commitment by Waters Corporation. Waters Corporation assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Waters Corporation be liable for incidental or consequential damages in connection with, or arising from, its use.

Trademarks

ACQUITY UPLC, Millennium, UPLC, and Waters are registered trademarks, and eCord, Empower, MassLynx, nanoACQUITY UPLC, and “THE SCIENCE OF WHAT’S POSSIBLE” are trademarks of Waters Corporation.

Keps is a registered trademark of Illinois Tool Works, Inc.

PEEK is a trademark of Victrex Corporation.

PharMed and Tygon are registered trademarks of Saint-Gobain Ceramics & Plastics, Inc.

Phillips is a registered trademark of Phillips Screw Company.

TORX is a registered trademark of Textron Corporation.

Windows is a registered trademark of Microsoft Corporation.

Other trademarks or registered trademarks are the sole property of their respective owners.

Customer comments

Waters' Technical Communications department invites you to tell us of any errors you encounter in this document or to suggest ideas for otherwise improving it. Please help us better understand what you expect from our documentation so that we can continuously improve its accuracy and usability.

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

Contacting Waters

Contact Waters[®] with enhancement requests or technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us via the Internet, telephone, or conventional mail.

Waters contact information

Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com .
Telephone and fax	From the USA or Canada, phone 800 252-HPLC, or fax 508 872 1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.
Conventional mail	Waters Corporation 34 Maple Street Milford, MA 01757 USA

Safety considerations

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

Considerations specific to ACQUITY UPLC instruments

High voltage hazard



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

Safety advisories

Consult [Appendix A](#) for a comprehensive list of warning and caution advisories.

Operating the ACQUITY UPLC instruments

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

Applicable symbols

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

Audience and purpose

This guide is intended for personnel who install, operate, and maintain ACQUITY UPLC instruments.

Intended use of the ACQUITY UPLC system

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

Calibrating

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

Quality-control

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

ISM classification

ISM Classification: ISM Group 1 Class B

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

EC Authorized Representative



Waters Corporation
Stamford Avenue
Altrincham Road
Wilmslow SK9 4AX
United Kingdom

Telephone: +44-161-946-2400
Fax: +44-161-946-2480
Contact: Quality manager

Table of Contents

Copyright notice	ii
Trademarks	ii
Customer comments	iii
Contacting Waters	iii
Safety considerations	iv
Considerations specific to ACQUITY UPLC instruments	iv
Safety advisories	iv
Operating the ACQUITY UPLC instruments	v
Applicable symbols	v
Audience and purpose	v
Intended use of the ACQUITY UPLC system	v
Calibrating	v
Quality-control	vi
ISM classification	vi
ISM Classification: ISM Group 1 Class B	vi
EC Authorized Representative	vi
1 System Overview	1-1
Instruments, components, and data systems	1-2
Examples of Waters ACQUITY UPLC systems	1-3
UPLC system guidelines	1-4
ACQUITY UPLC columns calculator	1-5
Binary solvent manager	1-5
How the binary solvent manager works	1-6
Sample manager	1-7
How sample flows	1-7

High temperature column heater	1-8
Column manager	1-8
Column heater/cooler	1-9
30-cm column heater/cooler	1-9
Optional sample organizer	1-9
Detectors	1-10
TUV detector	1-10
PDA detector	1-10
ELS detector.....	1-10
FLR detector.....	1-11
Median baseline filter.....	1-11
Mass spectrometers	1-11
SQ detector	1-11
TQ detector.....	1-11
Data systems	1-12
Empower software	1-12
MassLynx software.....	1-12
Columns	1-12
eCord column chip	1-13
FlexCart	1-13
For additional information	1-14
2 System Setup	2-1
Before you begin	2-2
Tools.....	2-3
Assembling the FlexCart	2-3
Adjusting the FlexCart's height.....	2-6
Locking the FlexCart in place	2-7
Moving the assembled FlexCart	2-9

Unpacking and installing the sample organizer	2-9
Installing the binary solvent manager	2-10
Installing the sample manager	2-10
Installing the HT column heater	2-11
Installing the optional column manager	2-12
Installing the optional column heater/cooler	2-13
Installing the detector	2-13
Installing the 30-cm column heater/cooler	2-14
Plumbing the system	2-20
Installation recommendations for fittings.....	2-21
Plumbing a TUV detector.....	2-22
Plumbing a PDA detector.....	2-25
Plumbing an ELS detector.....	2-25
Plumbing an FLR detector.....	2-25
Plumbing the binary solvent manager and sample manager.....	2-26
Plumbing the column manager.....	2-33
Plumbing the 30-cm column heater/cooler.....	2-34
Routing system drainage through the FlexCart.....	2-36
Installing the column stabilizer assembly in an HT column heater.....	2-37
Installing the column stabilizer assembly in a column heater/cooler.....	2-40
Installing the column in-line filter in a HT column heater.....	2-43
Installing the column in-line filter in a column manager or column heater/cooler.....	2-44
Installing the column in an HT column heater.....	2-46
Installing the column in a column manager or column heater/cooler.....	2-49
Installing the column in a system with a mass spectrometer.....	2-53
Connecting to the solvent supply.....	2-55
Making Ethernet and signal connections	2-57
Ethernet connections.....	2-57
HT column heater connections.....	2-57
30-cm column heater/cooler connections.....	2-58
Binary solvent manager I/O signal connectors.....	2-59

Sample manager I/O signal connectors	2-61
TUV detector I/O signal connectors	2-63
PDA detector signal connectors	2-64
ELS detector signal connectors	2-64
FLR detector signal connectors	2-64
Signal connections	2-64
Connecting to the electricity source	2-65
Calibrating the XYZ mechanism using the teach block	2-67
3 Preparing System Hardware	3-1
Powering-on the system	3-1
Monitoring startup tests	3-3
Monitoring system instrument LEDs	3-3
Power LED	3-4
Status LEDs	3-4
Enabling the leak sensors	3-6
Preparing the binary solvent manager	3-7
Performing a seal wash prime	3-7
Priming the binary solvent manager	3-9
Priming a dry binary solvent manager	3-10
Priming a wetted binary solvent manager	3-12
Preparing the sample manager	3-15
Selecting weak wash and strong wash solvents	3-15
Priming the sample manager	3-17
Washing the sample manager needle	3-19
Characterizing the needle seal	3-21
Characterizing the needle and sample loop volumes	3-22
Using the extended puncture needle	3-23
Loading sample plates in the sample manager	3-24
Selecting the optimum sample injection mode	3-25
Installing the optional sample manager shade	3-28

Preparing the column manager	3-30
Preparing the sample organizer	3-30
Initiating communications	3-30
Loading sample plates	3-31
Displaying sample plate information	3-35
Starting the TUV detector	3-36
Starting the TUV detector.....	3-37
Conditioning the column	3-39
Shutting down the system	3-40
Shutting down for less than 24 hours.....	3-40
Shutting down for more than 24 hours.....	3-41
Running HPLC methods on an ACQUITY UPLC system	3-42
System considerations	3-42
Choosing fittings	3-44
4 Configuring System Software	4-1
Configuring Empower software	4-1
Starting Empower software and logging in.....	4-1
Selecting system instruments	4-2
About the binary solvent manager control panel.....	4-4
About the sample manager control panel.....	4-6
About the TUV detector control panel.....	4-9
About the column manager control panel	4-11
Starting the ACQUITY UPLC Console from Empower software	4-12
Configuring MassLynx software	4-13
Starting the ACQUITY UPLC Console from MassLynx software	4-14
5 Verifying System Operation	5-1
Preparing the system	5-2
Creating the test methods	5-5
Creating the instrument method	5-5

Creating the sample set method	5-9
Performing the gradient performance test	5-10
6 Maintaining the System	6-1
Maintenance schedule	6-2
Spare parts	6-5
Contacting Waters technical service	6-6
Locating system serial numbers	6-6
Maintenance considerations	6-7
Safety and handling.....	6-7
Proper operating procedures.....	6-8
Configuring maintenance warnings	6-8
Maintaining the binary solvent manager	6-9
Resolving binary solvent manager leak sensor errors.....	6-10
Replacing the binary solvent manager's leak sensor.....	6-15
Replacing the mixer.....	6-19
Replacing the i ² Valve actuator	6-21
Replacing the i ² Valve cartridge	6-32
Replacing the in-line filter cartridge on the <i>i²Valve</i> actuator.....	6-42
Replacing the accumulator check valve.....	6-46
Replacing solvent filters	6-49
Cleaning the air filters in the binary solvent manager door.....	6-50
Replacing the air filters in the binary solvent manager door	6-51
Removing and replacing the primary head seals.....	6-52
Removing and replacing the accumulator head seals.....	6-70
Replacing the primary head plungers	6-83
Replacing the accumulator head plungers	6-103
Replacing the vent valve cartridge	6-117
Replacing the fuses.....	6-120
Cleaning the instrument's exterior.....	6-122
Maintaining the sample manager	6-122
Defrosting the sample compartment	6-122

Resolving sample manager leak sensor errors.....	6-123
Replacing the sample manager's leak sensor.....	6-128
Replacing the sample needle assembly	6-131
Calibrating the needle Z-axis	6-139
Characterizing the needle seal.....	6-140
Characterizing the needle and sample loop volumes.....	6-140
Replacing the puncture needle holder	6-142
Replacing the sample loop.....	6-145
Replacing the sample syringe	6-149
Replacing the wash syringes.....	6-152
Modifying sample syringe configuration parameters	6-155
Replacing the injection valve cartridge	6-156
Testing the sample manager backpressure regulator	6-159
Replacing the fuses.....	6-159
Cleaning the instrument's exterior.....	6-161
Maintaining the HT column heater	6-161
Resolving HT column heater leak sensor errors	6-162
Resolving HT column heater leak sensor errors (door fully opened)	6-167
Replacing the HT column heater's leak sensor	6-171
Replacing the HT column heater's leak sensor (door fully opened)	6-174
Replacing the column in the HT column heater	6-176
Replacing the column stabilizer assembly in the HT column heater	6-177
Replacing the frit in the HT column heater's in-line filter unit.....	6-178
Maintaining the column manager and column heater/cooler	6-180
Resolving column manager and column heater/cooler leak sensor errors	6-180
Replacing the column manager's or column heater/cooler's leak sensor ..	6-184
Replacing the column in the column manager.....	6-186
Replacing the column in the column heater/cooler.....	6-193
Replacing the column stabilizer assembly in the column heater/cooler ...	6-195
Replacing the frit in the column manager's in-line filter unit	6-196
Replacing the filter in the column heater/cooler's in-line filter unit.....	6-199
Cleaning the column manager and column heater/cooler	6-201
Maintaining the TUV detector	6-203
Resolving detector leak sensor errors.....	6-204

Replacing the detector's leak sensor	6-208
Maintaining the flow cell.....	6-210
Cleaning the flow cell	6-212
Performing a system acid cleansing flush	6-215
Replacing the flow cell	6-217
Replacing the lamp	6-221
Replacing the fuses	6-223
Cleaning the instrument's exterior	6-225
Maintaining the sample organizer	6-225
Defrosting the sample organizer	6-225
Cleaning the air filter	6-225
Replacing the fuses	6-226
Cleaning the instrument's exterior	6-228
Maintaining the 30-cm column heater/cooler	6-228
Replacing the fuses	6-228
Cleaning the instrument's exterior	6-230
7 Troubleshooting	7-1
Troubleshooting with Connections Insight	7-2
Proper operating procedures	7-3
Contacting Waters technical service.....	7-3
Locating system serial numbers	7-4
Solving baseline noise and drift problems	7-5
Binary solvent manager troubleshooting	7-10
Power LED	7-10
Flow LED	7-11
Sample manager troubleshooting	7-14
Power LED	7-14
Run LED.....	7-14
Sample organizer troubleshooting	7-17
Power LED	7-17
Run LED.....	7-17

HT column heater troubleshooting	7-21
Column manager and column heater/cooler troubleshooting	7-21
Power LED	7-21
Run LED.....	7-21
TUV detector troubleshooting	7-24
Power LED	7-24
Lamp LED.....	7-24
Clearing bubbles from the flow cell	7-24
PDA detector troubleshooting	7-27
Power LED	7-27
Lamp LED.....	7-28
ELS detector troubleshooting	7-32
Power LED	7-32
Lamp LED.....	7-32
FLR detector troubleshooting	7-33
Power LED	7-33
Lamp LED.....	7-34
Chromatography troubleshooting	7-35
A Safety Advisories	A-1
Warning symbols	A-2
Task-specific hazard warnings.....	A-2
Warnings that apply to particular instruments, instrument components, and sample types.....	A-3
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-12
Electrical symbols.....	A-12
Handling symbols	A-13

B Specifications	B-1
Binary solvent manager specifications	B-2
Sample manager specifications	B-4
Sample organizer specifications	B-7
HT column heater specifications	B-11
Column manager and column heater/cooler specifications	B-13
30-cm column heater/cooler specifications	B-15
TUV detector specifications	B-17
C Solvent Considerations	C-1
Introduction	C-2
Preventing contamination	C-2
Clean solvents	C-2
Solvent quality	C-2
Solvent preparation	C-2
Water	C-3
Solvent recommendations	C-4
General solvent guidelines	C-4
Solvents to use	C-4
Solvents to avoid	C-6
ACQUITY UPLC system recommendations	C-6
Binary solvent manager recommendations	C-8
Sample manager recommendations	C-8
Detector recommendations	C-8
Solvent miscibility	C-8
Using miscibility numbers (M-numbers)	C-9
Solvent stabilizers	C-10
Solvent viscosity	C-10
Wavelength selection	C-11
UV cutoffs for common solvents	C-11

Mixed mobile phases.....	C-11
Mobile phase absorbance.....	C-12
Index	Index-1

1 System Overview

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

Contents

Topic	Page
Instruments, components, and data systems	1-2
UPLC system guidelines	1-4
Binary solvent manager	1-5
Sample manager	1-7
High temperature column heater	1-8
Column manager	1-8
Column heater/cooler	1-9
30-cm column heater/cooler	1-9
Optional sample organizer	1-9
Detectors	1-10
Mass spectrometers	1-11
Data systems	1-12
Columns	1-12
FlexCart	1-13
For additional information	1-14

Instruments, components, and data systems

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

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

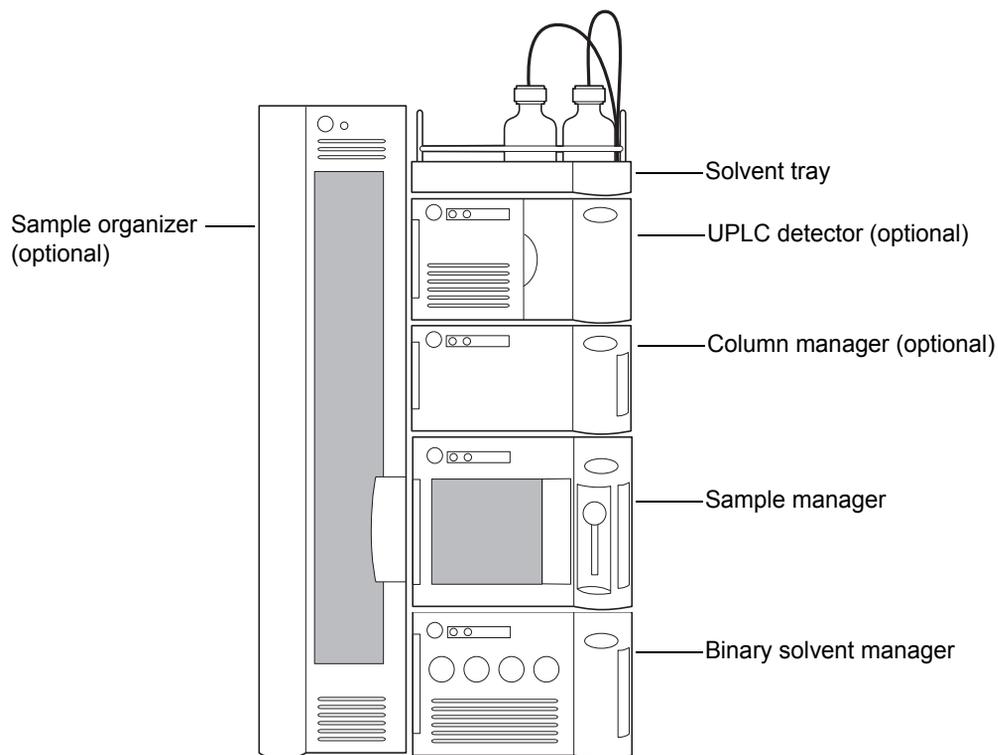
The binary solvent manager and injector can sustain pressures up to 103,421 kPa (1034 bar, 15,000 psi) and can generate high-pressure gradients with minimal gradient delay. The upper limit of the flow rate range is 2 mL/min.

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

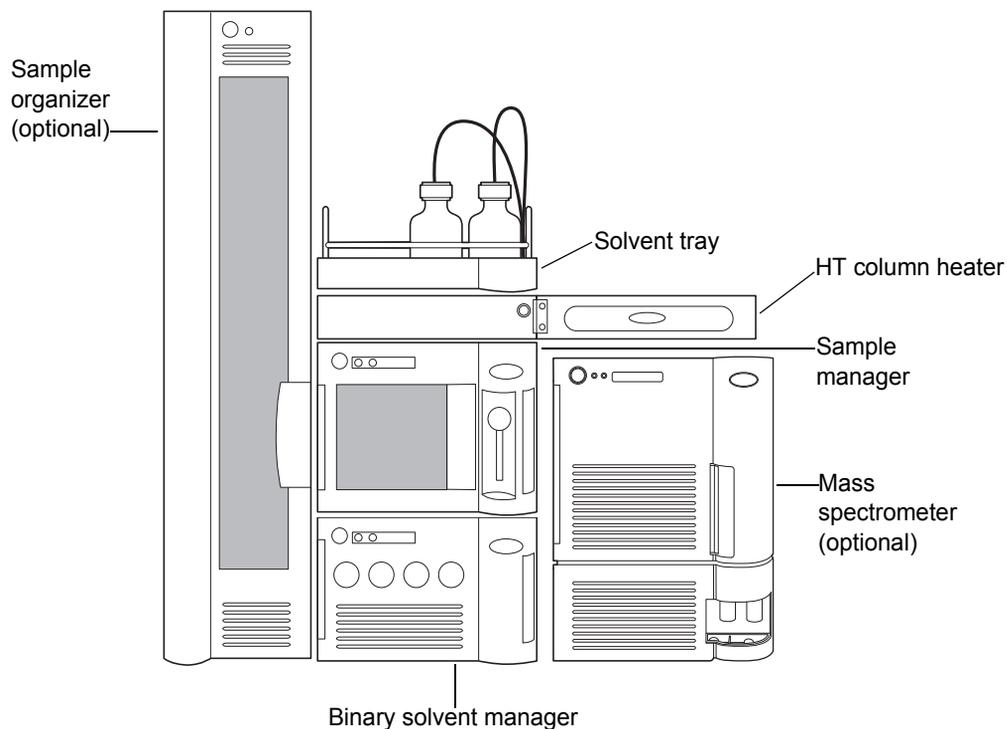
Waters® Empower™ chromatography software, MassLynx™ mass spectrometry software, or certain third-party software controls the ACQUITY UPLC systems.

Examples of Waters ACQUITY UPLC systems

Single detector system with column manager, no mass spectrometer



Single detector system with a mass spectrometer and column heater



UPLC system guidelines

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

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

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

Optimal flow rates for molecular weight range

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

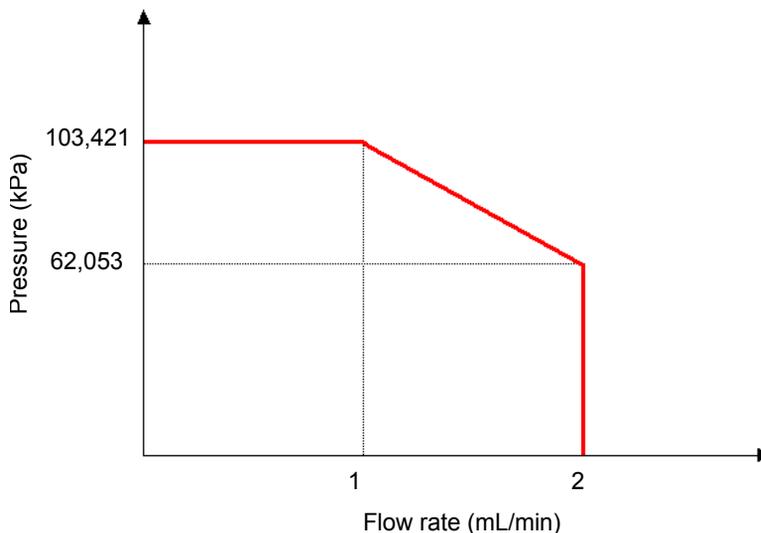
ACQUITY UPLC columns calculator

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

Binary solvent manager

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

Pressure flow envelope



How the binary solvent manager works

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

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

Sample manager

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

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

How sample flows

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

High temperature column heater

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

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

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

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

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

Column manager

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

Column heater/cooler

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

30-cm column heater/cooler

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

Optional sample organizer

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

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

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

Detectors

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

TUV detector

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

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

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

PDA detector

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

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

ELS detector

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

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

FLR detector

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

Median baseline filter

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

See also: ACQUITY UPLC Console online Help.

Mass spectrometers

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

SQ detector

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

TQ detector

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

Data systems

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

Empower software

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

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

See also: Empower online Help.

MassLynx software

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

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

Columns

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

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

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

eCord column chip

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

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

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

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

FlexCart

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

For additional information

Refer to the following documents for further information:

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

2 System Setup

Contents

Topic	Page
Before you begin	2-2
Assembling the FlexCart	2-3
Unpacking and installing the sample organizer	2-9
Installing the binary solvent manager	2-10
Installing the sample manager	2-10
Installing the HT column heater	2-11
Installing the optional column manager	2-12
Installing the optional column heater/cooler	2-13
Installing the detector	2-13
Installing the 30-cm column heater/cooler	2-14
Plumbing the system	2-20
Making Ethernet and signal connections	2-57
Connecting to the electricity source	2-65
Calibrating the XYZ mechanism using the teach block	2-67

Before you begin

Requirement: To install the system, you must know how to set up and operate laboratory instruments and computer-controlled devices and how to handle solvents.

Before installing the system, ensure that

- it is not positioned under a heating or cooling vent.
- the required components are present.
- none of the shipping containers or unpacked items is damaged.

Recommendation: Because the system is heavy (140.6 kg, or 310 pounds), put it on the optional FlexCart before assembly.



Warning:

- To avoid back injuries, two or more people must unpack the sample organizer and transfer it to its final position. Also, if only one person will install the sample manager, binary solvent manager, or other system instrument, he or she must do so using a mechanical lift.
- To avoid overheating, and to provide clearance for cable connections, make sure there is at least 15.24 cm (6 inches) of clearance at the rear of the system.
- To maintain proper drainage and leak control, the system must be within ± 1 degree of ground level.

If you discover any damage or discrepancy when you inspect the contents of the cartons, immediately contact the shipping agent and your local Waters representative.

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800 252-4752). If you are located anywhere else, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Waters' site includes phone numbers and e-mail addresses for Waters locations worldwide. Visit www.waters.com, and click Waters Division > Regional/Global Contacts.

For complete information on reporting shipping damages and submitting claims, see the document *Waters Licenses, Warranties, and Support Services*.

Tools

You will need the following tools and materials to install the Waters ACQUITY UPLC[®] system.

- 5/16-inch open-end wrench (2)
- 5/8-inch open-end wrench
- 1/4-inch open-end wrench
- Phillips[®] screwdriver
- Small mirror
- T25 TORX[®] driver

Assembling the FlexCart

If your system includes the optional FlexCart, follow the procedure below to unpack and assemble it.

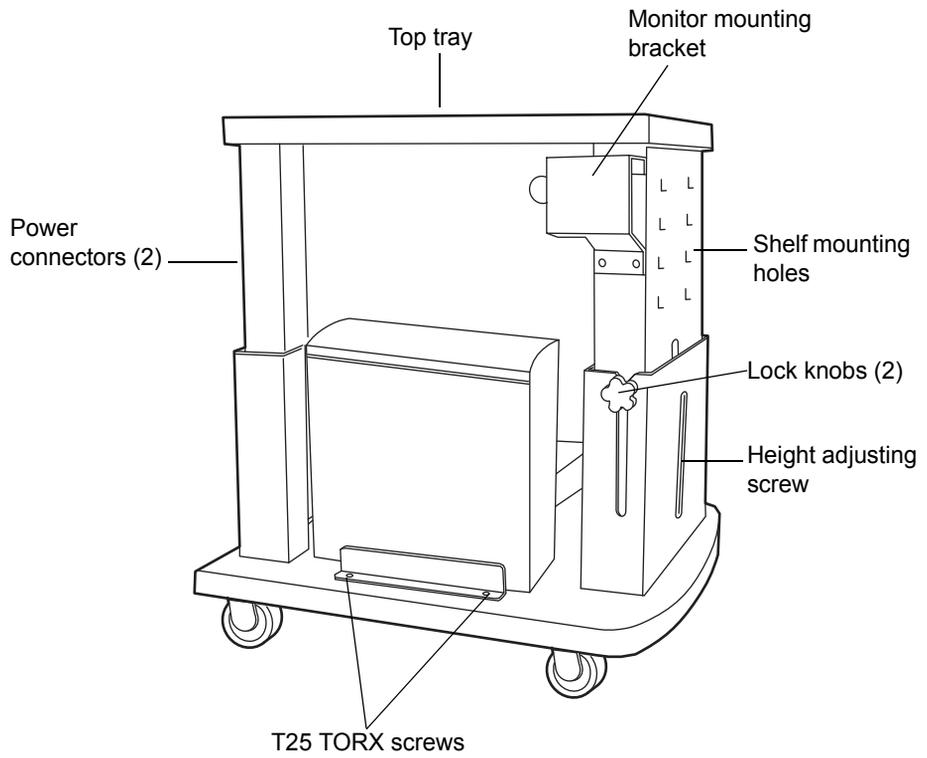
Recommendation: Because the assembled system weighs at least 140.6 kg (310 pounds), Waters recommends that you assemble the instruments and components on the FlexCart.

To assemble the FlexCart

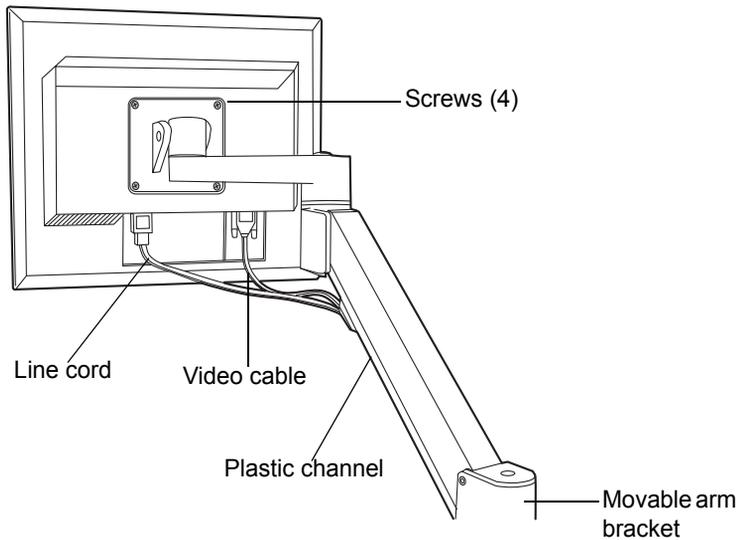
1. Remove the contents from the box packaged in the FlexCart.

Tip: The box contains the monitor arm, the bolts used to attach the monitor arm to the base, and an IBM instruction book for converting the monitor from stand-alone to arm-mounted.

FlexCart components

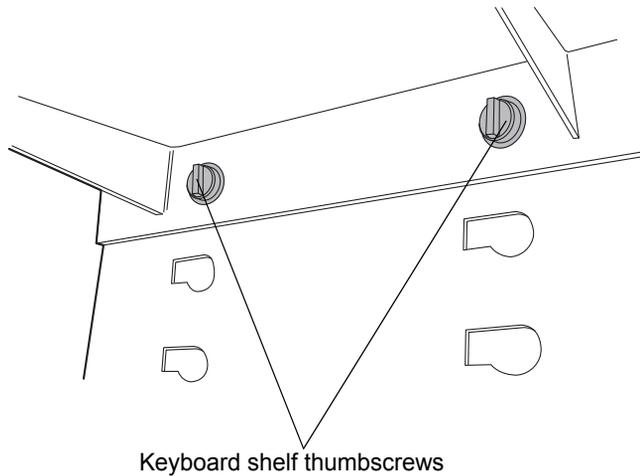


2. Remove the 4 Phillips screws from the support plate at the rear of the monitor, and mount the monitor onto the movable arm assembly. Refer to the IBM instruction book, if necessary.



3. Loosen the 2 T25 TORX screws, at the bottom left-hand side of the cart, that secure the bracket for the CPU.
4. Attach the power and video cables to the CPU, and place it in position on the bottom shelf of the cart.
5. Route the monitor's power and video cables through the plastic channel provided, and plug them into the monitor. Refer to the IBM instruction book, if necessary.

6. Insert the keyboard shelf's 2 captive thumbscrews into slots on the cart's front panel at a level that affords comfortable and safe operation. Turn the thumbscrews 1/4-turn clockwise to lock the position of the shelf.

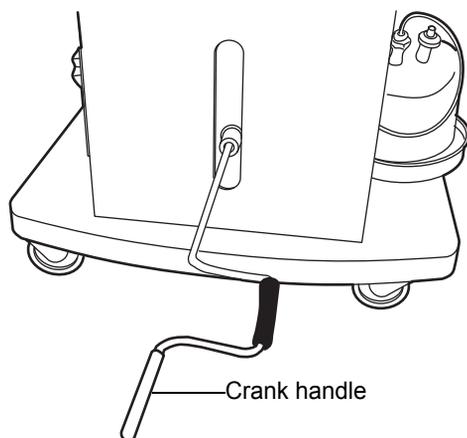


Adjusting the FlexCart's height

To adjust the cart's height

1. Loosen the side lock knobs before raising or lowering the top portion of the cart.
2. Remove the crank handle from its storage brackets on the lower right-hand side of the cart.

3. Insert the crank handle into the bottom front of the cart, and turn it to raise or lower the cart.



Tip: If your system includes a mass spectrometer and it is positioned on the right-hand side of the system stack, set the cart to a height that minimizes the length of tubing needed between the instruments stacked on the cart and the mass spectrometer.

4. Tighten the side lock knobs after reaching the desired height.
5. Loosen the keyboard shelf's 2 captive thumbscrews on the cart's front panel.
6. Move the keyboard to a level that provides comfortable and safe operation. Turn the thumbscrews 1/4-turn clockwise to lock the position of the shelf.

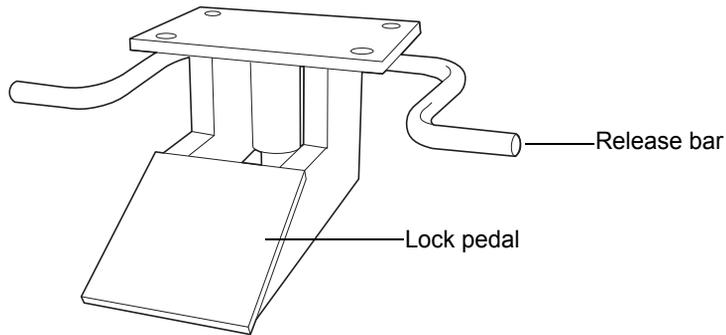
Locking the FlexCart in place

To lock the FlexCart in place

Lock the cart by depressing the lock pedal located at the front of the cart.

To release the FlexCart floor lock brake

Release the cart by depressing the brake release bar located at the front of the cart.



Moving the assembled FlexCart



Caution:

- To avoid spills, remove all solvent reservoirs from the solvent tray before moving the cart.
- To avoid striking low doorways, lower the cart fully before moving it.
- To avoid toppling the instruments stacked on the cart, do not move the cart by pushing on them.

Once it is assembled, you can move the cart to other areas of a lab to minimize tubing runs between the ACQUITY UPLC instruments and a mass spectrometer. Use the lip on top of the cart to pull it.



Unpacking and installing the sample organizer



Warning: To avoid back injuries, two or more people must unpack the sample organizer and transfer it to its final position.

The optional sample organizer is available for situations requiring increased throughput capacity. If your system includes the sample organizer, a Waters service technician unpacks and installs it.

Installing the binary solvent manager

To install the binary solvent manager



Warning: To avoid back injuries, two or more people must lift the binary solvent manager.

Lift the binary solvent manager onto the bench top. Or, if your system includes the optional FlexCart, lift the binary solvent manager onto the cart.

If your system includes the optional sample organizer,

1. unscrew the existing 7/8-inch-high feet from the bottom of the binary solvent manager and replace them with the 1/4-inch high feet from the startup kit.
2. attach the 2 self-adhesive rubber pads from the startup kit to the 2 front feet on the binary solvent manager.
3. place the binary solvent manager onto the sample organizer's base plate.

Installing the sample manager

To install the sample manager



Warning: To avoid back injuries, two or more people must lift the sample manager.

1. Unpack the sample manager, and place it on top of the binary solvent manager.

Alternative: If your system includes the optional sample organizer, place the sample manager on the sample organizer shelf.

2. Open the front access door of the sample manager, and remove the foam block from the sample compartment.
3. Open both the sample manager fluidics tray and the binary solvent manager door to ensure that the drip management system is properly aligned.

Tip: PEEK™ fittings, sample syringes, and wash syringes inside the sample manager may have loosened during shipping. To prevent leaks, ensure all PEEK fittings, sample syringes, and wash syringes are tight.

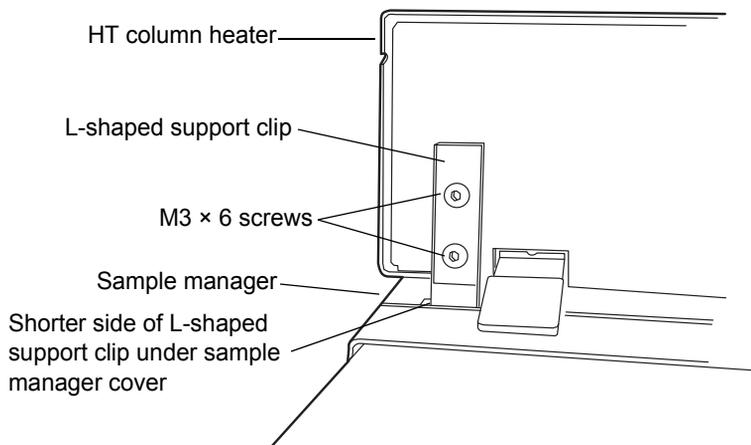
Installing the HT column heater

Required materials

- T10 TORX® screwdriver
- L-shaped support hold-down clip (startup kit)
- M3 × 6 recessed TORX pan head screws (2) (startup kit)

To install the HT column heater

1. Place the HT column heater atop the sample manager, ensuring that the feet are properly positioned in the indentations of the sample manager.
2. Slide the shorter side of the L-shaped support clip under the cover of the sample manager.
3. Position the HT column heater so the 2 holes on the L-shaped support clip align with the threaded holes on its chassis.
4. Using the T10 TORX screwdriver, attach the L-shaped support clip to HT column heater chassis with the two M3 × 6 screws.



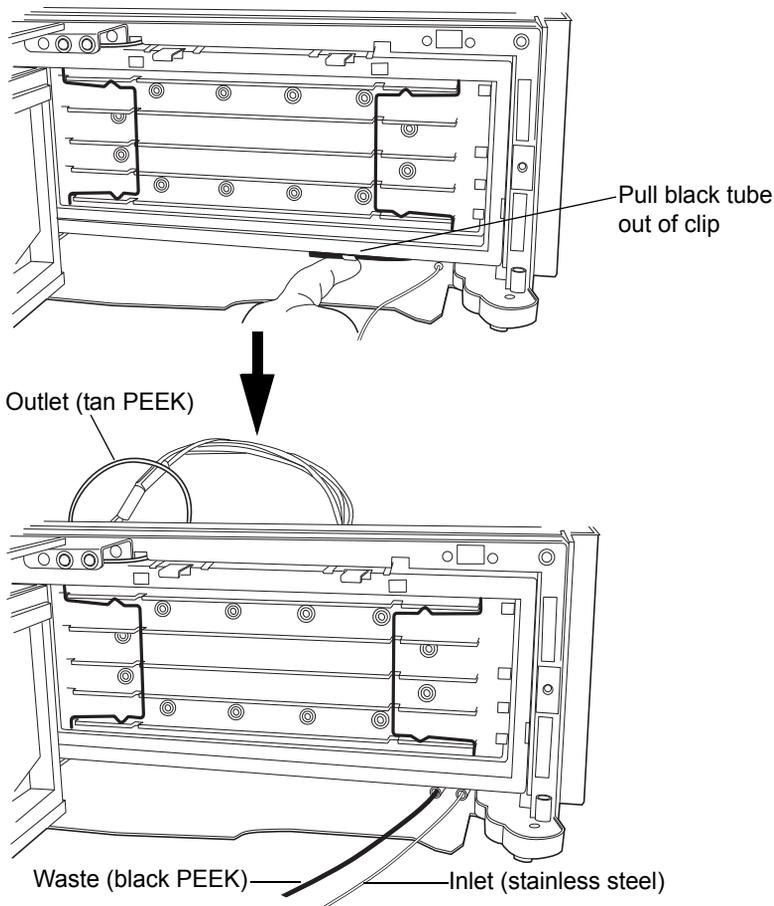
Installing the optional column manager

To install the optional column manager

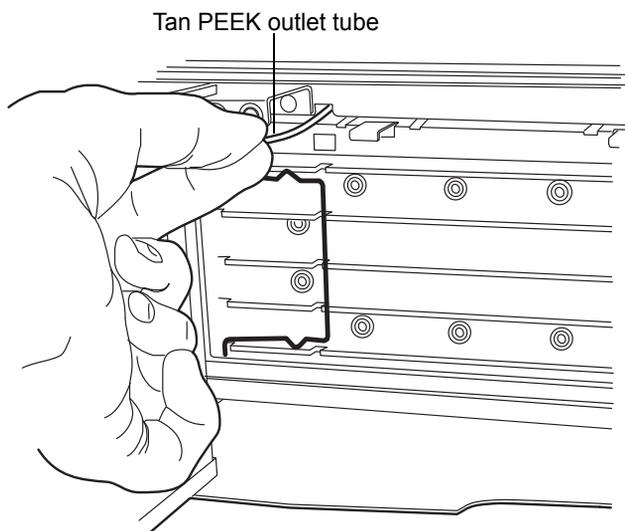
1. Place the column manager on a flat surface.
2. Open the column manager door.

 **Caution:** To avoid poor chromatographic performance, do not remove the switching valve fittings. They are preset and tested at the factory.

3. Pull the black waste tube out of the tie clip on the bottom of the drawer until it extends to the front.



4. Grasp the tan PEEK outlet tube on the upper left-hand side of the drawer and gently pull it forward until you feel it tighten.



5. Place the column manager atop the sample manager, ensuring that the feet are properly positioned in the indentations of the sample manager. Leave the column manager door open.

Installing the optional column heater/cooler

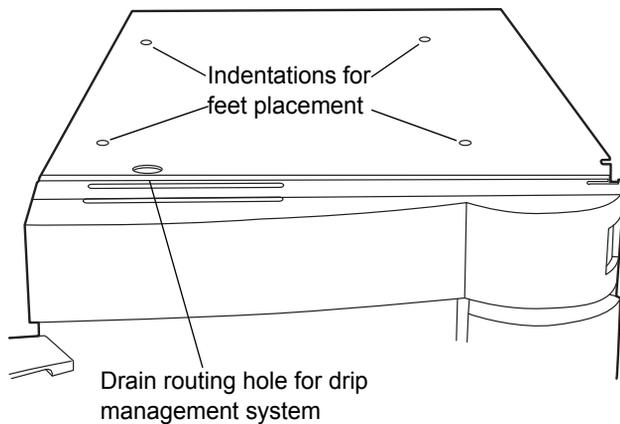
If your system includes a column heater/cooler, place it atop the sample manager, ensuring that the feet are properly positioned in the indentations of the sample manager.

Installing the detector

To install the detector

1. Place the detector atop the HT column heater, column manager, or column heater/cooler, ensuring that the feet are properly positioned in the indentations of the instrument. This position aligns the detector's

drip tray over the drain routing hole, on the top left-hand side of the HT column heater, column manager, or column heater/cooler.



2. Place the solvent tray atop the detector.

Installing the 30-cm column heater/cooler

If your system includes a 30-cm column heater/cooler, follow the procedure below to install it.

 **Caution:** If your system includes a high-temperature column heater with a rear DSUB cable, to avoid damaging the instrument's electronics, unplug the cable from the sample manager before performing this procedure.

Required materials

- 1/4-inch bit driver
- 7-mm adjustable wrench
- T20 TORX screwdriver

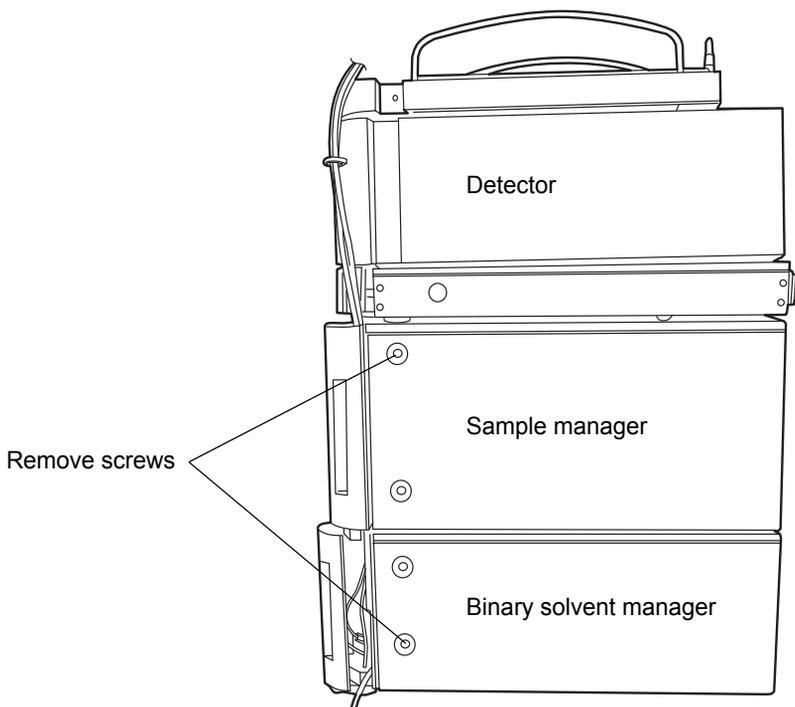
To install the 30-cm column heater/cooler



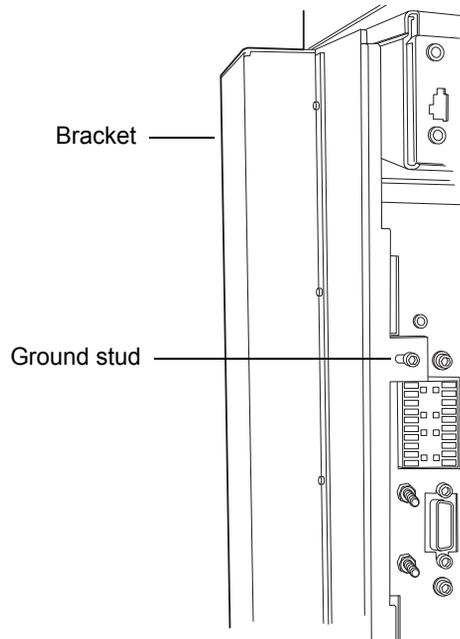
Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To completely interrupt power to an instrument, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds thereafter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

1. Power-off the sample manager and column heater.
2. Using the T20 TORX screwdriver, remove the top screw from the right-hand side of the sample manager, and the bottom screw from the right-hand side of the binary solvent manager.

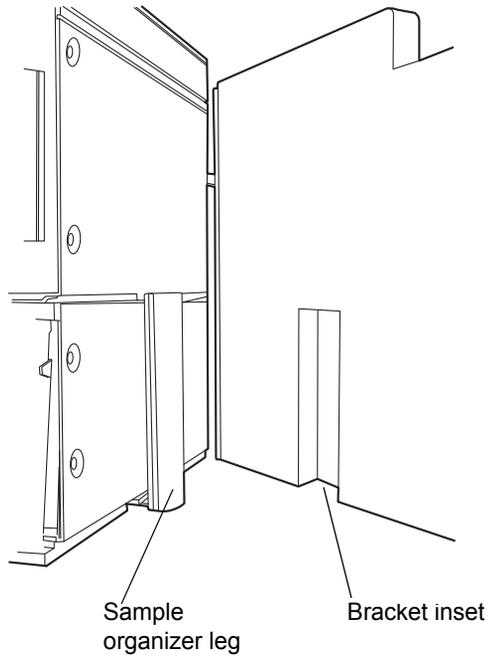
Right-hand side of system stack



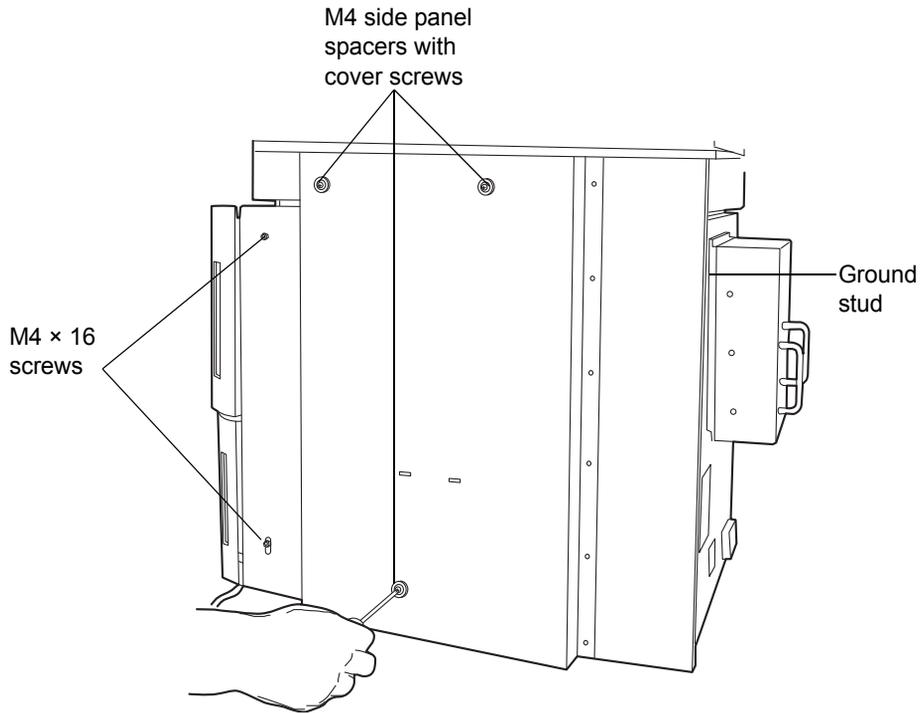
3. Mount the rear tab of the adaptor bracket onto the ground stud at the rear of the sample manager.



4. If your system includes a sample organizer, position the bracket so that its inset area fits over the sample organizer leg.

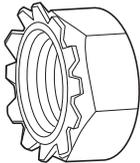


5. Insert 2 M4 × 16 screws into the slots on the bracket's left-hand panel, leaving the first screw loose until both screws are inserted. Then, tighten both screws.

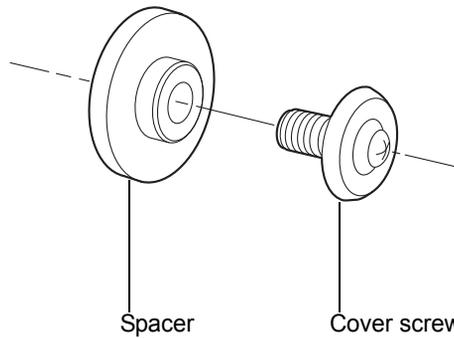


6. Add an M4 Keps[®] nut to the ground stud at the rear of the sample manager and tighten with a 7-mm adjustable wrench.

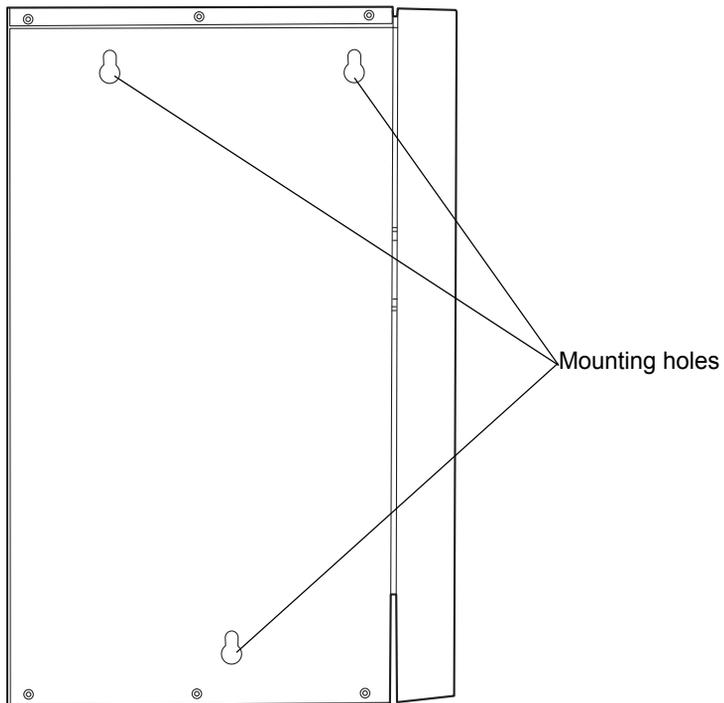
M4 Keps Nut



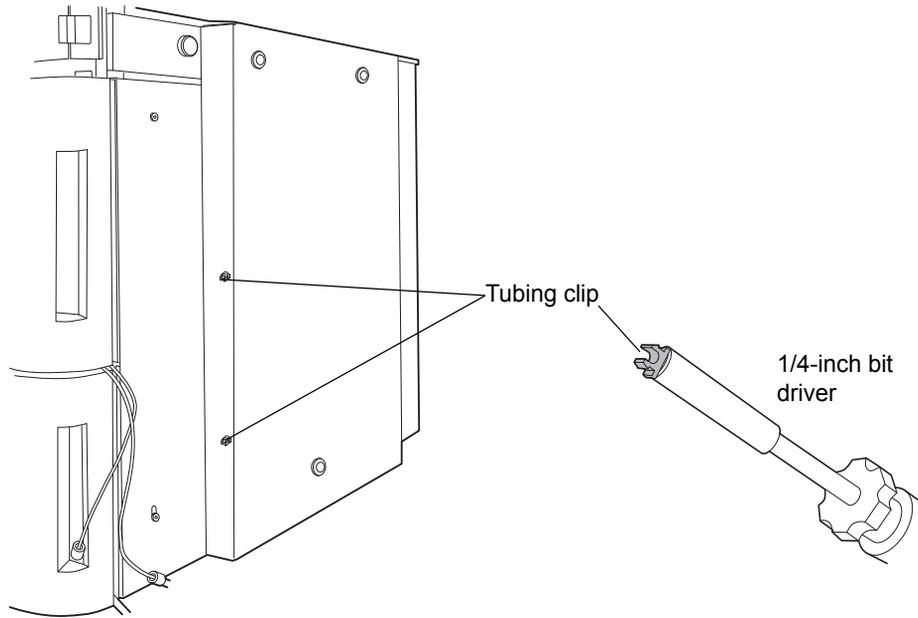
7. Insert the 3 M4 blue cover screws through the 3 side panel spacers, and then screw them into the 3 threaded holes in the bracket's center panel.



8. Mount the 30-cm column heater/cooler onto the bracket, fitting the column heater/cooler mounting holes over the M4 cover screws.



- Using a 1/4-inch bit driver, attach the black tubing clips to the front of the bracket. Load the clip into the bit driver in the orientation desired, and then snap into the bracket.



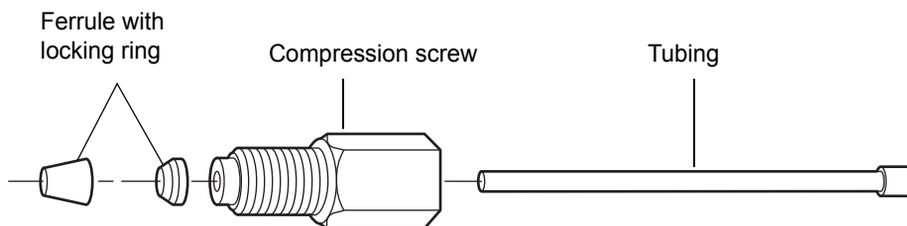
Plumbing the system

 **Caution:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when plumbing the system.

When all the components are stacked, make the plumbing connections. Compression fittings and ferrules are already fitted to tubing assemblies, but they must be properly set.

Installation recommendations for fittings

The system uses gold-plated compression screws and two-piece ferrules. See the diagram below for assembly orientation.

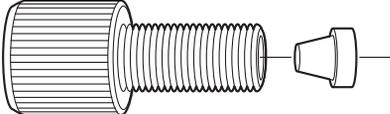
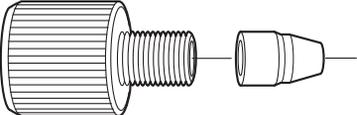
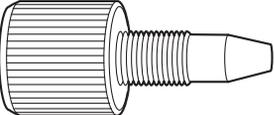


Recommendations:

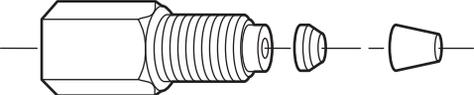
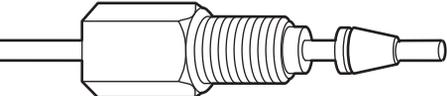
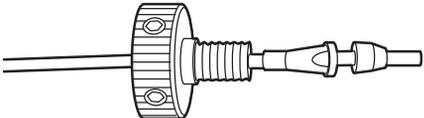
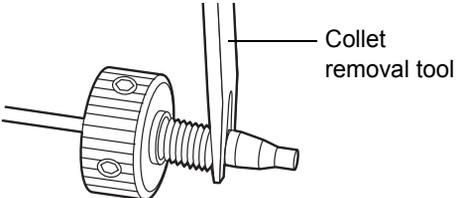
- To prevent bandspreading, ensure the tube is fully bottomed in the fitting hole before tightening the compression screw.
- For easier accessibility, use long compression screws to attach tubes to the injector and vent valve.

When tightening system fittings, consult the following table.

Installation recommendations for ACQUITY UPLC fittings

Fitting	Recommended tightening
1/4-28 flangeless with ferrule 	Snug plus 1/4-turn
10-32 LT135 PEEK with ferrule 	Snug plus 1/4-turn; if leaking, tighten another 1/8-turn
10-32 one-piece PEEK 	Finger-tight

Installation recommendations for ACQUITY UPLC fittings (Continued)

Fitting	Recommended tightening
Stainless steel with 2-piece stainless steel ferrule (first use) 	Finger-tight plus 3/4-turn with wrench
Stainless steel with 2-piece stainless steel ferrule (re-installed) 	Finger-tight plus 1/4-turn with wrench
Reusable finger-tight (first use) 	Snug plus 1/4-turn
Reusable finger-tight (re-installed) 	Snug plus 1/4-turn; if leaking, tighten another 1/8-turn

Plumbing a TUV detector



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when plumbing the detector.

Plumbing a TUV detector involves connecting the flow cell and installing a backpressure regulator.

Although the in-line degasser removes most of the gas (air) from solvents, some gas is unavoidably introduced into the system during partial loop injections. Under pressure, this gas remains in solution. However, because the post-column pressure is normally much lower than the pre-column pressure, the gas can come out of solution and produce an unstable baseline characterized by large, unexpected spikes. The backpressure regulator

maintains a minimum post-column pressure of 1724 kPa (17 bar, 250 psi), eliminating post-column outgassing and ensuring a smooth baseline.

Tip: When the backpressure regulator is installed, the system maintains at least 1724 kPa (17 bar, 250 psi) backpressure, regardless of the outlet tubing configuration and flow rate, provided that there is some positive flow.

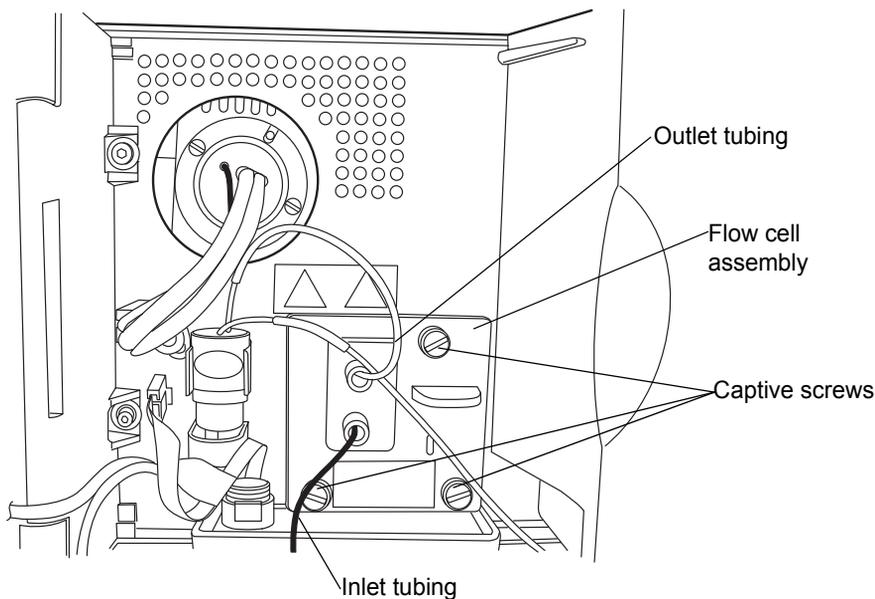
To plumb a TUV detector

1. Open the front panel door of the TUV detector, and install the flow cell so that the 3 captive screws align with their holes in the bulkhead.

Requirement: Ensure the dust cover has been removed from the bulkhead before you install the flow cell.

2. Finger tighten the captive screws.

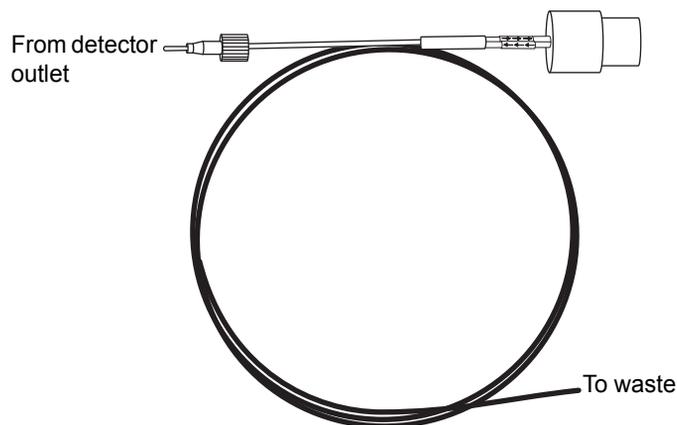
TUV detector flow cell



3. Remove the protective cover from the PEEK cell inlet tubing, and connect the tubing to the flow cell inlet.
4. Attach the short length of outlet tubing from the backpressure regulator to the outlet of the flow cell.

Rule: Do not install the backpressure regulator if you are connecting to a second detector or mass spectrometer.

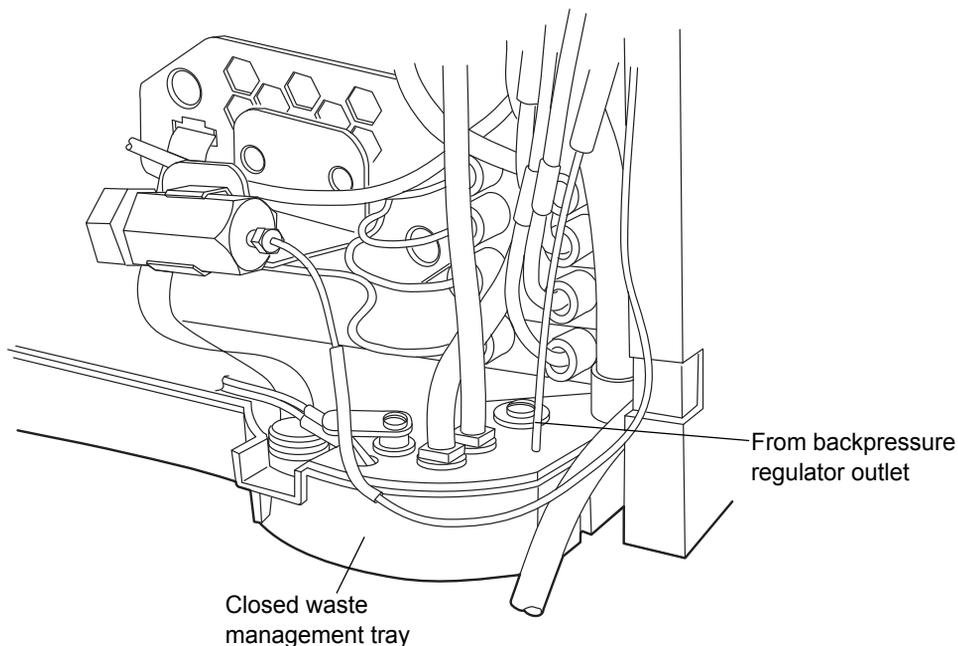
Backpressure regulator



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

5. Route the long end of the outlet tubing from the backpressure regulator through the channel clips along the front right-hand side of the system

and into the closed waste management tray on the binary solvent manager.



6. Close the front panel door of the TUV detector.

Plumbing a PDA detector

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

Plumbing an ELS detector

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

Plumbing an FLR detector

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

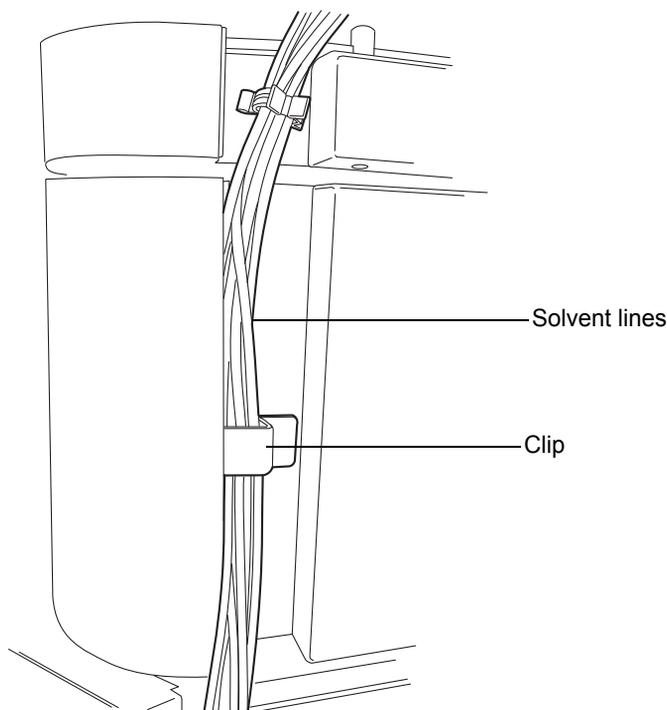
Plumbing the binary solvent manager and sample manager



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when plumbing the binary solvent manager and sample manager.

To plumb the binary solvent manager and sample manager

1. Route the solvent inlet tubing attached to the in-line degasser and seal wash pump through the channel clips of the sample manager.
2. Continue routing the lines between the column heater door and hinge through the detector's clip, and then place the lines into the solvent tray.

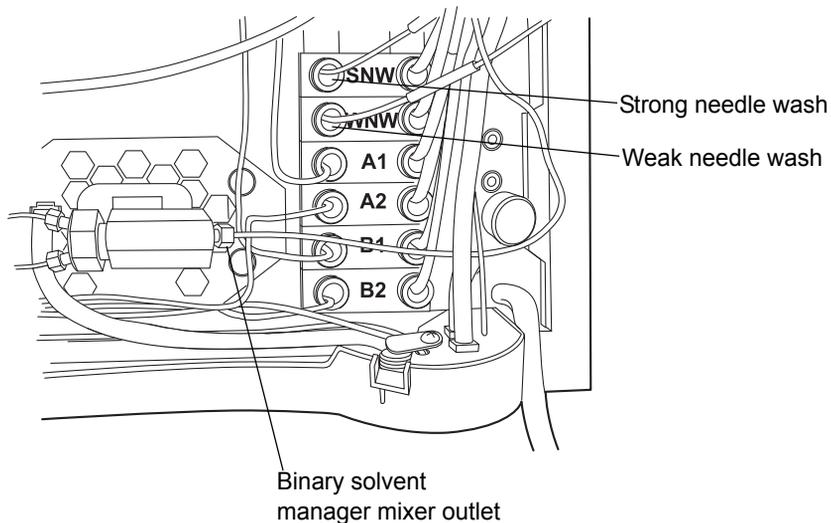


Exception: If your system uses a mass spectrometer positioned on the right-hand side of the system stack, ensure that the column heater door is in the “away” (swung to the right-hand side) position. Then route the lines in front of the column heater door, not between the door and hinge.

3. Remove the 2 protective caps from the orange and white color-coded wash lines.

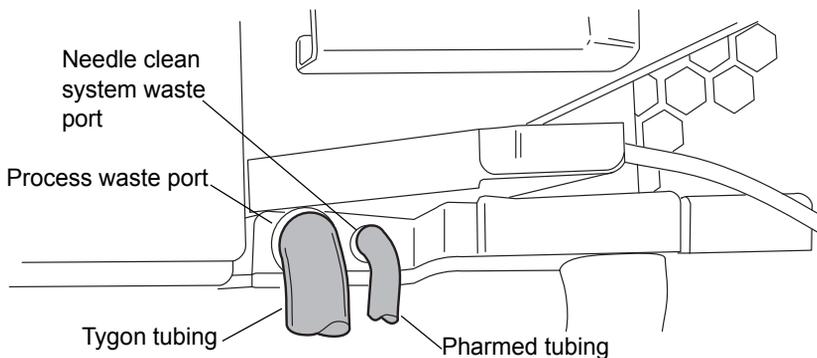
4. Remove the 2 protective screws from the corresponding wash ports on the in-line degasser of the binary solvent manager.
5. From the sample manager, route the weak and strong needle wash lines, indicated by the orange and white labels, to their corresponding ports on the in-line degasser in the binary solvent manager. Finger tighten the knurled nut.

Needle wash and pump outlet connections

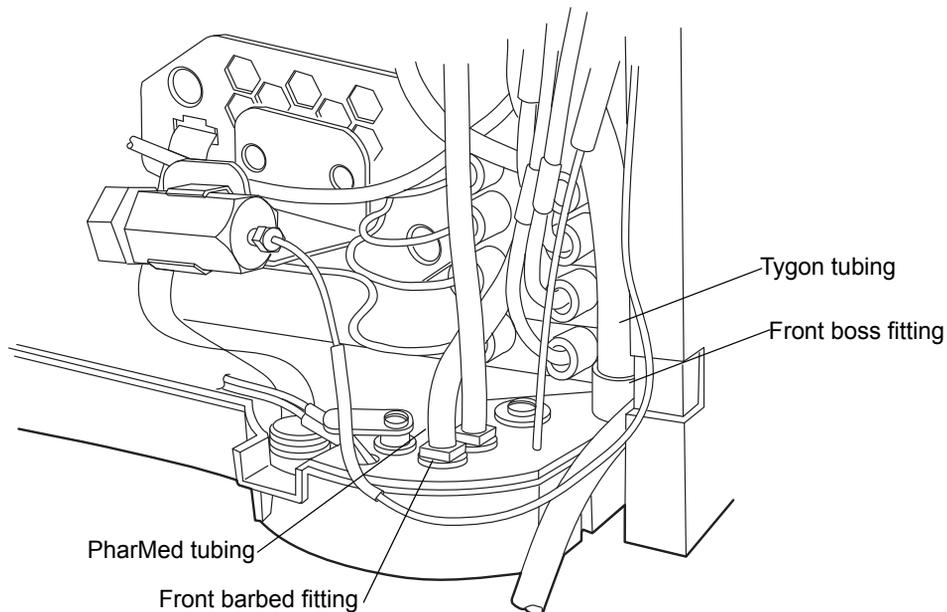


6. Locate the pre-installed Tygon[®] tubing running from the process waste port and the PharMed[®] tubing running from the needle-clean system waste port (found on the lower drip tray of the sample manager), and

route them through the pass-through of the upper binary solvent manager drip tray.



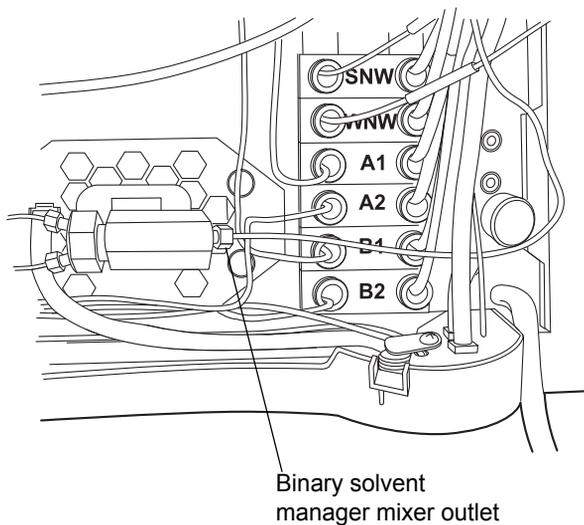
7. Connect the PharMed tubing to the front barbed fitting and the Tygon tubing to the front boss fitting, both of which are on the lower binary solvent manager drip tray.



8. Route the seal wash and solvent inlet line tubing attached to the in-line degasser through the channel clips of both the binary solvent manager and sample manager.
9. Continue routing the lines between the column heater door and hinge and through the detector's clips. Then place the lines in the solvent tray.

10. Remove the protective O-ring from the stainless steel pump outlet tubing.
11. Seat the end of the tubing with the shorter compression screw into the binary solvent manager mixer outlet, and tighten the compression fitting using the 1/4-inch and 5/8-inch open-end wrenches.

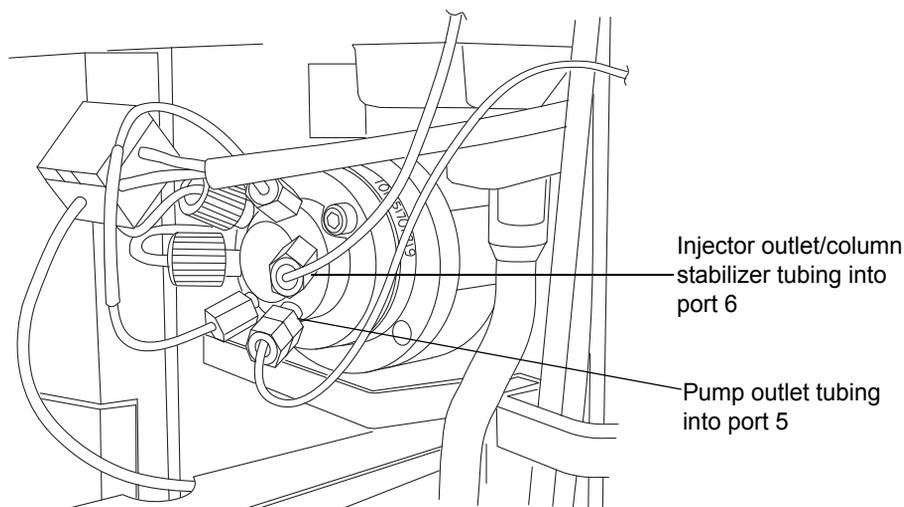
Tip: When using new fittings, tighten 3/4-turn beyond finger-tight. For older or previously used fittings, tighten 1/4-turn beyond finger-tight.



12. Route the other end of the tubing through the channel clips to the sample manager injection valve.

13. Remove the O-ring, and seat the end of the tubing with the longer compression screw into port 5 on the injection valve. Tighten the compression fitting using the 1/4-inch open-end wrench.

Injection valve connections



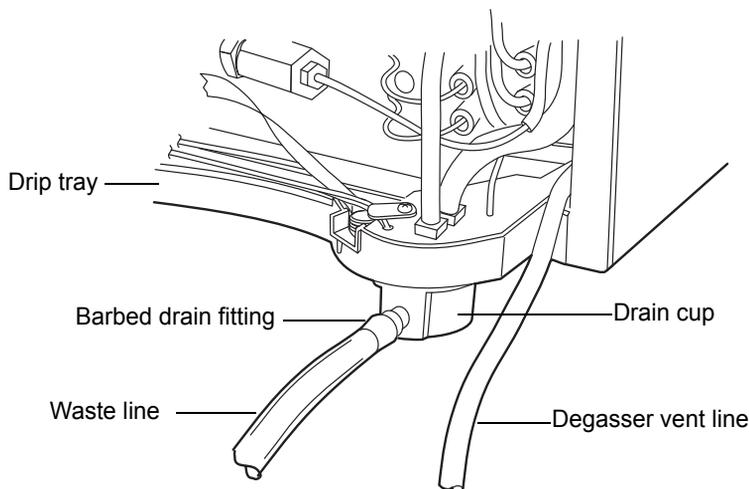
14. Remove the protective cover from the injector outlet tube, and then seat the tube and ferrule into port 6 of the injection valve. Tighten the compression fitting using a 1/4-inch open-end wrench.



Caution: To avoid distorting the drip tray or causing the drain cup to leak, restrain the drain cup when attaching or removing the waste line.

15. Wet the barbed drain fitting located at the bottom of the binary solvent manager with methanol.

16. Hold the back of the drain cup, and then slide a waste line over the barbed drain fitting and route it to a suitable waste container.



 **Warning:** To avoid releasing solvent vapors into the room, route the degasser vent line to a fume hood or other suitable exhaust system, or to a suitable waste container, ensuring the tubing's discharge end is at all times above the fluid level.

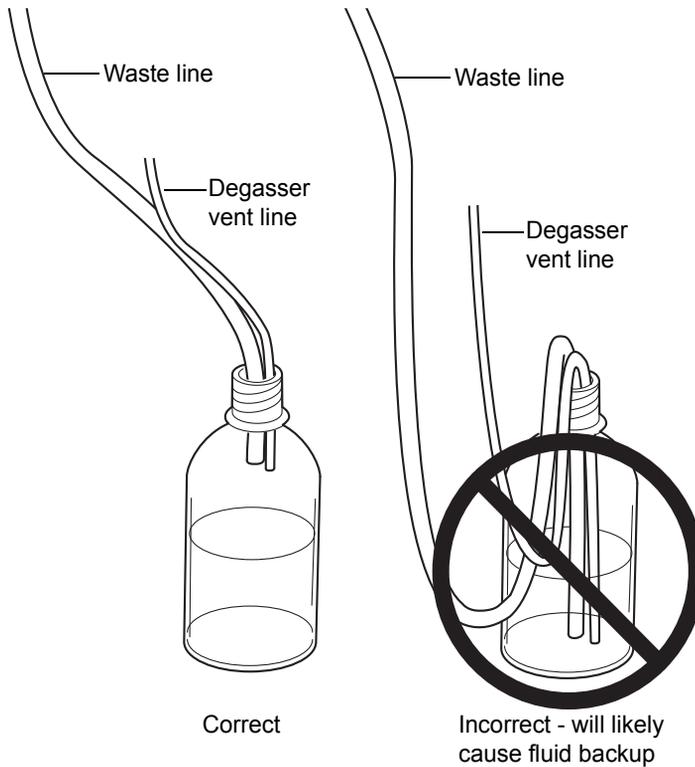
 **Warning:** To avoid spills, empty the waste container at regular intervals.

 **Caution:** To avoid fluid backup, you must ensure proper drainage of waste:

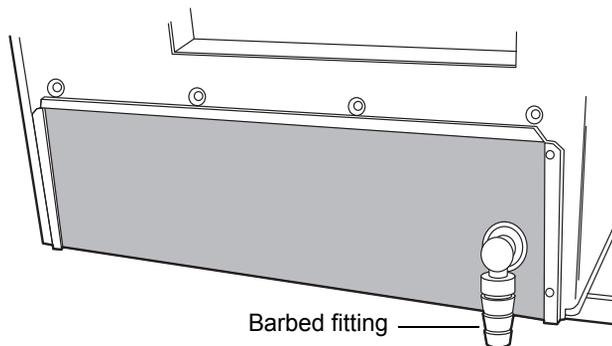
- Place the waste container below the system stack.
- Ensure that the waste and degasser vent lines do not crimp or bend. A crimp or bend can impede flow to the waste container.
- Ensure the exit of the waste and degasser vent lines is not immersed in waste solvent. If necessary, shorten each line so that no portion of it drops below the top of the waste container (see next figure).

17. Route the degasser vent line to a suitable waste container.

Correct positioning of waste and degasser vent lines



18. Attach a waste line to the barbed fitting on the filter drainage assembly (on the lower front side of the sample organizer), and route it to a suitable waste container.





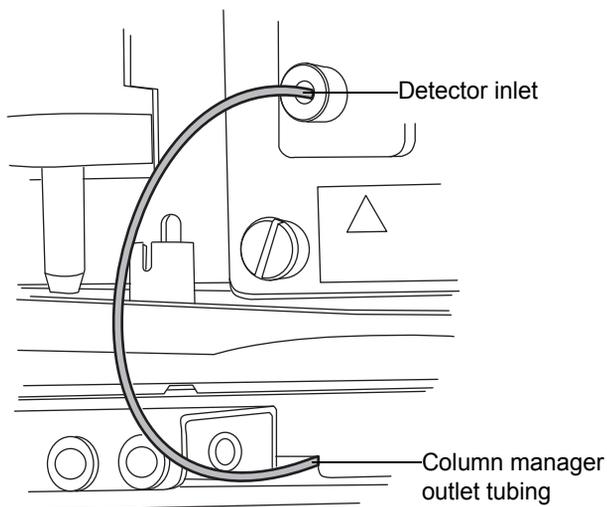
Warning: To avoid spills, empty the waste container at regular intervals.

19. Route a waste line from the barbed fitting on the rear of the solvent tray to a suitable waste container.
20. Close the sample organizer door, sample manager door, sample manager fluidics tray, and the binary solvent manager door.

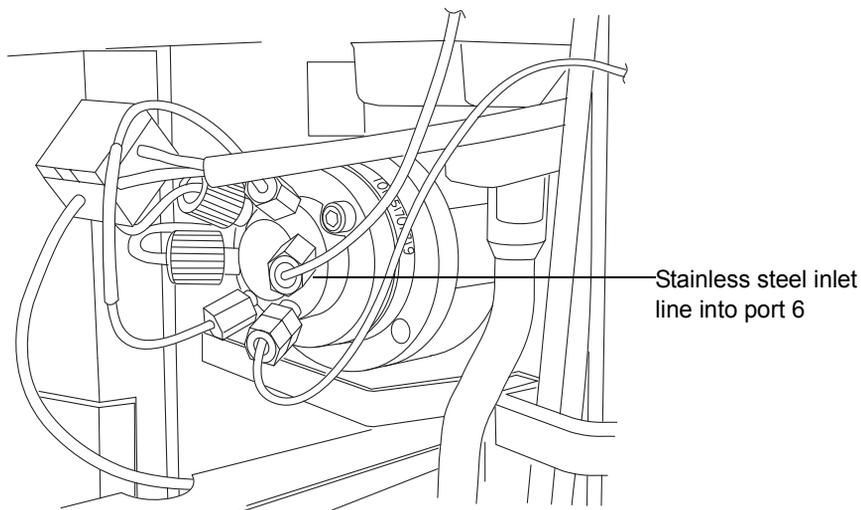
Plumbing the column manager

To plumb the column manager

1. Run the tan PEEK outlet tubing to the flow cell inlet on the detector.



2. Use the gold fittings to connect the stainless steel inlet line to port 6 on the injector valve.



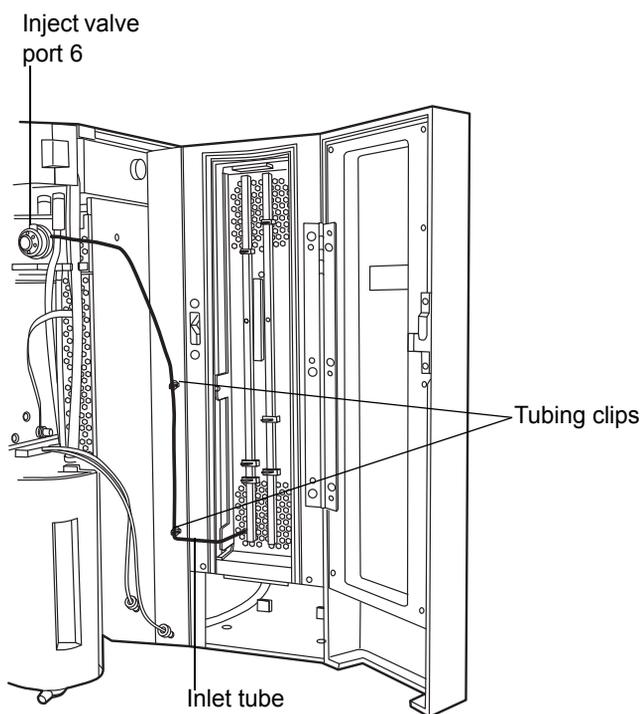
Plumbing the 30-cm column heater/cooler

If your system includes a 30-cm column heater/cooler, follow the procedure below to plumb it.

 **Caution:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when plumbing the 30-cm column heater/cooler.

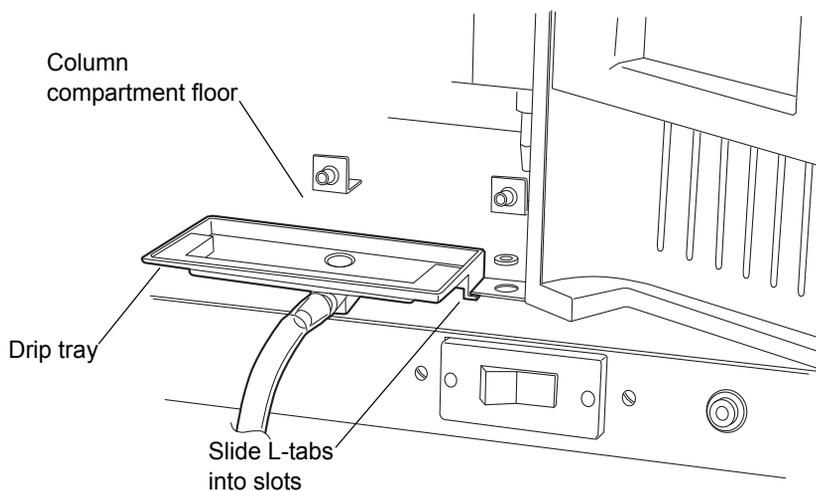
To plum the 30-cm column heater/cooler

1. Connect the stainless steel inlet tube from the column to the inject valve port 6.



2. Connect the red PEEK tubing from the outlet of the column to the detector.

3. Slide the drip tray onto the bottom of the column compartment, inserting the L-tabs on each side of the drip tray into the slots in the column compartment floor.



4. Route the tubing to a suitable waste container.

Routing system drainage through the FlexCart

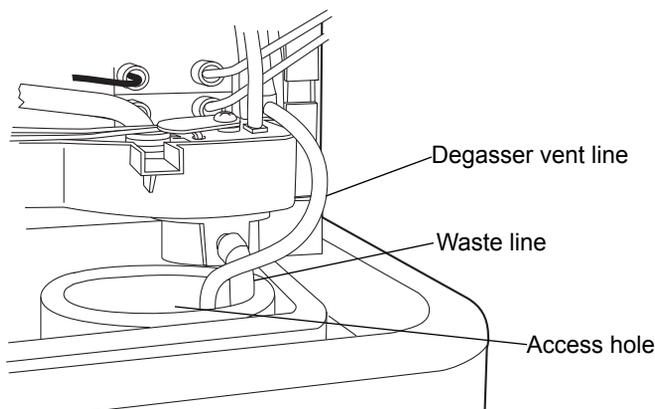
To route system drainage through the FlexCart

1. Place a suitable waste container in the bottom right-hand side of the cart.
2. Open the sample organizer, detector, sample manager, and binary solvent manager doors.
3. Route the long end of the outlet tubing from the detector's backpressure regulator through the channel clips along the front right-hand side of the system and down through the cart's top access hole to the waste container.



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

4. Route the waste and degasser vent lines (located at the front, bottom right-hand side of the binary solvent manager) down through the cart's top access hole to the waste container.



5. Route the waste line (located at the front, bottom right-hand side of the sample organizer) down through the cart's top access hole to the waste container.
6. Close the sample organizer, detector, sample manager, and binary solvent manager doors.

Installing the column stabilizer assembly in an HT column heater

For instruction on how to replace the column stabilizer assembly, see [“Replacing the column stabilizer assembly in the HT column heater”](#) on page 6-177.

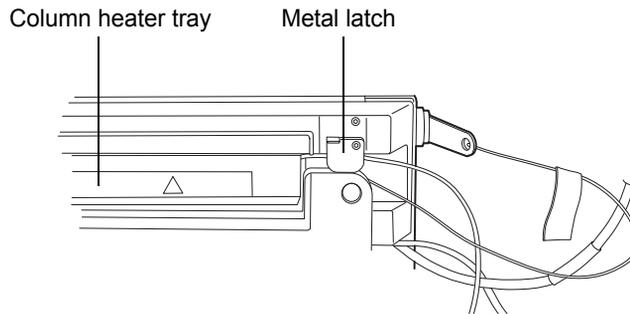
Required materials

Column stabilizer kit:

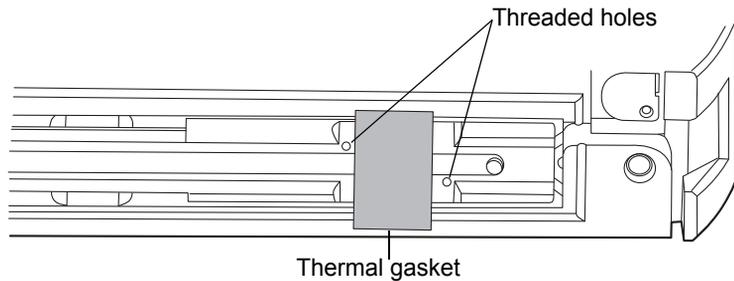
- Collet and compression screw multi-tool (collet separator)
- Column stabilizer assembly
- PEEK[®] tubing for connecting the column to the detector's flow cell
- 3 Phillips screws (1 is a spare)
- 3 thermal gaskets (2 are spares)

To install the column stabilizer assembly

1. Open the HT column heater door, lift up and rotate the metal latch clockwise, and tilt the column heater tray 45 degrees downward.

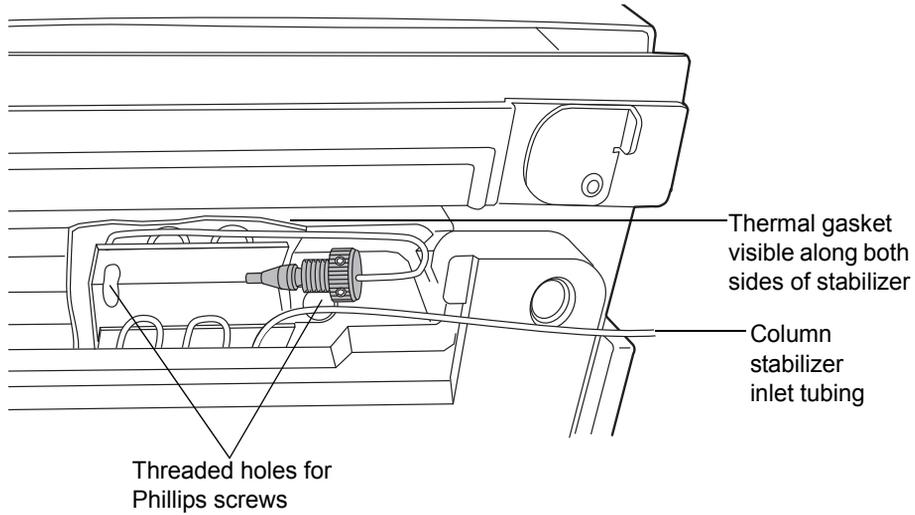


2. Remove the clear, protective backing from the thermal gasket, and with the thermal gasket oriented vertically, center it between the two threaded holes.



3. Press the thermal gasket into the tray. Ensure that the gasket is centered and formed into the bottom of the tray with equal lengths on both sides.

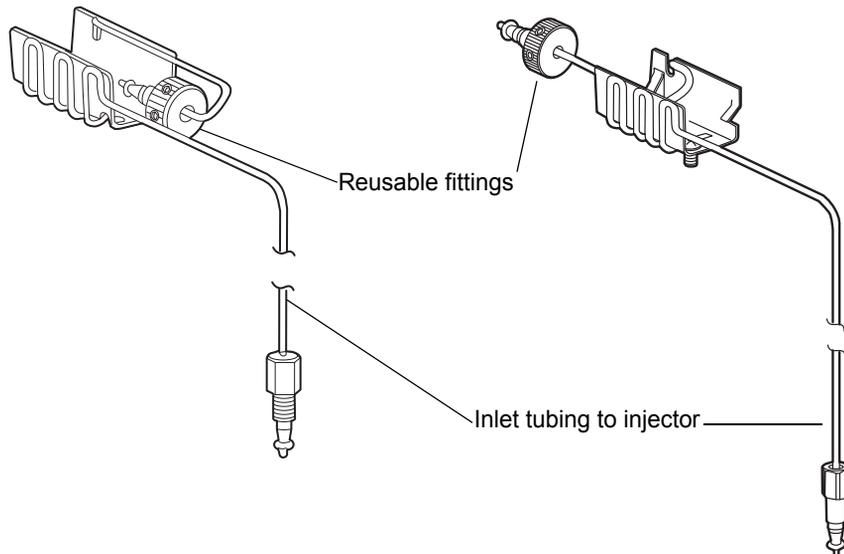
4. With the column stabilizer assembly's inlet tubing on the right-hand side, firmly press the assembly into the thermal gasket. Ensure that the thermal gasket uniformly envelops the sides of the column stabilizer and does not bunch beneath it.



Column stabilizer assembly (two styles)

For 150-mm column

For 50-mm or 100-mm column

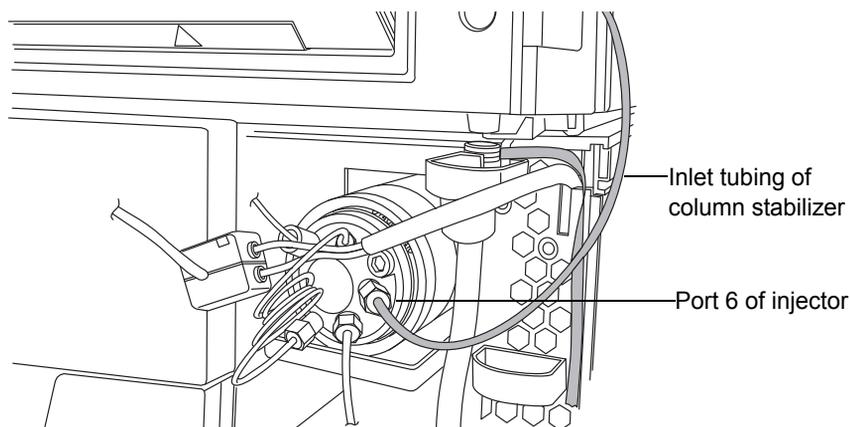


- Carefully route the column stabilizer inlet tubing through the groove on the right-hand side of the tray.



Caution: To avoid stripping the threads, do not overtighten screws.

- Insert 2 Phillips screws into the threaded holes, and tighten them gently using the Phillips screwdriver.
- Remove the O-ring retainer from the inlet end of the column stabilizer tube, and then connect the inlet tube to injector port 6 of the sample manager.



Installing the column stabilizer assembly in a column heater/cooler

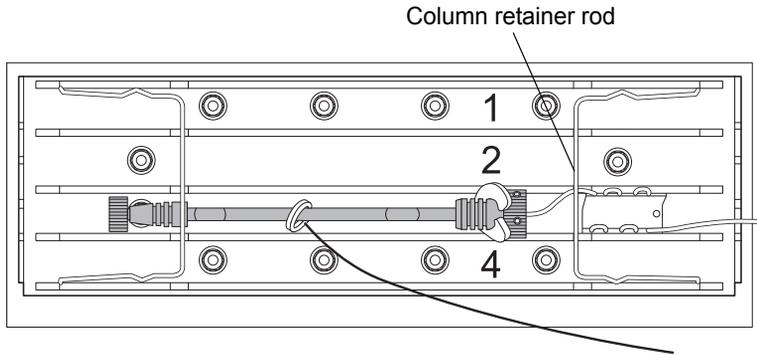
For instruction on how to replace the column stabilizer assembly, see [“Replacing the column stabilizer assembly in the HT column heater”](#) on page 6-177.

Required materials

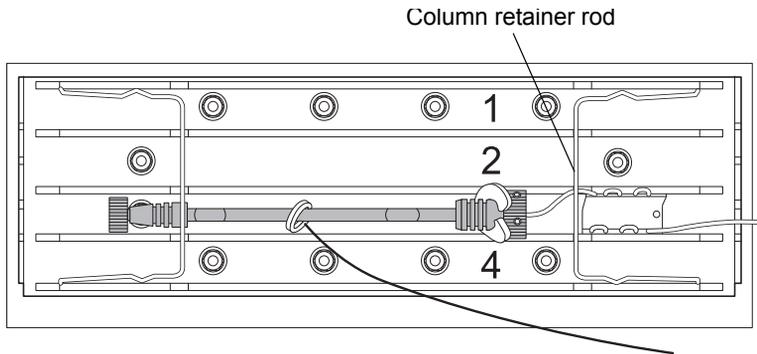
- Column stabilizer kit
 - Column stabilizer assembly
 - Collet and compression screw multi-tool (collet separator)
 - 3 thermal gaskets (2 are spares)
 - PEEK tubing for connecting the column to the detector’s flow cell
- Phillips[®] screwdriver

To install the column stabilizer assembly

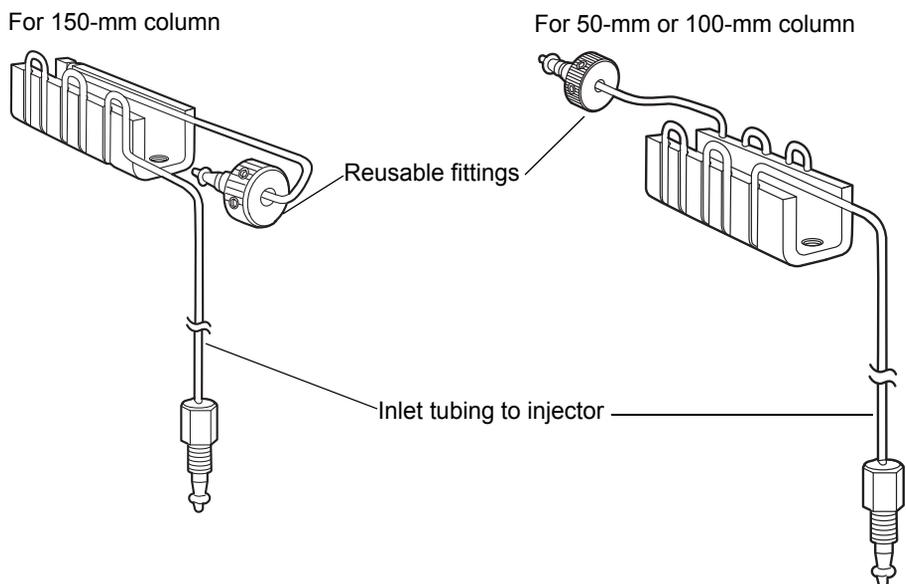
1. Swing the column retainer rod out to the right.



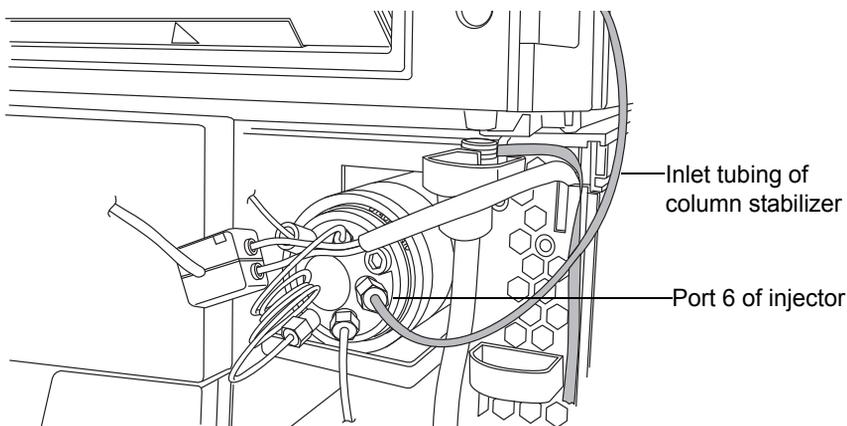
2. Remove the clear, protective backing from the thermal gasket, and then center the thermal gasket vertically between the two threaded holes.
3. Press the thermal gasket into the column compartment sleeve. Ensure that the gasket is centered and formed into the bottom of the sleeve with equal lengths on both sides.
4. With the column stabilizer assembly's inlet tubing on the right-hand side, firmly press the assembly into the thermal gasket. Ensure that the thermal gasket uniformly envelops the sides of the column stabilizer and does not bunch beneath it.



Column stabilizer assembly (two styles)



5. Carefully route the column stabilizer inlet tubing through the groove on the right-hand side of the tray.
6. Remove the O-ring retainer from the inlet end of the column stabilizer tube, and then connect the inlet tube to injector port 6 of the sample manager.



Installing the column in-line filter in a HT column heater

You can install a 0.2- μm in-line filter to protect the analytical column against contamination.

Required materials

- Column in-line filter
- Reusable fitting

For instruction on how to replace the filter in the HT column heater's in-line filter unit, see [“Replacing the frit in the HT column heater's in-line filter unit” on page 6-178.](#)

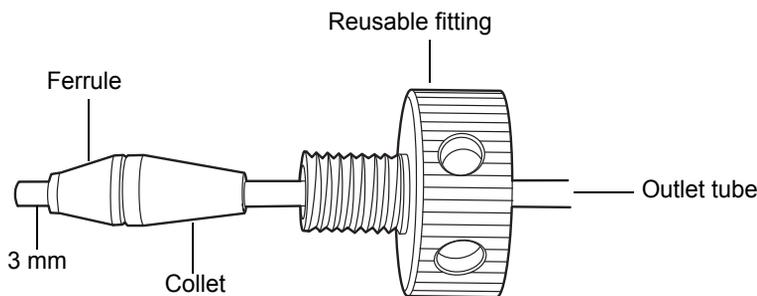


Caution: To prevent contamination, wear powder-free, non-latex gloves when connecting tubing and tightening fittings.

To install the column in-line filter

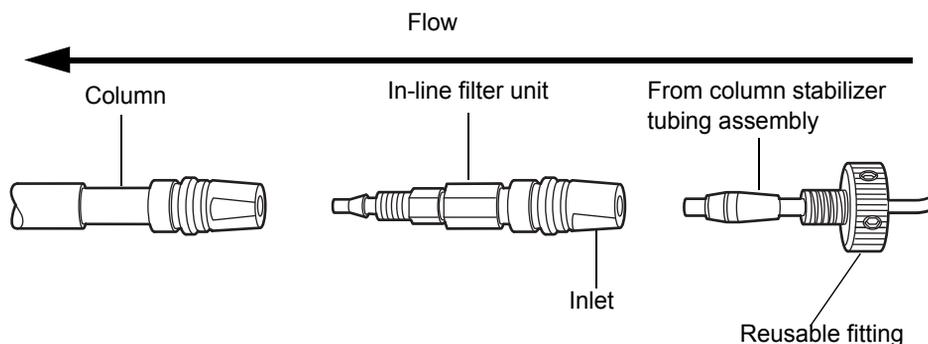
1. Remove the O-ring retainer from the outlet end of the column stabilizer tube.

Tip: Before connecting, make sure that the ferrule is approximately 3 mm from the end of the outlet tube and that the collet is touching the back of the ferrule.



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

2. Remove the column in-line filter unit from its packaging, and then push the outlet end of the column stabilizer tubing assembly into the in-line filter inlet until it stops.



3. Holding the reusable fitting in place, rotate the in-line filter unit onto the fitting until it is snug.



Caution: To avoid damaging the tubing, use only your fingers to tighten the tubing fittings.

4. Finger tighten the fitting until it is snug, and then add 1/4-turn.

Installing the column in-line filter in a column manager or column heater/cooler

You can install a 0.2- μm in-line filter to protect the analytical column against contamination. The maximum size that fits in the column manager or column heater/cooler is 30 mm.

Required materials

- 5/16-inch open-end wrench
- Collet and reusable fitting multi-tool (collet separator)
- Reusable fitting

For instruction on how to replace the filter in the column manager's or column heater/cooler's in-line filter unit, see [“Replacing the filter in the column heater/cooler's in-line filter unit”](#) on page 6-199.

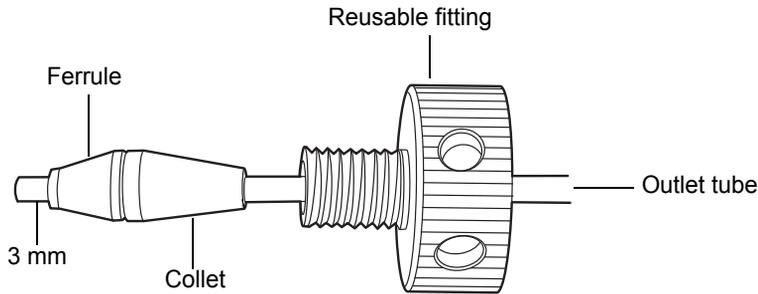


Caution: To prevent contamination, wear powder-free, non-latex gloves when connecting tubing and tightening fittings.

To install the column in-line filter

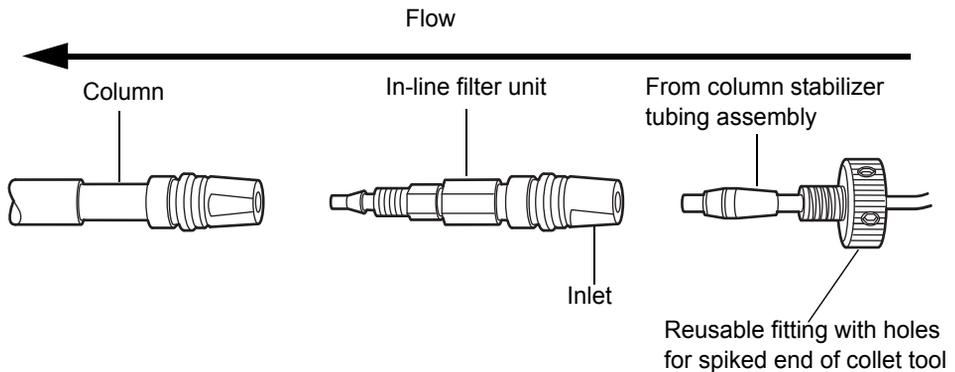
1. Assemble the reusable fitting, collet, and ferrule (in that order) on the outlet end of the column stabilizer tubing assembly, leaving the ferrule 3 mm from the end.

Tip: Before connecting, make sure that the ferrule is approximately 3 mm from the end of the outlet tube and that the collet is touching the back of the ferrule.



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

2. Remove the column in-line filter unit from its packaging, and then push the outlet end of the column stabilizer tubing assembly into the in-line filter inlet until it stops.



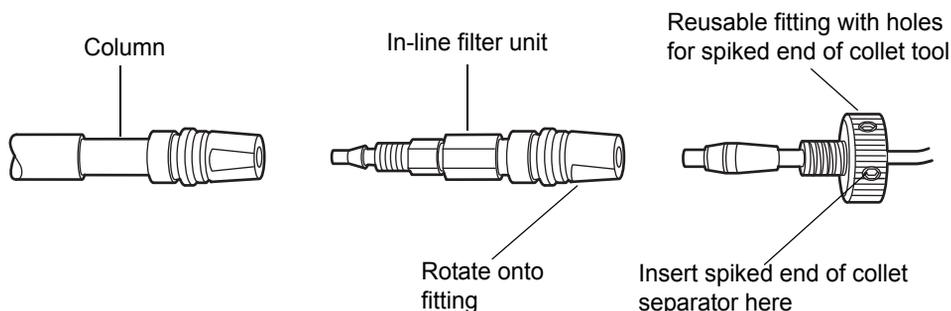
Note: Older reusable fittings do not have holes in the side of them.

3. Holding the reusable fitting in place, loosely thread the in-line filter unit onto the fitting.

4. Insert the spiked end of the collet separator into one of the holes in reusable fitting, and push the reusable fitting into the column compartment sleeve.

 **Caution:** To avoid damaging the tubing, use only your fingers to tighten the tubing fittings.

5. Holding the reusable fitting in place with the spiked end of the collet separator, rotate the in-line filter onto the fitting.



6. Using the spiked end of the collet separator to hold the reusable fitting, twist the column until it is snug.

 **Caution:** To avoid damaging the tubing, do not overtighten the reusable fitting.

7. Using the 5/16-inch open-end wrench to hold the column, use the spiked end of the collet separator to turn the reusable fitting until it is snug, and then add 1/4-turn.

Installing the column in an HT column heater

Required materials

- 5/16-inch open-end wrench
- High-temperature outlet fitting
- High-temperature outlet fitting wrench
- Reusable fitting

For instruction on how to replace the column in the HT column heater, see [“Replacing the column in the HT column heater”](#) on page 6-176.

To connect the column in-line filter unit and the column inlet

1. Remove the O-ring from the outlet end of the column in-line filter unit.
2. Remove the plug from the column inlet.
3. Push the outlet end of the column in-line filter unit into the column inlet until it stops.



Caution: To avoid damaging the tubing, use only your fingers to tighten the fittings.

4. Holding the finger-tight reusable fitting in place, rotate the column onto the fitting until it is snug, and then add 1/4-turn.

To install the PEEK tubing in the column outlet



Caution: To avoid bandspreading, ensure that the tubing is fully bottomed in the fitting hole before tightening it.

1. Insert the PEEK tubing into the high-temperature outlet fitting until it stops.



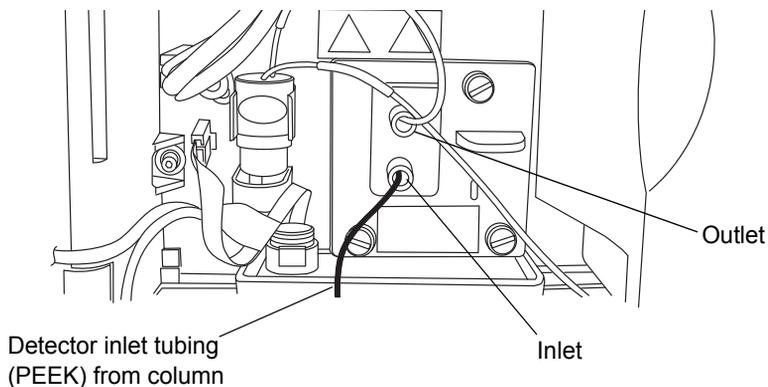
Caution: To avoid damaging the outlet fitting, do not overtighten it with the outlet fitting wrench.

2. Holding the PEEK tubing, thread the high-temperature outlet fitting into the column outlet (located on the left-hand side of the column) until it is finger-tight.
3. Using a 5/16-inch wrench to hold the column steady, use the high-temperature outlet fitting wrench to give the outlet fitting 1/4-turn.
4. Push the PEEK tubing into the left-hand side of the column tray.

To connect the PEEK tubing to the detector

1. Remove the protective cover from the PEEK tubing from the flow cell inlet in the detector.
2. Check the label on the flow cell inlet tubing to ensure it matches the type of detector and flow cell in your system. Attach the 0.004-inch or

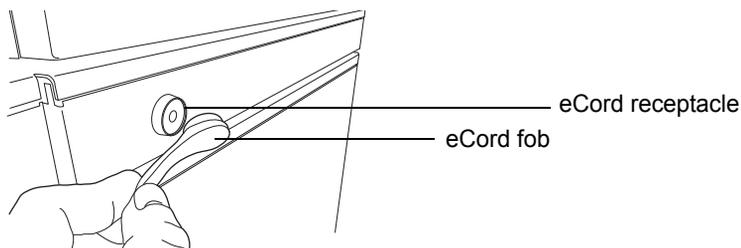
.0025-inch ID inlet tubing (included with the flow cell or the stabilizer kit) to the column outlet.



3. Snap the column support clips onto the body of the column (one between the reusable fitting and column). Rotate the clips so that the openings face forward.
4. Place the column in the tray.
5. Close the column heater tray, and then push down the metal latch to secure the column stabilizer tubing.
6. Close the HT column heater door, and then inspect the seal around the front panel to ensure it aligns correctly.

Note: Proper alignment of the seal is critical for a stable thermal environment.

7. Attach the eCord fob to the receptacle on the right-hand side of the HT column heater.





Warning: To avoid electric shock or possible damage to the components, power-off the sample manager before you connect or disconnect the external communication cable (between the sample manager and the HT column heater).



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

Requirement: The external communication cable must be connected to the HD port on the rear of the column manager or column heater/cooler and the QSPI port on the rear of the sample manager, so that the sample manager can power and communicate with the column manager or column heater/cooler.

Installing the column in a column manager or column heater/cooler

Required materials

- 5/16-inch open-end wrench
- High-temperature outlet fitting
- High-temperature outlet fitting wrench
- Reusable fitting

For instruction on how to replace the column in the column manager or column heater/cooler, see [“Replacing the column in the column manager”](#) on page 6-186 or [“Replacing the column in the column heater/cooler”](#) on page 6-193.

To connect the column in-line filter unit and the column inlet

1. Remove the O-ring from the outlet end of the column in-line filter unit.
2. Remove the plug from the column inlet.

3. Push the outlet end of the column in-line filter unit into the column inlet until it stops.

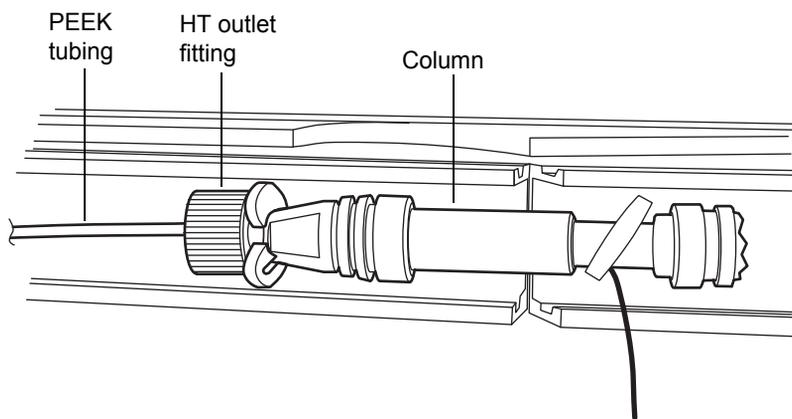
 **Caution:** To avoid damaging the tubing, use only your fingers to tighten the fittings.

4. Tighten the fitting until it is snug, and then add 1/4-turn.

To install the PEEK tubing in the column outlet

 **Caution:** To avoid bandspreading, ensure that the tubing is fully bottomed in the fitting hole before tightening it.

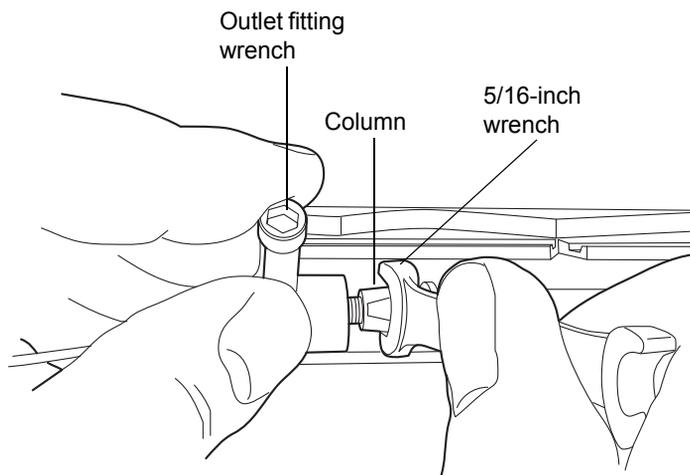
1. Insert the PEEK tubing into the high-temperature outlet fitting until it stops.



2. Holding the PEEK tubing, thread the high-temperature outlet fitting into the column outlet (located on the left-hand side of the column) until it is finger-tight.

 **Caution:** To avoid damaging the outlet fitting, do not overtighten it with the outlet fitting wrench.

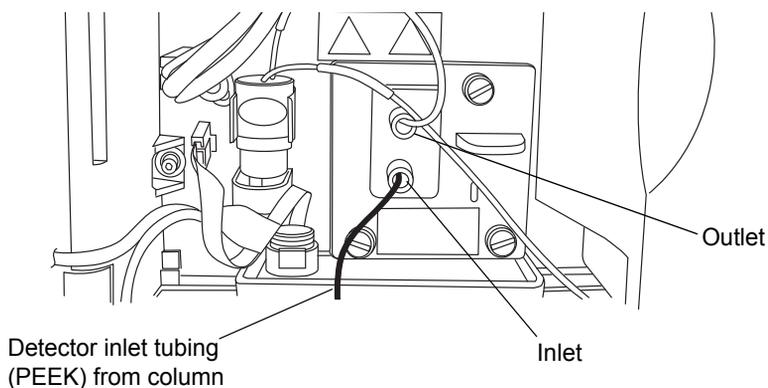
- Using a 5/16-inch wrench to hold the column steady, use the high-temperature outlet fitting wrench to give the outlet fitting a 1/4-turn.



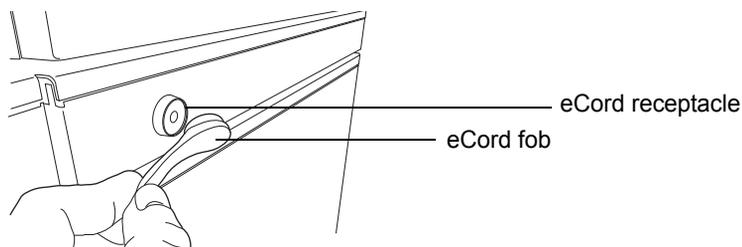
- Push the PEEK tubing into the left-hand side of the column tray.

To connect the PEEK tubing to the detector

- Remove the protective cover from the PEEK tubing from the flow cell inlet in the detector.
- Check the label on the flow cell inlet tubing to ensure it matches the type of detector and flow cell in your system. Attach the 0.004-inch or .0025-inch ID inlet tubing (included with the flow cell or the stabilizer kit) to the column outlet.



3. Snap the column support clips onto the body of the column (one between the reusable fitting and column). Rotate the clips so that the openings face forward.
4. Place the column in the column compartment sleeve.
5. Swing the column retainer rod to the left to fasten it.
6. Attach the eCord fob to the receptacle on the right-hand side of the column manager or column heater/cooler.



 **Warning:** To avoid electric shock or possible damage to the components, power-off the sample manager before you connect or disconnect the external communication cable (between the sample manager and the HT column heater).

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

Requirement: The external communication cable must be connected to the HD port on the rear of the HT column heater and the QSPI port on the rear of the sample manager, so that the sample manager can power and communicate with the HT column heater.

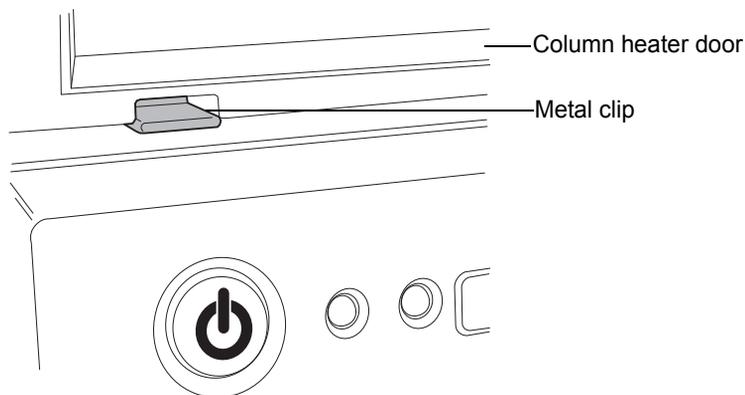
Installing the column in a system with a mass spectrometer

If your system includes a mass spectrometer positioned to the right-hand side of the system stack, swing the column heater door to the “away” position (that is, swing it rightward). In this way, you connect to the mass spectrometer’s inlet without increasing the tubing length. You must also install the MS drip tray to ensure any solvent leaked from the column or column connections is routed into the drip management system.

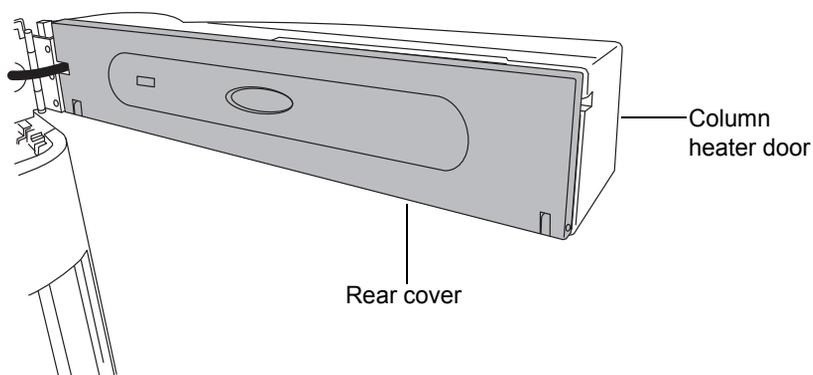
To install the column in an MS detection system

1. Open the column heater door.
2. Remove any solvent lines that are routed between the column heater door and hinge.
3. Push down on the metal clip at the bottom, left-hand side of the column heater to release the door, and then pull the door toward you. The door swings from the left-hand side to the right-hand side. Swing it fully to the right-hand side.

Metal clip on left-hand side of column heater

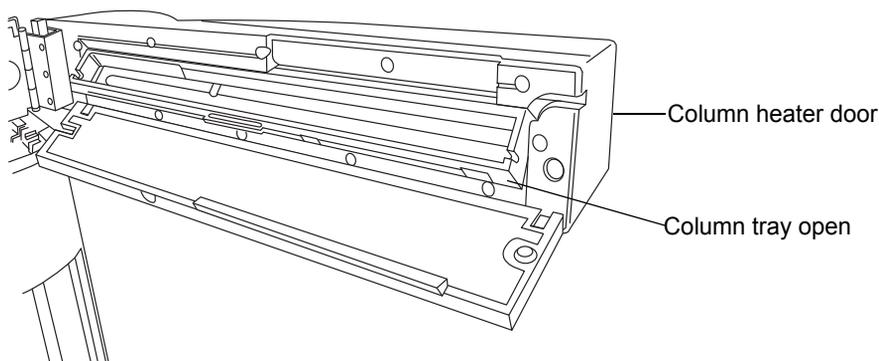


Column heater door swung to right the right-hand side



4. Pull off the rear cover, and then tilt the column tray downward.

Column heater door with rear cover removed and column tray open



5. Snap the plastic column clips onto the body of the column.
6. Attach the column inlet to the stainless steel tubing from port 6 of the injection valve.
7. Attach the mass spectrometer inlet tubing included with the system to the column outlet.
8. Set the column into the column tray. Ensure that the stainless steel tubing from the sample manager is routed through the front notch on the left-hand side of the column tray.
9. Tilt the column tray upward, to the closed position, and refit the rear cover. The cover snaps into place.

10. Attach the eCord column chip holder to the receptacle on the side of the column heater. See the figure on [page 2-52](#).
11. Attach the column outlet tubing to the mass spectrometer's inlet. Consult the instrument's documentation for more information about connecting to the inlet.

Installing a guard column

You can install a guard column between the injector and the analytical column to protect the analytical column against contamination by sample particulates and by strongly retained species. The guard column is a short column that is usually packed with the same material as the analytical column and is often of the same internal diameter. A guard column removes particulate contaminants from samples by adsorption.

Replace the guard column when it becomes contaminated or the system pressure rises above expected levels.

Connecting to the solvent supply



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when connecting to the solvent supply.

The solvent tray located on top of the system holds up to 2 L of spilled solvent. You need a suitable waste container to collect any spill from the waste line at the rear of the tray.

To connect the solvent supply



Warning: To avoid spills, do not place solvent reservoirs atop the sample organizer.



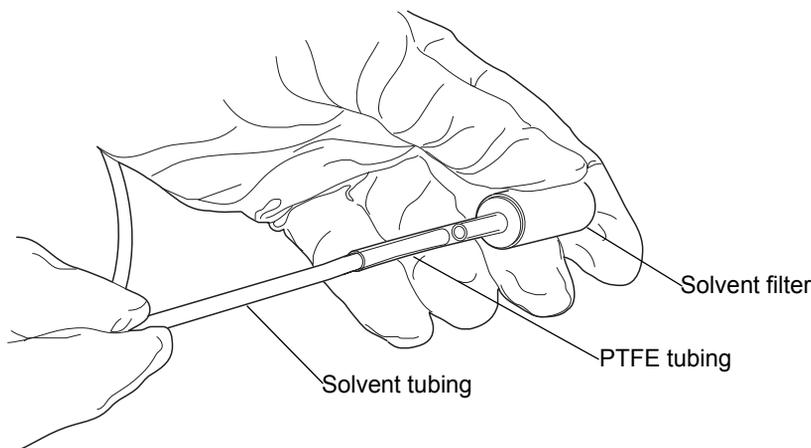
Caution: To maintain adequate solvent head pressure and ensure proper solvent delivery, position the solvent reservoirs in the solvent tray at the top of the system stack.

1. Choose solvent reservoirs that snugly fit the reservoir caps supplied in the startup kit. Waters recommends 1-L reservoirs.

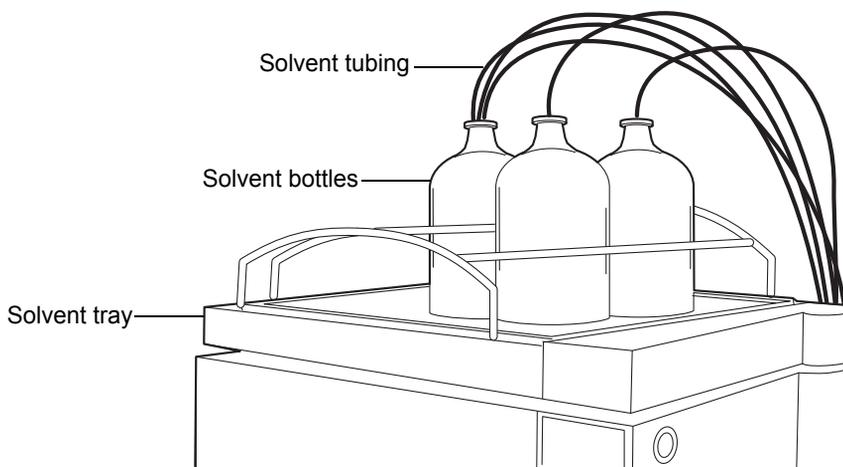


Caution: Wear clean, chemical-resistant, powder-free gloves when handling the solvent filter. Skin oils can contaminate the solvent filter.

2. Remove the solvent filter from the short piece of PTFE tubing.



3. Insert the solvent tubing through the solvent reservoir cap.
4. Re-install the solvent filter on the short piece of PTFE tubing.
5. Insert the filtered end of the solvent tubing into the solvent bottle, in the tray, on top of the sample manager or optional detector.



6. Repeat [step 2](#) through [step 5](#) for the remaining solvent supply lines.

2. Connect the external communication cable to the High Density (HD) port on the rear of the HT column heater.
3. Connect the other end of the external communication cable to the QSPI port on the rear of the sample manager.

30-cm column heater/cooler connections

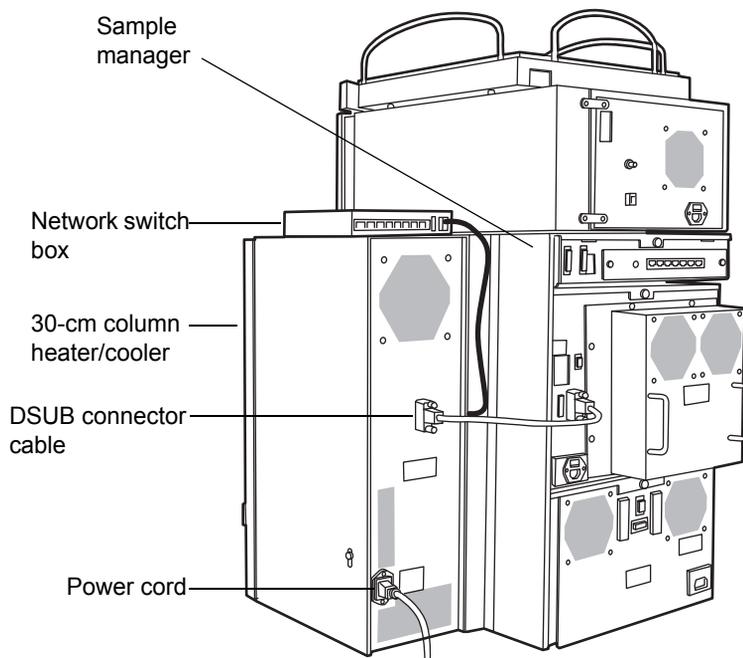
To make 30-cm column heater/cooler connections

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

1. Make sure the sample manager and the 30-cm column heater/cooler are powered-off.

 **Caution:** To avoid damaging the 30-cm column heater/cooler and sample manager electronics, never connect or disconnect the DSUB cable when either instrument is powered-on.

2. Connect the DSUB connector cable to the High Density (HD) port on the rear of the 30-cm column heater/cooler.

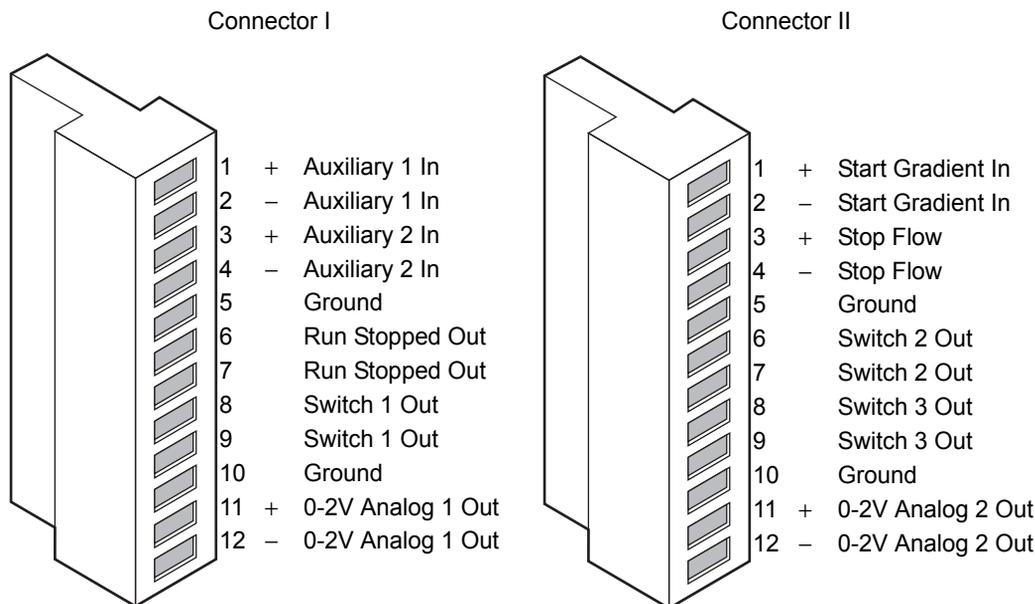


3. Connect the other end of the DSUB connector cable to the QSPI port on the rear of the sample manager.
4. Place an 8-port network switch box on top of the column heater/cooler and connect one end of a shielded Ethernet cable to the network switch, and the other end to the Ethernet card on the workstation.
See also: *Waters Ethernet Instrument Getting Started Guide*.
5. Plug the 8-port network switch box into a suitable wall outlet.

Binary solvent manager I/O signal connectors

The rear panel of the binary solvent manager includes two removable connectors that hold the screw terminals for I/O signal cables. These connectors are keyed so that they can be inserted only one way.

Binary solvent manager I/O signal connectors



For electrical specifications, see the [table titled “Binary solvent manager electrical specifications” on page B-2](#).

Binary solvent manager analog-out/event-in connections

Signal connection	Description
Auxiliary 1 In	Reserved for future use.
Auxiliary 2 In	Reserved for future use.
Run Stopped Out	Indicates (with a contact closure) the binary solvent manager has ceased operation because of an error condition or operator request.
Switch 1 Out	Used to send time-based contact closure signals to external devices.
0–2V Analog 1 Out	Analog chart output functionality.
Gradient In	Initiates the pumps to begin gradient operation by either contact closure input or 0-volt input.
Stop Flow In	Allows you to stop the flow from the binary solvent manager when an error condition or hardware failure occurs on another instrument.

Binary solvent manager analog-out/event-in connections (Continued)

Signal connection	Description
Switch 2 Out	Used to send time-based contact closure signals to external devices.
Switch 3 Out	Used to send time-based contact closure signals to external devices.
0–2V Analog 2 Out	Outputs the analog signal to a device such as an integrator or strip-chart recorder. Through Empower or MassLynx software, you can select one of the following signals as the chart out signal: <ul style="list-style-type: none"> • flow rate • system pressure • composition (%A, %B)

Chart-out signal conditions

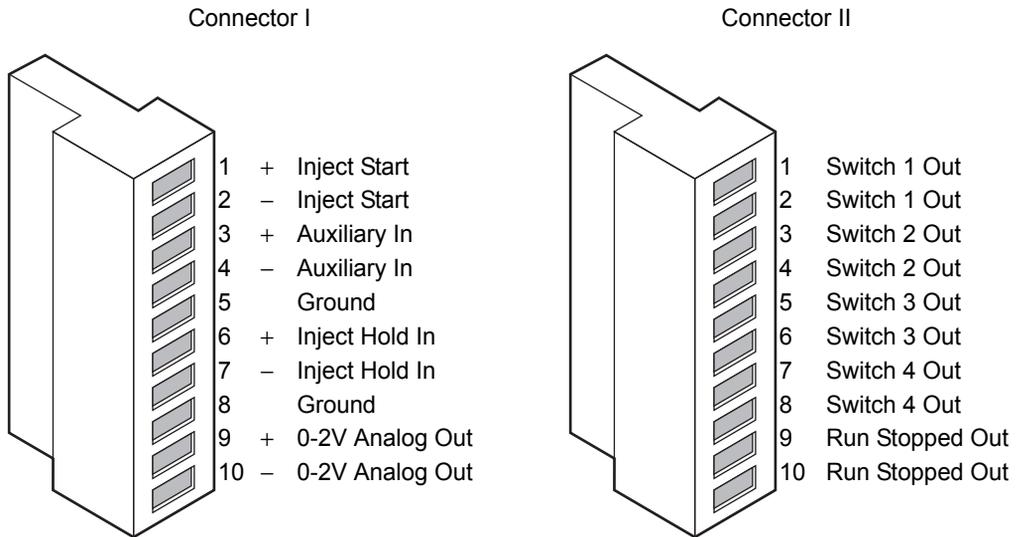
Signal	Parameter Setting at 0 Volts (Minimum)	Parameter Setting at 2.000 Volts (Maximum)
Flow Rate	0.000 mL/min	2 mL/min
System Pressure	–345 kPa (–3.45 bar, –50 psi)	103,421 kPa (1034 bar, 15,000 psi)
Composition	0.0%	100.0%

Sample manager I/O signal connectors

The rear panel of the sample manager includes two removable connectors that hold the screw terminals for I/O signal cables. These connectors are keyed so that they can receive a signal cable inserted only one way.

Requirement: A contact closure input connection from the sample manager is required to trigger a mass spectrometer, an ACQUITY 2996 PDA detector, or an ACQUITY ELS detector running under MassLynx software control to start.

Sample manager I/O signal connectors



For electrical specifications, see the [table titled “Sample manager electrical specifications” on page B-4.](#)

Sample manager analog-out/event-in connections

Signal connections	Description
Inject Start Out	Indicates (with a contact closure) that an injection has started.
Auxiliary In	Reserved for future use.
Inject Hold In	Input signals from other system instruments to delay the injection.
0–2V Analog 2 Out	Analog chart output functionality.
Switch 1 Out	Used to send time-based contact closure signals to external devices.
Switch 2 Out	Used to send time-based contact closure signals to external devices.
Switch 3 Out	Used to send time-based contact closure signals to external devices.
Switch 4 Out	Used to send time-based contact closure signals to external devices.

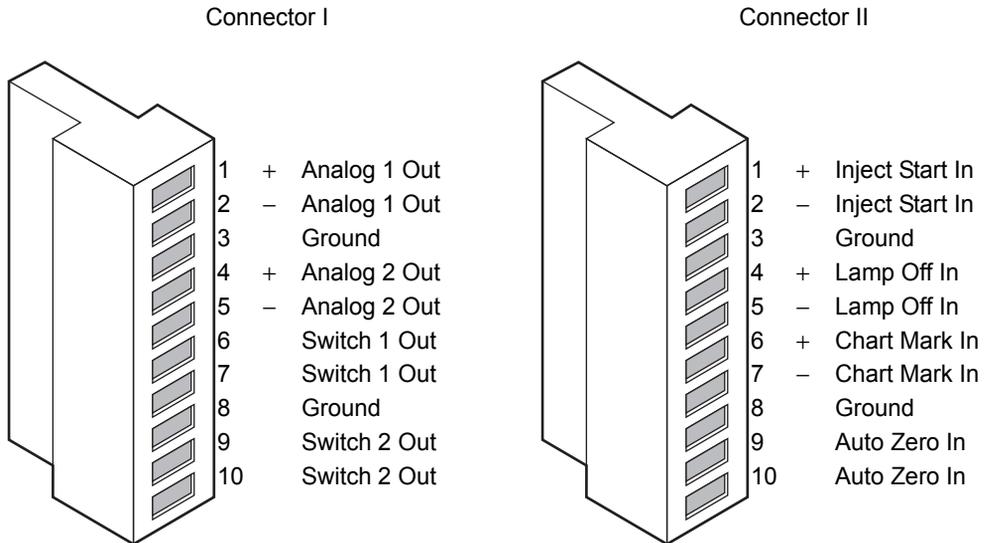
Sample manager analog-out/event-in connections (Continued)

Signal connections	Description
Run Stopped Out	Indicates (with a contact closure) that the sample manager ceased operation because of an error condition or an operator request.

TUV detector I/O signal connectors

The rear panel of the TUV detector includes two removable connectors that hold the screw terminals for I/O signal cables. These connectors are keyed so that they can be inserted only one way.

TUV detector I/O signal connectors



For electrical specifications, see the [table titled “TUV detector electrical specifications” on page B-17](#).

TUV detector analog-out/event-in connections

Signal connections	Description
Analog 1 (Out)	Not used.
Analog 2 (Out)	Not used.

TUV detector analog-out/event-in connections (Continued)

Signal connections	Description
Switch 1 (Out)	Controlled by threshold and timed events.
Switch 2 (Out)	Controlled by threshold and timed events.
Inject Start (In)	Not used.
Lamp Off (In)	When triggered, it extinguishes the lamp.
Chart Mark (In)	When triggered, causes both analog output channels and the digital data sent to the data system to increase their value for a period of time.
Auto Zero (In)	Calculates an offset value that, when added to the sample signal, makes the resulting baseline signal zero.

PDA detector signal connectors

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

ELS detector signal connectors

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

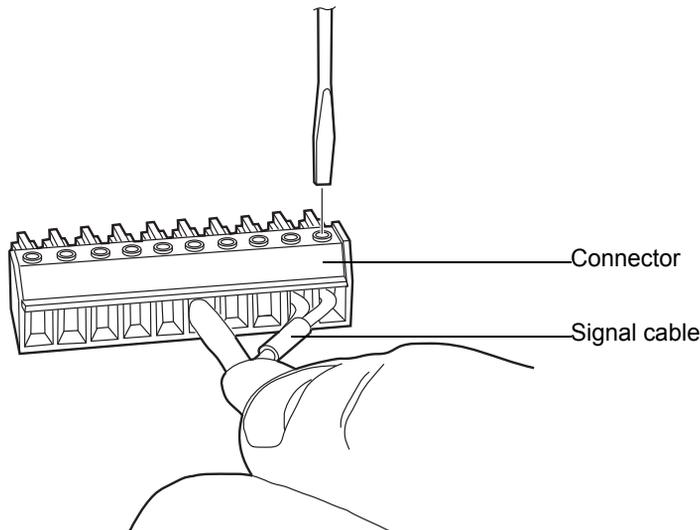
FLR detector signal connectors

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

Signal connections

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

To make signal connections, attach the positive and negative leads of the signal cable to the connector.



Connecting to the electricity source

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

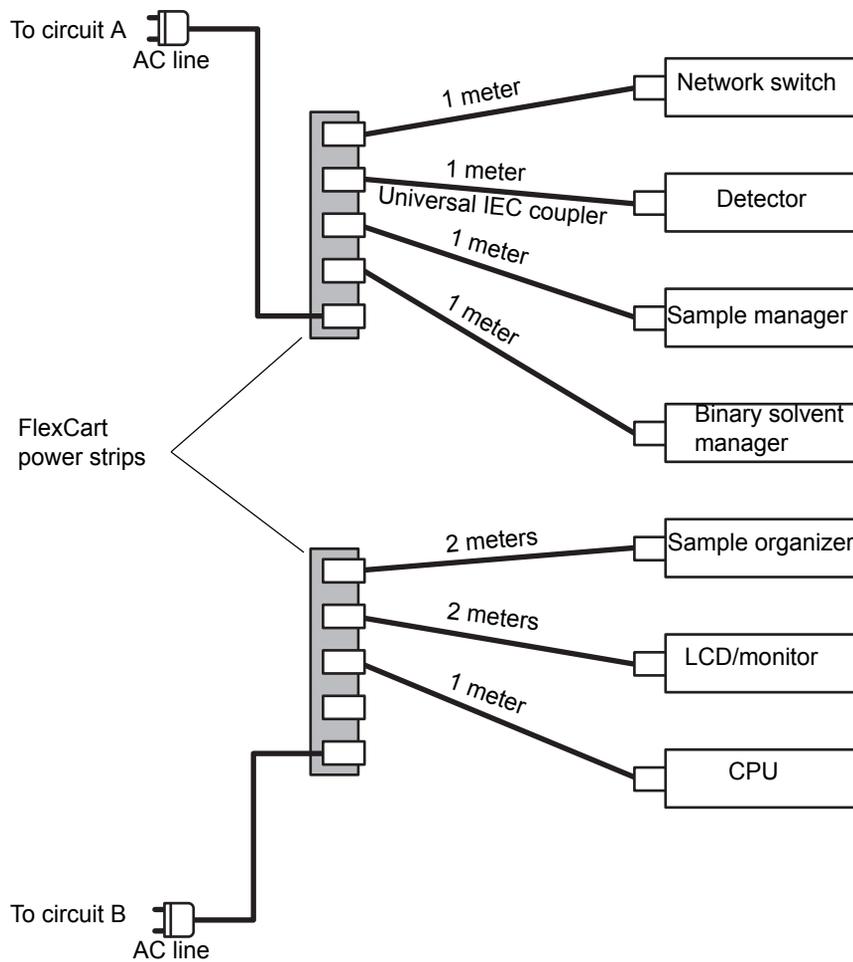
To connect to the electricity source

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

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

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

FlexCart power connections



Calibrating the XYZ mechanism using the teach block

Before performing any chromatography, you must verify that the XYZ mechanism positions the needle properly in the sample chamber.

Requirement: If your system has a sample organizer, you must install the organizer before calibrating the XYZ mechanism.

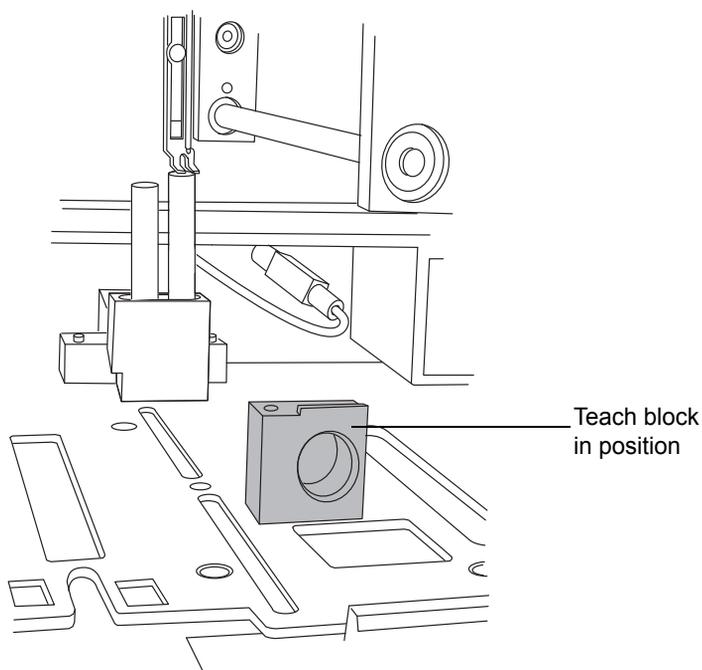


Warning: To avoid puncture wounds, keep hands or loose clothing clear of the needle assembly mechanism while it is moving. Note that the sample manager beeps three times whenever the door is open and the needle assembly mechanism is about to move.

To calibrate the XYZ mechanism

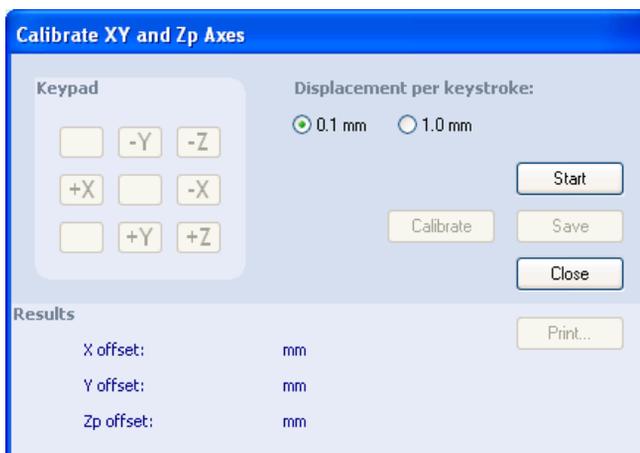
1. Open the sample manager door.
2. Remove the sample plate from the sample tray.
3. Loosen the screw holding the sample tray in place by turning it counterclockwise a 1/4-turn. Then slide the sample tray out. If your system does not include a sample organizer, you must remove two sample trays from the sample chamber.
4. With your finger, remove the teach block from the sample manager chassis floor.

5. Insert the teach block into the locating holes.



6. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
7. Click Troubleshoot > Calibrate XY and Zp axes.

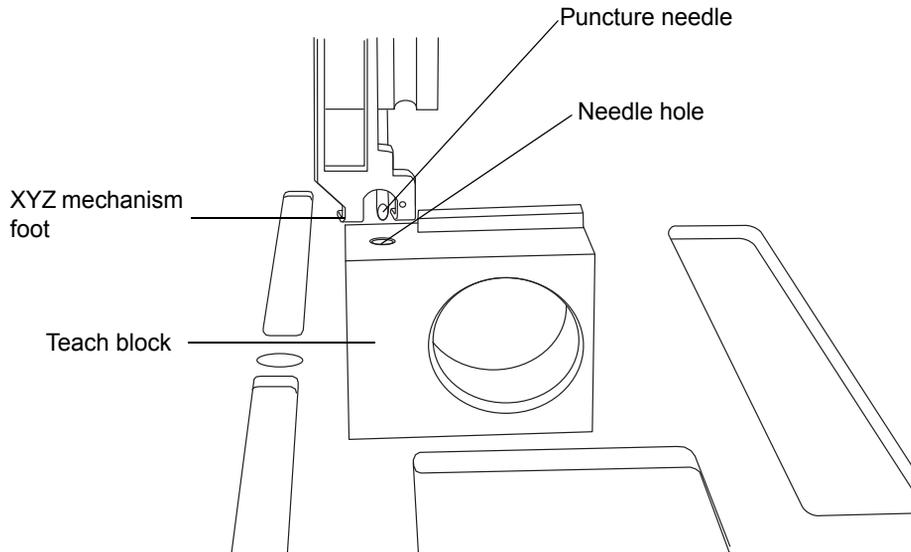
Calibrate XY and Zp Axes dialog box



- In the Calibrate XY and Zp Axes dialog box, click Start to begin.

Result: A warning appears, prompting you to keep clear of the sample chamber. Confirm that all sample plates and trays are removed, and ensure the teach block is installed.

- In the warning dialog box, click OK. The sample manager beeps 3 times to indicate the needle assembly mechanism is about to move, and the XYZ mechanism positions the puncture needle above the teach block.



- Examine the location of the puncture needle relative to the needle hole in the teach block.
- Select the 1.0-mm displacement increment, and then click the +Z button to position the XYZ mechanism foot close to the top of the teach block. Each click of the mouse moves the needle 1 mm.
- Select the 0.1-mm displacement increment, and then fine tune the adjustment so that the XYZ mechanism foot is just above the teach block, but not touching it.



Caution:

- To avoid triggering the top-of-plate sensor prematurely, do not allow the mechanism foot to touch the teach block.
- To avoid damaging the puncture needle, do not move it to the left-hand or right-hand side while it is in the teach block.

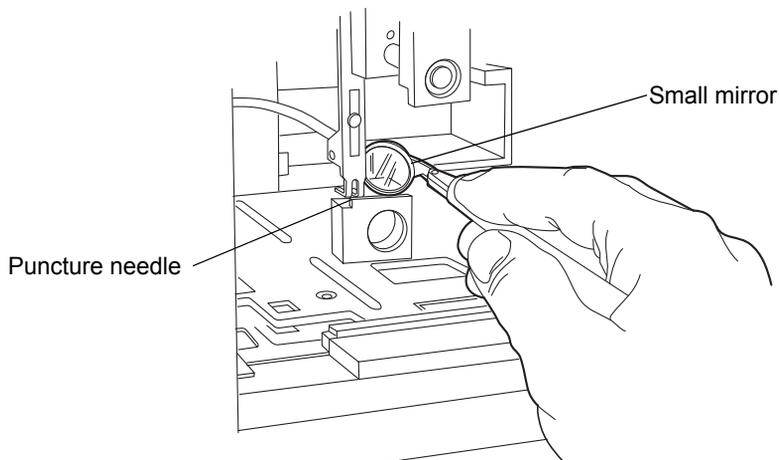
13. To laterally position the puncture needle, use the +X and –X buttons to move it to the left-hand or right-hand side, respectively.

Alternative: Press the keyboard arrow keys instead of clicking the on-screen buttons to adjust the needle position. Doing so lets you adjust the position without taking your eyes off the needle.



Caution: Avoid holding down an arrow key, as doing so stores keystrokes and causes the needle to continue moving, even after you release it.

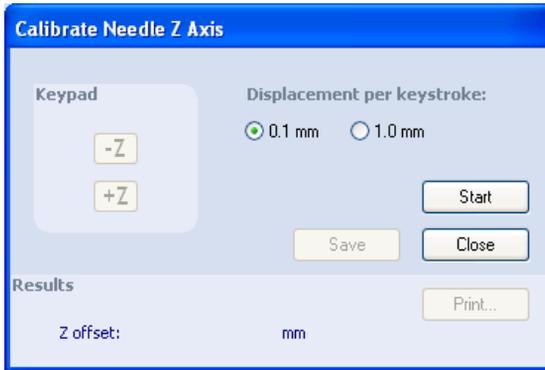
14. Position a small mirror so that you can see the location of the puncture needle, and then use the +Y and –Y buttons to adjust the front-to-back location of the puncture needle so that it is centered over the needle hole.



15. Using the 0.1-mm displacement increment, lower the needle into the teach block hole to confirm an accurate X and Y alignment.
16. Click Calibrate. A warning appears, indicating you must keep clear of the sample chamber. If your sample manager has an extended puncture needle installed, the XYZ mechanism automatically recognizes it.
17. Click OK. The puncture needle fully lowers into the teach block to calibrate the top-of-plate sensor.
18. Click Save. When the confirmation window appears, click Yes.
19. Remove the teach block, place it in the storage location in the floor of the sample compartment, and re-insert the sample trays.

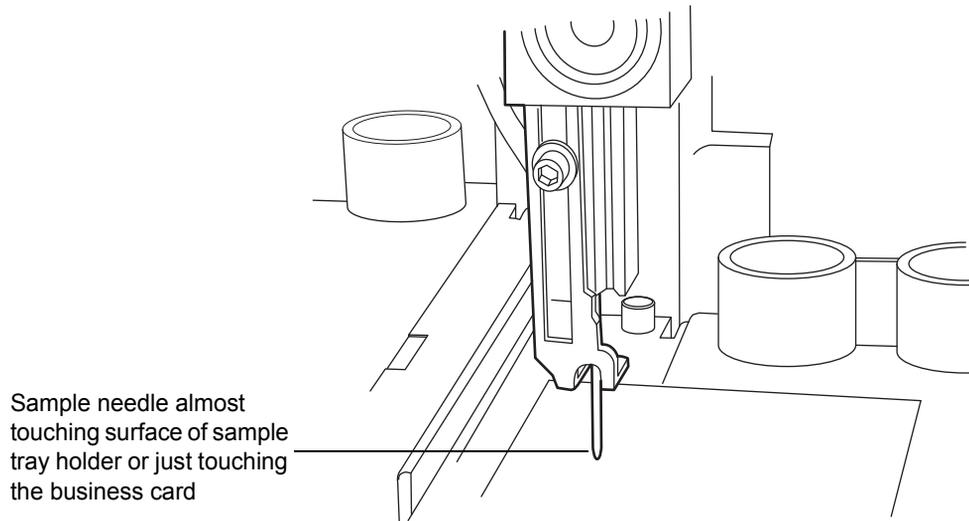
20. Click Maintain > Calibrate needle Z axis.

Calibrate Needle Z Axis dialog box



21. In the Calibrate Needle Z Axis dialog box, click Start, and then click OK in the confirmation window.
22. Use the +Z button to drive the sample needle down to within 1 mm of the tray surface.
23. Switch the displacement increment to 0.1 mm, and lower the sample needle until it almost touches the surface of the sample tray holder.

Tip: To easily and efficiently achieve the correct needle setting, slide a business card under the needle. Then lower the needle until it touches the card lightly but does not restrict the card's free movement.



24. Click Save. The confirmation window appears.
25. Click Yes.

3 Preparing System Hardware

Contents

Topic	Page
Powering-on the system	3-1
Monitoring startup tests	3-3
Monitoring system instrument LEDs	3-3
Enabling the leak sensors	3-6
Preparing the binary solvent manager	3-7
Preparing the sample manager	3-15
Preparing the column manager	3-30
Preparing the sample organizer	3-30
Starting the TUV detector	3-36
Conditioning the column	3-39
Shutting down the system	3-40
Running HPLC methods on an ACQUITY UPLC system	3-42

Powering-on the system

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

To power on the system

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

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

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

The power and status LEDs change as follows:

- Each system instrument's power LED shows steady green. The column manager's and column heater/cooler's run LEDs all show red for a few seconds.
 - During initialization, each system instrument's power LED shows steady green. The column manager's and column heater/cooler's run LEDs all show flashing green.
 - After the instruments are successfully powered-on, each one's power LED shows steady green. The column manager's and column heater/cooler's run LEDs are unlit.
3. Press the power switch on the top, left-hand side of the binary solvent manager's, sample organizer's, and sample manager's door. Each system instrument beeps 3 times and runs a series of startup tests.

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

The power and status LEDs change as follows:

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

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

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

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

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

Monitoring startup tests

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

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

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

Monitoring system instrument LEDs

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

Power LED

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

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

Status LEDs

Flow LED (binary solvent manager)

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

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

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

Lamp LED (detector)

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

Status LED indications

LED mode and color	Description
Unlit	<ul style="list-style-type: none">• Binary solvent manager, sample manager, column manager, column heater/cooler, and sample organizer – Indicates the instrument is currently idle.• Detector – Indicates the detector lamp is extinguished.

Status LED indications (Continued)

LED mode and color	Description
Steady green	<ul style="list-style-type: none">• Binary solvent manager – Indicates solvent is flowing.• Sample manager, column manager, column heater/cooler, and sample organizer – Indicates the sample manager, column manager, column heater/cooler, or sample organizer is operating normally, attempting to complete any outstanding samples or diagnostic function requests. When sample and diagnostic function requests are finished, the LED reverts to the unlit mode.• Detector – Indicates the detector lamp is ignited.
Flashing green	<ul style="list-style-type: none">• Binary solvent manager, sample manager, and sample organizer – Indicates the system is waiting for at least one instrument to become operable. Detector lamp warm-up and column temperature equilibration times typically cause such a delay.• Column manager and column heater/cooler – Indicates the system is waiting for the instrument to reach the temperature set point before operating. Column temperature equilibration times typically cause such a delay. The LED also flashes green during initialization or while waiting for initialization.• Detector – Indicates the detector is initializing or calibrating.
Flashing red	Indicates that an error stopped the instrument. Refer to the ACQUITY UPLC Console for information regarding the error.
Steady red	Indicates an instrument failure that prevents further operation. Power-off the instrument, and then power-on. If the LED is still steady red, contact your Waters service representative.

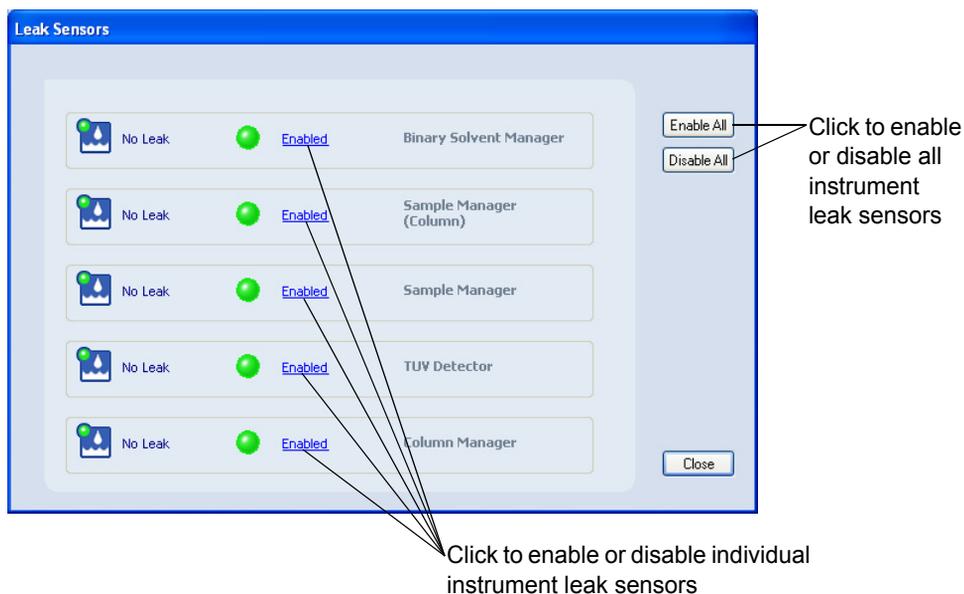
Enabling the leak sensors

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

To enable the leak sensors

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

Leak Sensors dialog box



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

Preparing the binary solvent manager

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

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



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

Requirement: To maintain the efficiency of the binary solvent manager and to obtain accurate, reproducible chromatograms, use only MS-grade solvents, water, and additives. For details, see [Appendix C](#).



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

Performing a seal wash prime

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

Prime the plunger seal wash

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



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

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

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

Recommendations:

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

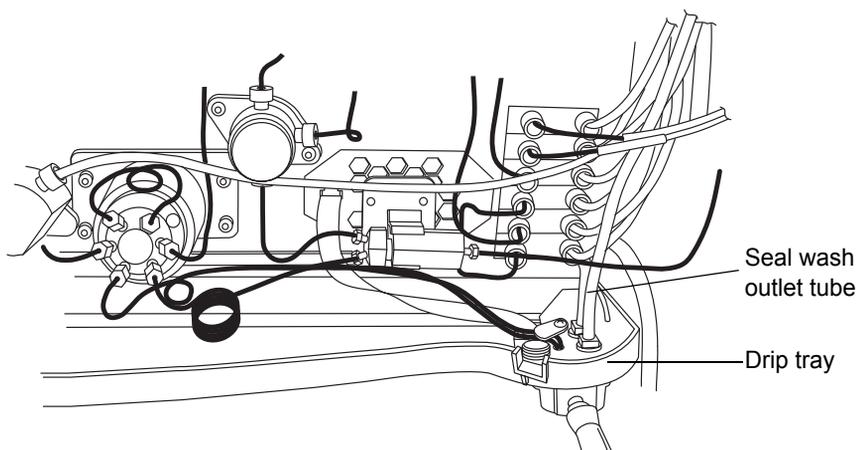
See also: *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD.

Required materials

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

To perform a seal wash prime

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



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

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

Priming the binary solvent manager

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

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



Caution: To prevent salts from precipitating in the system, introduce an intermediate solvent, such as water, when changing from buffers to high-organic-content solvents. Be sure to consult the solvent miscibility tables on [page C-8](#).

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



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

Priming a dry binary solvent manager

To prime a dry binary solvent manager

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

Default: 1.0 minute

Recommendations: Prime the binary solvent manager until a steady flow exits the vent tube (typically 7 to 10 minutes).

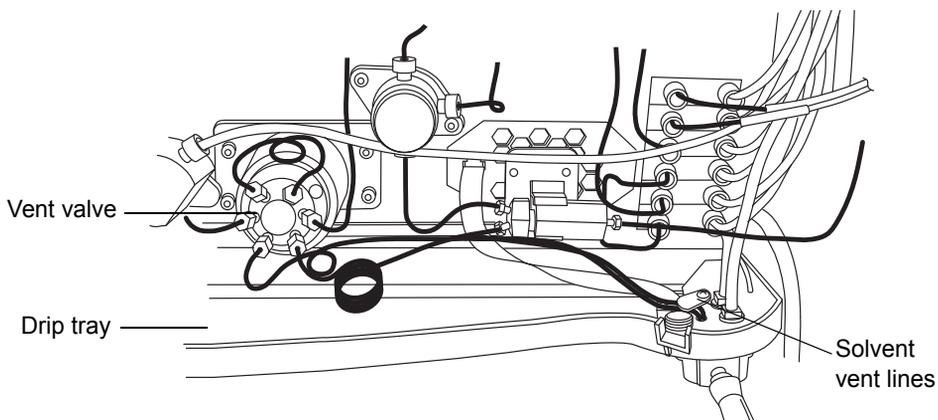
7. Click Start. When solvent flows out of the vent line without bubbles, the path is primed.
8. Repeat [step 3](#) through [step 7](#) to prime the other solvents.

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

To prime a dry binary solvent manager using a syringe

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

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



3. Push the syringe plunger fully into the syringe barrel.
4. Connect the tubing adapter to the syringe.
5. Connect the syringe assembly to the short length of PharMed tubing, and then connect the short length of PharMed tubing to the solvent vent line you located in [step 2](#).
6. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
7. In the binary solvent manager information window, click Control > Prime A/B Solvents.
8. In the Prime A/B Solvents dialog box, select solvent A1.
9. In the Time box, specify the number of minutes from 0.1 through 60.0.
Default: 1.0 minute
Recommendation: Prime the binary solvent manager until a steady flow exits the vent tube (typically 3 minutes).
10. Click Start.
11. Slowly draw back on the syringe plunger to pull solvent through the solvent path. When solvent flows out of the vent line without bubbles, the path is primed.

12. Remove the syringe from the vent line, and reconnect the vent line to the drip tray.
13. Repeat [step 2](#) through [step 12](#) for solvent A2, B1, and B2.
Requirement: The reservoirs and solvent lines for solvents A1, A2, B1, and B2 must not be empty. Otherwise the degasser does not function correctly.

Priming a wetted binary solvent manager

Two functions help prepare the system for operation:

- Refresh system (Sys Prep)
- Start up

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

Refreshing the system

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

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

Recommendations:

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

To refresh the system

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

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

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

Starting up the system

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

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

To start up the system

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

Allowed values: 0.1 to 60.0 minutes

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

Defaults: Solvents A1, A2, B1, and B2 prime for 1.0 minute each.

3. Select or clear priming of the seal wash, strong wash, weak wash, and/or sample syringe.

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

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

Default: 10 cycles for each syringe selected

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

Equilibrate to Method tab values

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

6. If you changed the sample needle or loop, in the Configuration area, click Change. In the Volume Configuration dialog box, select the new size of loop and/or needle, and then click OK.
7. If you changed the sample needle, click the Optional: Characterize Volume tab, and then select “Characterize seal” and “Characterize needle and loop volumes”.
8. If you changed the sample loop, in the Optional: Characterize Volume tab, select Characterize needle and loop volumes.
9. Click Start.

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

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

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

Preparing the sample manager

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

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



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

Selecting weak wash and strong wash solvents

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

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

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

See [Appendix C](#) for further information about solvents.



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

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

Wash solvent effects

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

Wash solvent effects (Continued)

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

Priming the sample manager

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

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

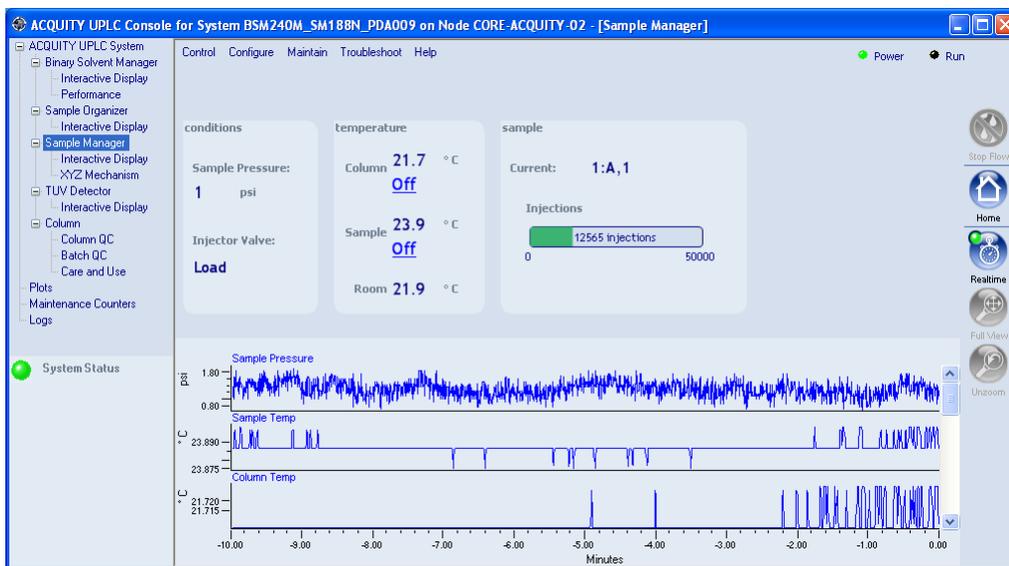
Ensure that the priming solvent is correctly composed and that it is high in quality and miscible with any other solvents used in your system. Use filters in all solvent reservoirs, and ensure the volumes of solvents are sufficient for priming.

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

To prime the sample manager

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

Sample manager information window



2. Click Control > Prime syringes.

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

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

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

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

Default: 1

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



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

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

Tip: Each prime takes approximately 2 to 4 minutes.

Washing the sample manager needle

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

Rule: Do not use buffered solvents as wash solvents.

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

Observing wash solvent recommendations

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

- To ensure that the strong wash solvent is completely removed, the system washes the needle with 500 μL of weak wash solvent after you use strong wash solvent. You can increase, but not decrease, the default value of 500 μL .
- The analytes and sample matrix must be soluble in both weak and strong solvents. Proteins do not dissolve in solvents whose organic component is greater than 40%. Do not use buffers in any wash solvent.
Example: If the weak wash solvent is 30% acetonitrile and 70% water, the strong wash solvent must contain a greater concentration of acetonitrile in water.
- The weak wash solvent must be the same as the initial eluting solvent, and the strong wash solvent must at least equal the composition of the final eluting solvent.
- Use a weak wash solvent based on the sample and mobile phase chemistries of your application. Make sure all solutions are miscible and soluble. For best results, weak wash solvent must match the initial gradient conditions and mobile phase composition (isocratic). High sample concentrations can require additional weak wash solvents.
- For buffered aqueous, reversed-phase chromatography, use weak wash solvent consisting of 100% water or up to 25% methanol or acetonitrile. For strong wash solvent, use 50% to 100% methanol or acetonitrile.

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

Needle recommendations

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

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

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

To wash the sample manager needle

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree. The sample manager information window appears.
2. Click Control > Wash Needle.
Alternative: Right-click in the Empower or MassLynx sample manager control panel, and then click Wash Needle.
3. In the Strong Wash box, specify the volume for the strong wash solvent. Or, to omit strong wash solvent, enter 0 in the Strong Wash box, or leave it blank.

Range: 0.0 through 99,999 μL

Default: 0.0 μL

Recommendation: 100 through 500 μL

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



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

4. In the Weak Wash box, specify the volume for the weak wash solvent.
Range: 1.0 through 99,999 μL
Default: 200.0 μL without strong wash or 500 μL with strong wash
Recommendation: 200 through 500 μL or three times the strong wash volume.
5. Click OK. The needle wash begins.
6. When needle washing is complete, the status returns to Idle.

To stop a needle wash routine before it finishes

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

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



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

Characterizing the needle seal

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

Requirements:

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

To characterize the needle seal

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

Characterizing the needle and sample loop volumes

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

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

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

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

Requirements:

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

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

To characterize the needle and sample loop volumes

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

Tip: This procedure takes at least 5 minutes.

4. Click Results to view the results of the needle and loop volumes characterization operation.

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

Using the extended puncture needle

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

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

Loading sample plates in the sample manager

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

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

Observing vial and plate recommendations

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

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

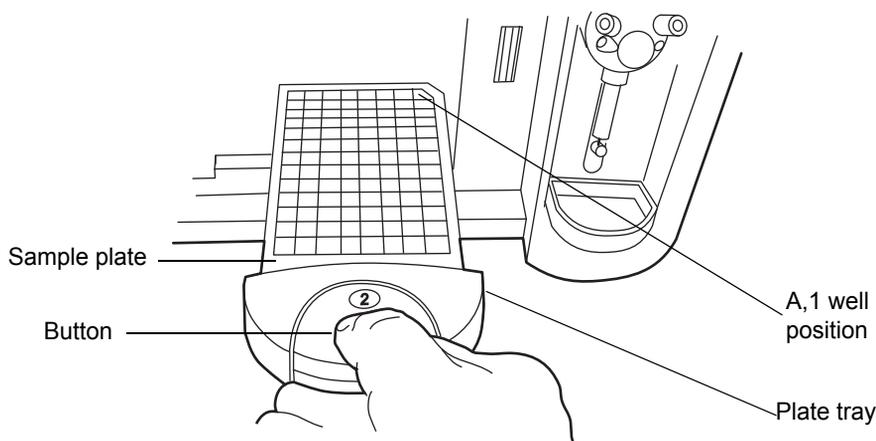
To load a sample plate

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

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

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

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



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

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

Selecting the optimum sample injection mode

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

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

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

Partial loop needle overfill mode

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

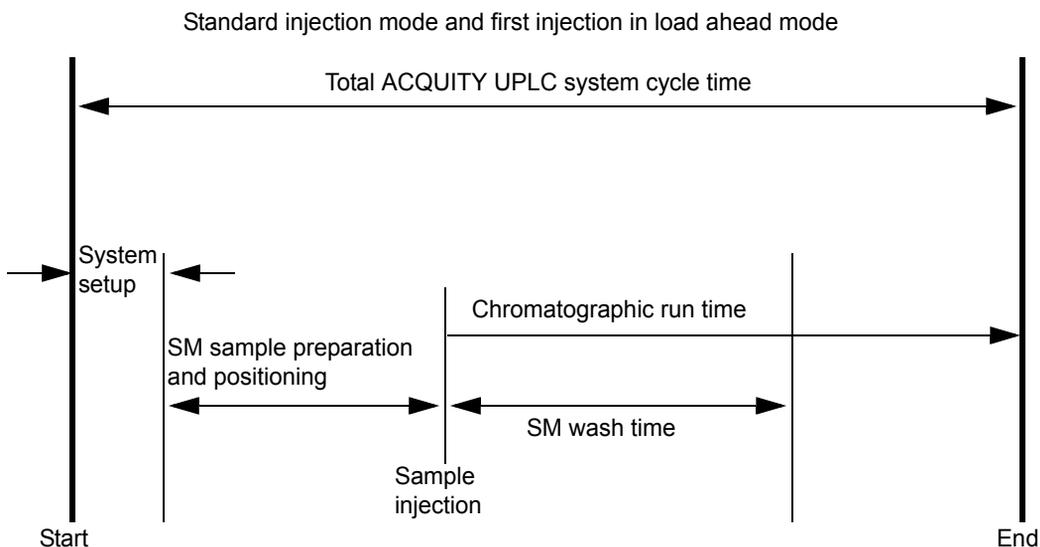
See also: The [table titled “Selecting the injection mode and loop volume” on page 3-28](#).

Load ahead mode

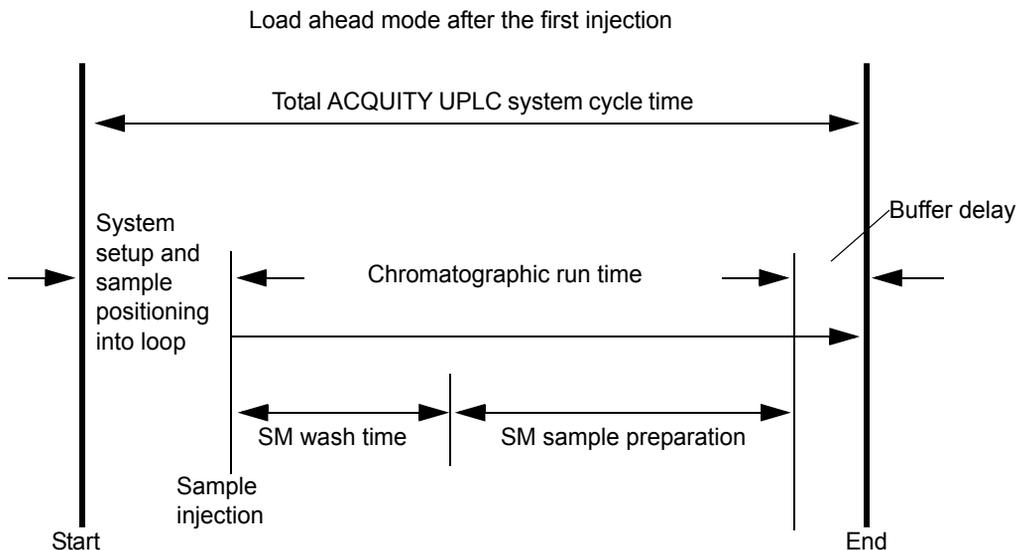
The first injection of a sample set and injection sets with different methods do not utilize load ahead mode. The minimum cycle time is the lesser of either the two run times or sample preparation and wash.

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

Standard ACQUITY UPLC system cycle time definition



ACQUITY UPLC system load-ahead cycle time definition



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

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

Selecting the injection mode and loop volume

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

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

Installing the optional sample manager shade

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

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

Required material

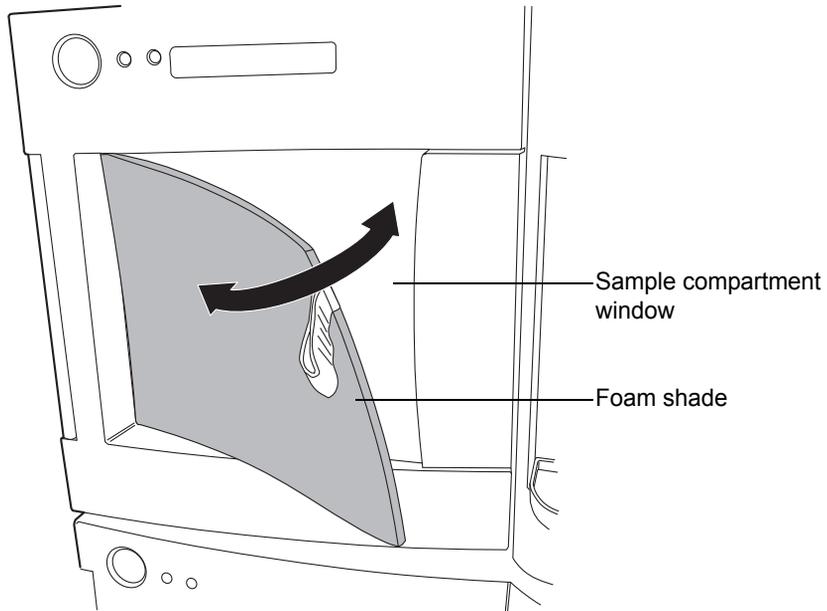
Sample manager foam shade

To install the sample manager foam shade

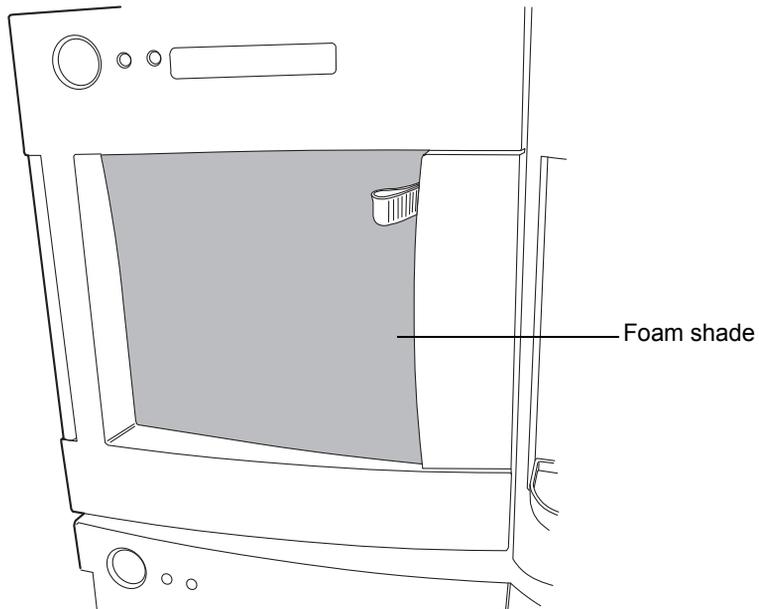
1. Turn off the sample manager compartment lights.

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

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



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



Preparing the column manager

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

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

Preparing the sample organizer

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

Initiating communications

To initiate communications between the sample manager and sample organizer

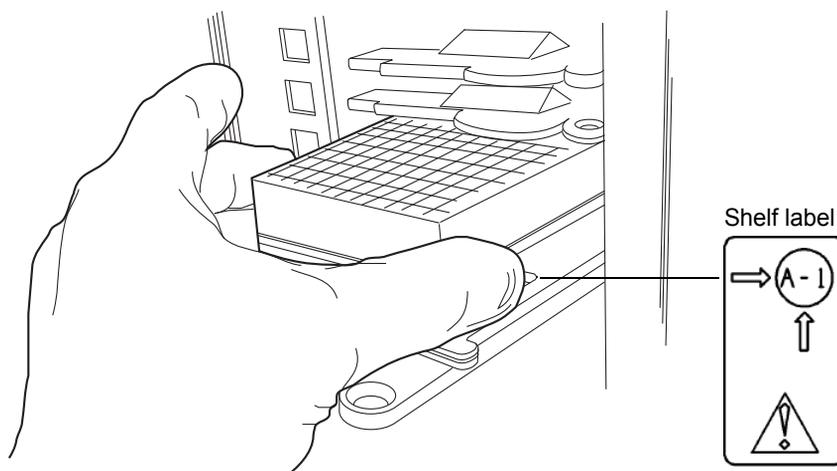
1. Open the sample manager door, load a plate onto the right-hand tray, and then close the sample manager door.
Tip: When the system has both a sample manager and a sample organizer, the right-hand tray of the sample manager becomes position 1, and the left-hand tray is not available. The bottom shelf in the sample organizer becomes position 2.
Exception: If the system does not include a sample organizer, the left-hand tray in the sample manager is designated position 1 and the right-hand tray is position 2.
2. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
3. In the sample manager information window, click Configure > Sample Organizer.
4. In the Sample Organizer Configuration dialog box, select the sample organizer from the list of serial numbers, and then click OK.
5. The sample organizer automatically detects which shelves contain plates and illuminates their corresponding LEDs.

Loading sample plates

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

To load sample plates

1. Open the sample organizer door.
Tip: To keep the sample compartment from freezing, open its door only when necessary. (Opening the door admits humid air into the sample compartment, which causes condensation and freezing.)
2. Pull the shelf toward you.
3. Load the plate onto the shelf so that position A,1 is at the rear, right-hand corner and the forward edge of the plate is behind the stop at the front, left-hand corner.



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

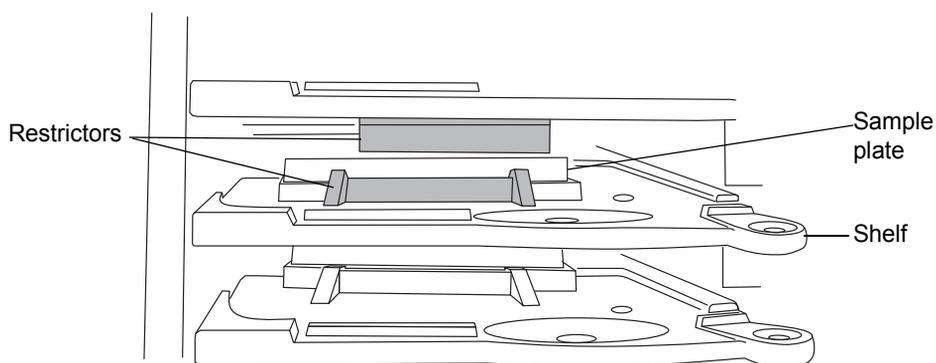
4. Ensure that the plate does not extend beyond the plate stop at the rear of the shelf.



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

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

Shelf/plate combination and restrictors



6. Repeat [step 2](#) through [step 5](#) for the remaining plates.



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

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

To remove and replace the same plate on the same shelf

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



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

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



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

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



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

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

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

To rearrange the shelves for a different plate configuration

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

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



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

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



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

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



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

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

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

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

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

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

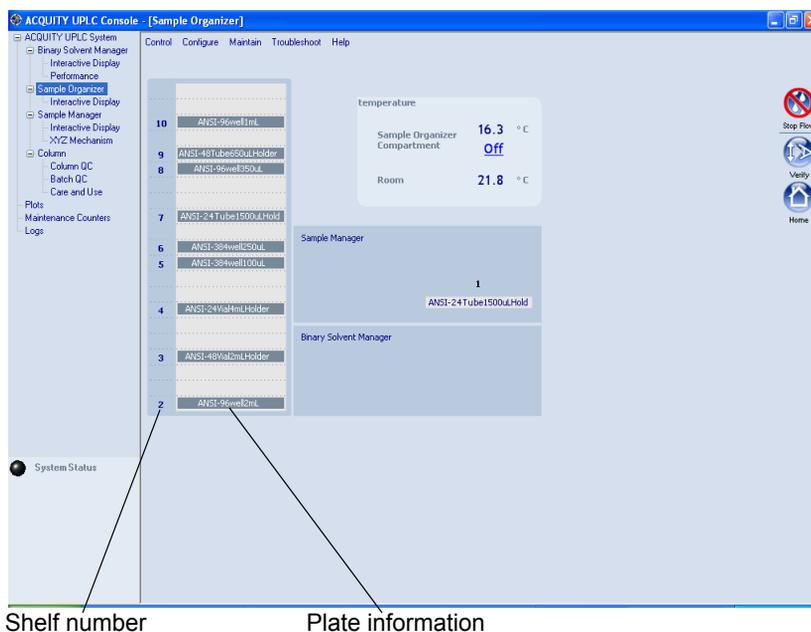
Displaying sample plate information

To display sample plate information

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

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

Sample organizer information window



Tips:

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

Starting the TUV detector

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

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

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

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

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

Starting the TUV detector



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

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

To start the TUV Detector

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

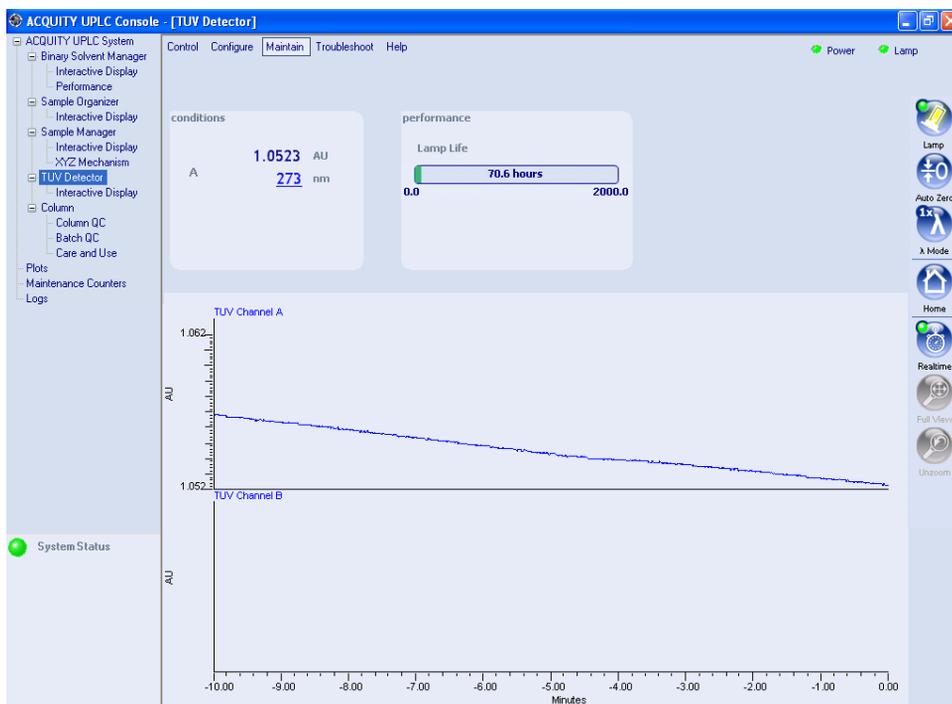
Tip: Initialization usually requires approximately 2 minutes, and lamp warm-up requires approximately 3 minutes.

4. When the lamp LED shows steady green, start the Empower or MassLynx software. You can monitor the ACQUITY UPLC Console for messages and LED indications. For best results, allow at least 30 minutes to equilibrate the detector and stabilize the baseline.

Tips:

- The absorbance value appears in the ACQUITY UPLC Console and also in Empower's Run Samples window or MassLynx's Inlet Editor window. If the detector is in dual wavelength mode, two absorbance values appear.
 - Absorbance values have a resolution of 0.0001 AU.
 - When the lamp is extinguished, "Lamp Off" appears in the software instead of absorbance values.
5. Configure the detector according to the instructions in the Empower or MassLynx online Help, or as provided in ["Configuring Empower software" on page 4-1](#) and ["Configuring MassLynx software" on page 4-13](#).

TUV detector information window



Recording sample and reference energies

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

To record sample and reference energies

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



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

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

4. Ensure the detector cell is filled with solvent and free of air bubbles.
5. When both LEDs show steady green, initialization is complete.
6. Start the Empower or MassLynx software.
7. Launch the ACQUITY UPLC Console from the sample manager control panel.
For additional information, see [“Starting the ACQUITY UPLC Console from Empower software” on page 4-12](#) and [“Starting the ACQUITY UPLC Console from MassLynx software” on page 4-14](#).
8. Select the TUV detector view in the ACQUITY UPLC Console.
9. Set the wavelength to 230 nm.
10. In the Console, select TUV Detector > Interactive Display from the system tree.
11. Record the sample and reference energies at 230 nm.

Conditioning the column

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



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

To condition the column

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

3. If MassLynx software controls the system, proceed as follows:
 - a. Open the Sample Set window, and select an inlet method that includes the chromatographic conditions you want to use.
 - b. Run the sample set line. The system runs the separation method.

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

Shutting down the system



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

Tips:

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

Shutting down for less than 24 hours

To shut down the system for less than 24 hours

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

Tip: If you are running under MassLynx control, ensure that Auto-Shutdown for your shutdown method is deactivated.

3. If a few hours will pass before the next injection, slow the flow rate in the interim to a few tenths of a mL/min to conserve solvent.

Tip: Ensure that the shutdown method is deactivated.

4. Keep the detector operating and the column heater at operating temperature during this period.

Shutting down for more than 24 hours

To shut down the system for more than 24 hours

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

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



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

4. Power-off the system.

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



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

5. Cap the flow cell inlet and outlet ports.

Running HPLC methods on an ACQUITY UPLC system

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

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

System considerations

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

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

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

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

Requirements for using an HPLC column on an ACQUITY UPLC system

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

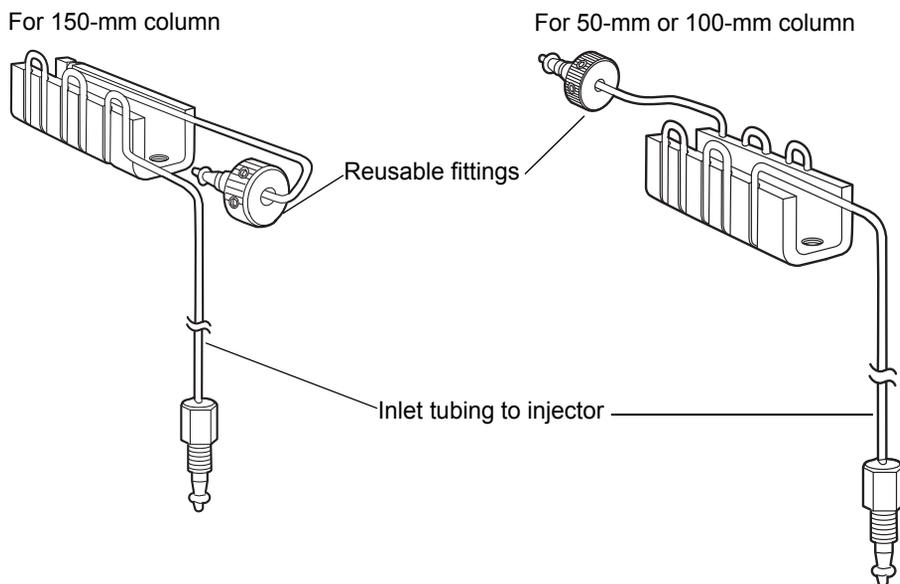
- When running columns without an eCord, verify that the console software is configured to ignore the absence of the eCord.

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

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

For detailed instructions on how to install the column stabilizer, see [“Installing the column stabilizer assembly in an HT column heater” on page 2-37](#) and [“Installing the column stabilizer assembly in a column heater/cooler” on page 2-40](#).

Column stabilizer assembly (two styles)



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

Recommendation: To reduce variability from temperature fluctuations, use a column oven with a thermostat. For columns longer than 150 mm, use the ACQUITY UPLC system 30-cm column heater/cooler or another appropriate external oven.

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

Choosing fittings

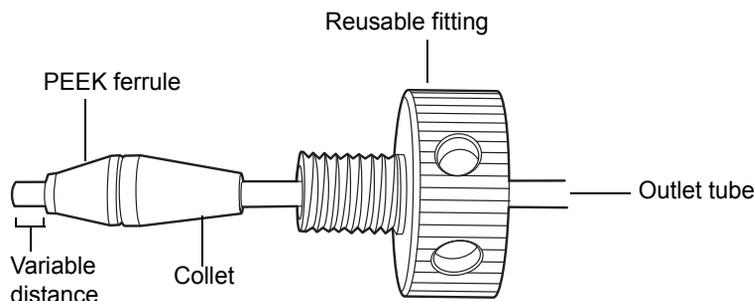
Column inlet

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

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

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

Column stabilizer reusable fitting



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

For more information on replacing fittings and installing columns, see [“Plumbing the system” on page 2-20](#).

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



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

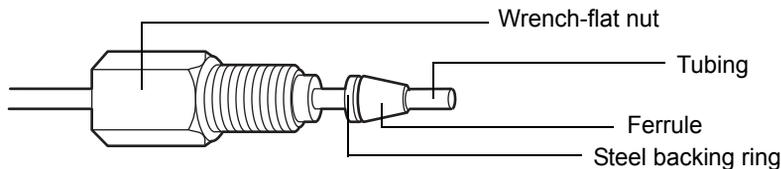
You can also use stainless-steel fittings recommended by your HPLC column’s manufacturer. For stainless-steel fittings, the ferrule position remains fixed (swaged) once it is set. The distance from the ferrule to the end of the tubing when connected to a UPLC column is approximately 3 mm. Your HPLC column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

Column outlet

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

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

Wrench-flat column outlet fitting



For information on tubing and fittings for post-column tubing connections, see [“Plumbing the system” on page 2-20](#) and the appropriate detector user documentation.

4 Configuring System Software

Contents

Topic	Page
Configuring Empower software	4-1
Starting the ACQUITY UPLC Console from Empower software	4-12
Configuring MassLynx software	4-13
Starting the ACQUITY UPLC Console from MassLynx software	4-14

Configuring Empower software

Perform these tasks to configure Empower software:

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

Starting Empower software and logging in

To start Empower software and log in

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

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

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

Selecting system instruments

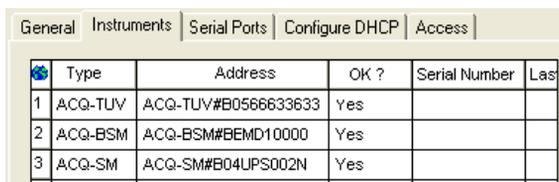
To select system instruments

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

Tip: If you are using Empower 1154 software to control the system, the node name is referred to as the acquisition server name.

3. In the Acquisition Server dialog box, click the Instruments tab. The system instruments that are successfully communicating with your system are shown with a Yes in the “OK?” column.

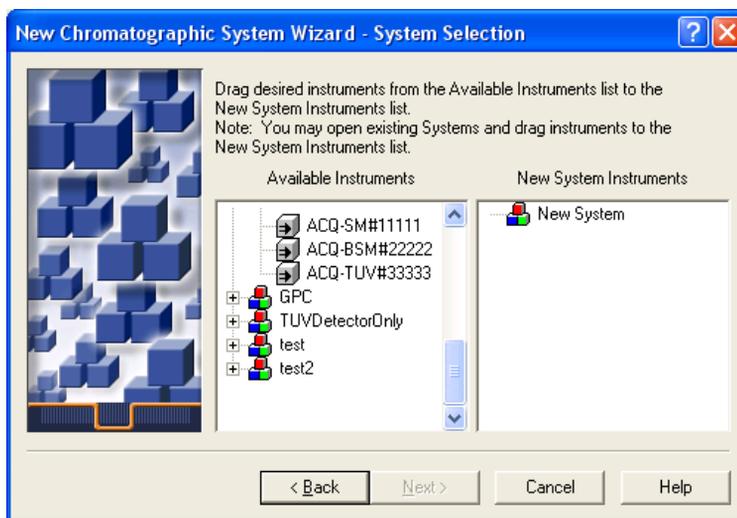
Instruments tab



Type	Address	OK ?	Serial Number	Last
1 ACQ-TUV	ACQ-TUV#B0566633633	Yes		
2 ACQ-BSM	ACQ-BSM#BEMD10000	Yes		
3 ACQ-SM	ACQ-SM#B04UPS002N	Yes		

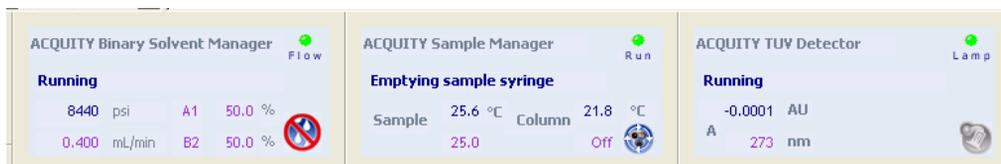
4. Ensure that a binary solvent manager (ACQ-BSM), sample manager (ACQ-SM), and detector (ACQ-TUV, ACQ-PDA, or ACQ-ELS) appear in the instrument list and are successfully communicating with your system, and then click OK.
5. Right-click Systems, and then select New > Chromatographic System.
6. In the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

System Selection dialog box



7. In the System Selection dialog box, drag the name of the instrument(s) you want to include in the new system from the Available Instruments pane to the New System Instruments pane. Click Next.
 8. When the Access Control dialog box appears, click Next.
 9. In the Name Selection dialog box, specify a name for your system. Enter comments, if any, and then click Finish. A confirmation dialog box appears.
 10. Click Projects, right-click a project, and then select Open.
 11. In the Project window, click Run Samples .
- Alternative:** Access the Run Samples window via the Empower QuickStart menu.
12. On the Run Samples window, you can monitor control panels for the binary solvent manager, sample manager, detector, and optional column manager.

Control panels



About the binary solvent manager control panel

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

Binary solvent manager control panel



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

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

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

Binary solvent manager control panel items

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

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

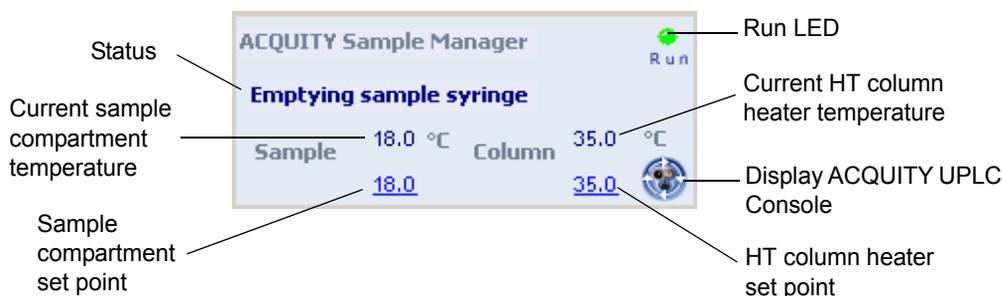
Additional functions in the binary solvent manager control panel

Control panel function	Description
Refresh system (Sys Prep)	Refreshes the fluid-carrying lines according to the current method conditions. See “Refreshing the system” on page 3-12.
Startup	Brings the system to operational conditions after an extended idle period or when switching to different solvents. See “Starting up the system” on page 3-13.
Prime A/B solvents	Displays the Prime A/B Solvents dialog box. See “Priming the binary solvent manager” on page 3-9.
Prime seal wash	Displays the Prime Seal Wash dialog box. See “Performing a seal wash prime” on page 3-7.
Reset BSM	Resets the binary solvent manager after an error condition.
Help	Displays the ACQUITY UPLC Console online Help.

About the sample manager control panel

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

Sample manager control panel



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

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

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

Sample manager control panel items

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

Sample manager control panel items (Continued)

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

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

Additional functions in the sample manager control panel

Control panel function	Description
Run Sys Prep	Primes the sample manager with one weak wash prime (wash and sample syringes). See “Refreshing the system” on page 3-12.
Prime syringes	Displays the Prime Syringes dialog box. See “Priming the sample manager” on page 3-17.
Wash needle	Displays the Wash Needle dialog box. See “Washing the sample manager needle” on page 3-19.
Turn lights on/off	Turns the sample manager compartment and optional sample organizer lights on or off.

Additional functions in the sample manager control panel (Continued)

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

About the TUV detector control panel

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

TUV detector control panel



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

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

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

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

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

TUV detector control panel items

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

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

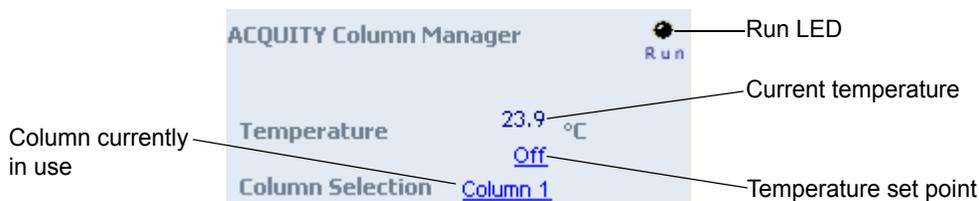
Additional functions in the detector control panel

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

About the column manager control panel

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

Column manager control panel



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

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

Column manager control panel items

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

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

Additional functions in the column manager control panel

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

Starting the ACQUITY UPLC Console from Empower software

To start the ACQUITY UPLC Console from Empower software

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

ACQUITY UPLC Console window

Configuring MassLynx software

Perform these tasks to configure MassLynx software:

- Start the application
- Select system instruments

To start MassLynx software

1. Select Start > Programs > MassLynx > MassLynx.

Alternative: Use the MassLynx desktop shortcut.

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

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

To select system instruments

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

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

Starting the ACQUITY UPLC Console from MassLynx software

To start the ACQUITY UPLC Console from MassLynx software

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

5 Verifying System Operation

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

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

Contents

Topic	Page
Preparing the system	5-2
Creating the test methods	5-5
Performing the gradient performance test	5-10

Restrictions:

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

Preparing the system

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

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

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

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

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

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

To prepare the system for verification



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

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

- d. Place the reservoir bottle in the solvent tray.



Caution: Never change directly between immiscible eluents or between buffered solutions and organic eluents. Immiscible eluents form emulsions in the flow path. Buffered solutions and organic eluents in combination can result in salt precipitation in the gradient proportioning valves, pump heads, check valves, or other parts of the system. Confirm that all fluids in the system are miscible with acetonitrile. If you need additional information about priming your system, see [“Priming the binary solvent manager” on page 3-9](#).

3. Install the ACQUITY UPLC column in the column heater, close the column tray, and replace the column heater’s front cover. If you need more information about installing the column, see [“Installing the column in an HT column heater” on page 2-46](#).
4. Access the ACQUITY UPLC Console and perform these tasks:
 - a. Prime each solvent line of the binary solvent manager for 10 minutes. See [“Priming a wetted binary solvent manager” on page 3-12](#).
 - b. Prime the seal wash of the binary solvent manager for several minutes. See [“Priming a wetted binary solvent manager” on page 3-12](#).
 - c. Prime the sample manager at least 5 times. See [“Priming the sample manager” on page 3-17](#).
 - d. Perform the seal characterization procedure in the sample manager. See [“Characterizing the needle seal” on page 3-21](#).
 - e. Calibrate the needle and loop volume in the sample manager.
Alternative: Use the Refresh (Sys Prep) function to prime the binary solvent manager. See [“Refreshing the system” on page 3-12](#).
5. Prepare the sample as listed on the sample instructions, using 10:90 acetonitrile/water.
6. Place the sample in the vial plate, noting the vial position, and put the plate in position 2 of the sample manager.

Creating the test methods

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

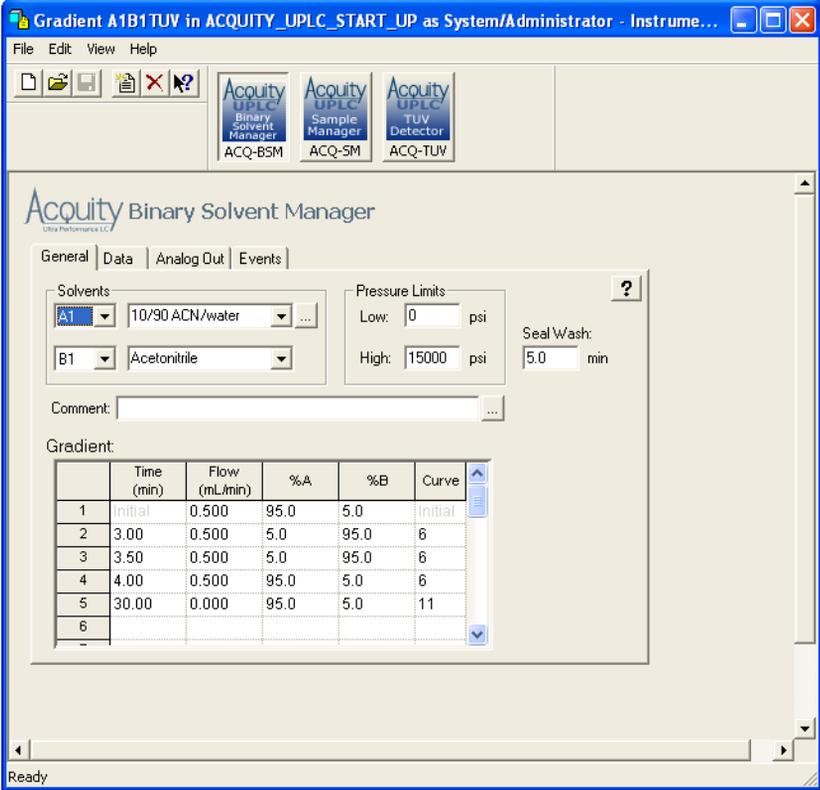
Tip: Click  on the tabs to display online Help.

Creating the instrument method

To create the instrument method

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

Binary solvent manager instrument parameters

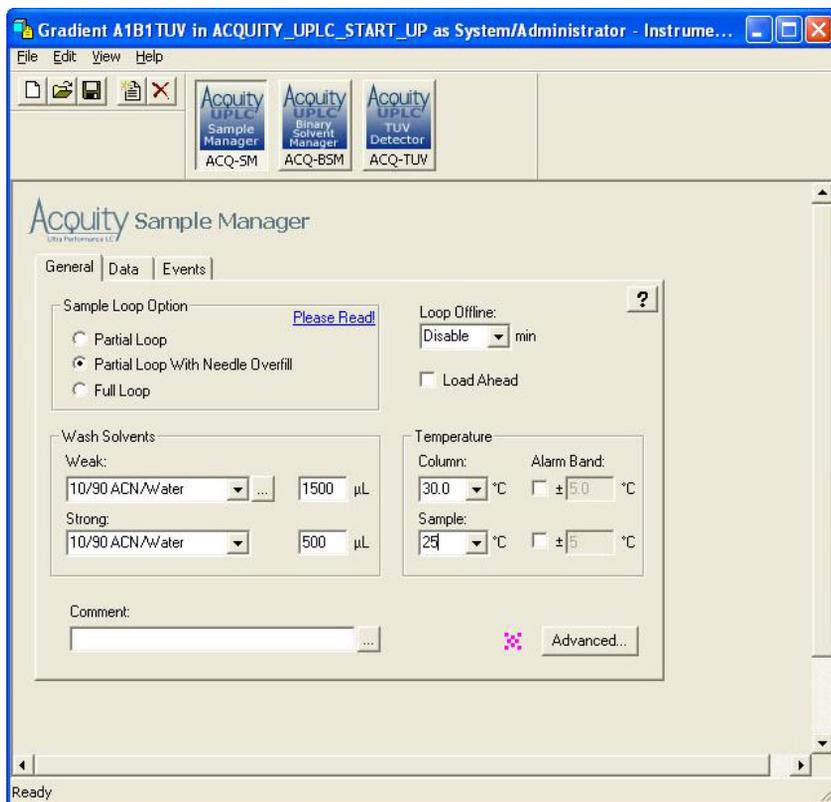


	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.500	95.0	5.0	Initial
2	3.00	0.500	5.0	95.0	6
3	3.50	0.500	5.0	95.0	6
4	4.00	0.500	95.0	5.0	6
5	30.00	0.000	95.0	5.0	11
6	-	-	-	-	-

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

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

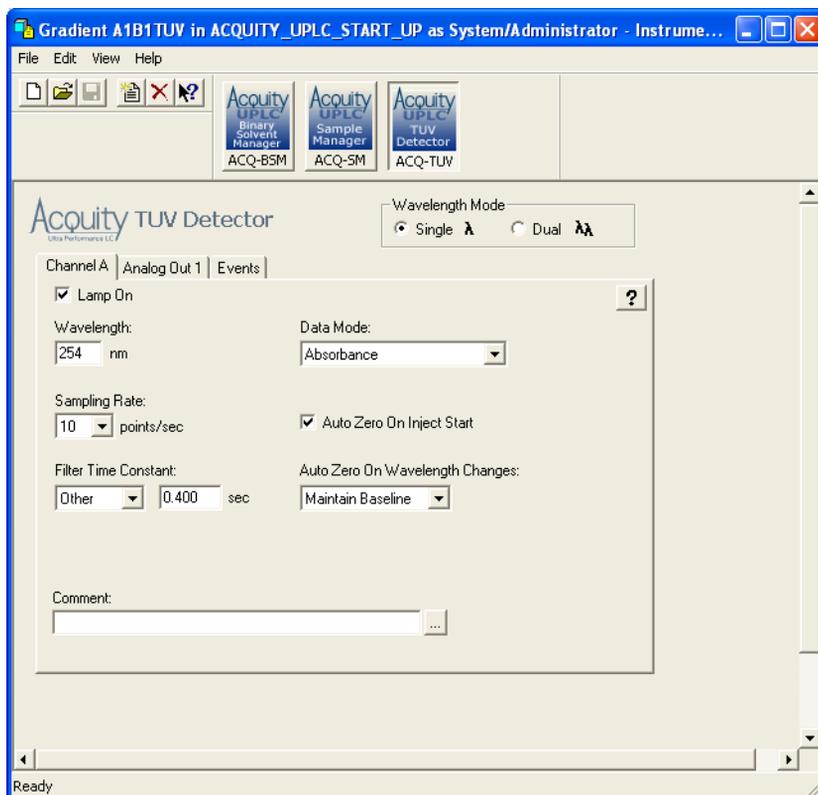
Sample manager instrument parameters



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

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

TUV detector instrument parameters



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

See also:

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

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

Creating the sample set method

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

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

To create the sample set method

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

Performing the gradient performance test

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

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

To perform the test

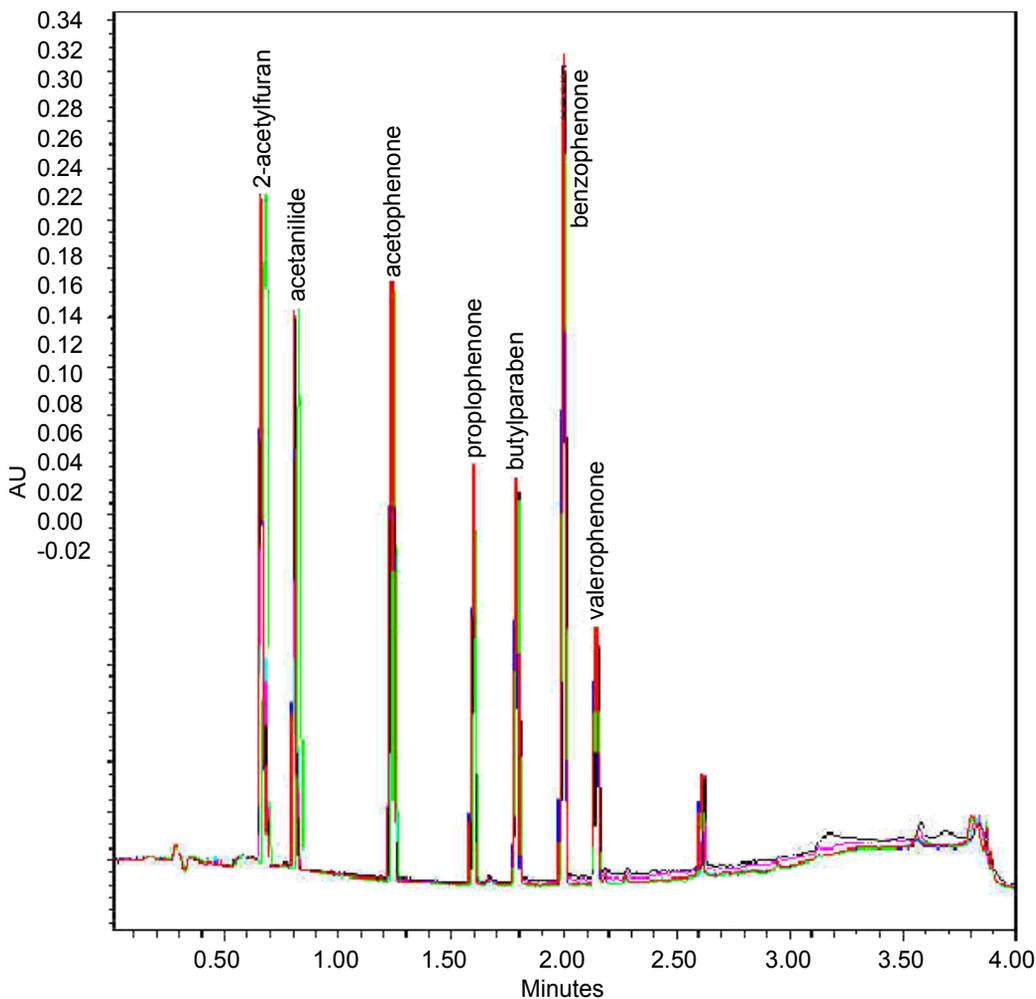
1. Start the run:
 - If the system is controlled by Empower software, open the project in Run Samples, select the gradient performance test sample set, and then select Run and Report.
 - If the system is controlled by MassLynx software, access the MassLynx main page, and select Start from the Run menu.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

Retention time reproducibility (three replicates)

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

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

Sample gradient performance test chromatogram



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

6 Maintaining the System

Contents

Topic	Page
Maintenance schedule	6-2
Spare parts	6-5
Contacting Waters technical service	6-6
Maintenance considerations	6-7
Configuring maintenance warnings	6-8
Maintaining the binary solvent manager	6-9
Maintaining the sample manager	6-122
Maintaining the HT column heater	6-162
Maintaining the column manager and column heater/cooler	6-180
Maintaining the TUV detector	6-203
Maintaining the sample organizer	6-225
Maintaining the 30-cm column heater/cooler	6-229

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

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

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

Maintenance schedule

Waters recommends that you perform the following routine maintenance on the ACQUITY UPLC[®] system to ensure reliable operation and accurate results. When using the system throughout the day (and on nights and weekends), or when using aggressive solvents such as buffers, perform these maintenance tasks more frequently.

Recommended routine maintenance schedule

Maintenance procedure	Frequency	For information...
Clear leak sensor error message	As needed	See page 6-10 , page 6-123 , page 6-162 , page 6-167 , page 6-180 , and page 6-204
Replace the leak sensors on the system instruments	As needed	See page 6-15 , page 6-128 , page 6-171 , page 6-174 , page 6-184 , and page 6-208
Replace the mixer on the binary solvent manager	During scheduled preventive maintenance or as needed	See page 6-19
Replace the <i>i</i> ² Valve actuator on the binary solvent manager	5 years from the date of manufacture or as needed	See page 6-21
Replace the <i>i</i> ² Valve cartridge on the binary solvent manager	During scheduled preventive maintenance or as needed	See page 6-32
Replace the in-line filter on the binary solvent manager	During scheduled preventive maintenance or as needed	See page 6-42

Recommended routine maintenance schedule (Continued)

Maintenance procedure	Frequency	For information...
Replace the accumulator check valve on the binary solvent manager	During scheduled preventive maintenance or as needed	See page 6-46
Replace solvent filters	During scheduled preventive maintenance or as needed	See page 6-49
Clean the air filters in the binary solvent manager door	As needed	See page 6-50
Replace the air filters in the binary solvent manager door	During scheduled preventive maintenance or as needed	See page 6-51
Replace the binary solvent manager head seals	During scheduled preventive maintenance or as needed	See page 6-52
Replace the binary solvent manager plungers	During scheduled preventive maintenance or as needed	See page 6-83
Replace the vent valve cartridge	As needed	See page 6-117
Defrost the sample compartment	As needed	See page 6-122
Replace the sample needle assembly on the sample manager	During scheduled preventive maintenance or as needed	See page 6-131
Calibrate the needle Z-axis on the sample manager	Whenever the needle is replaced	See page 6-139
Characterize the needle seal on the sample manager	Whenever the needle or needle seal is replaced	See page 6-140

Recommended routine maintenance schedule (Continued)

Maintenance procedure	Frequency	For information...
Characterize the needle and sample loop volumes on the sample manager	Whenever the needle or sample loop is replaced	See page 6-140
Replace the puncture needle holder on the sample manager	As needed	See page 6-142
Replace the sample loop on the sample manager	During scheduled preventive maintenance or as needed	See page 6-145
Replace the sample syringe on the sample manager	During scheduled preventive maintenance or as needed	See page 6-149
Replace the wash syringes on the sample manager	During scheduled preventive maintenance or as needed	See page 6-152
Modify the sample syringe configuration parameters on the sample manager	As needed	See page 6-155
Replace the injection valve cartridge on the sample manager	During scheduled preventive maintenance or as needed	See page 6-156
Test the backpressure regulator	As needed	See page 6-159
Replace the filter in the column in-line filter unit on the column heater, HT column heater, column manager, or column heater/cooler	During scheduled preventive maintenance or as needed	See page 6-178
Replace the column in-line filter unit on the column heater or HT column heater	After you have used the six filters that accompanied the unit	See page 2-43

Recommended routine maintenance schedule (Continued)

Maintenance procedure	Frequency	For information...
Replace the column in-line filter unit on the column manager or column heater/cooler	After you have used the six filters that accompanied the unit	See page 2-44
Clean the flow cell on the TUV detector	As needed	See page 6-212
Perform a system acid cleansing flush	As needed	See page 6-215
Replace the flow cell on the TUV detector	As needed	See page 6-217
Replace the lamp on the TUV detector	During scheduled preventive maintenance or as needed	See page 6-221
Defrost the sample organizer	As needed	See page 6-225
Clean the air filter in the sample organizer	Twice every year under normal operating conditions or as needed	See page 6-225
Replace the fuses on the system instruments	As needed	See page 6-120 , page 6-159 , page 6-223 , page 6-226 , and page 6-229
Clean the system instruments with a soft, lint-free cloth, or paper dampened with water	As needed	See page 6-122 , page 6-161 , page 6-225 , page 6-228 , and page 6-230

Spare parts

Replace only the component parts mentioned in this document. See the Waters Quality Parts Locator on the Waters Web site's Services & Support page.

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). Otherwise, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Waters' site includes phone numbers and e-mail addresses for Waters locations worldwide. Visit www.waters.com, and click Waters Division > Regional/Global Contacts.

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

- Error message (if any)
- Nature of the symptom
- Instrument serial numbers (see [“Locating system serial numbers” on page 6-6](#))
- Flow rate
- Operating pressure
- Solvent(s)
- Detector settings (sensitivity and wavelength)
- Type and serial number of column(s)
- Sample type
- Empower or MassLynx software version and serial number
- ACQUITY UPLC system workstation model and operating system version

For complete information on reporting shipping damages and submitting claims, see the document *Waters Licenses, Warranties, and Support Services*.

Locating system serial numbers

Each system instrument's unique serial number facilitates service and support. Serial numbers also provide a way to create single log entries for each instrument so that you can review the usage history of only that instrument.

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

To view instrument information

1. In the ACQUITY UPLC Console, select an instrument from the system tree.
2. Click Configure > View module information. The Module Information dialog box displays this information:
 - Serial Number
 - Firmware Version
 - Firmware Checksum
 - Component Software Version

Alternatives:

- From the main window, place the pointer over the visual representation of the system instrument you want information for.
- Obtain the serial number from the printed labels on the rear panels of instruments or inside their front doors.

To view column information

1. In the ACQUITY UPLC Console, select Column from the system tree.
2. View the column part and serial numbers on the Column screen.

Alternative: View column part and serial numbers on the column.

Maintenance considerations

Safety and handling

Observe these warning and caution advisories when you perform maintenance operations on your system.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the system. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



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



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

Proper operating procedures

To ensure your system runs efficiently, follow the operating procedures and guidelines in [Chapter 3](#) and [Chapter 5](#).

Configuring maintenance warnings

Maintenance counters provide real-time usage status information that can help you determine when to schedule routine maintenance for specific components. You can set usage thresholds and maintenance warnings that alert you when a component reaches the designated threshold limit. By setting threshold limits and monitoring these usage counters regularly, you can minimize unexpected failures and unscheduled downtime during important work. For information on setting maintenance warnings, consult the ACQUITY UPLC Console online Help.

Maintenance warnings can be set for these conditions:

- Solvent manager pumped volume in liters – The binary solvent manager requires regular maintenance, the frequency of which depends on the volume of fluid it pumps. You can set a maintenance warning to alert you when the pumped fluid reaches a specified volume (pumped fluid volume threshold).
- Number of sample manager injection cycles – The sample manager requires regular maintenance, the frequency of which depends on how many injections it makes. You can set a maintenance warning to alert you when the injection count reaches a specified count (injections threshold).
- Detector lamp life in hours – The detector's lamp requires regular replacement, the frequency of which depends on its operating time. You can set a maintenance warning to alert you when the lamp has operated for a specified number of hours (time threshold).

- Total number of injections on the column – Column life expectancy varies with the kinds of samples and solvents you use and on your usage patterns, including the number of sample injections loaded onto the column. You can set a maintenance warning to alert you when the number of injections on the column reaches a threshold limit. Doing so helps prevent chromatographic results from degrading during an important run and protects the system against sudden pressure changes that sample buildup in the column can cause.
- Number of column manager switching valve cycles – The column manager's switching valve requires regular replacement, the frequency of which depends on how many cycles the switching valves make. You can set a maintenance warning to alert you when the valve reaches that number of cycles.

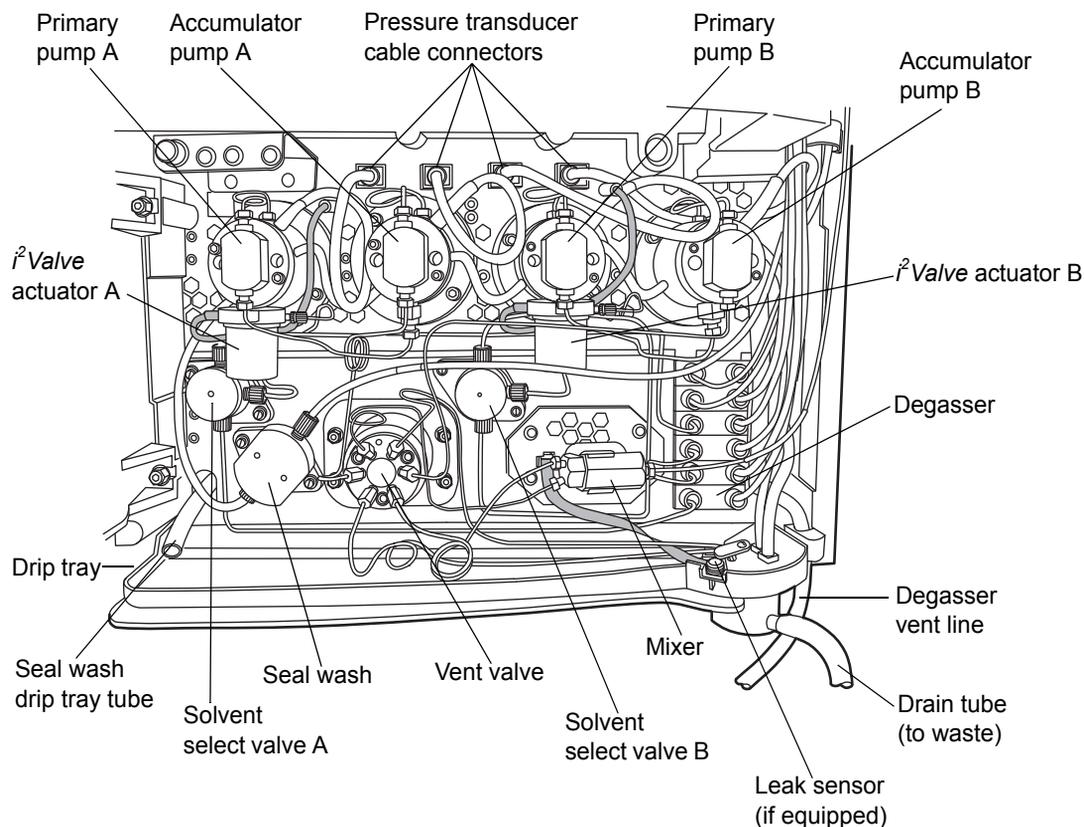
Maintaining the binary solvent manager

Perform the procedures in this section when you discover a problem with a binary solvent manager component or during preventive maintenance. For information about isolating problems in the binary solvent manager, consult the ACQUITY UPLC Console online Help.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.

Binary solvent manager major components



Resolving binary solvent manager leak sensor errors

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

To resolve a binary solvent manager leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to confirm that the binary solvent manager leak sensor detected a leak.

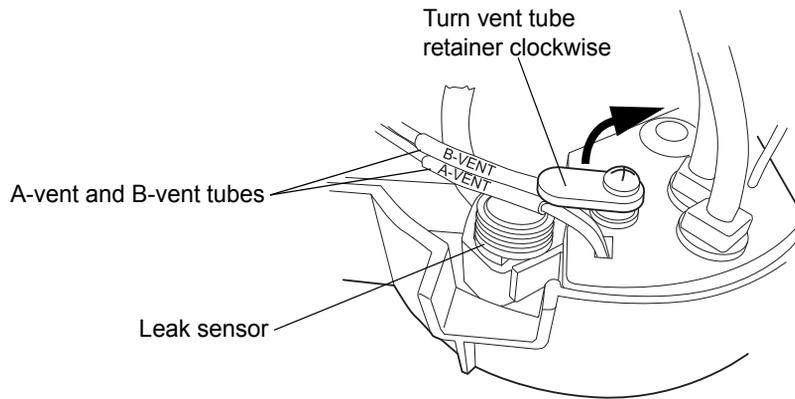
Tip: If a leak is detected, a “Leak Detected” error message appears.



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

2. Power-off the binary solvent manager.
3. Open the binary solvent manager’s door, gently pulling its right-hand edge toward you.
4. Locate the source of the leak, and make the repairs necessary to stop the leak.

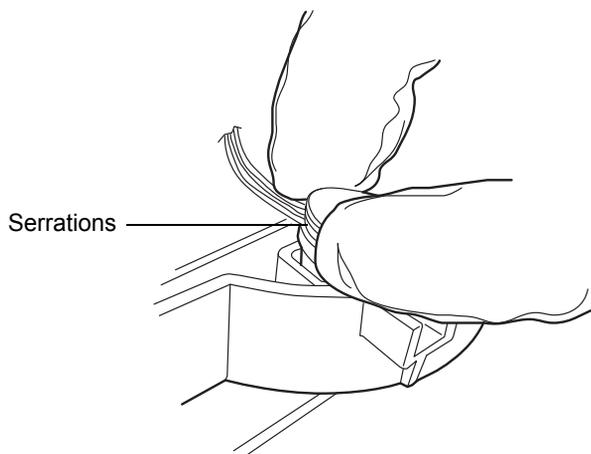
5. Turn the vent tube retainer clockwise, and then lift the A-vent and B-vent tubes out of the drip tray by pulling up on them and moving them to the left-hand side of the leak sensor.



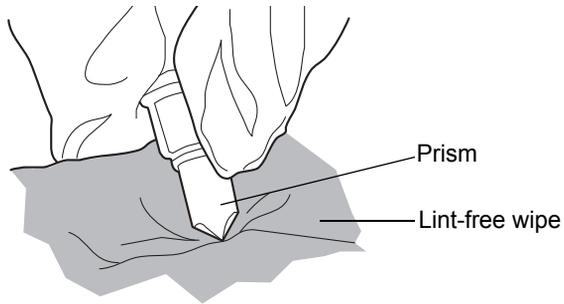
 **Caution:** To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

6. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

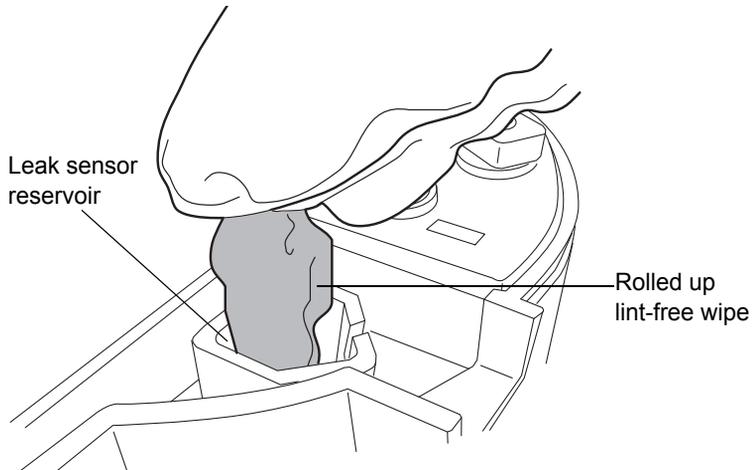
Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-16](#)).



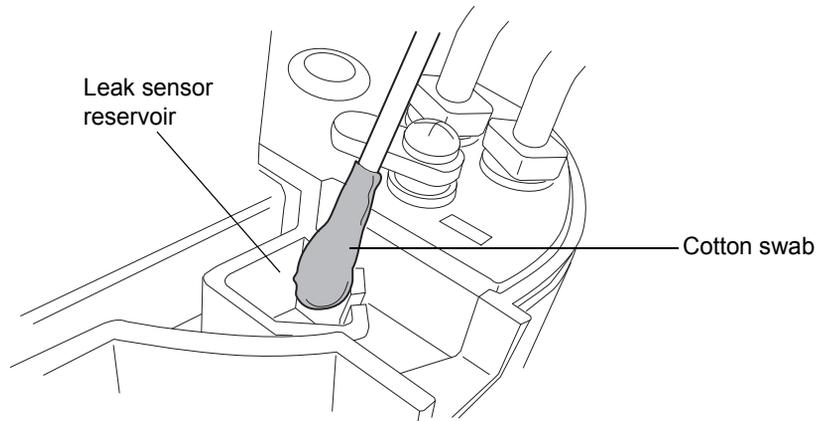
7. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



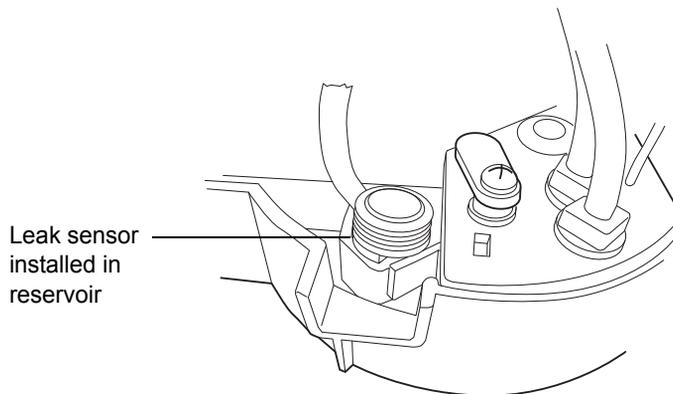
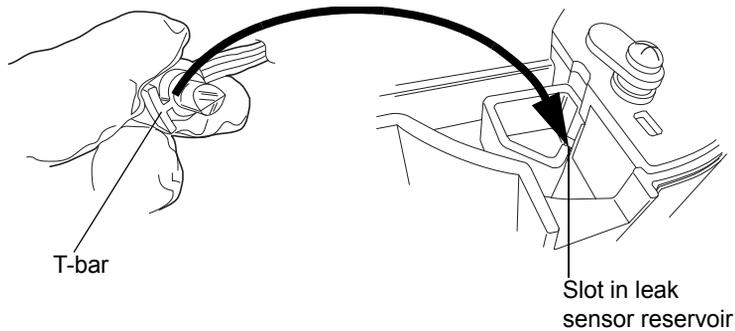
8. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



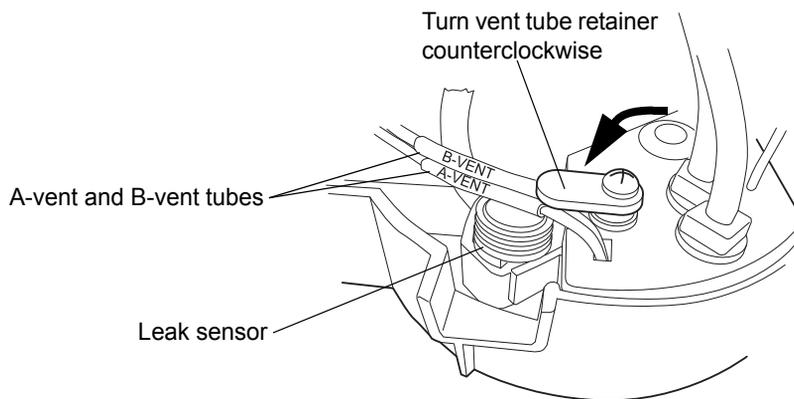
9. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



10. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



11. Reinsert the A-vent and B-vent tubes into the drip tray.
12. Turn the vent tube retainer, which holds the A-vent and B-vent tubing in place, counterclockwise.



13. If you detached the connector from the front of the instrument, reattach it.
14. Power-on the binary solvent manager.
15. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
16. In the binary solvent manager information window, click Control > Reset BSM, to reset the binary solvent manager.

Replacing the binary solvent manager's leak sensor



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

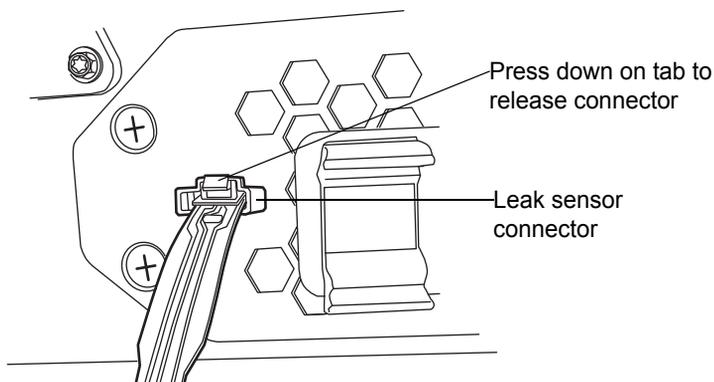
Required materials

- Clean, chemical-resistant, powder-free gloves
- Leak sensor

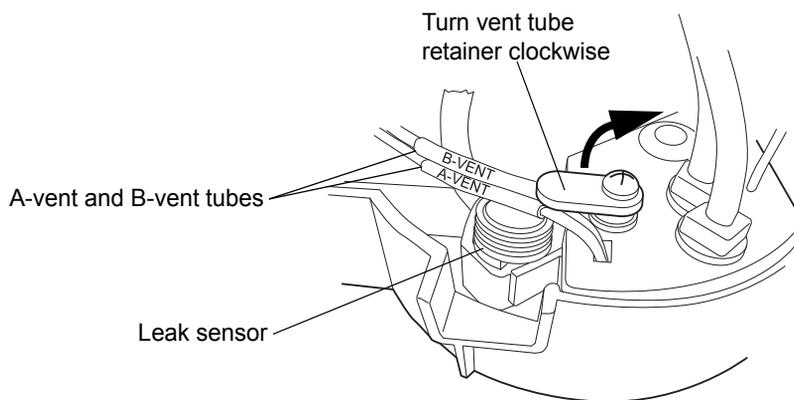
To replace the binary solvent manager's leak sensor

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

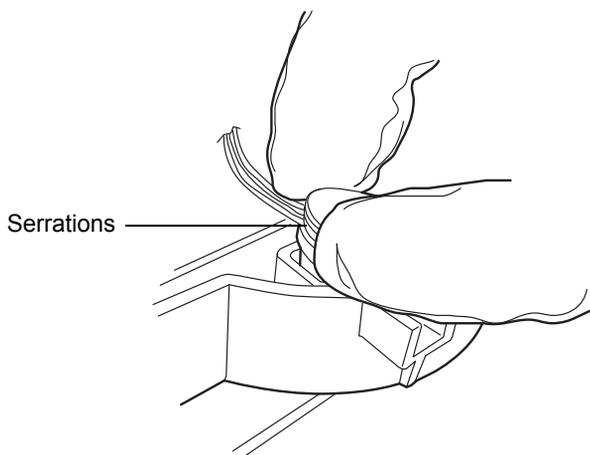
1. Power-off the binary solvent manager.
2. Open the binary solvent manager's door, gently pulling its right-hand edge toward you.
3. Press down on the tab to detach the leak sensor connector from the front of the instrument.



4. Turn the vent tube retainer clockwise, and then lift the A-vent and B-vent tubes out of the drip tray by pulling upward on them and moving them to the left-hand side of the leak sensor.

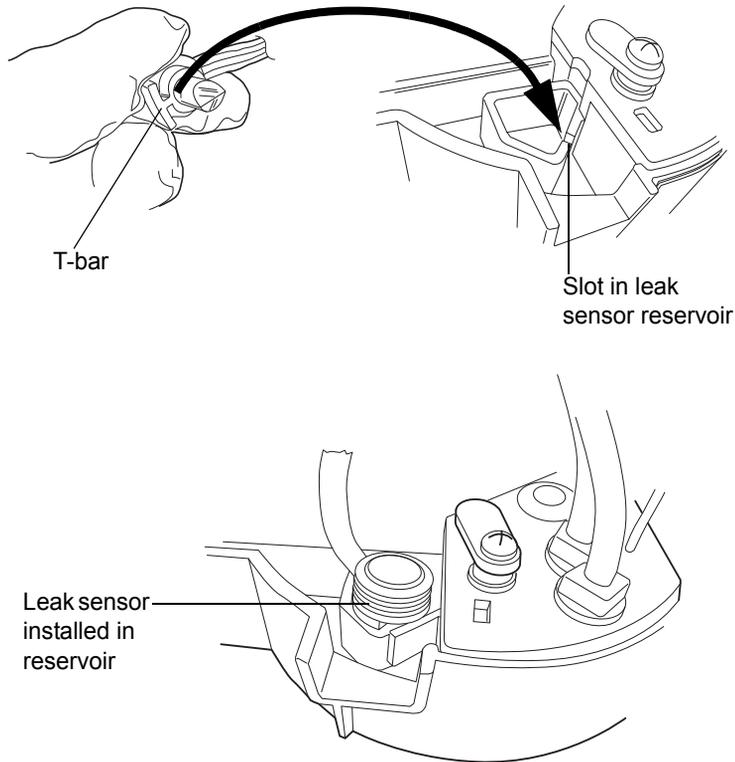


5. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

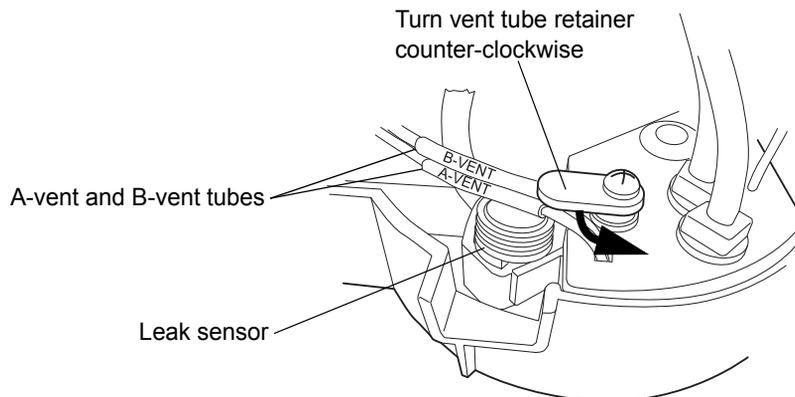


6. Unpack the new leak sensor.

7. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



8. Reinsert the A-vent and B-vent tubes into the drip tray.
9. Turn the vent tube retainer, which holds the A-vent and B-vent tubing in place, counterclockwise.



10. Connect the leak sensor connector to the front of the instrument.
11. Power-on the binary solvent manager.
12. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
13. In the binary solvent manager information window, click Control > Reset BSM, to reset the binary solvent manager.

Replacing the mixer



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the mixer.

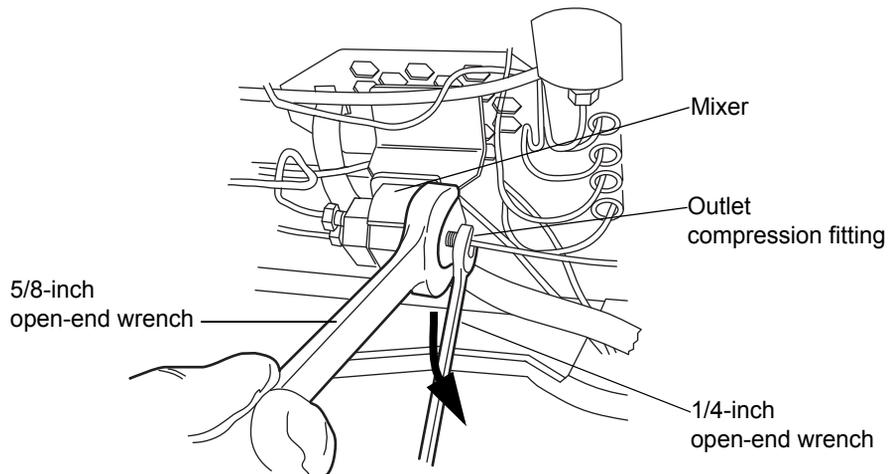
Required materials

- 1/4-inch open-end wrench
- 5/8-inch open-end wrench
- ACQUITY UPLC mixer
- Clean, chemical-resistant, powder-free gloves

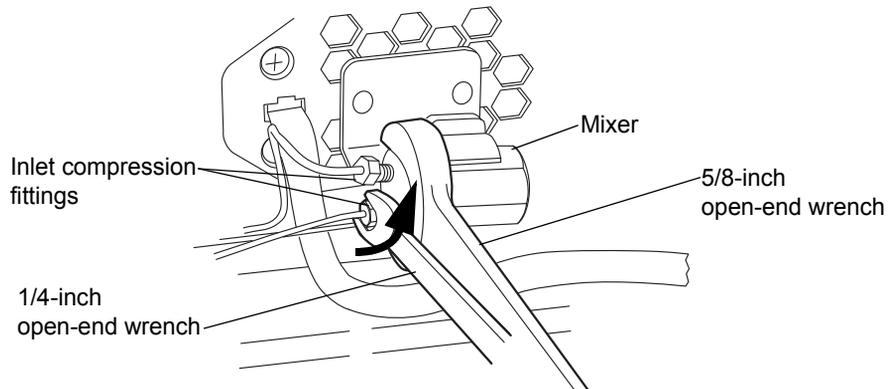
To replace the mixer

1. Flush the binary solvent manager with non-hazardous solvent.
2. Stop the solvent flow.

- Using the 5/8-inch open-end wrench to hold the mixer in place, disconnect the outlet compression fitting with the 1/4-inch open-end wrench.



- Again using the 5/8-inch open-end wrench to hold the mixer, disconnect the inlet compression fittings with the 1/4-inch wrench.



- Remove the old mixer from the bracket.
- Unpack the new mixer.
- Insert the new mixer into the bracket.
- Reattach the compression fittings to the mixer, and tighten them finger-tight plus an additional 1/4-turn for existing fittings or 3/4-turn for new fittings.

Replacing the *i*²Valve actuator



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution:

- To avoid damaging the *i*²Valve actuator, do not attempt to push or pull liquid or gas through the valve's inlet or outlet ports.
- To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the *i*²Valve actuator.

Required materials

- T8 TORX driver
- 5/16-inch open-end wrench
- Clean, chemical-resistant, powder-free gloves
- *i*²Valve actuator
- *i*²Valve cartridge (recommended)

To replace the *i*²Valve actuator



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

1. Flush the binary solvent manager with non-hazardous solvent.
2. Power-off the binary solvent manager.

Tip: The binary solvent manager is referred to as “pump” on the warning label affixed to the *i*²Valve actuator.



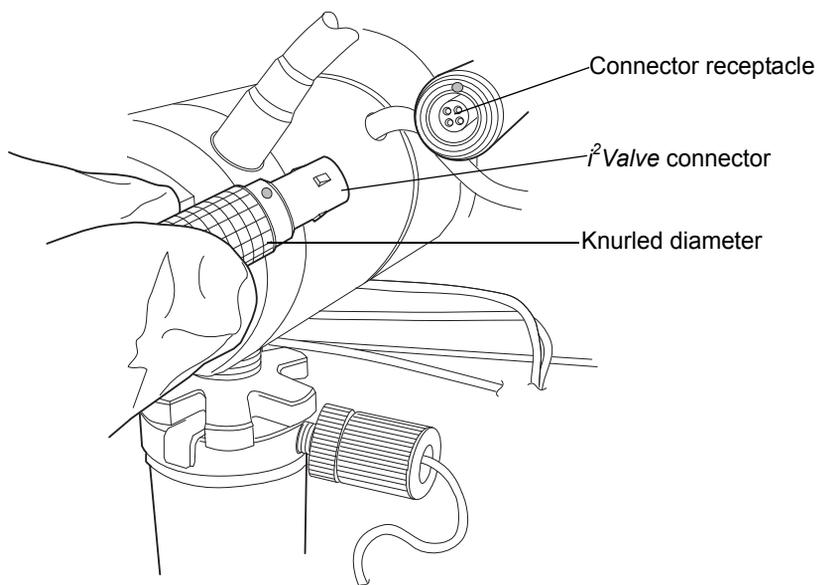
Warning: To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

3. Move the solvent bottles to a location below the binary solvent manager.

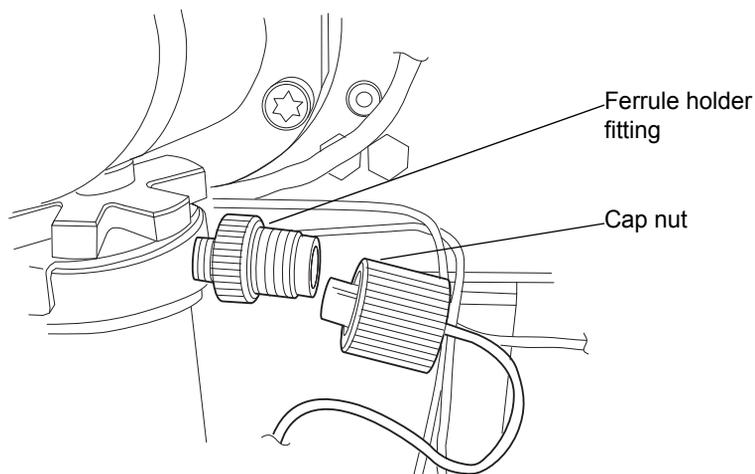
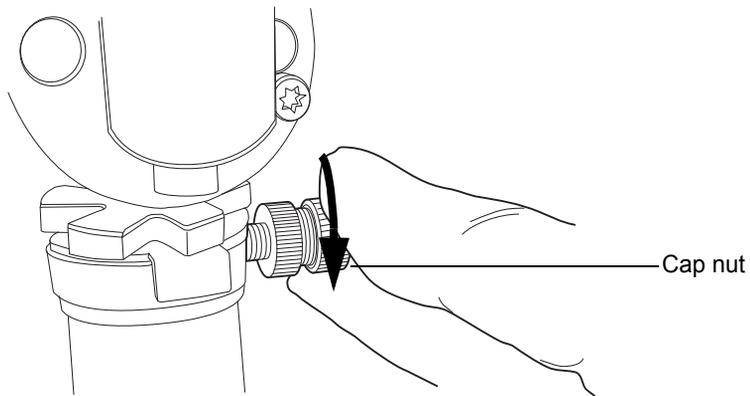


Caution: To avoid damage to the connector or cable, grasp the *i*²Valve connector by the knurled diameter.

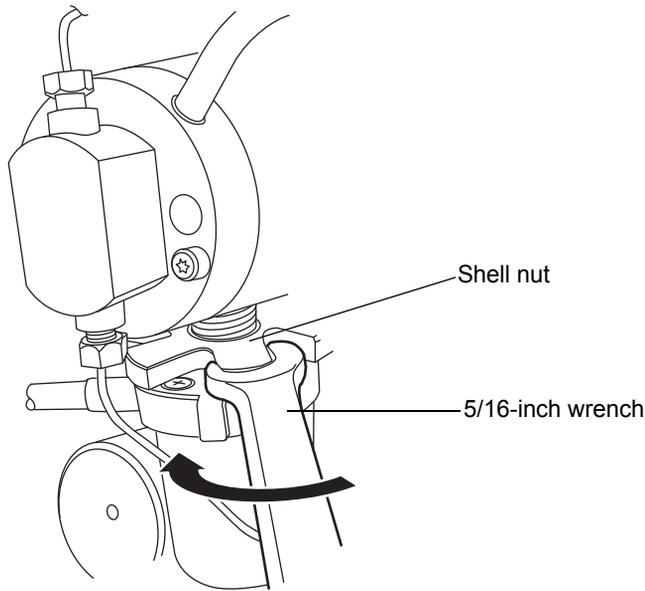
4. Grasp the *i*²Valve connector by the knurled diameter and pull it toward you to disconnect it from the receptacle.



5. Loosen the cap nut on the in-line filter so that it is all the way off the ferrule holder fitting threads.



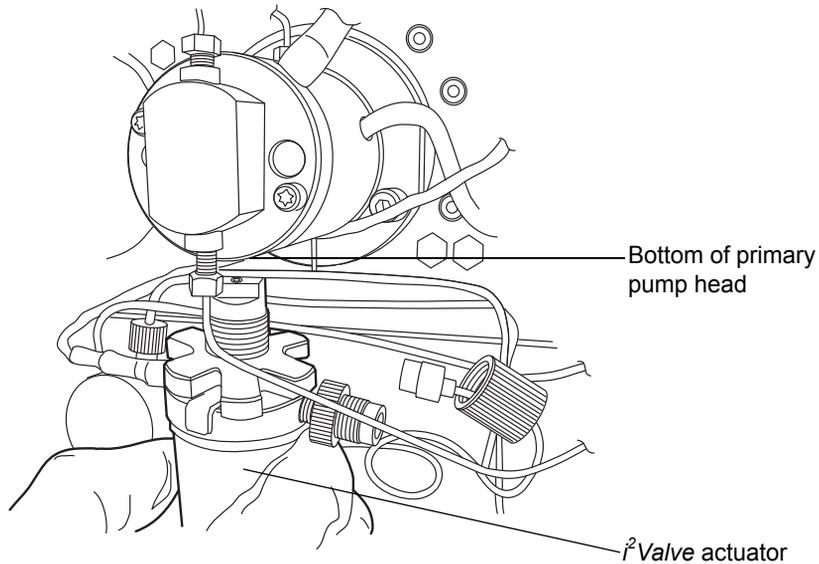
6. Use the 5/16-inch open-end wrench to loosen the shell nut and then fully unscrew it.



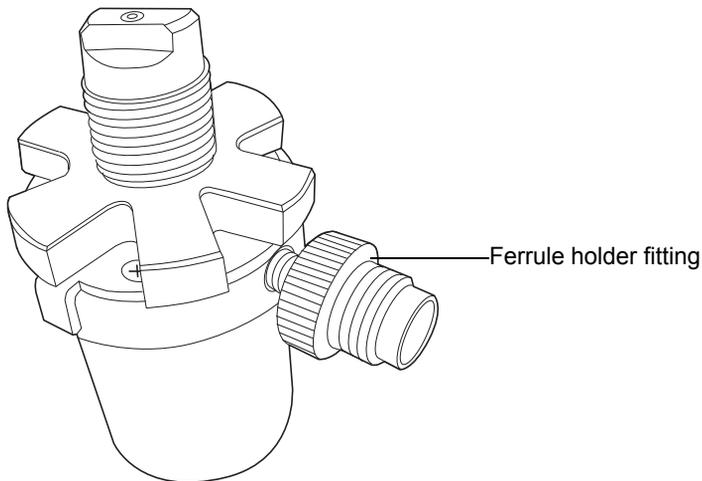
Caution:

- When you remove the valve assembly, ensure the PEEK washer, which is normally on the top face of the *i²Valve* cartridge, does not remain in the head. See [page 6-26](#).
- Never place the actuator assembly or electrical connector in the drip tray.

7. Remove the *i*²Valve actuator from the bottom of the primary pump head.



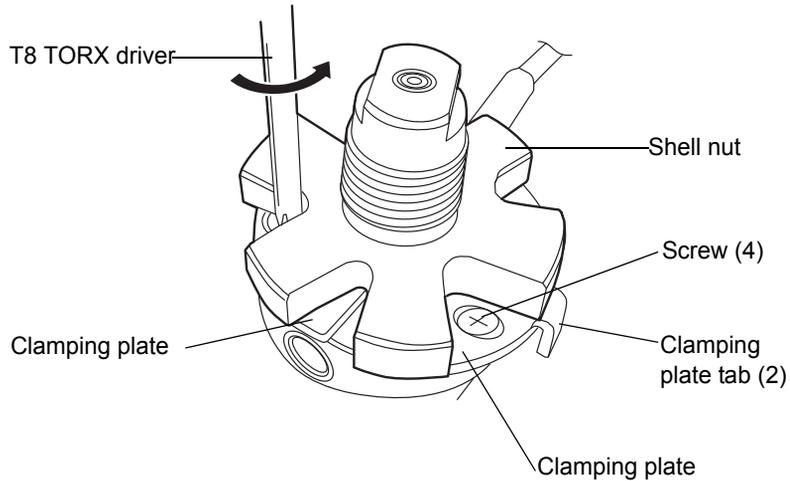
8. Remove the ferrule holder fitting from the old *i*²Valve actuator.



9. Use the T8 TORX driver to loosen 1/2-turn the 4 screws that secure the clamping plates. The shell nut must still be free to rotate and the plates should slide open.

Tips:

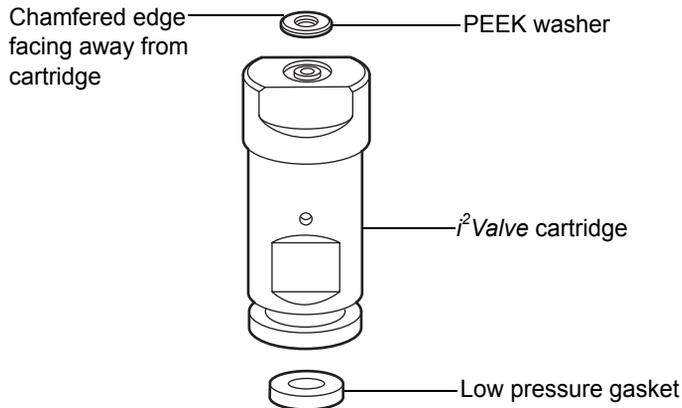
- Avoid touching the clamping plate tabs when loosening the screws.
- The shell nut can be rotated to access all 4 screws.



10. Remove the cartridge from the *i*² Valve actuator when both plates are in the maximum open position. Ensure that the low pressure gasket has been removed with the cartridge.

Tip: If you cannot remove the cartridge from the valve actuator, rotate the cartridge 1/2-turn and then remove it.

11. Make sure the PEEK washer is inserted into the cartridge so that its chamfered edge faces away from the cartridge.

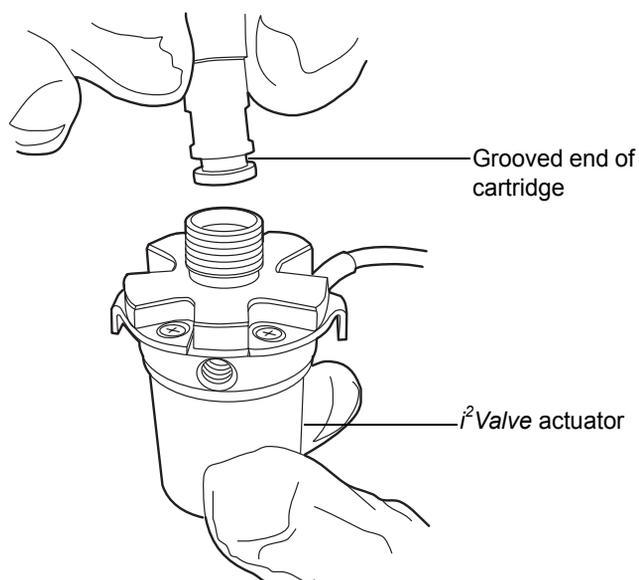


12. Use the T8 TORX driver to loosen 1/2-turn the 4 screws that secure the clamping plates on the new i^2 Valve actuator. Ensure the plates are loose and in their maximum open position.

Tips:

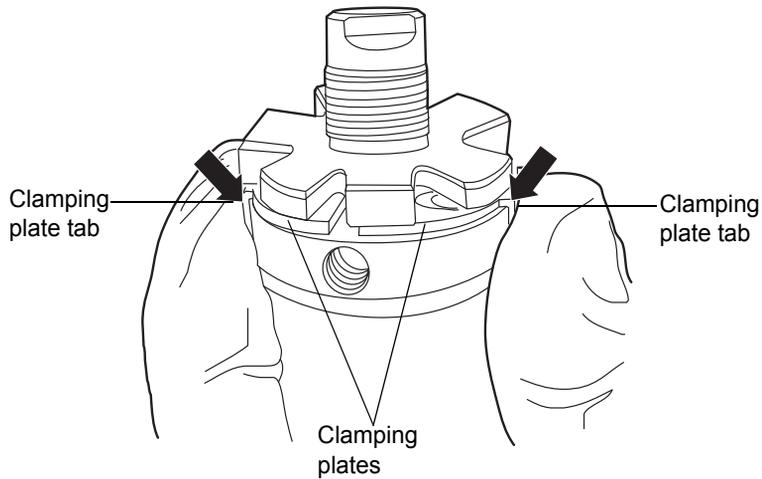
- Avoid touching the clamping plate tabs when loosening the screws.
 - The shell nut can be rotated to access all 4 screws.
13. Insert the cartridge you removed from the old i^2 Valve actuator into the new actuator, with the grooved end first.

Recommendation: Replace the cartridge whenever you replace the i^2 Valve actuator. See [step 11](#) on [page 6-37](#).

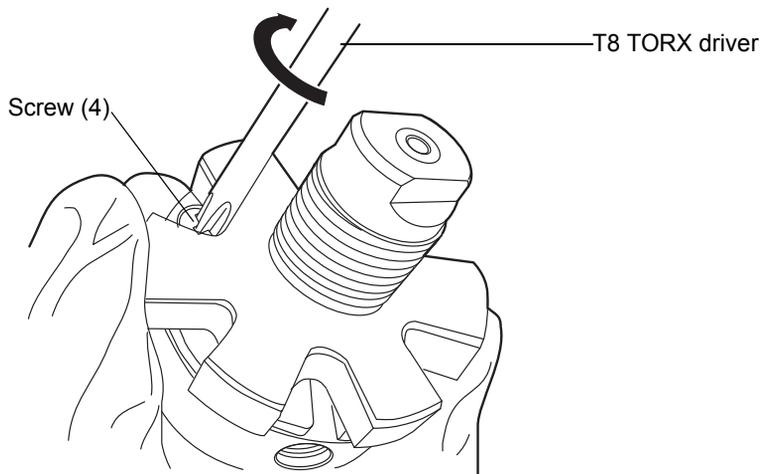


14. With one hand, squeeze the 2 clamping plate tabs on the i^2 Valve actuator to hold the clamping plates against the cartridge.

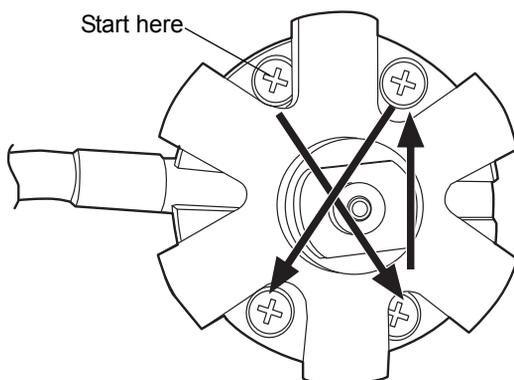
Requirement: The clamping plates must be fully engaged in the cartridge groove.



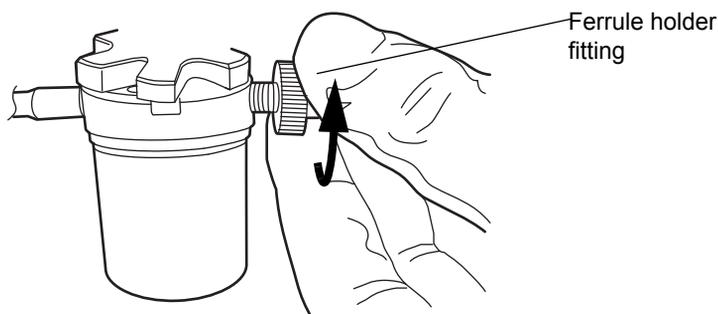
15. While squeezing the clamping plate tabs, use the T8 TORX driver to tighten the 4 screws that secure the plates, following the torquing pattern shown in [step 16](#).



16. Follow the pattern shown below when tightening the screws that secure the plates, and repeat it at least 3 times, gradually increasing the torque until the screws are tight.

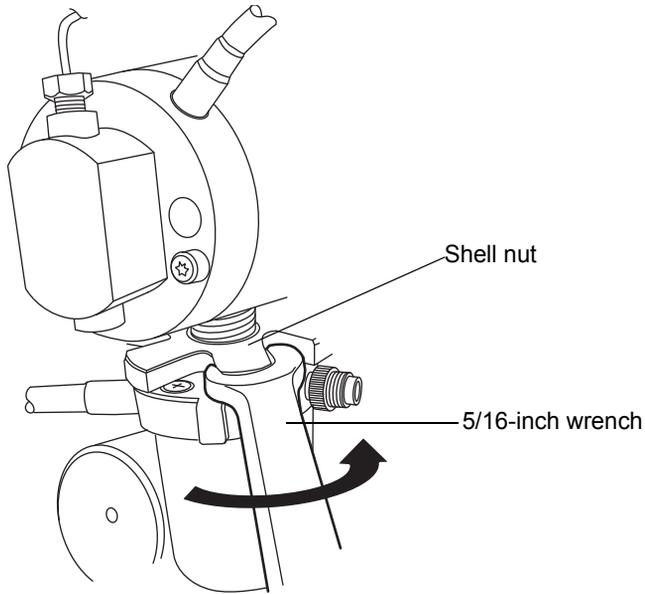


17. Insert the ferrule holder fitting into the inlet port on the *i*²Valve assembly, and tighten it finger-tight.

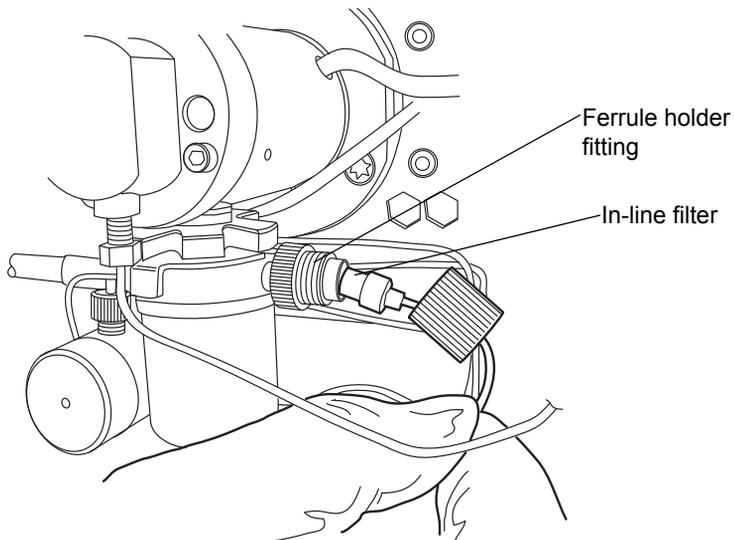


18. Orient the *i*²Valve assembly so that the cable exits from the left-hand side. Insert the *i*²Valve assembly into the bottom of the primary pump head, and route the cable behind the valve actuator.
19. Finger-tighten the shell nut to secure the valve. You should be able to rotate the shell nut approximately 5 full turns to reach the finger-tight

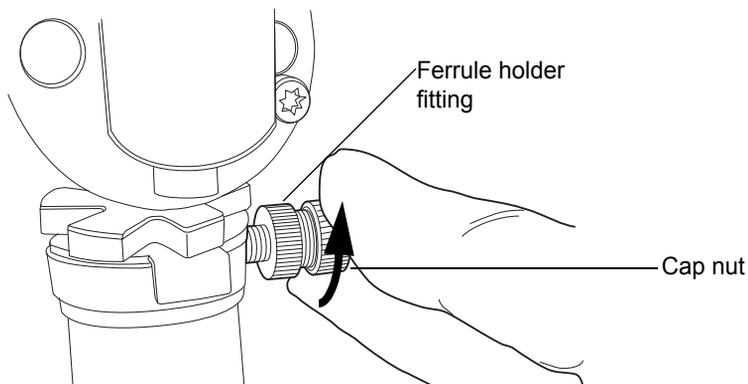
condition. Use the 5/16-inch open-end wrench to tighten the nut an additional 1/8-turn.



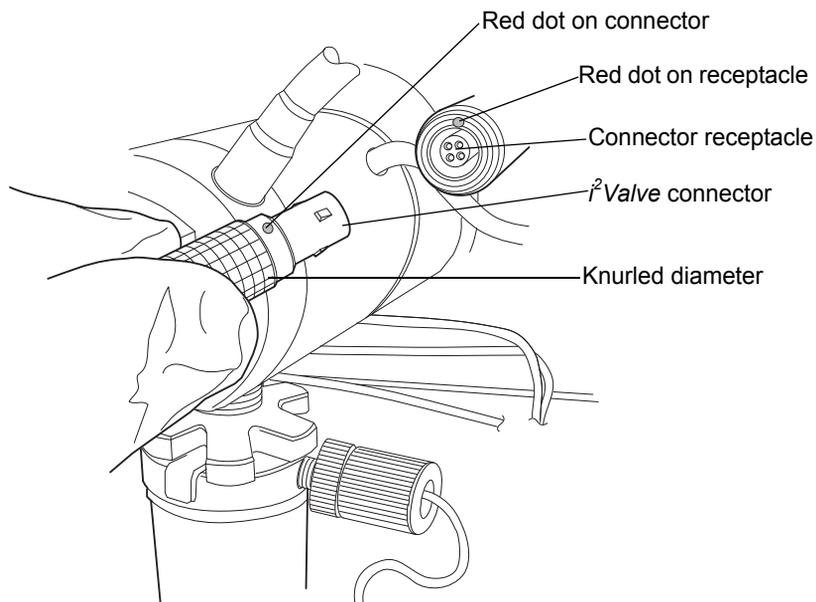
20. Reinsert the in-line filter and tube into the ferrule holder fitting.



21. Place the cap nut over the ferrule holder fitting and finger-tighten the cap nut to the extent possible.



22. Align the red dot on the *i*²Valve connector with the red dot on the receptacle, in the 12 o'clock position, and insert the connector into the receptacle.



23. Return the solvent bottles to their original location.
24. Power-on the binary solvent manager.
25. Prime the binary solvent manager (see [“Priming the binary solvent manager”](#) on page 3-9).

Replacing the *i*²Valve cartridge



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution:

- To avoid damaging the *i*²Valve actuator, do not attempt to push or pull liquid or gas through the valve's inlet or outlet ports.
- To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the *i*²Valve cartridge.

Required materials

- T8 TORX driver
- 5/16-inch open-end wrench
- Clean, chemical-resistant, powder-free gloves
- *i*²Valve cartridge

To replace the *i*²Valve cartridge



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

1. Flush the binary solvent manager with non-hazardous solvent.
2. Power-off the binary solvent manager.

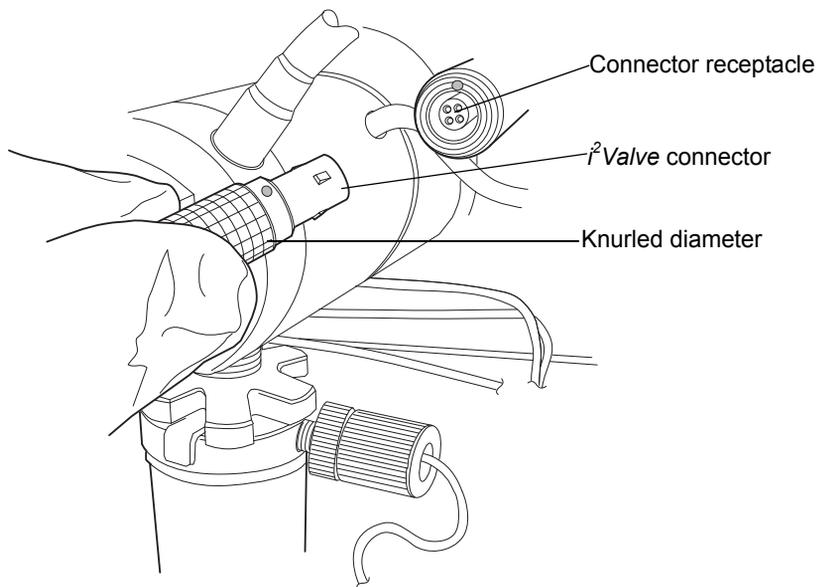
Tip: The binary solvent manager is referred to as “pump” on the warning label affixed to the *i*²Valve actuator.



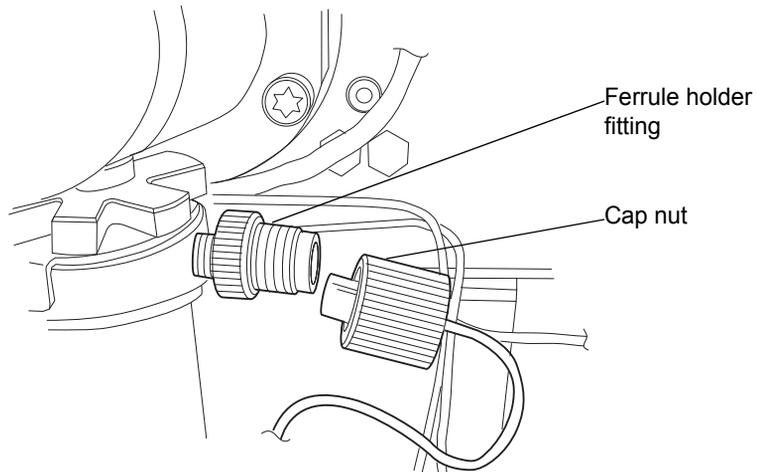
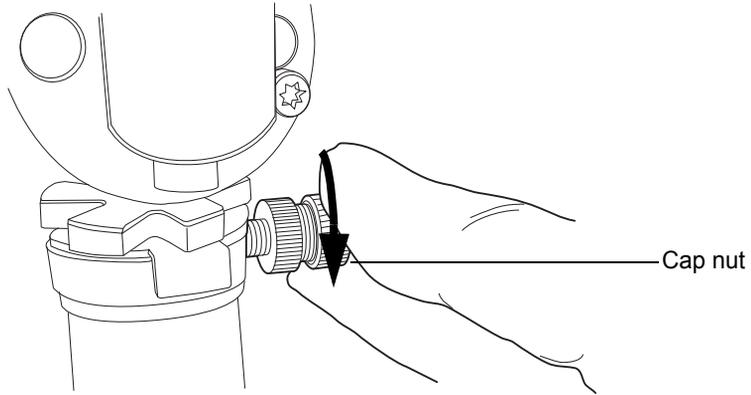
Warning: To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

3. Move the solvent bottles to a location below the binary solvent manager.

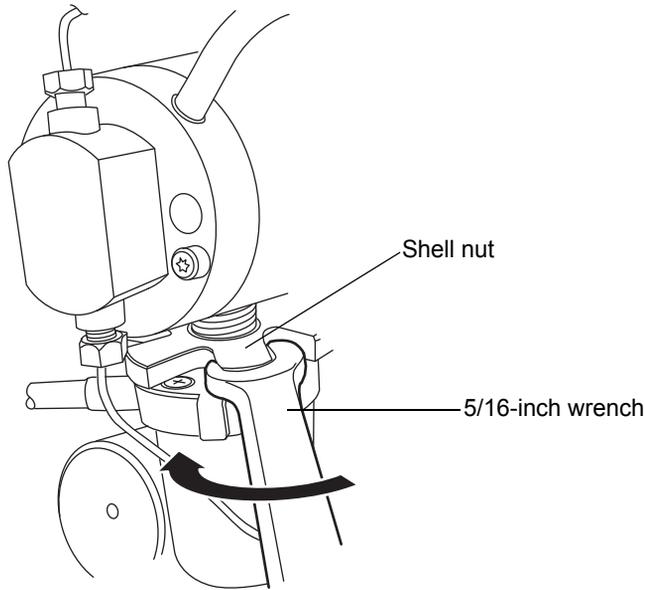
4. Grasp the *i*²Valve connector by the knurled diameter and pull it toward you to disconnect it from the receptacle.



5. Loosen the cap nut on the in-line filter so that it is all the way off the ferrule holder fitting threads.



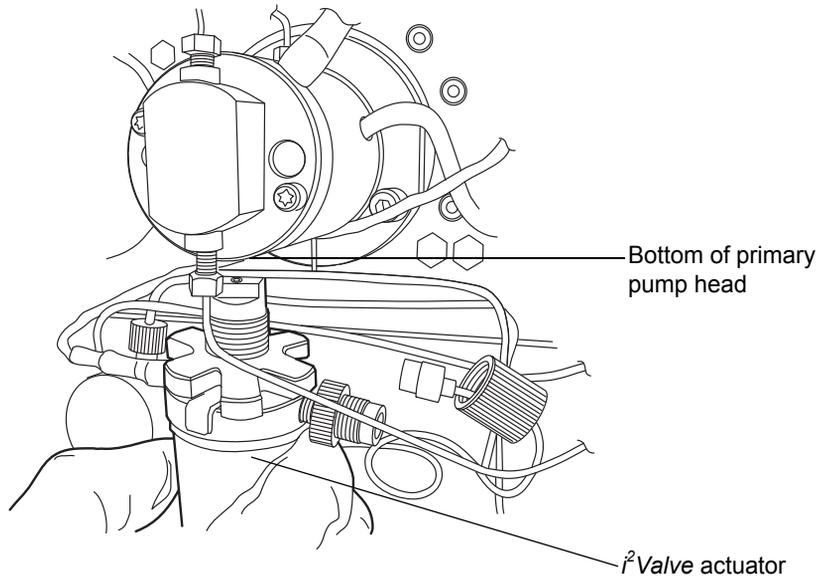
6. Use the 5/16-inch open-end wrench to loosen the shell nut and then fully unscrew it.



Caution:

- When you remove the valve assembly, ensure the PEEK washer, which is normally on the top face of the *i²Valve* cartridge, does not remain in the head. See [page 6-37](#).
- Never place the actuator assembly or electrical connector in the drip tray.

7. Remove the *i*²Valve actuator from the bottom of the primary pump head.

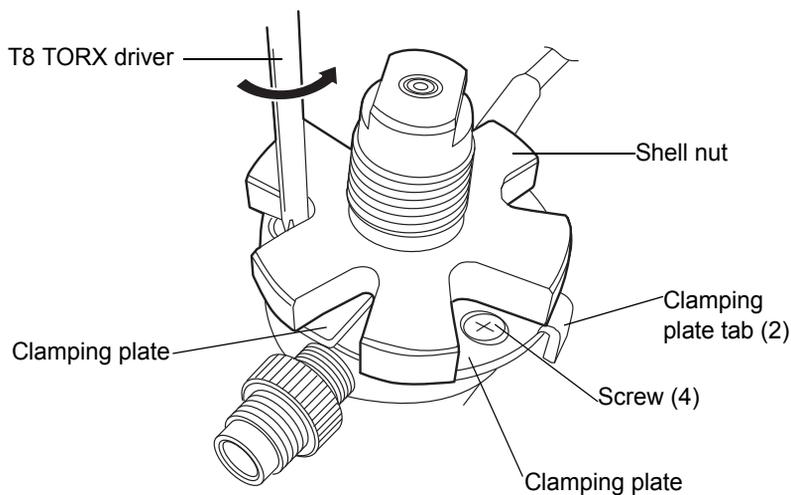


 **Caution:** To avoid damaging the *i*²Valve actuator, do not back the screws out all the way.

8. Use the T8 TORX driver to loosen 1/2-turn the 4 screws that secure the plates. The shell nut must still be free to rotate and the plates should slide open.

Tips:

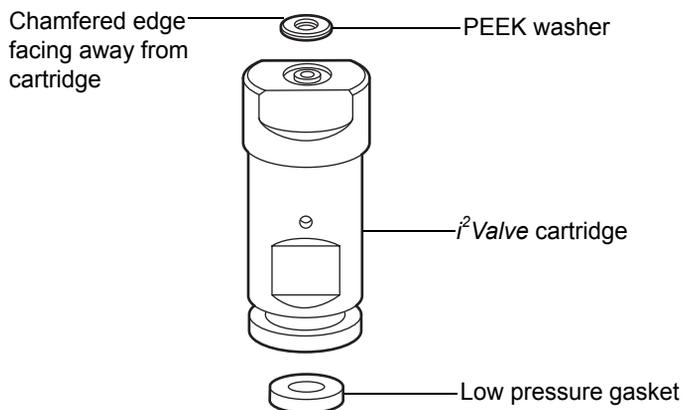
- Avoid touching the clamping plate tabs when loosening the screws.
- The shell nut can be rotated to access all 4 screws.



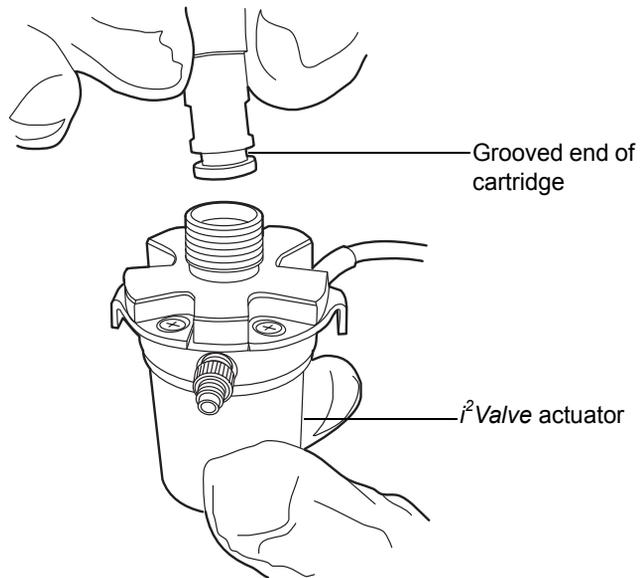
9. Remove the cartridge from the *i*² Valve actuator when both plates are in the maximum open position. Ensure that the low pressure gasket has been removed with the cartridge. See [page 6-37](#).

Tip: If you cannot remove the cartridge from the valve actuator, rotate the cartridge 1/2-turn and then remove it.

10. Unpack the new cartridge.
11. Make sure the PEEK washer is inserted into the cartridge so that its chamfered edge faces away from the cartridge.

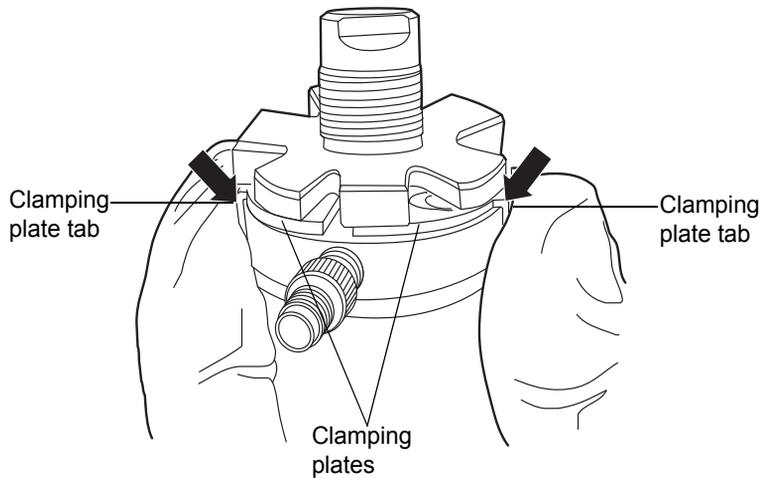


12. With the clamping plates still open, insert the cartridge into the *i*²Valve actuator, grooved end first.

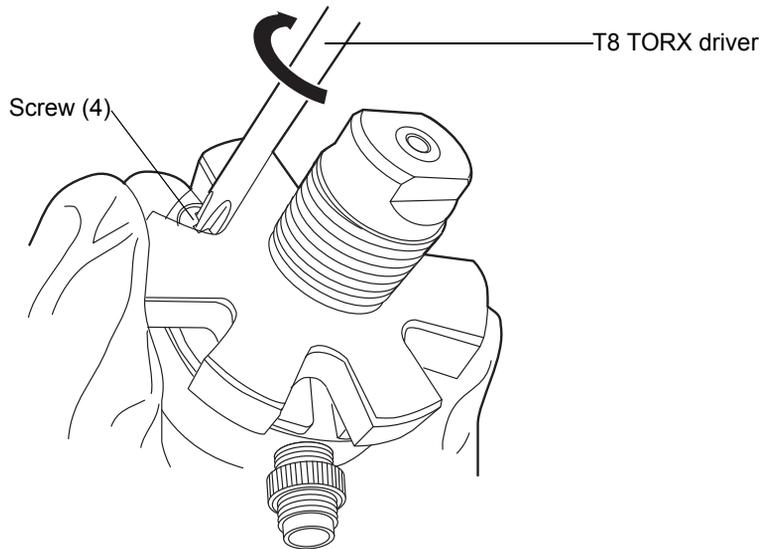


13. With one hand, squeeze the 2 clamping plate tabs on the *i*²Valve actuator to hold the clamping plates against the cartridge.

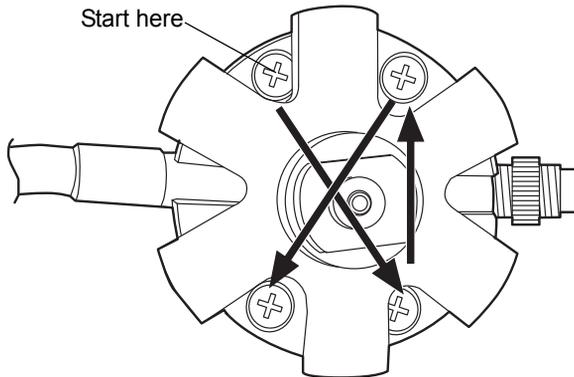
Requirement: The clamping plates must be fully engaged in the cartridge groove.



14. While squeezing the clamping plate tabs, use the T8 TORX driver to tighten the 4 screws that secure the plates, following the torquing pattern shown in [step 15](#).

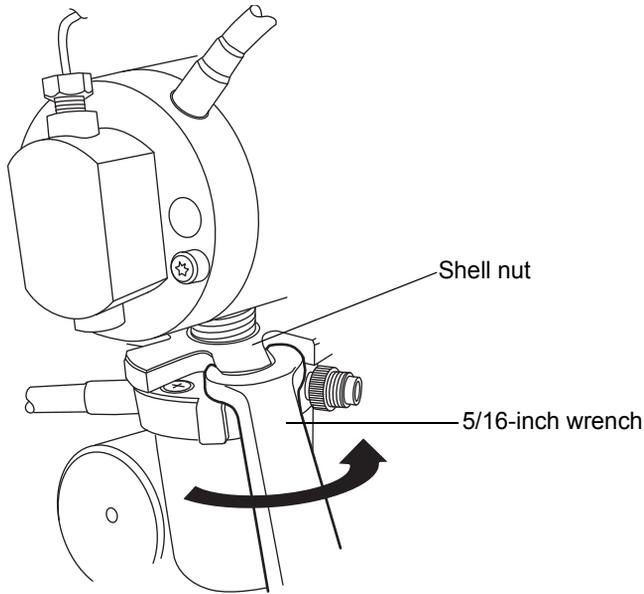


15. Follow the pattern shown below when tightening the screws that secure the plates, and repeat it at least 3 times, gradually increasing the torque until the screws are tight.

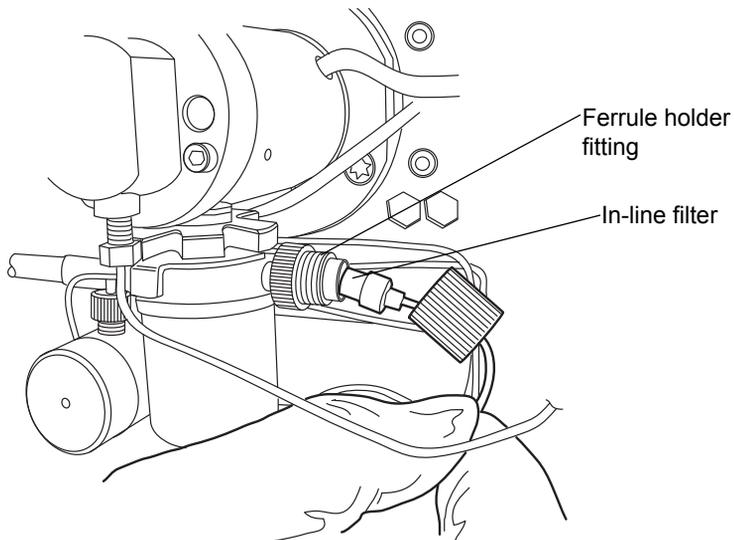


16. Orient the i^2 Valve assembly so that the cable exits from the left-hand side. Insert the i^2 Valve assembly into the bottom of the primary pump head, and route the cable behind the valve actuator.
17. Finger-tighten the shell nut to secure the valve. You should be able to rotate the shell nut approximately 5 full turns to reach the finger-tight

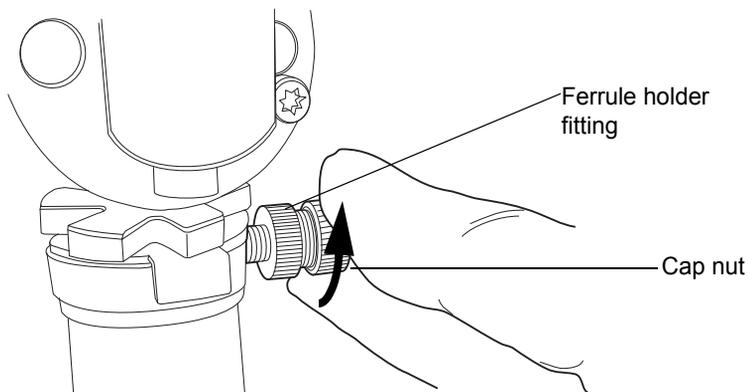
condition. Use the 5/16-inch open-end wrench to tighten the nut an additional 1/8-turn.



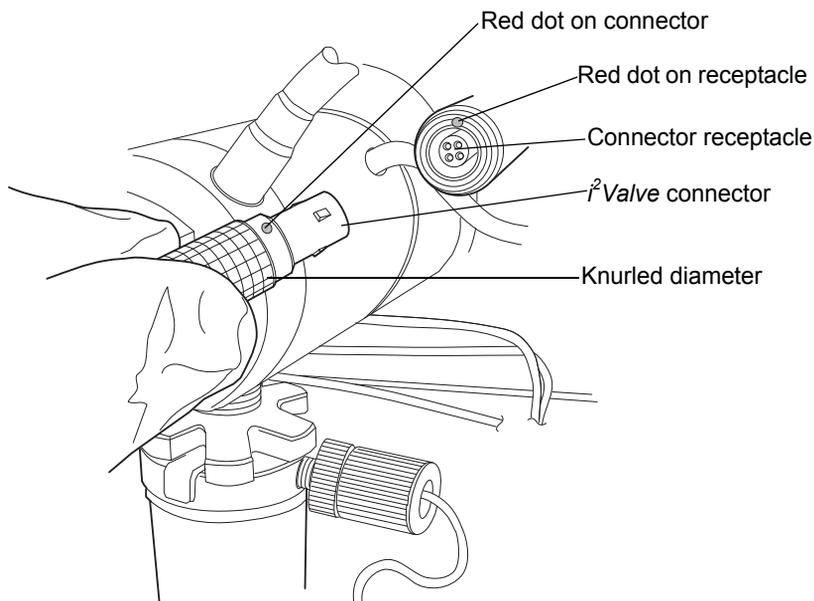
18. Reinsert the in-line filter and tube into the ferrule holder fitting.



19. Place the cap nut over the ferrule holder fitting and finger-tighten the cap nut to the extent possible.



20. Align the red dot on the *i*²Valve connector with the red dot on the receptacle, in the 12 o'clock position, and insert the connector into the receptacle.



21. Return the solvent bottles to their original location.
22. Power-on the binary solvent manager.
23. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Replacing the in-line filter cartridge on the *i*²Valve actuator



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: Wear clean, chemical-resistant, powder-free gloves when handling the in-line filter cartridge. Oil from your hands can contaminate the in-line filter cartridge.

Required materials

- Clean, chemical-resistant, powder-free gloves
- In-line filter cartridge

To replace the in-line filter



Caution: To avoid damaging the *i*²Valve actuator, do not attempt to push or pull liquid or gas through the valve's inlet or outlet ports.

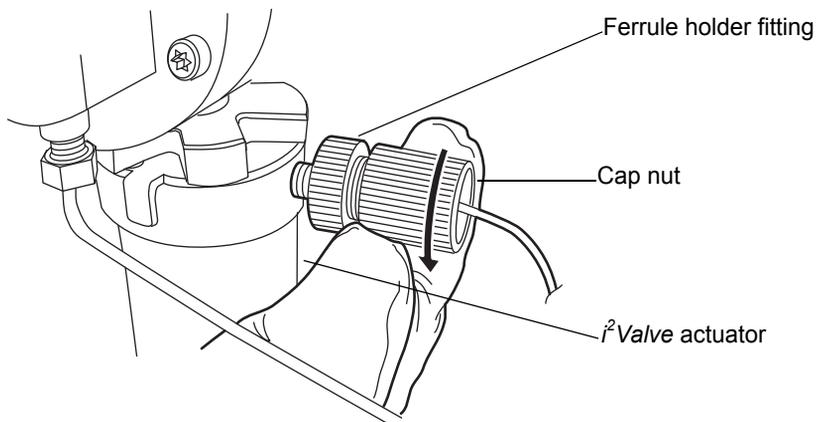
1. Flush the binary solvent manager with non-hazardous solvent.
2. Power-off the binary solvent manager.



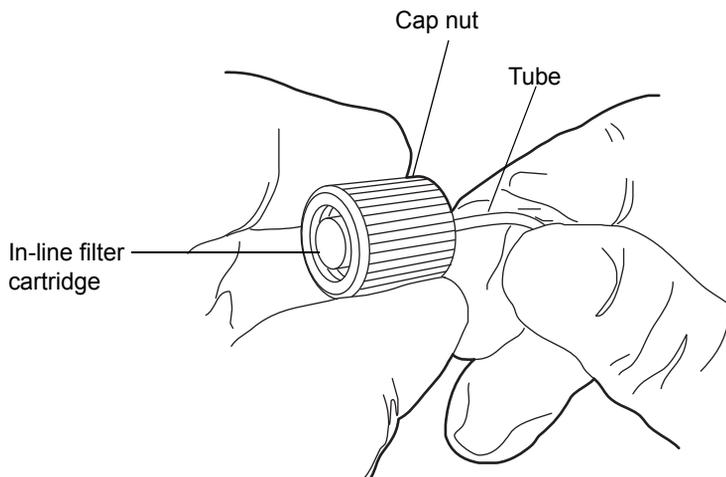
Warning: To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

3. Move the solvent bottles to a location below the binary solvent manager.

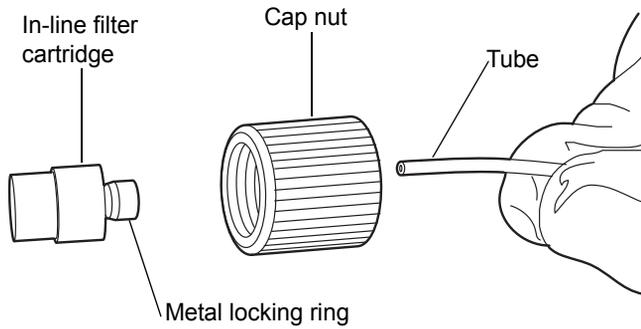
4. Unscrew the cap nut on the in-line filter assembly.



5. Pull the cap nut off the tube to remove the in-line filter cartridge.

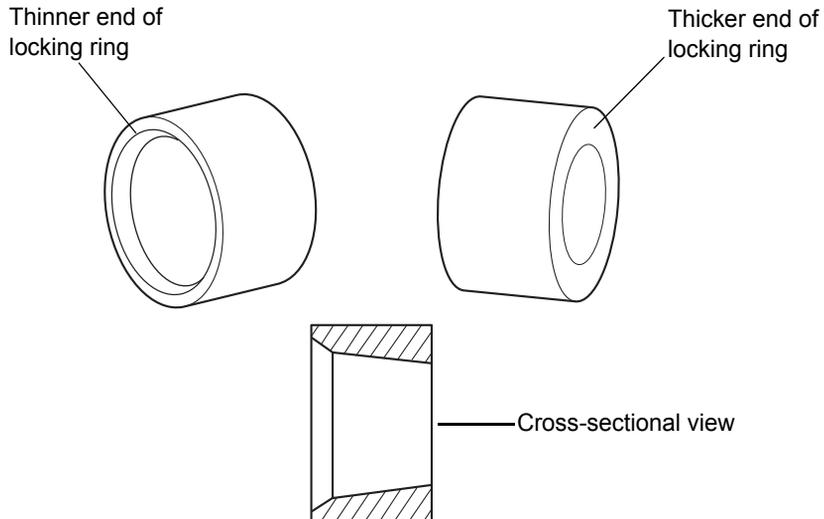


In-line filter cartridge and cap nut



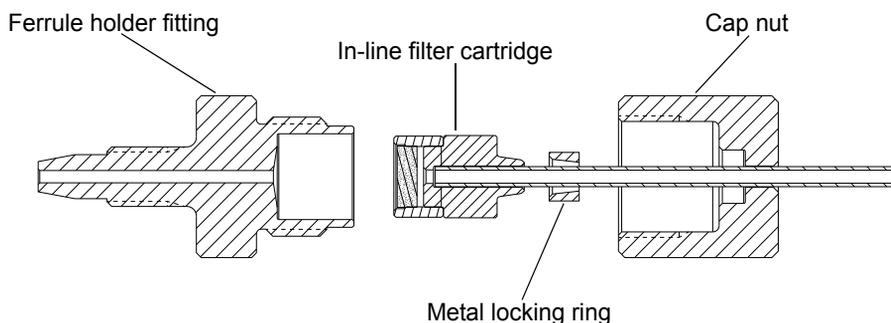
6. Put the cap nut over the end of the tube. See the [figure “Exploded view of in-line filter” on page 6-45](#).
7. Slide the metal locking ring onto the tube. Ensure that the thicker end of the metal locking ring is facing toward the cap nut.

Metal locking ring

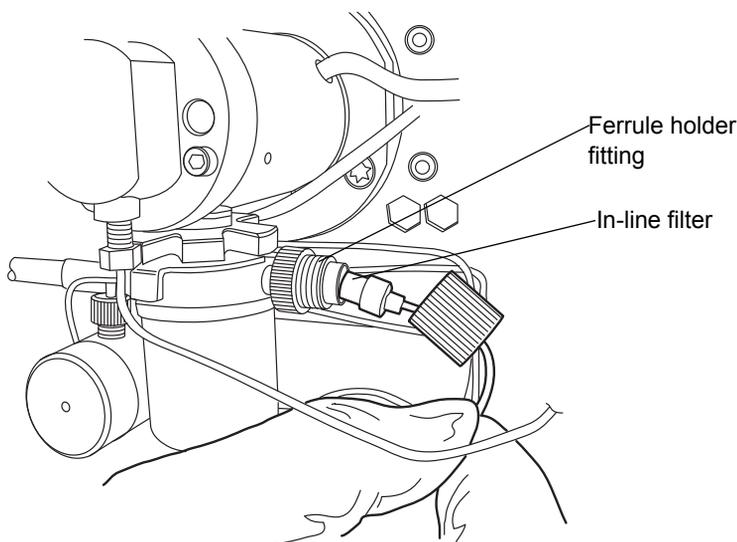


8. Slide the in-line filter cartridge onto the tube.

Exploded view of in-line filter



9. Insert the in-line filter cartridge with tubing into the ferrule holder fitting.



10. Screw the cap nut onto the ferrule holder fitting, ensuring that the tube is bottomed out in the in-line filter cartridge. Finger-tighten it to the extent possible.
11. Return the solvent bottles to their original location.
12. Power-on the binary solvent manager.
13. Prime the binary solvent manager (see [“Priming the binary solvent manager”](#) on page 3-9).

Replacing the accumulator check valve



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the check valve.

Required materials

- 1/2-inch open-end wrench
- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- Accumulator check valve assembly
- Clean, chemical-resistant, powder-free gloves

To replace the accumulator check valve



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

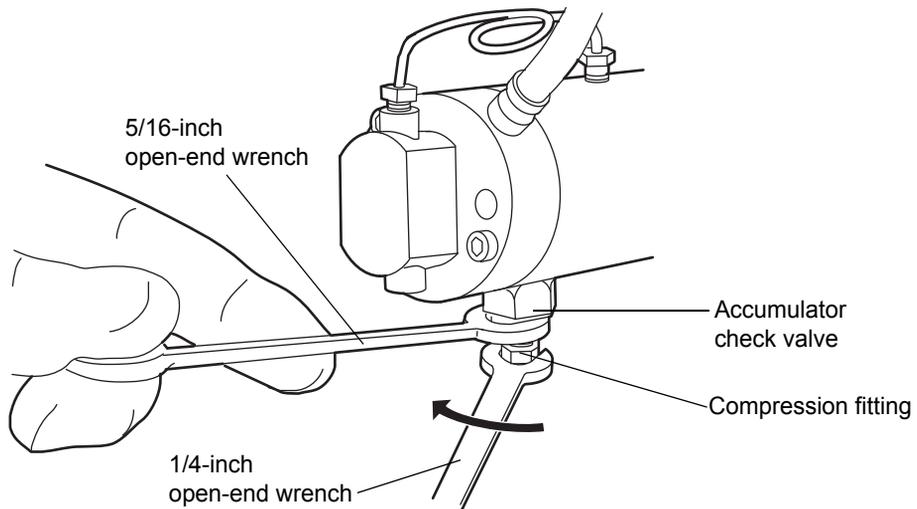
1. Flush the binary solvent manager with non-hazardous solvent.
2. Power-off the binary solvent manager.



Warning: To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

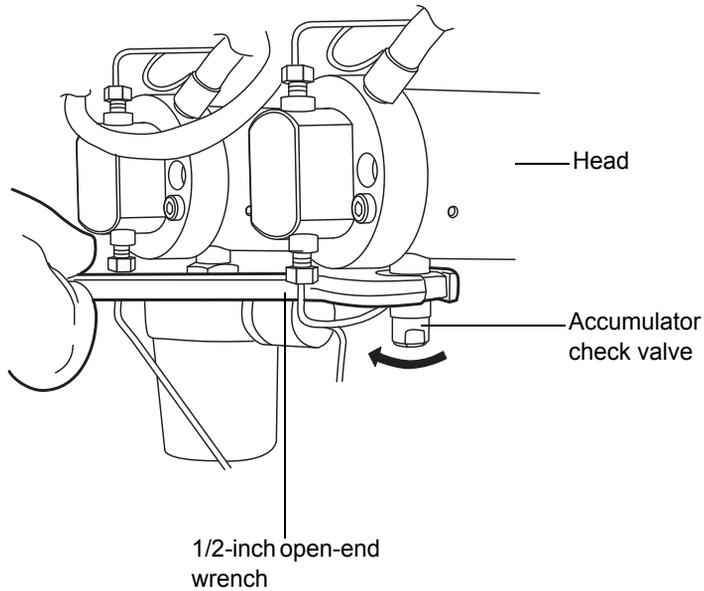
3. Move the solvent bottles to a location below the binary solvent manager.

- Using the 5/16-inch open-end wrench to hold the check valve in place, disconnect the compression fitting from the check valve with the 1/4-inch open-end wrench.

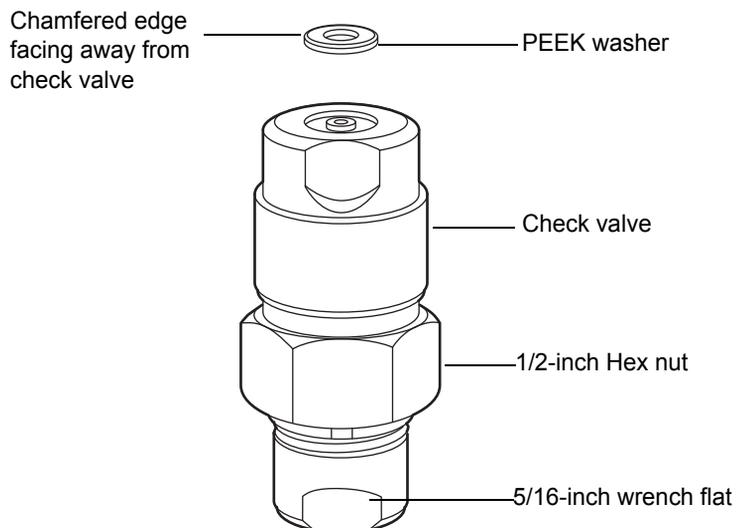


 **Caution:** When you remove the valve assembly, ensure the PEEK washer, which is normally on the top face of the check valve, does not remain in the head. See [page 6-48](#).

5. Use the 1/2-inch open-end wrench to loosen the check valve, and then remove the check valve assembly from the head.



6. Unpack the new check valve.
7. Make sure the new PEEK washer is inserted into the new check valve so that its chamfered edge faces away from the check valve.



8. Insert the check valve assembly into the head, and use the 1/2-inch wrench to tighten the check valve nut 1/8-turn beyond finger-tight.
9. Using the 5/16-inch open-end wrench to hold the check valve in place. Reattach the compression fitting to the check valve. Use the 1/4-inch wrench to tighten the compression screw 1/4-turn beyond finger-tight for existing stainless steel tubing assembly, or 3/4-turn beyond finger-tight for new stainless steel tubing assembly.
10. Return the solvent bottles to their original location.
11. Power-on the binary solvent manager.
12. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Replacing solvent filters



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: Wear clean, chemical-resistant, powder-free gloves when handling the solvent filter. Oil from your hands can contaminate the solvent filter.

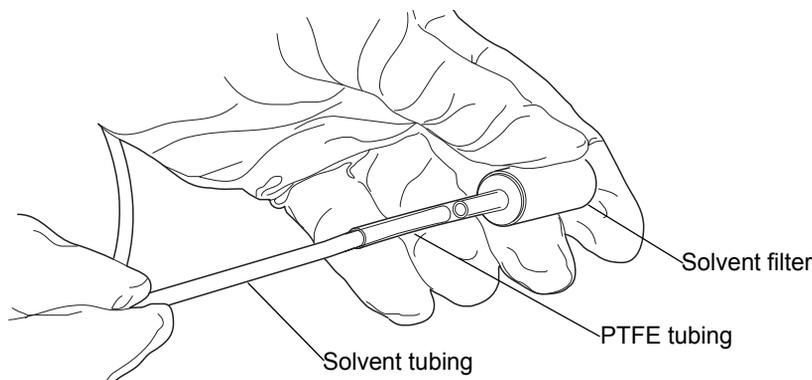
Required materials

- Clean, chemical-resistant, powder-free gloves
- New solvent filter

To replace a solvent filter

1. Remove the filtered end of the solvent tubing from the solvent bottle.
2. Remove the old solvent filter from the short piece of PTFE tubing.

3. Insert the new solvent filter into the PTFE tubing, pushing until it contacts the solvent tubing.



4. Insert the filtered end of the solvent tubing into the solvent bottle.
5. Shake the filter to remove any air from it.
6. Prime the binary solvent manager. See [“Priming the binary solvent manager” on page 3-9](#).

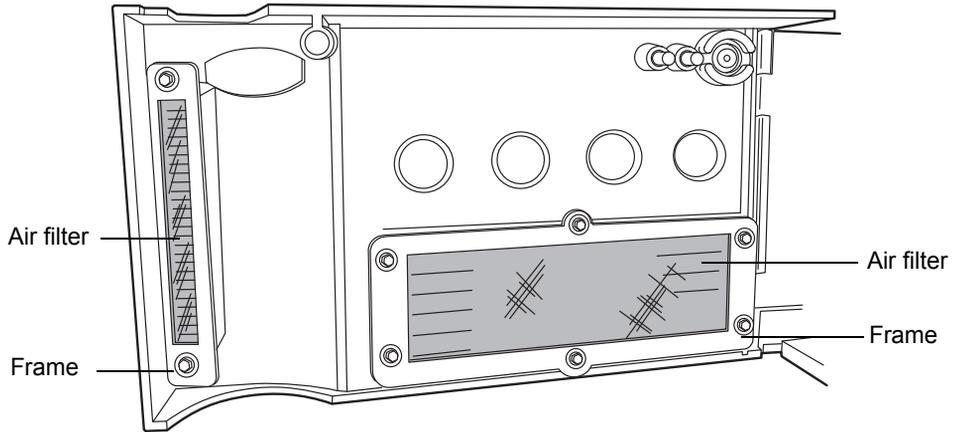
Cleaning the air filters in the binary solvent manager door

Required materials

- T10 TORX[®] driver
- Vacuum

To clean the air filters

1. Using the T10 TORX driver, remove the 8 screws that secure the air filter frames and air filters to the inside of the binary solvent manager door.



2. Remove the air filters from the air filter frames and use a vacuum to clean them.
3. Align the air filters with the air filter frames.
4. Using the T10 TORX driver, attach the air filters and frames to the inside of the binary solvent manager door with the 8 screws.

Replacing the air filters in the binary solvent manager door

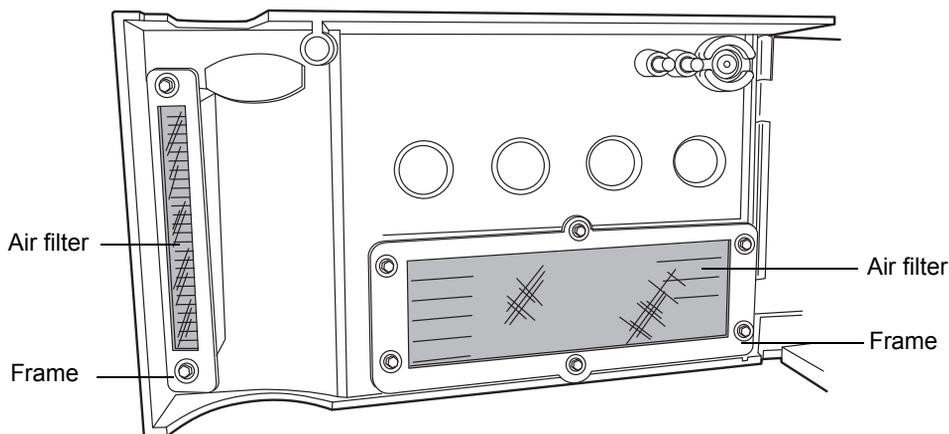
If the air filters cannot be cleaned by vacuuming, replace them with new filters.

Required materials

- T10 TORX[®] driver
- Binary solvent manager air filters

To replace the air filters

1. Using the T10 TORX driver, remove the 8 screws that secure the air filter frames and air filters to the inside of the binary solvent manager door.



2. Remove the old air filters from the air filter frames and discard them.
3. Align the new air filters with the air filter frames.
4. Using the T10 TORX driver, attach the air filters and frames to the inside of the binary solvent manager door with the 8 screws.

Removing and replacing the primary head seals

See the ACQUITY UPLC Console online Help to help determine whether you need to replace the primary head seals.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when removing and replacing the head seals.

Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- T27 TORX driver (startup kit)
- Clean, chemical-resistant, powder-free gloves
- Head seal and seal wash spacer
- Methanol
- Pliers
- PTFE O-ring
- Seal extraction tool
- Seal wash seal
- Sharp tool
- Plunger (recommended)

To remove the primary head

1. Flush the binary solvent manager with non-hazardous solvent.
2. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
3. In the binary solvent manager information window, click Maintain > Heads.
4. In the Head Maintenance dialog box, select the head (Primary) from the side of the solvent manager (A or B) that you plan to perform maintenance on.
5. Click Move Backward, and then wait for the plunger to stop.



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

6. Power-off the binary solvent manager.

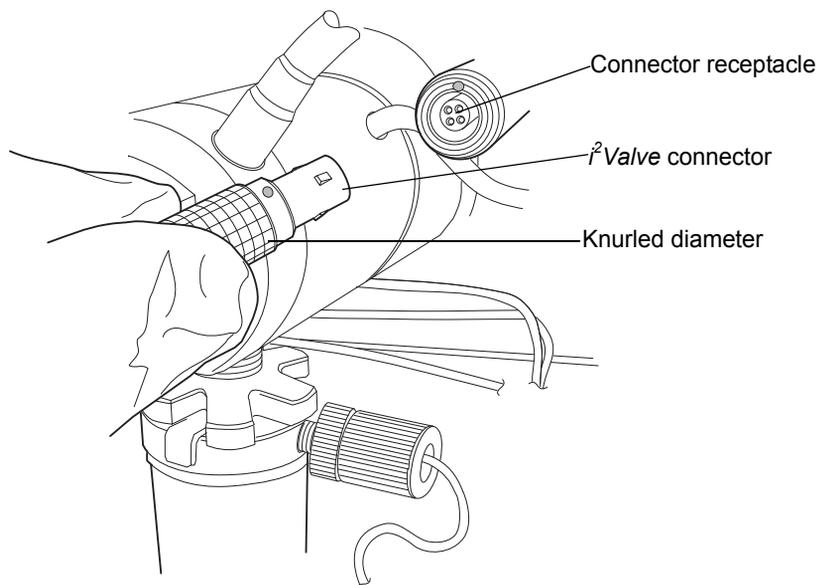
Tip: The binary solvent manager is referred to as “pump” on the warning label affixed to the *i*²Valve actuator.

 **Warning:** To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

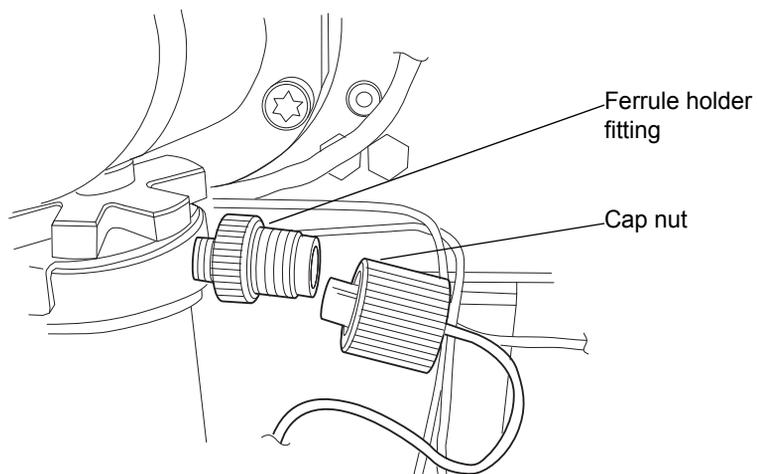
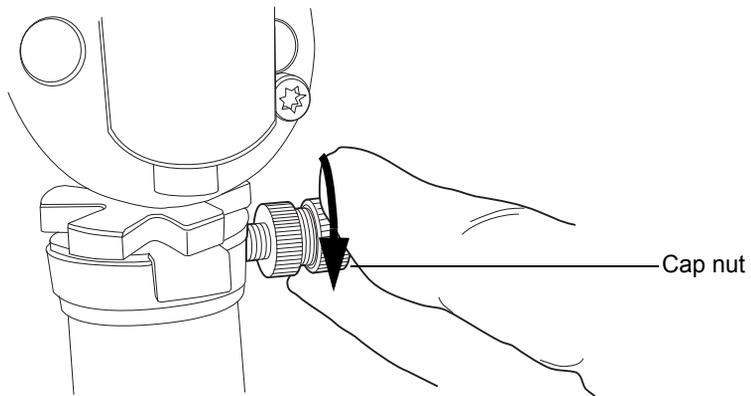
7. Move the solvent bottles to a location below the binary solvent manager.

 **Caution:** To avoid damage to the connector or cable, grasp the *i*²Valve connector by the knurled diameter.

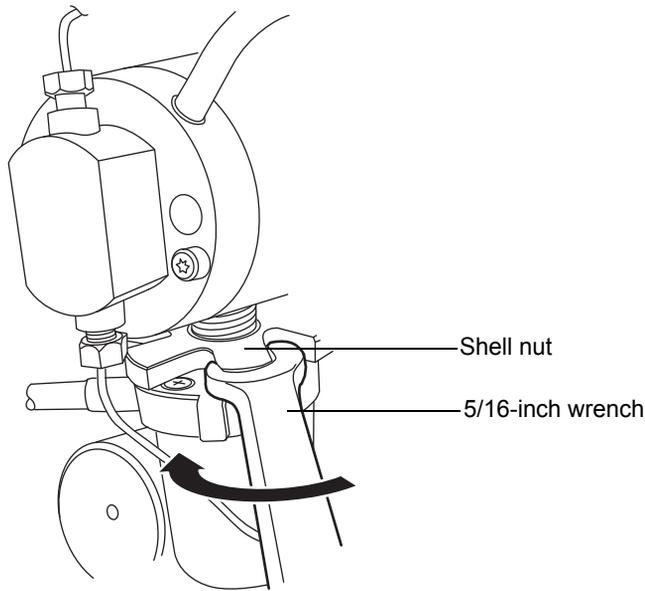
8. Grasp the *i*²Valve connector by the knurled diameter and pull it toward you to disconnect it from the receptacle.



9. Loosen the cap nut on the in-line filter so that it is all the way off the ferrule holder fitting threads.



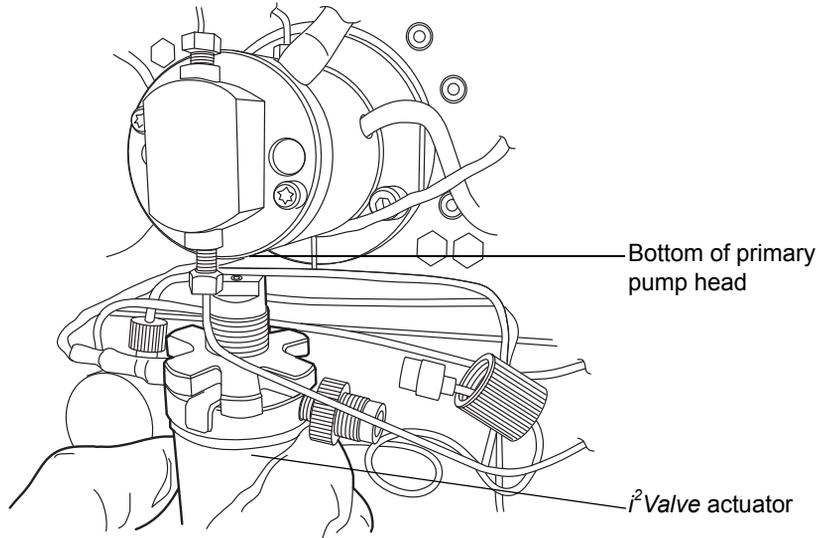
10. Use the 5/16-inch open-end wrench to loosen the shell nut and then fully unscrew it.



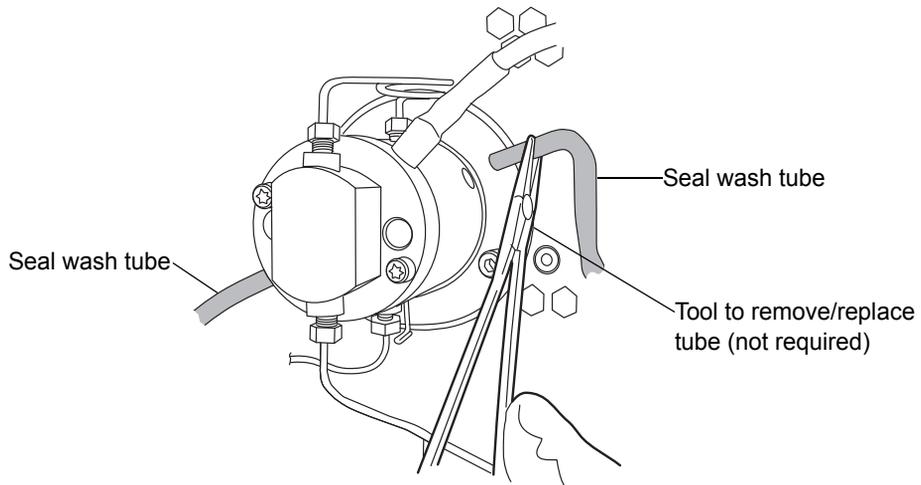
Caution:

- When you remove the valve assembly, ensure the PEEK washer, which is normally on the top face of the *i²Valve* cartridge, does not remain in the head. See [page 6-37](#).
- Never place the actuator assembly or electrical connector in the drip tray.

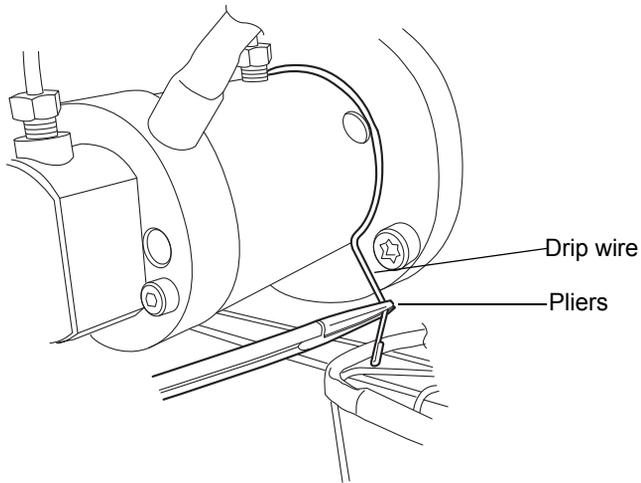
11. Remove the *i*²Valve actuator from the bottom of the primary pump head.



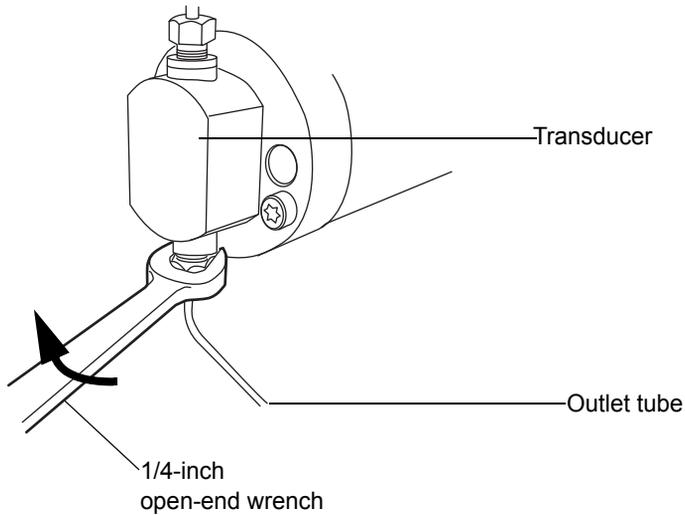
12. Remove both seal wash tubes secured to the seal wash housing by barbed fittings, using a tool or by pulling on the tubes as close to the head as possible.



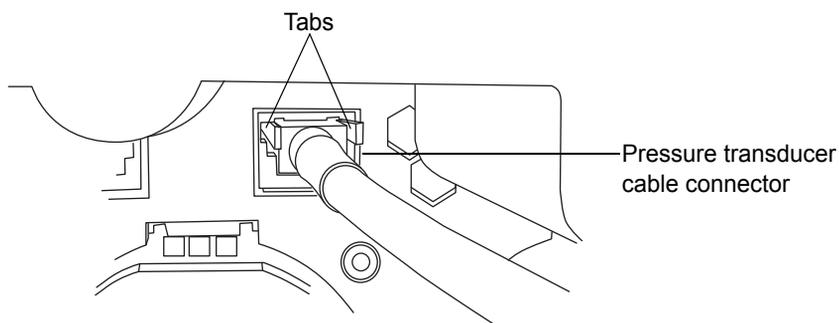
13. Using a pliers, remove the drip wire from the head assembly.



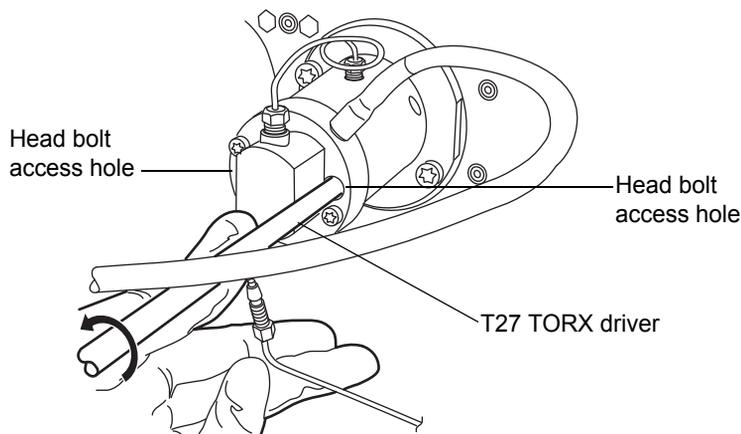
14. Use the 1/4-inch open-end wrench to disconnect the outlet tube from the transducer.



15. Disconnect the pressure transducer cable from the bulkhead by squeezing on the tabs and pulling gently.



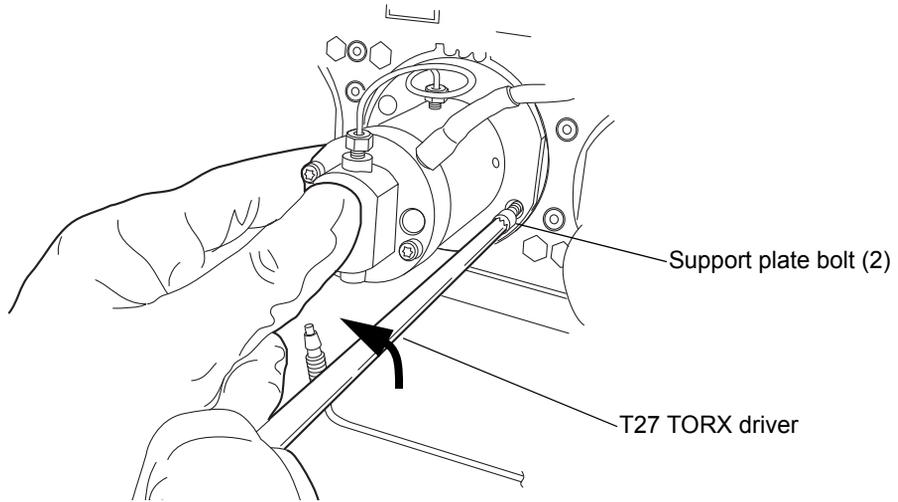
16. Using the T27 TORX driver, loosen the 2 head bolts 1/2-turn. The bolts are accessible from the front of the pressure transducer.



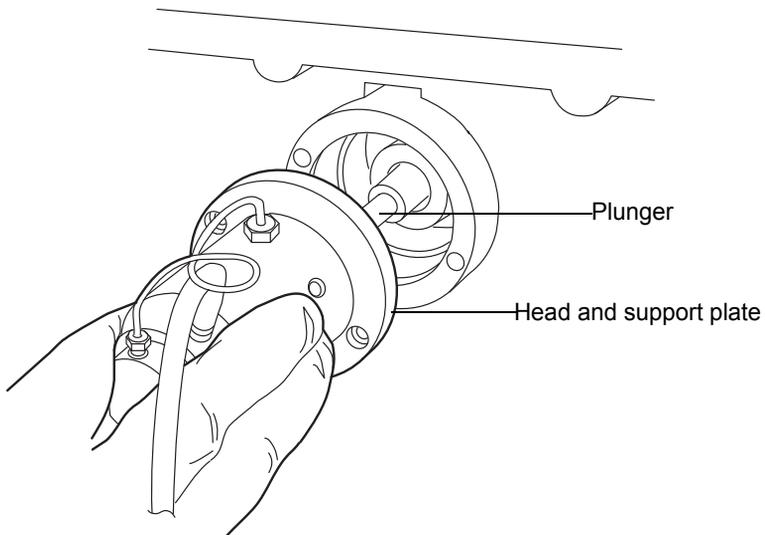
 **Caution:** To avoid damaging the plunger, support the head from below as you remove it.

17. Using the T27 TORX driver, loosen and remove the 2 support plate bolts, and then gently pull the head and support plate off the actuator housing, making sure not to tilt the head during the extraction.

Removing support plate bolts

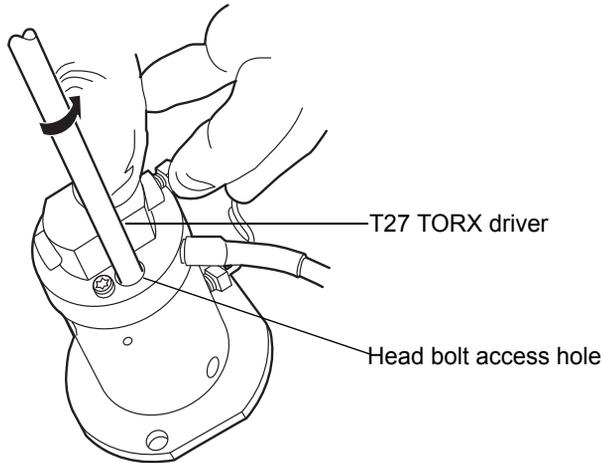


Pulling head and support plate off actuator housing

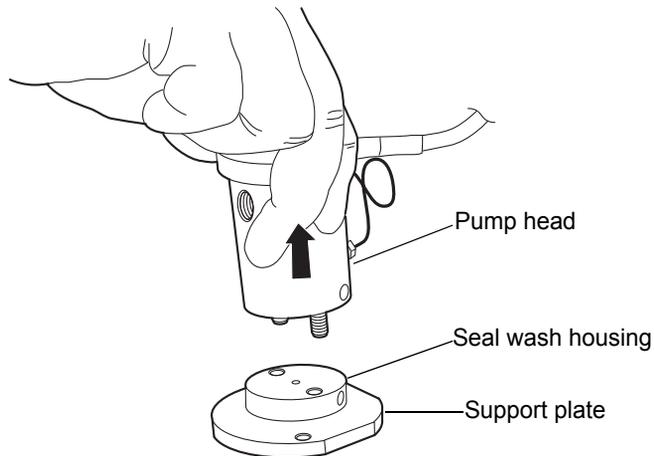


To remove the primary head seals

1. Stand the head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the 2 head bolts to release the support plate from the pump head.

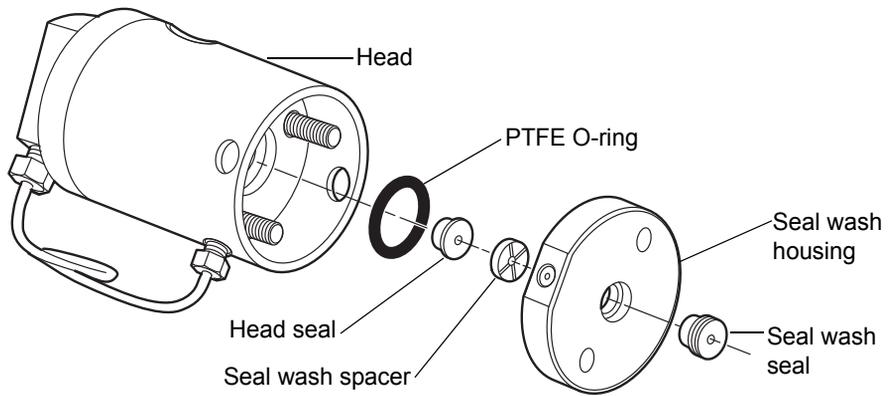


3. Lift the pump head from the support plate.

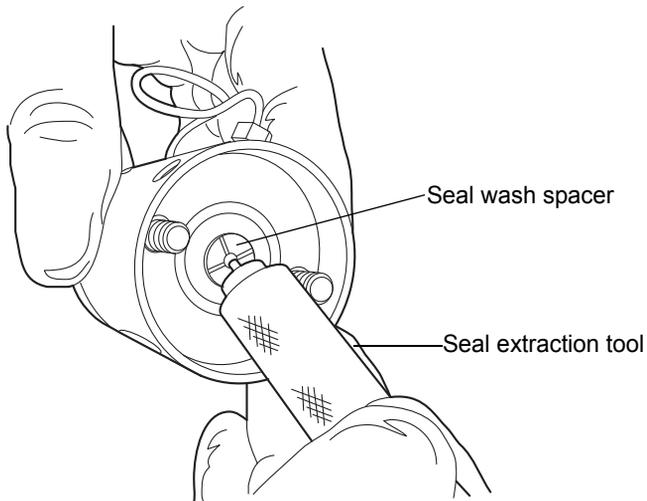


4. Remove the old seal wash seal and discard it.

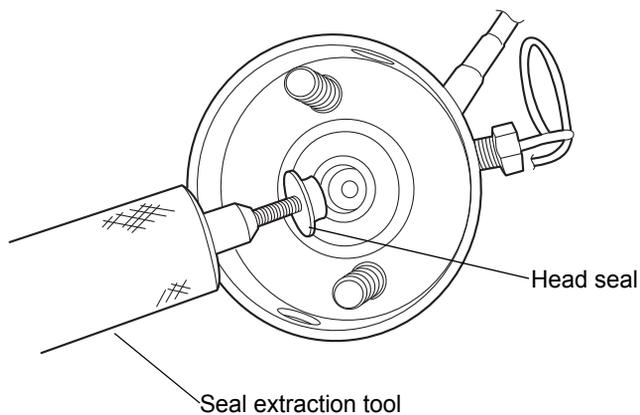
Head seals



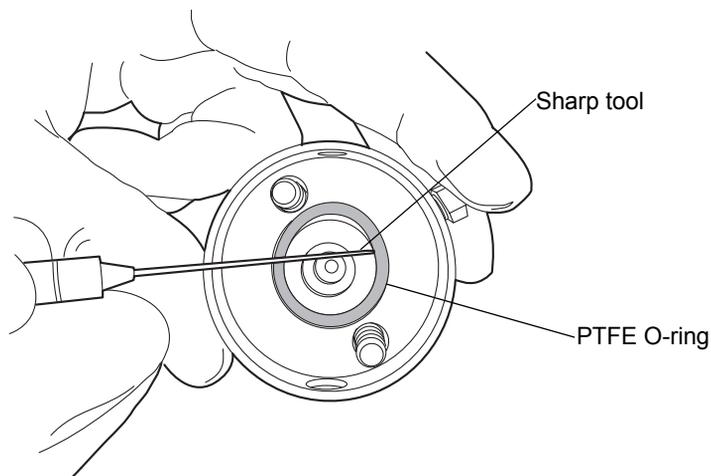
5. Using the smooth end of the seal extraction tool, pull the seal wash spacer from the head.



6. Taking care not to scratch any surfaces, screw the threaded end of the seal extraction tool into the head seal and carefully pull it out of the head.

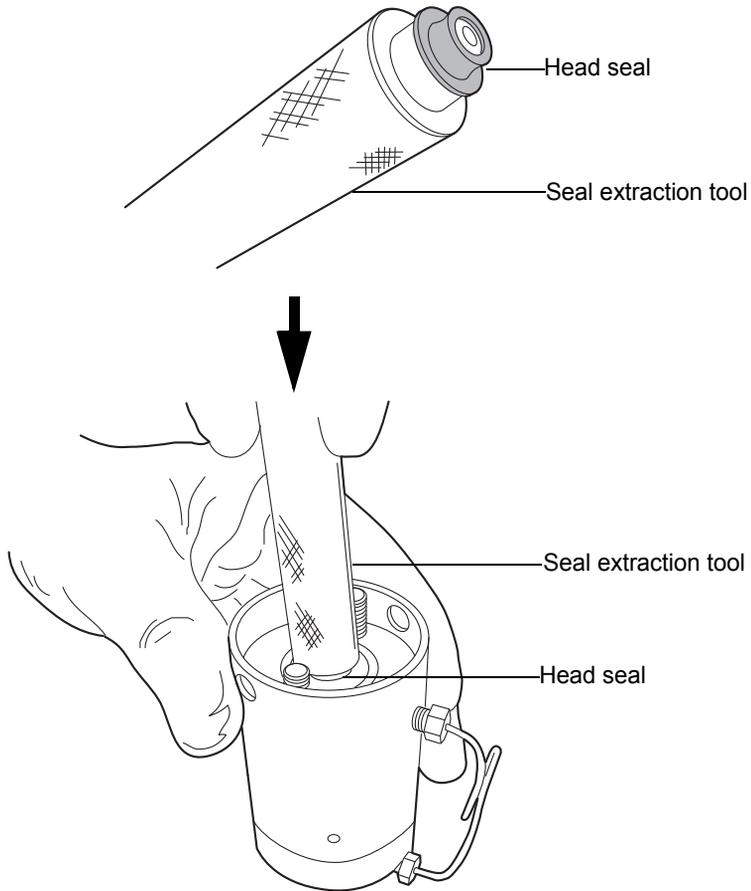


7. Taking care not to scratch any metal surfaces, use a sharp tool to remove the PTFE O-ring.

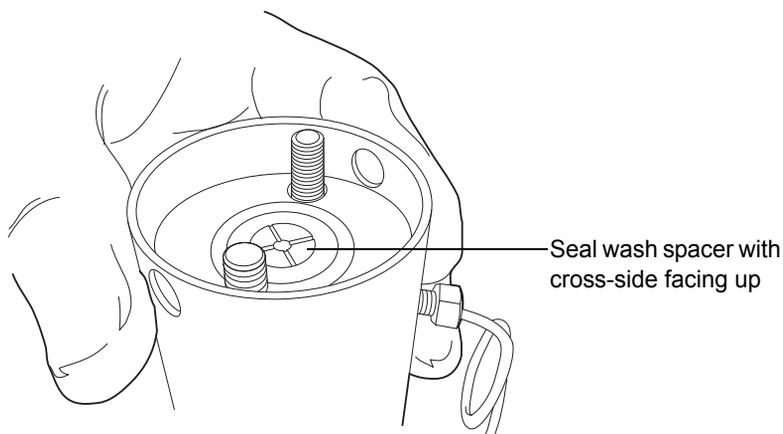


8. Lubricate the new PTFE O-ring with methanol and press the O-ring into its seat with your thumbs.

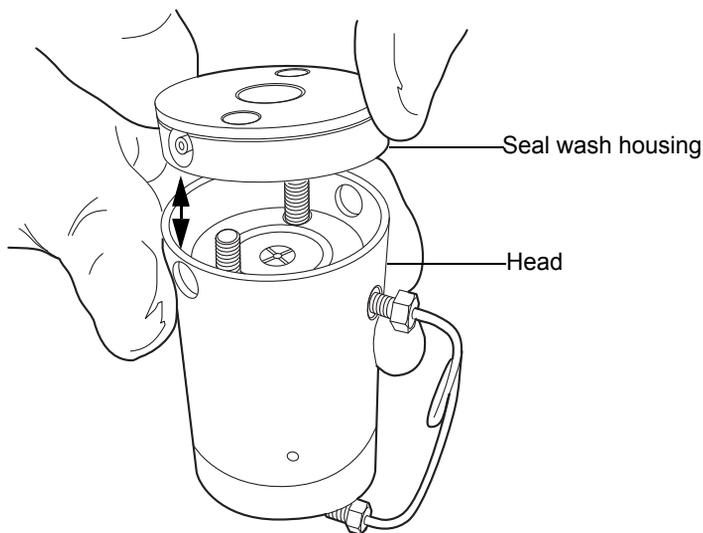
9. Lubricate the new head seal with methanol, and use the smooth end of the seal extraction tool to place it in the head.



10. Center the seal wash spacer over the head seal so that the cross-side faces upward.

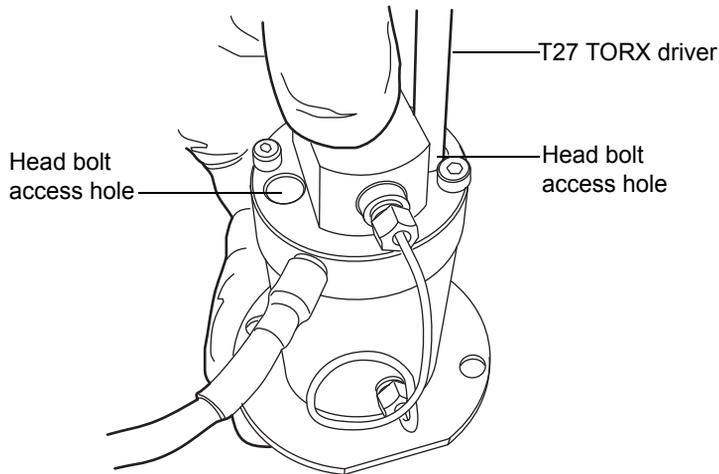


11. Orient the seal wash housing so that the holes on the side of it line up with the holes on the side of the head, and then drop it into place.



12. Install the new seal wash seal in the seal wash housing.
13. Place the support plate on top of the head.

14. Holding the assembly together, use the T27 TORX driver to tighten the 2 head bolts finger-tight.



Recommendation: Replace the plunger whenever you replace the head seal. See [“To replace the primary head plunger”](#) on page 6-97.

To reattach the primary head

1. Lubricate the seals and plunger with methanol.



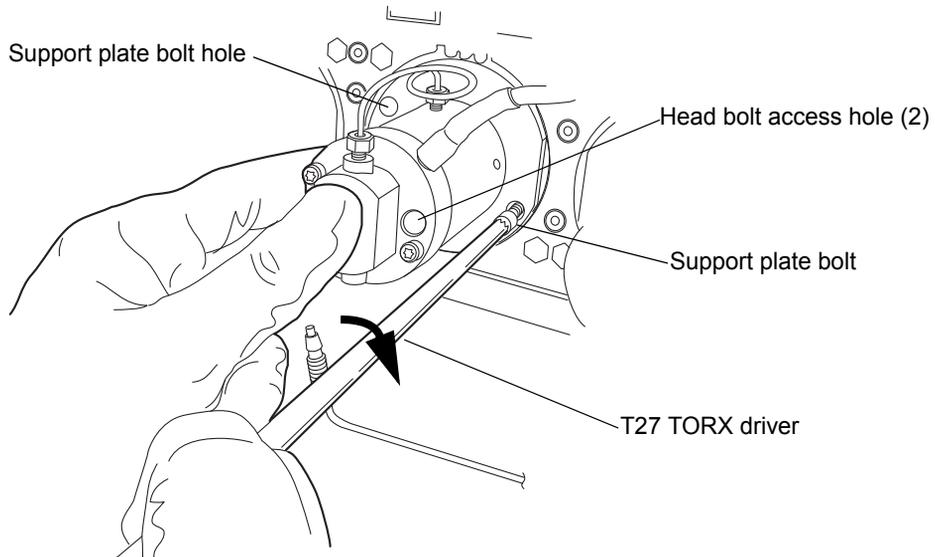
Caution: To avoid damaging the plunger, ensure that the head assembly is not tilted relative to the plunger when you position it on the mechanism.

2. Carefully slide the head assembly and support plate over the sapphire plunger, making sure not to tilt the head.

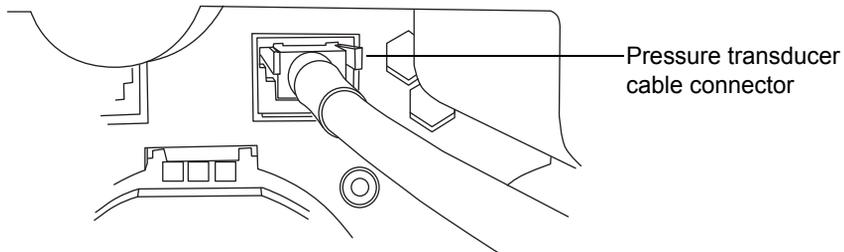


Caution: To avoid damaging the plunger, when tightening the support plate screws, alternate 1/4-turn between each screw so that they are tightened evenly.

3. Hold the head assembly securely against the actuator housing, and then use the T27 TORX driver to tighten the support plate screws securely.

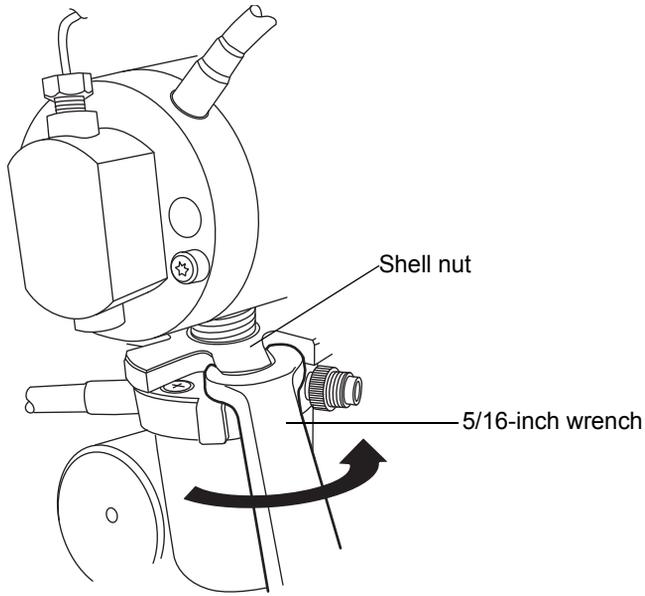


4. Tighten the head bolts evenly as well.
5. Reinstall the drip wire around the head assembly, ensuring that the tip is in the 6 o'clock position.
6. Connect the pressure transducer cable to the bulkhead.

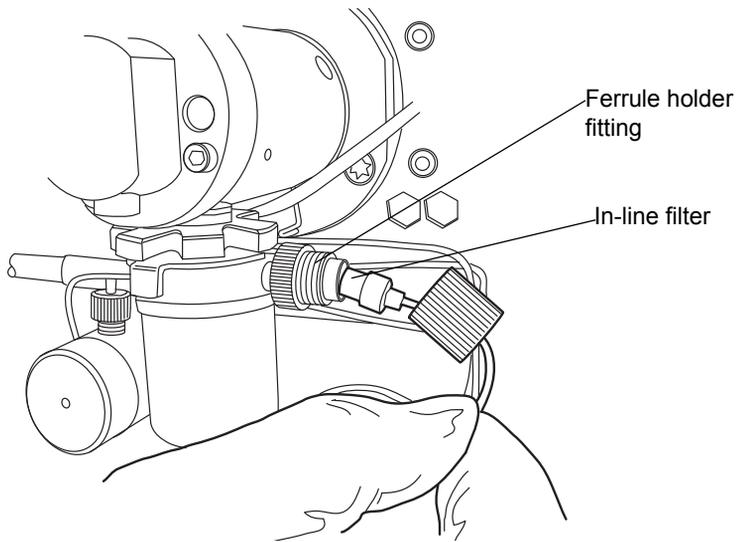


7. Orient the *i*²Valve assembly so that the cable exits from the left-hand side. Insert the *i*²Valve assembly into the bottom of the primary pump head, and route the cable behind the valve actuator.
8. Finger-tighten the shell nut to secure the valve. You should be able to rotate the shell nut approximately 5 full turns to reach the finger-tight

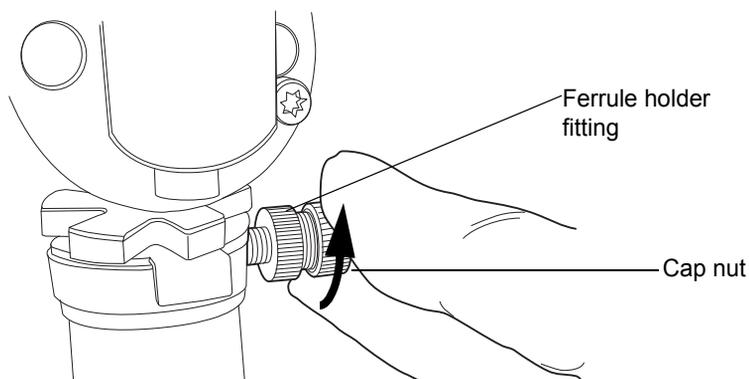
condition. Use the 5/16-inch open-end wrench to tighten the nut an additional 1/8-turn.



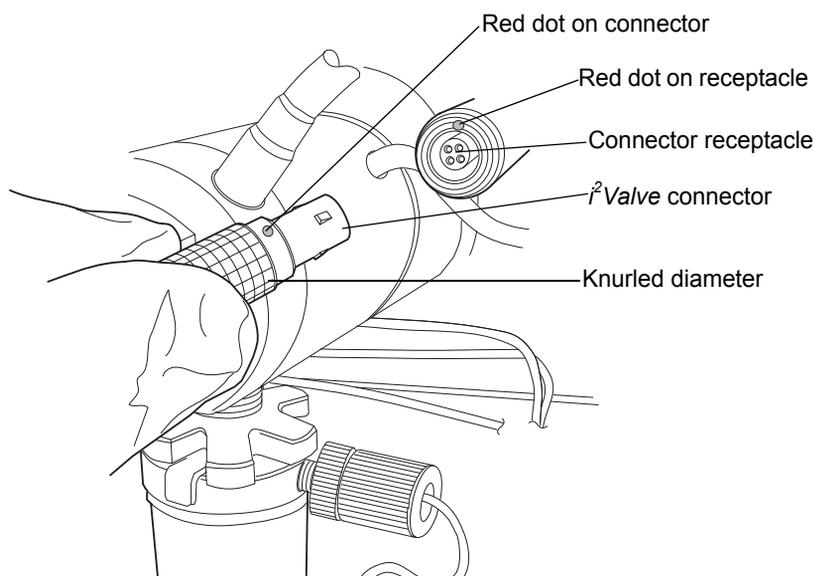
9. Reinsert the in-line filter and tube into the ferrule holder fitting.



10. Place the cap nut over the ferrule holder fitting and finger-tighten the cap nut to the extent possible.



11. Align the red dot on the *i*²Valve connector with the red dot on the receptacle, in the 12 o'clock position, and insert the connector into the receptacle.



12. Reattach all fittings and seal wash tubes.
13. Return the solvent bottles to their original location.
14. Power-on the binary solvent manager.
15. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Removing and replacing the accumulator head seals

See the ACQUITY UPLC Console online Help to help determine whether you need to replace the accumulator head seals.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when removing and replacing the head seals.

Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- T27 TORX driver (startup kit)
- Clean, chemical-resistant, powder-free gloves
- Head seal and seal wash spacer
- Methanol
- Pliers
- Plunger (recommended)
- Plunger removal tool (recommended)
- PTFE O-ring
- Seal extraction tool
- Seal wash seal
- Sharp tool

To remove the accumulator head

1. Flush the binary solvent manager with non-hazardous solvent.
2. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
3. In the binary solvent manager information window, click Maintain > Heads.

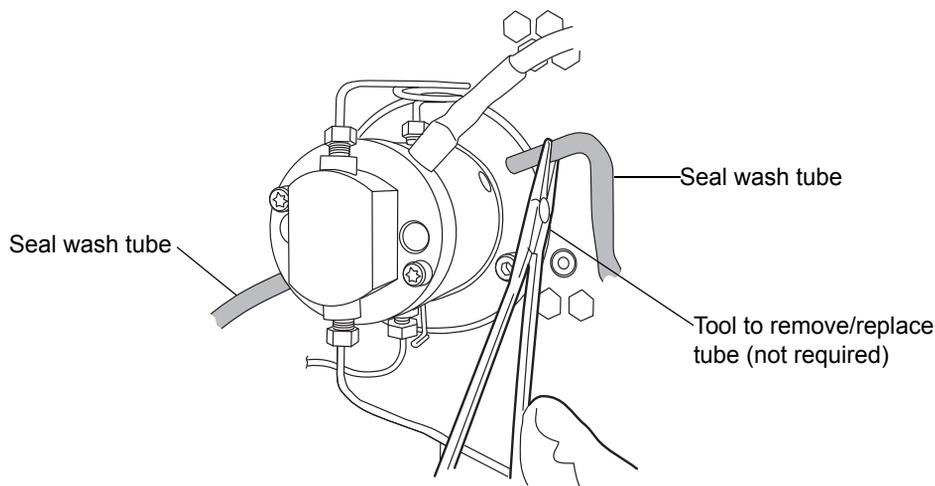
4. In the Head Maintenance dialog box, select the head (Accumulator) from the side of the solvent manager (A or B) that you plan to perform maintenance on.
5. Click Move Backward, and then wait for the plunger to stop.

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

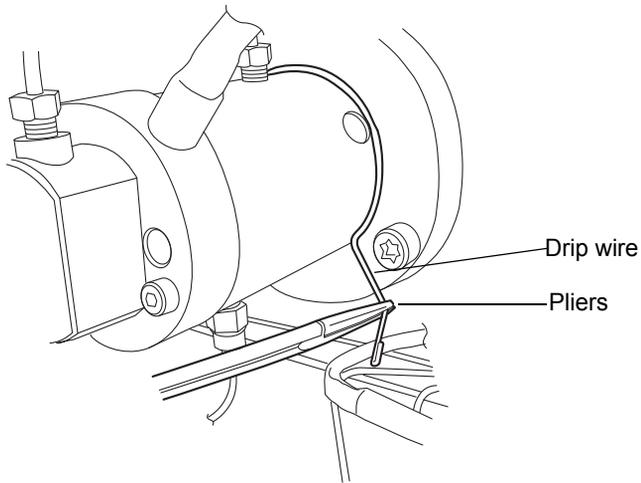
6. Power-off the binary solvent manager.

 **Warning:** To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

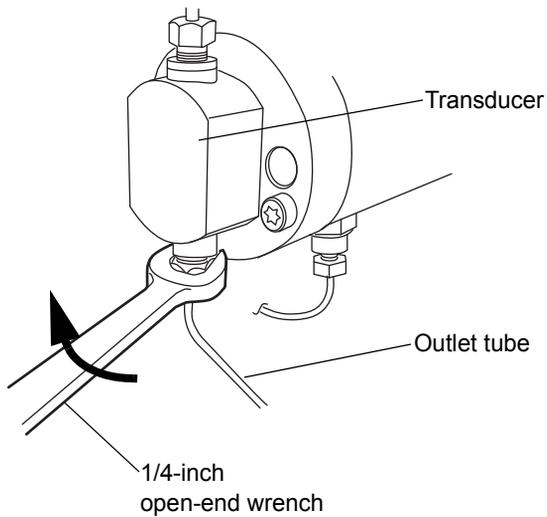
7. Move the solvent bottles to a location below the binary solvent manager.
8. Remove both seal wash tubes secured to the seal wash housing by barbed fittings, using a tool or by pulling on the tubes as close to the head as possible.



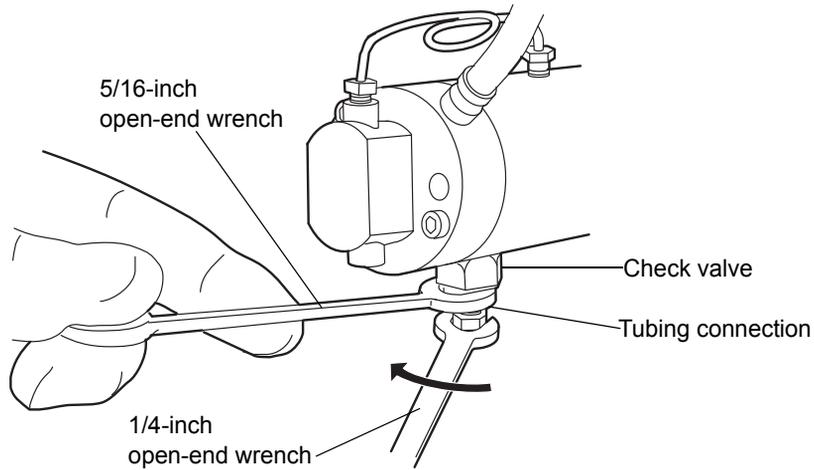
9. Using pliers, remove the drip wire from the head assembly.



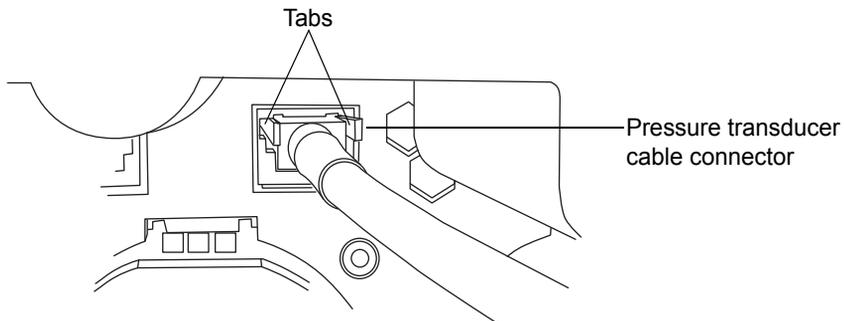
10. Use the 1/4-inch open-end wrench to disconnect the outlet tube from the transducer.



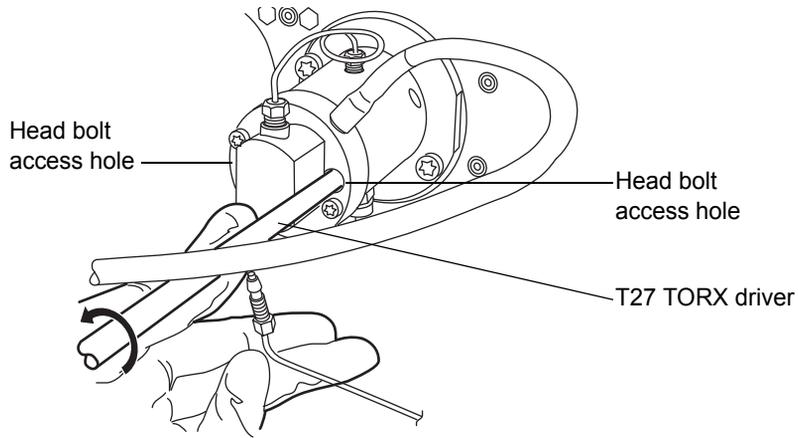
- Using the 5/16-inch open-end wrench to hold the check valve cartridge in place, disconnect the tubing connection from the check valve with the 1/4-inch open-end wrench.



- Disconnect the pressure transducer cable from the bulkhead by squeezing on the tabs and pulling gently.



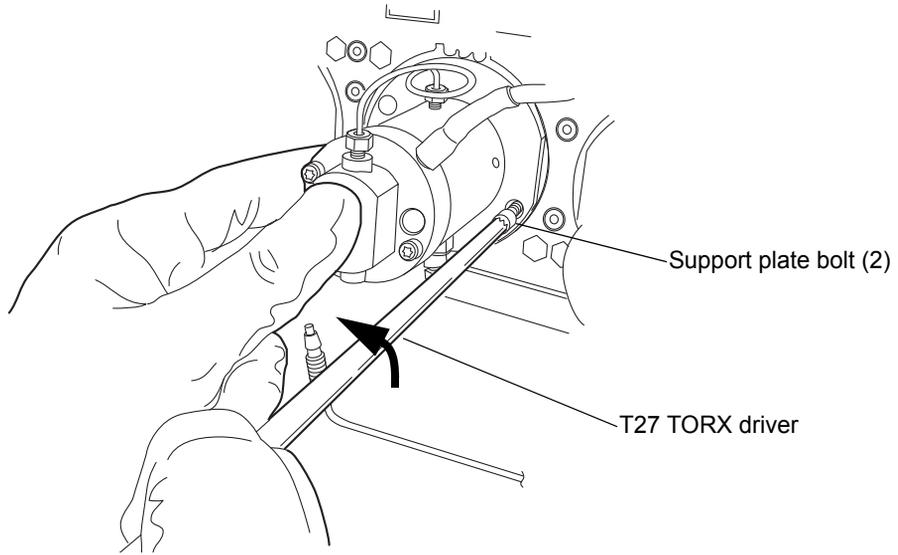
- Using the T27 TORX driver, loosen the 2 head bolts 1/2-turn. The bolts are accessible from the front of the pressure transducer.



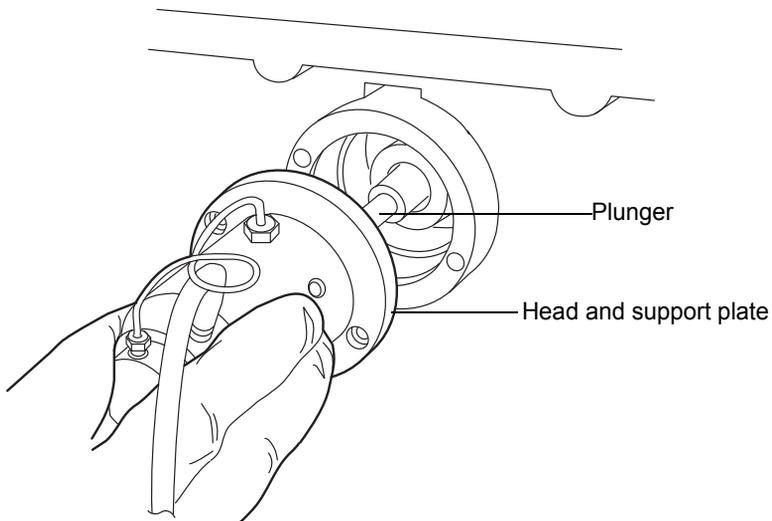
 **Caution:** To avoid damaging the plunger, support the head from below as you remove it.

- Using the T27 TORX driver, loosen and remove the 2 support plate bolts, and then gently pull the head and support plate off the actuator housing, making sure not to tilt the head during the extraction.

Removing support plate bolts

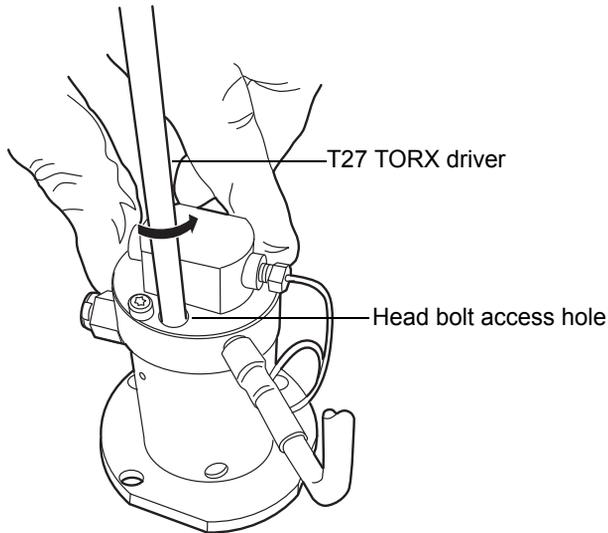


Pulling head and support plate off actuator housing

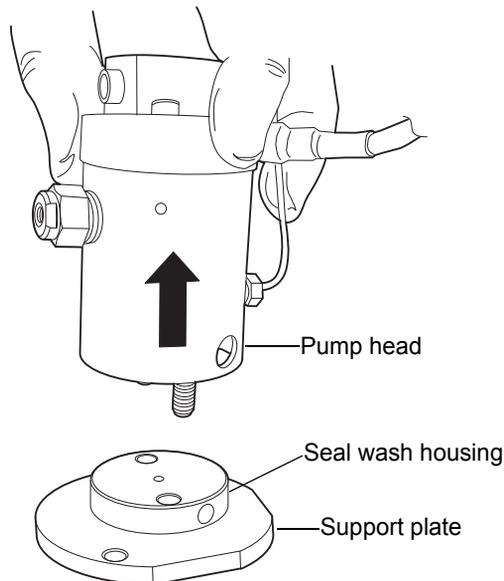


To remove the accumulator head seals

1. Stand the head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the 2 head bolts to release the support plate from the pump head.

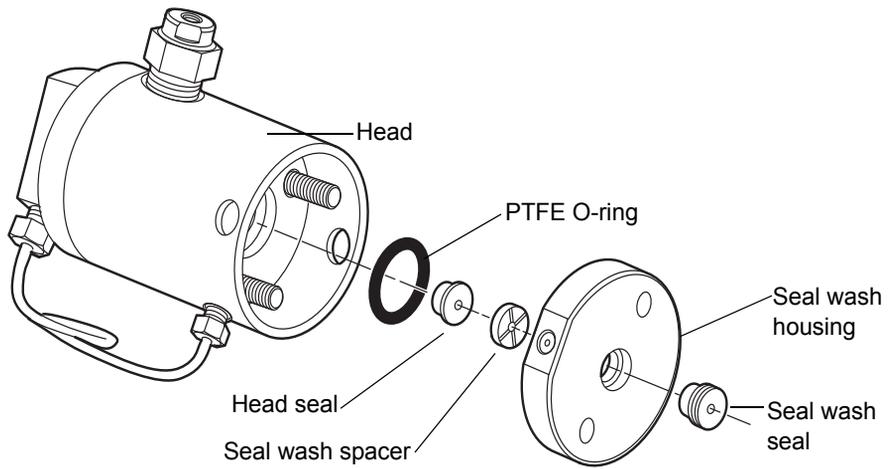


3. Lift the pump head from the support plate.

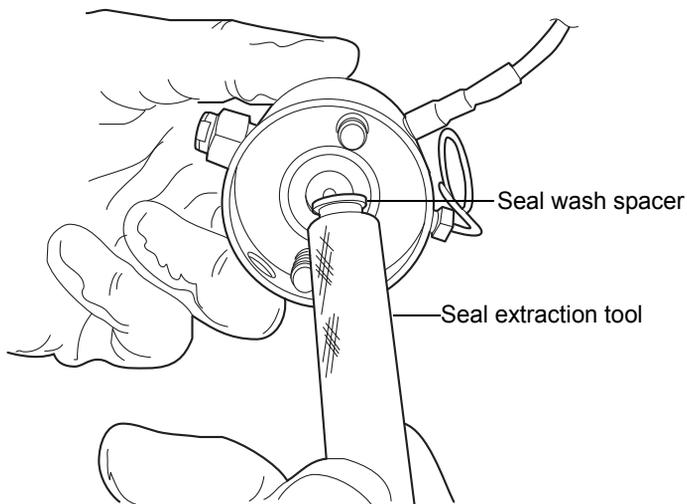


4. Remove the old seal wash seal and discard it.

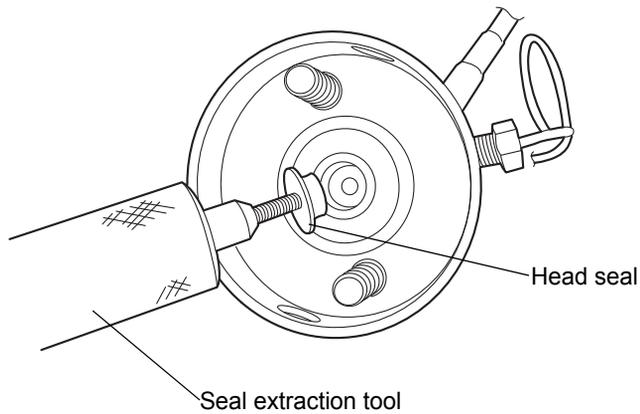
Head seals



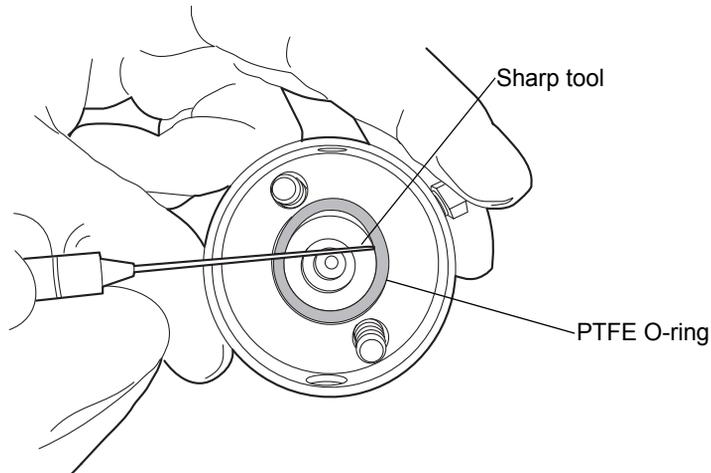
5. Using the smooth end of the seal extraction tool, pull the seal wash spacer from the head.



6. Taking care not to scratch any surfaces, screw the threaded end of the seal extraction tool into the head seal and carefully pull it out of the head.

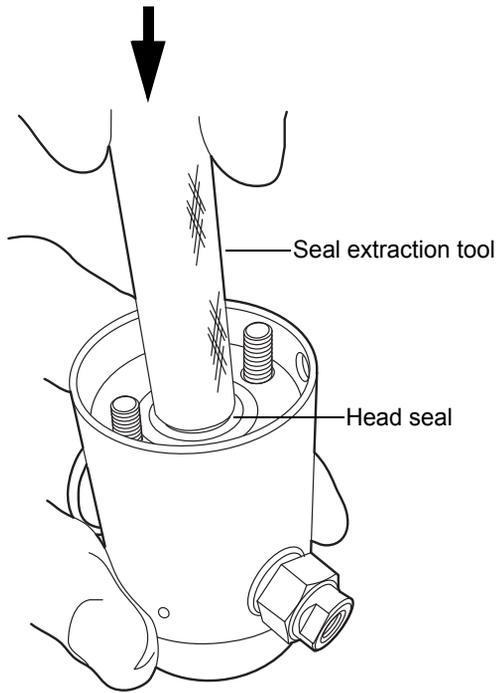
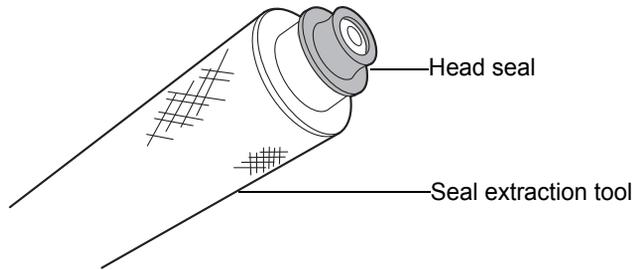


7. Taking care not to scratch any metal surfaces, use a sharp tool to remove the PTFE O-ring.

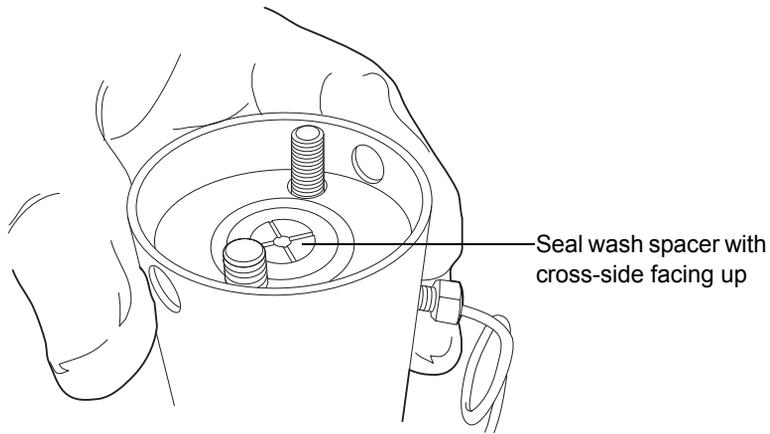


8. Lubricate the new PTFE O-ring with methanol and press the O-ring into its seat with your thumbs.

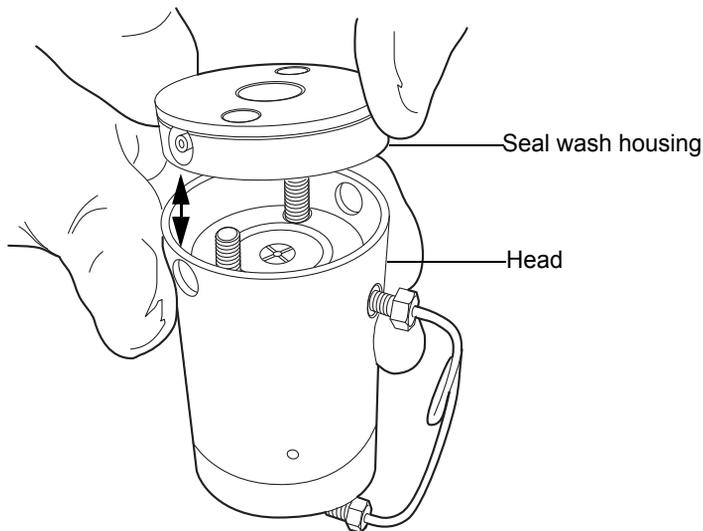
9. Lubricate the new head seal with methanol, and use the smooth end of the seal extraction tool to place it in the head.



10. Center the seal wash spacer over the head seal so that the cross-side faces upward.

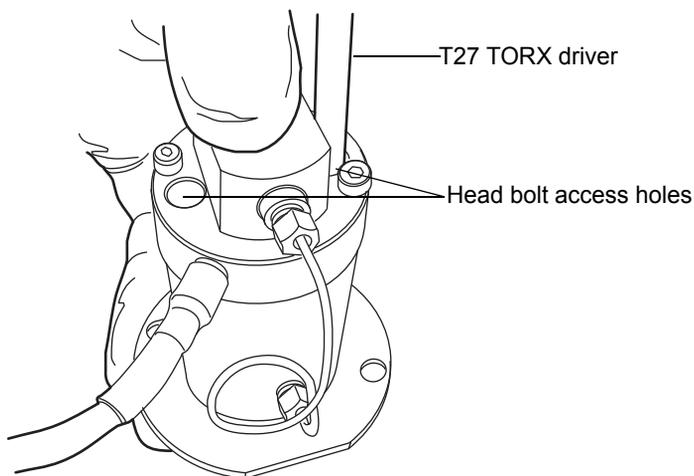


11. Orient the seal wash housing so that the holes on the side of it line up with the holes on the side of the head, and then drop it into place.



12. Install the new seal wash seal in the seal wash housing.
13. Place the support plate on top of the head.

14. Holding the assembly together, use the T27 TORX driver to tighten the 2 head bolts finger-tight.



Recommendation: Replace the plunger whenever you replace the head seal. See [“To replace the accumulator head plunger”](#) on page 6-114.

To reattach the accumulator head

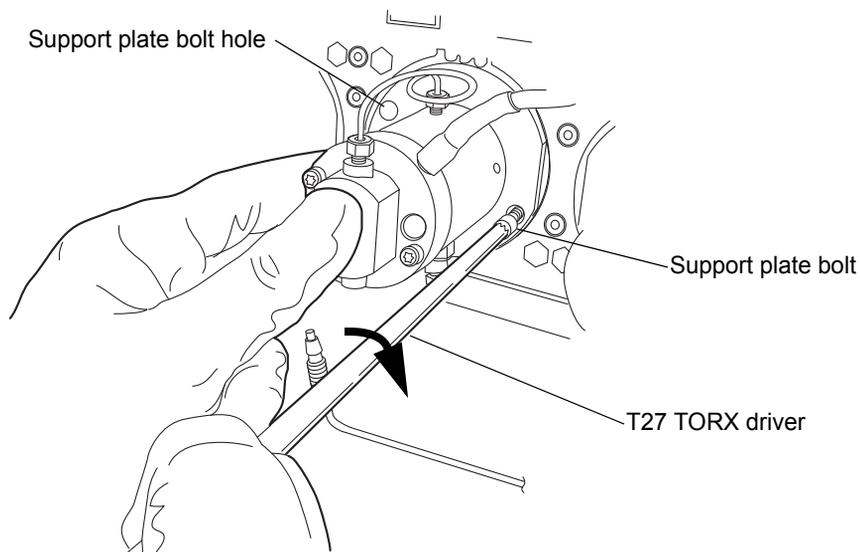
1. Lubricate the seals and plunger with methanol.

 **Caution:** To avoid damaging the plunger, ensure that the head assembly is not tilted relative to the plunger when you position it on the mechanism.

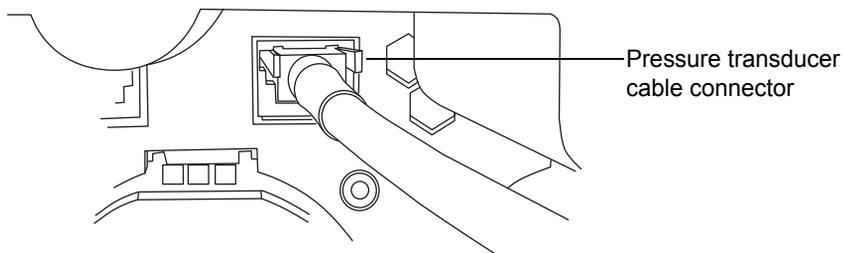
2. Carefully slide the head assembly and support plate over the sapphire plunger, making sure not to tilt the head.

 **Caution:** To avoid damaging the plunger, when tightening the support plate screws, alternate 1/4-turn between each screw so that they are tightened evenly.

3. Hold the head assembly securely against the actuator housing, and then use the T27 TORX driver to tighten the support plate screws securely.



4. Tighten the head bolts evenly as well.
5. Reinstall the drip wire around the head assembly, ensuring that the tip is in the 6 o'clock position.
6. Connect the pressure transducer cable to the bulkhead.



7. Reattach all fittings and seal wash tubes.
8. Return the solvent bottles to their original location.
9. Power-on the binary solvent manager.
10. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Replacing the primary head plungers



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the plungers.

Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- T27 TORX driver (startup kit)
- Clean, chemical-resistant, powder-free gloves
- Head seal and seal wash spacer (recommended)
- Methanol
- Pliers
- Plunger removal tool
- PTFE O-ring (recommended)
- Replacement plunger
- Seal extraction tool (recommended)
- Seal wash seal (recommended)
- Sharp tool (recommended)

To remove the primary head

1. Flush the binary solvent manager with non-hazardous solvent.
2. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
3. In the binary solvent manager information window, click Maintain > Heads.

4. In the Head Maintenance dialog box, select the head (Primary) from the side of the solvent manager (A or B) that you plan to perform maintenance on.
5. Click Move Backward, and then wait for the plunger to stop.



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

6. Power-off the binary solvent manager.

Tip: The binary solvent manager is referred to as “pump” on the warning label affixed to the *i*²Valve actuator.



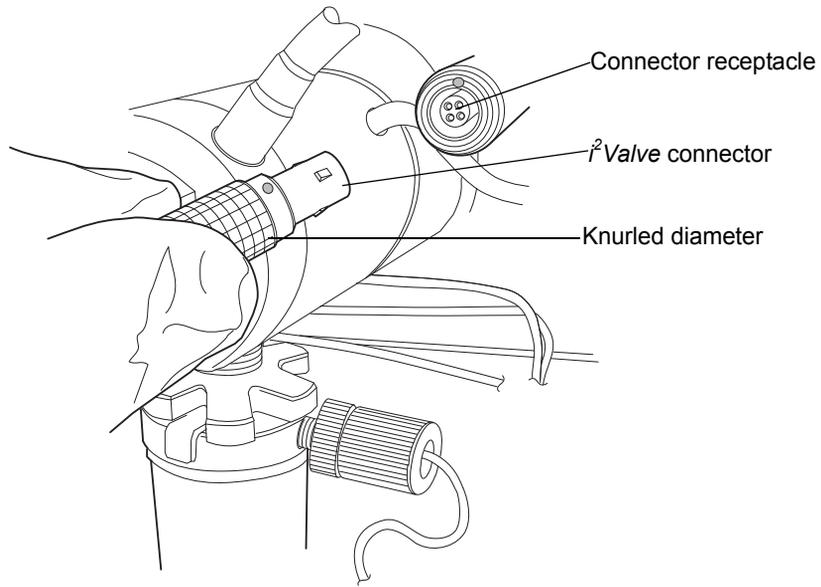
Warning: To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

7. Move the solvent bottles to a location below the binary solvent manager.

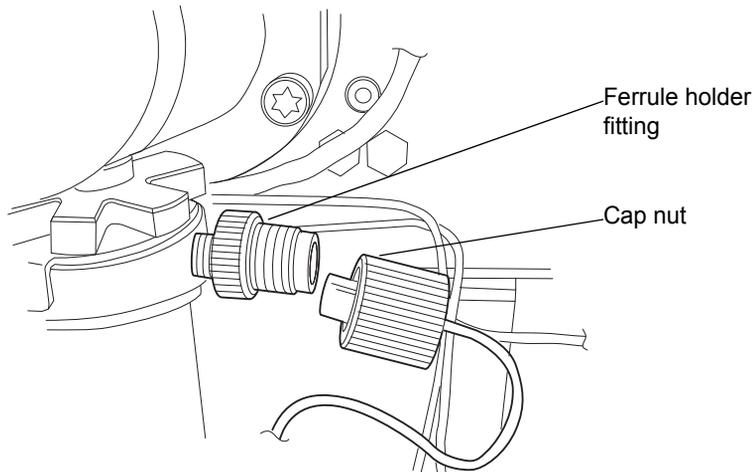
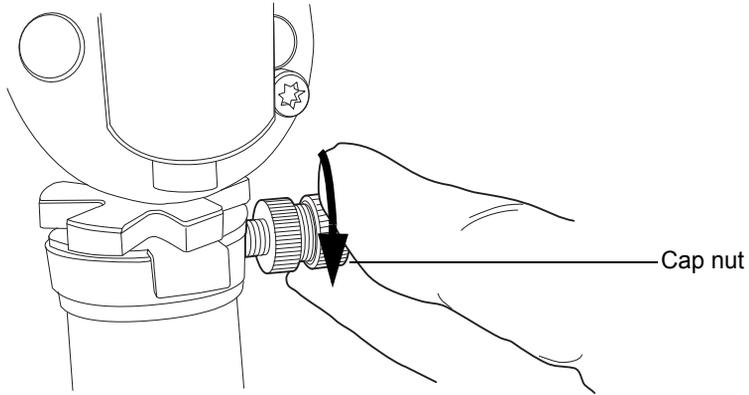


Caution: To avoid damage to the connector or cable, grasp the *i*²Valve connector by the knurled diameter.

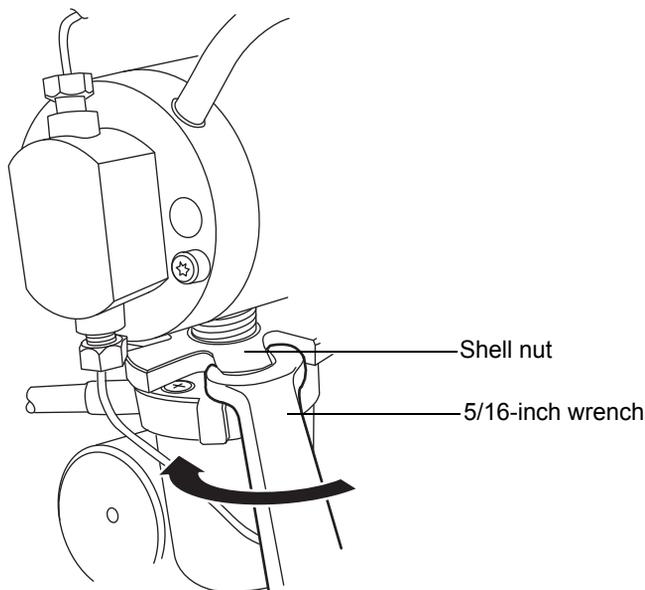
8. Grasp the *i*²Valve connector by the knurled diameter and pull it toward you to disconnect it from the receptacle.



9. Loosen the cap nut on the in-line filter so that it is all the way off the ferrule holder fitting threads.



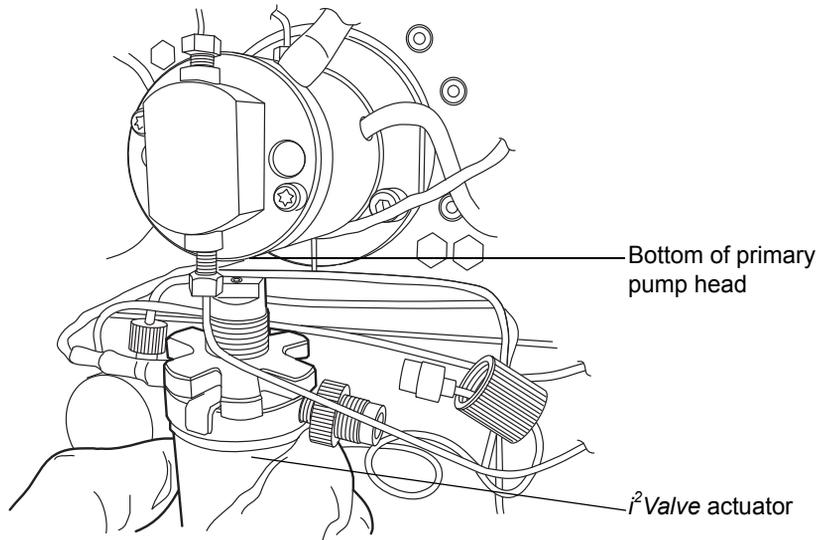
10. Use the 5/16-inch open-end wrench to loosen the shell nut and then fully unscrew it.



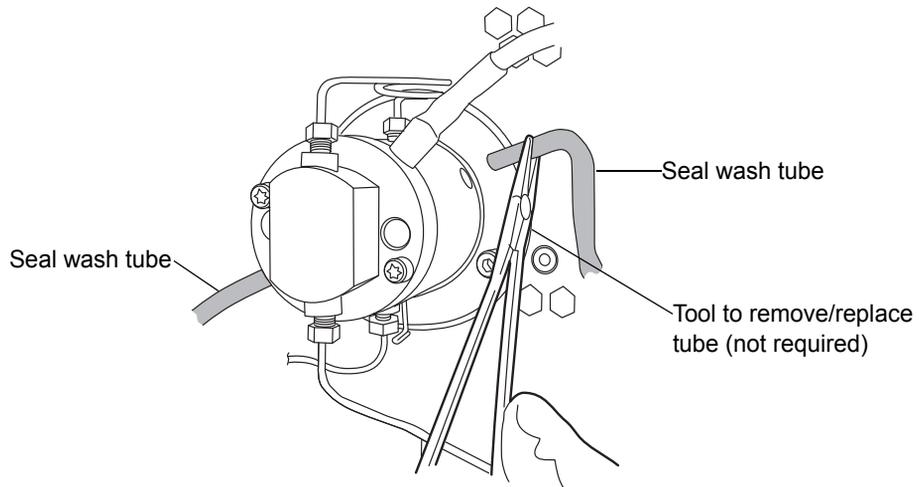
Caution:

- When you remove the valve assembly, ensure the PEEK washer, which is normally on the top face of the *i²Valve* cartridge, does not remain in the head. See [page 6-37](#).
- Never place the actuator assembly or electrical connector in the drip tray.

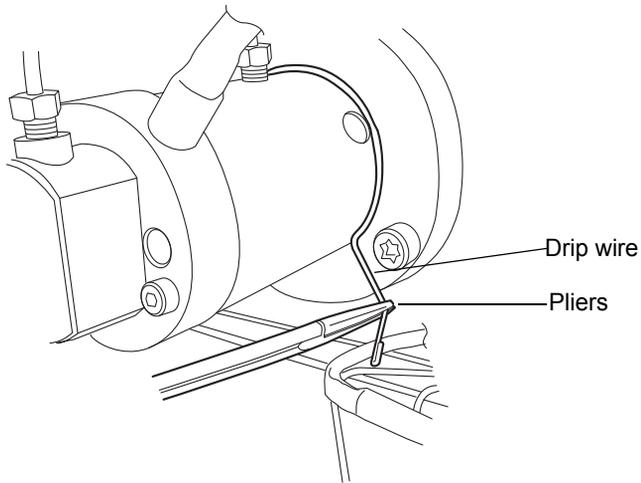
11. Remove the *i*² Valve actuator from the bottom of the primary pump head.



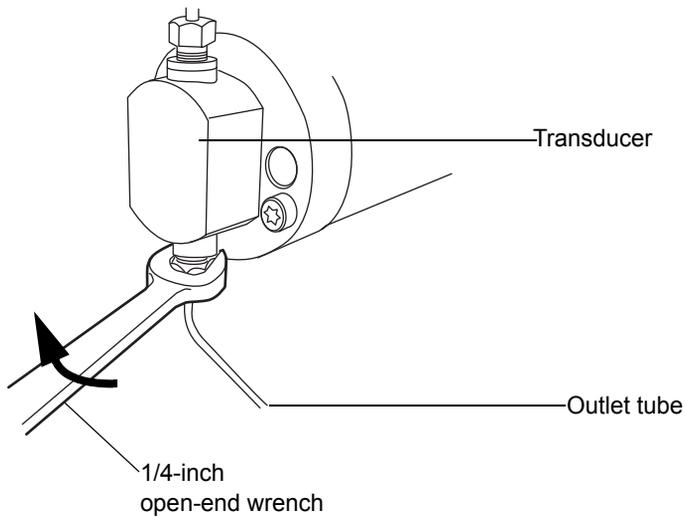
12. Remove both seal wash tubes secured to the seal wash housing by barbed fittings, using a tool or by pulling on the tubes as close to the head as possible.



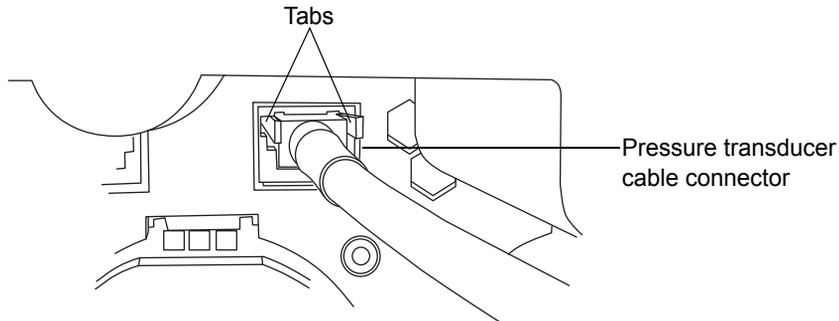
13. Using pliers, remove the drip wire from the head assembly.



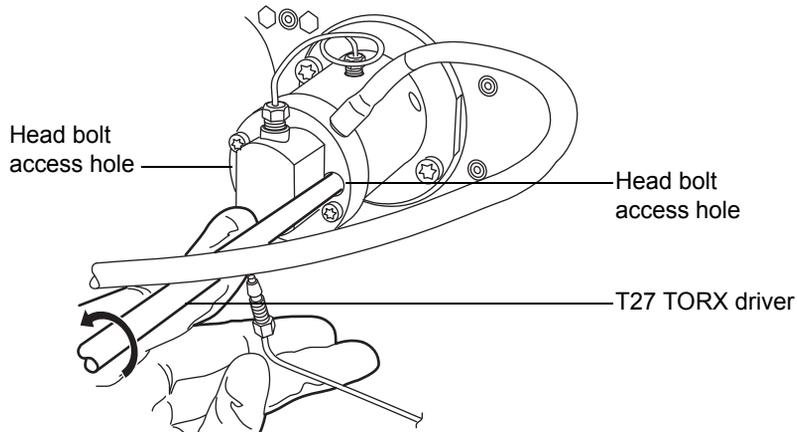
14. Use the 1/4-inch open-end wrench to disconnect the outlet tube from the transducer.



15. Disconnect the pressure transducer cable from the bulkhead by squeezing on the tabs and pulling gently.



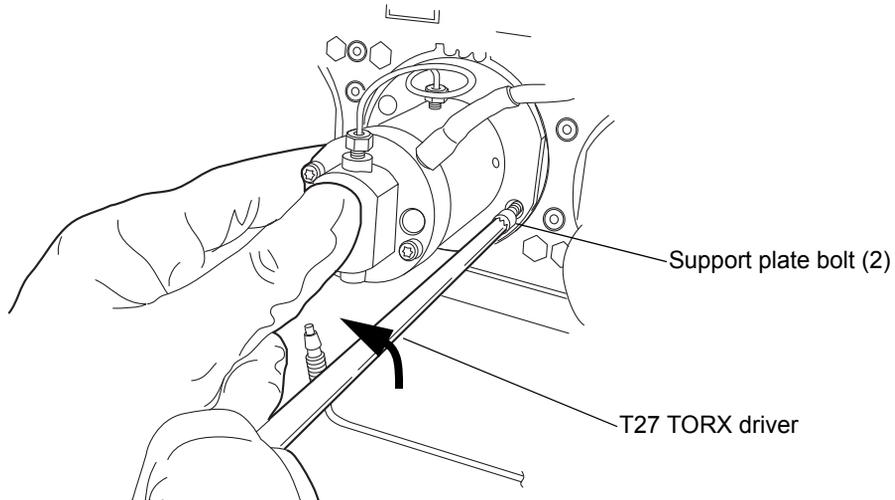
16. Using the T27 TORX driver, loosen the 2 head bolts 1/2-turn. The bolts are accessible from the front of the pressure transducer.



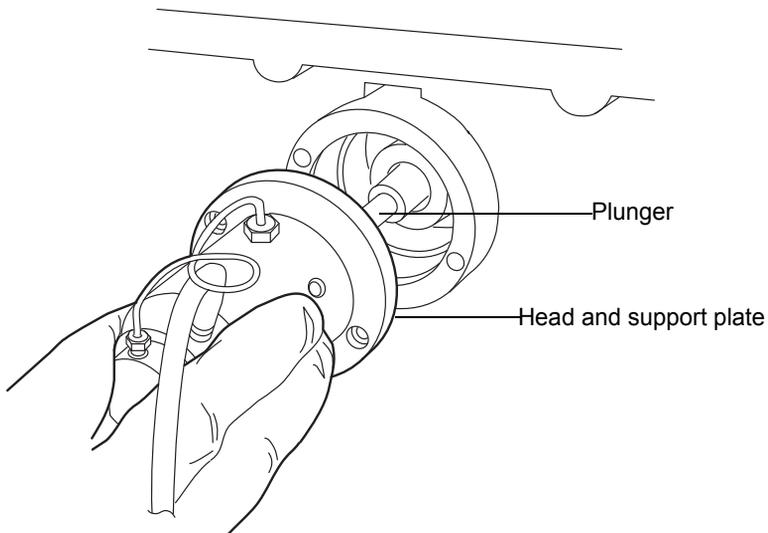
Caution: To avoid damaging the plunger, support the head from below as you remove it.

17. Using the T27 TORX driver, loosen and remove the 2 support plate bolts, and then gently pull the head and support plate off the actuator housing, making sure not to tilt the head during the extraction.

Removing support plate bolts



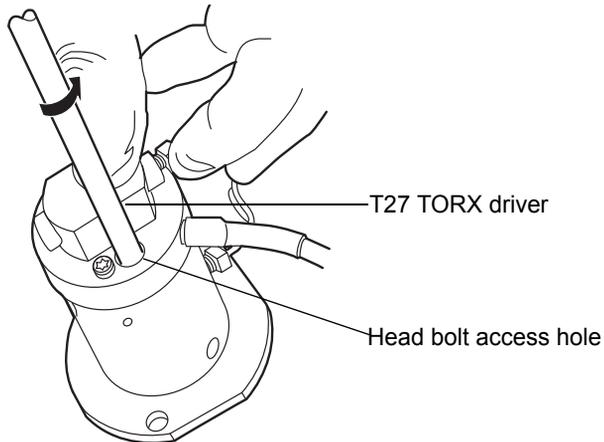
Pulling head and support plate off actuator housing



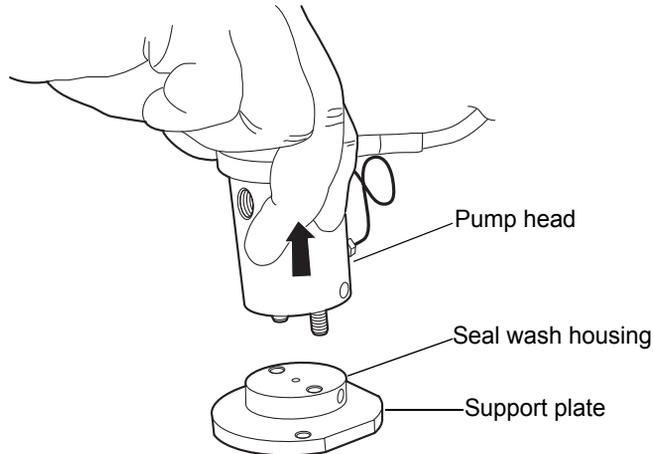
Recommendation: Waters strongly recommends that you replace the head seals when you replace the plunger. If you are not replacing any seals as part of this procedure, proceed to [“To replace the primary head plunger” on page 6-97.](#)

To remove the primary head seals

1. Stand the head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the 2 head bolts to release the support plate from the pump head.

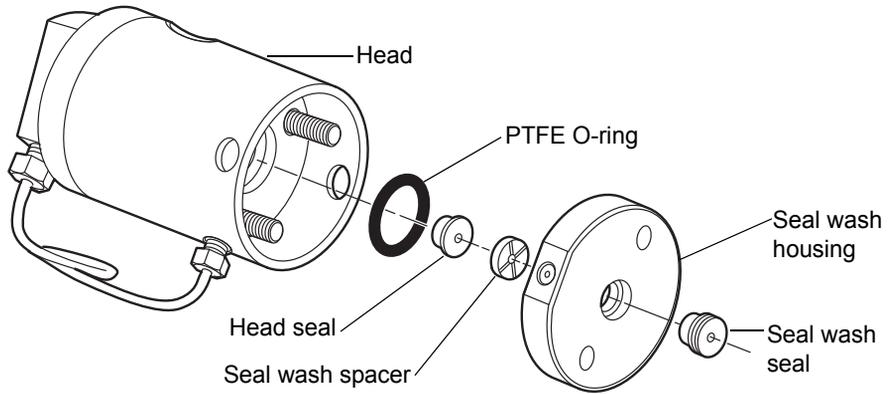


3. Lift the pump head from the support plate.

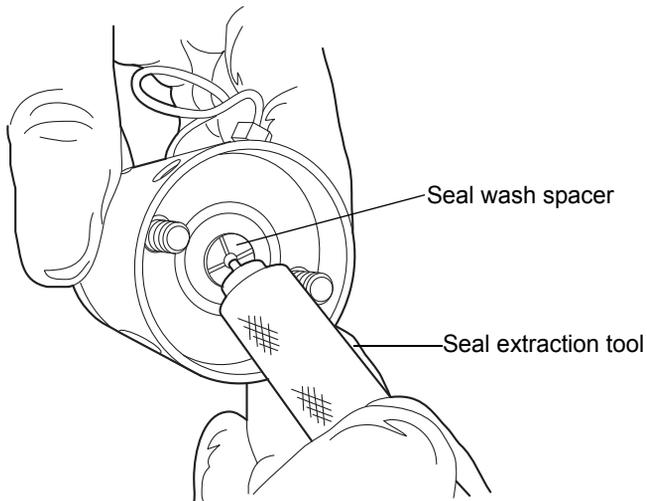


4. Remove the old seal wash seal and discard it.

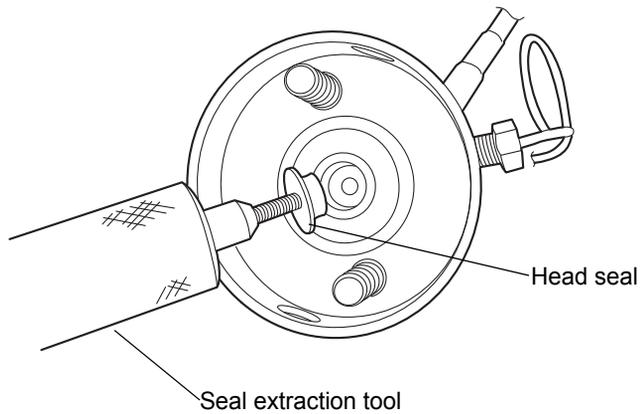
Head seals



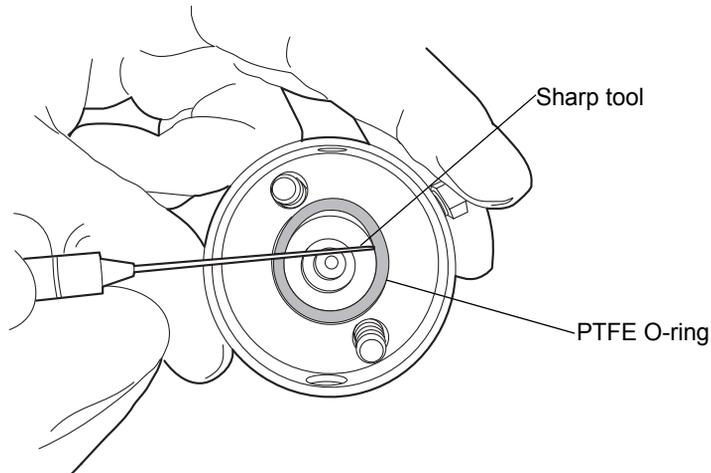
5. Using the smooth end of the seal extraction tool, pull the seal wash spacer from the head.



6. Taking care not to scratch any surfaces, screw the threaded end of the seal extraction tool into the head seal and carefully pull it out of the head.

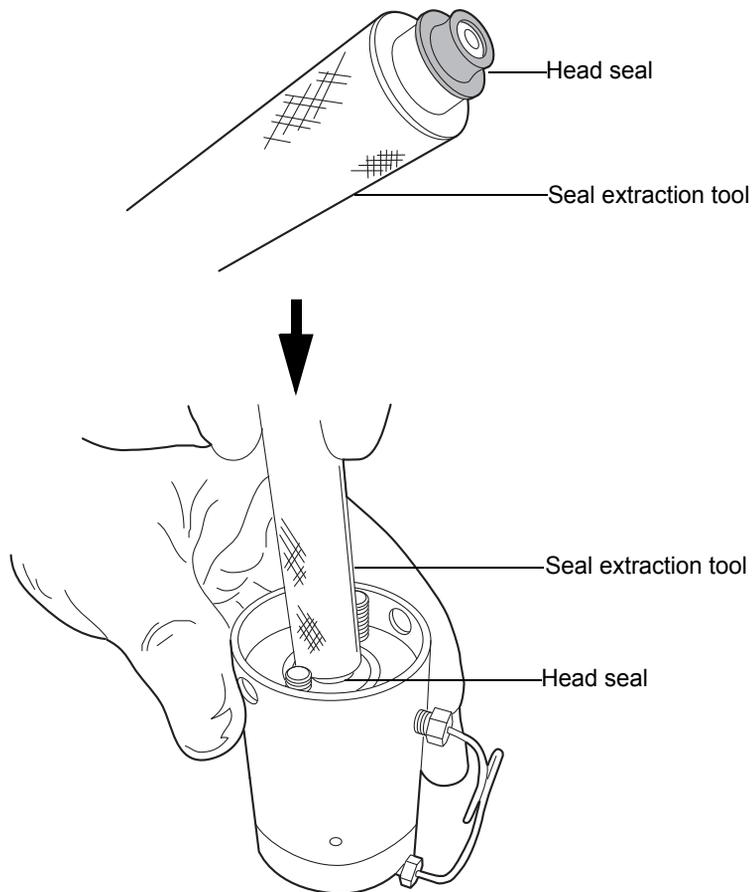


7. Taking care not to scratch any metal surfaces, use a sharp tool to remove the PTFE O-ring.

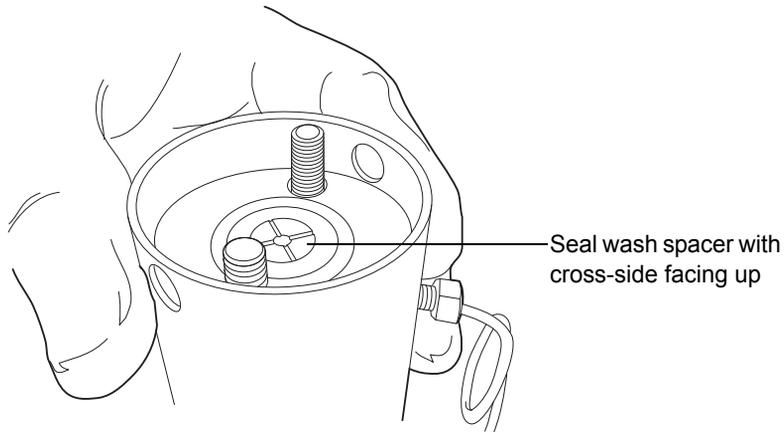


8. Lubricate the new PTFE O-ring with methanol and press the O-ring into its seat with your thumbs.

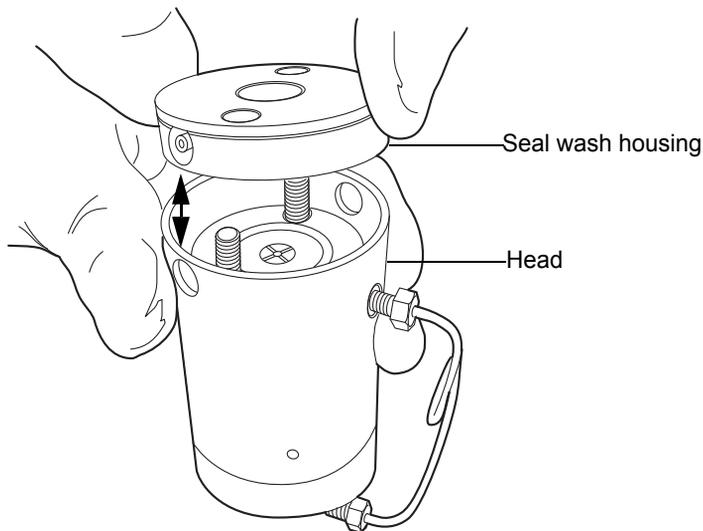
9. Lubricate the new head seal with methanol, and use the smooth end of the seal extraction tool to place it in the head.



10. Center the seal wash spacer over the head seal so that the cross-side faces upward.

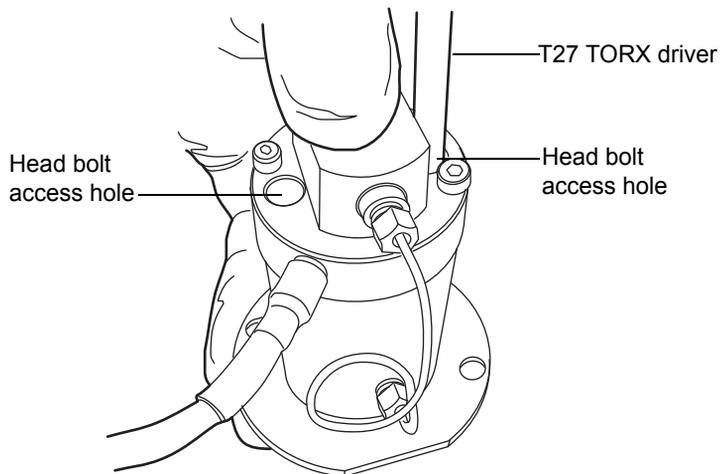


11. Orient the seal wash housing so that the holes on the side of it line up with the holes on the side of the head, and then drop it into place.



12. Install the new seal wash seal in the seal wash housing.
13. Place the support plate on top of the head.

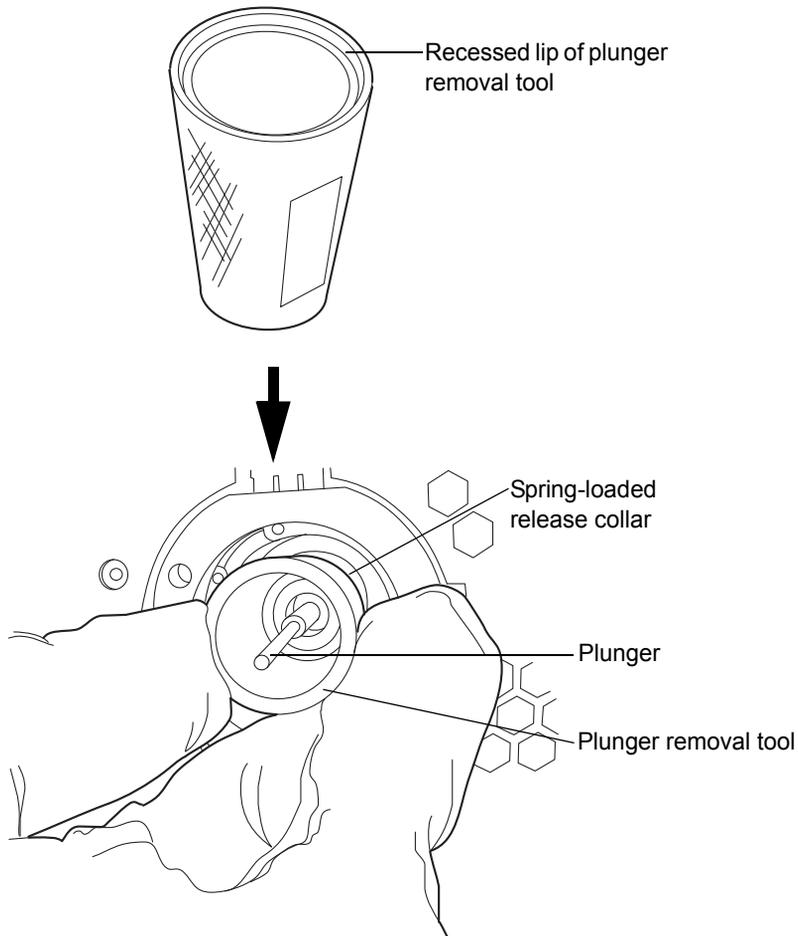
14. Holding the assembly together, use the T27 TORX driver to tighten the 2 head bolts finger-tight.



To replace the primary head plunger

1. Stand the head assembly upright on a clean surface, and set it aside.
Recommendation: Waters strongly recommends that you replace the head seals when you replace the plunger.

2. Use the recessed side of the plunger removal tool to apply pressure to both sides of the release collar, and then remove the old plunger.



3. While applying pressure to the release collar with the plunger removal tool, grasp the new plunger with a clean, lint-free cloth, and insert it.
4. Remove the plunger removal tool from the release collar.
5. Grasp the new plunger by the metal portion and make sure it is secure.

To reattach the primary head

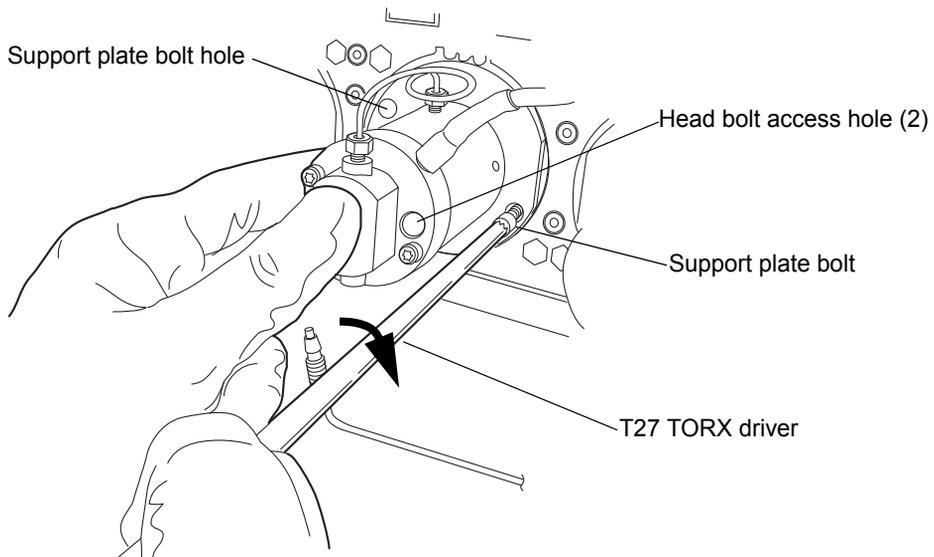
1. Lubricate the seals and plunger with methanol.

 **Caution:** To avoid damaging the plunger, ensure that the head assembly is not tilted relative to the plunger when you position it on the mechanism.

2. Carefully slide the head assembly and support plate over the sapphire plunger, making sure not to tilt the head.

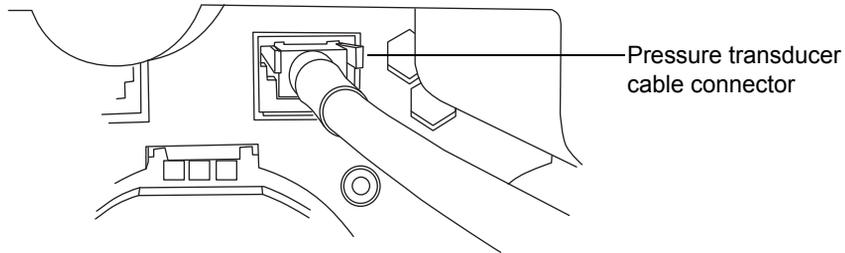
 **Caution:** To avoid damaging the plunger, when tightening the support plate screws, alternate 1/4-turn between each screw so that they are tightened evenly.

3. Hold the head assembly securely against the actuator housing, and then use the T27 TORX driver to tighten the support plate screws securely.

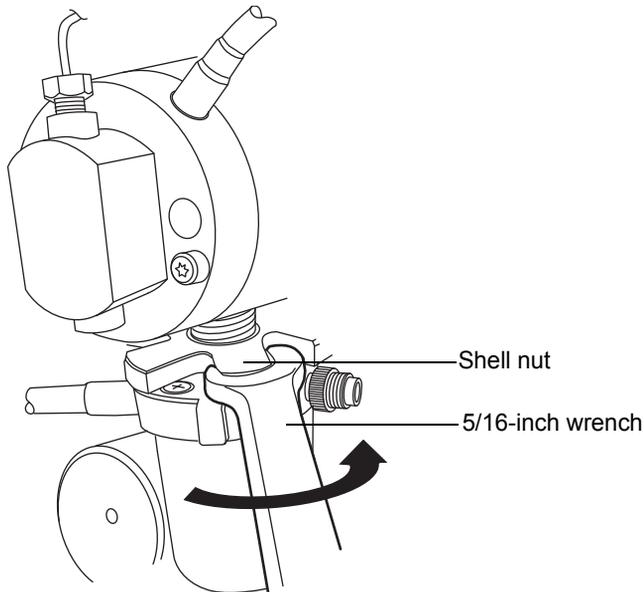


4. Tighten the head bolts evenly as well.
5. Reinstall the drip wire around the head assembly, ensuring that the tip is in the 6 o'clock position.

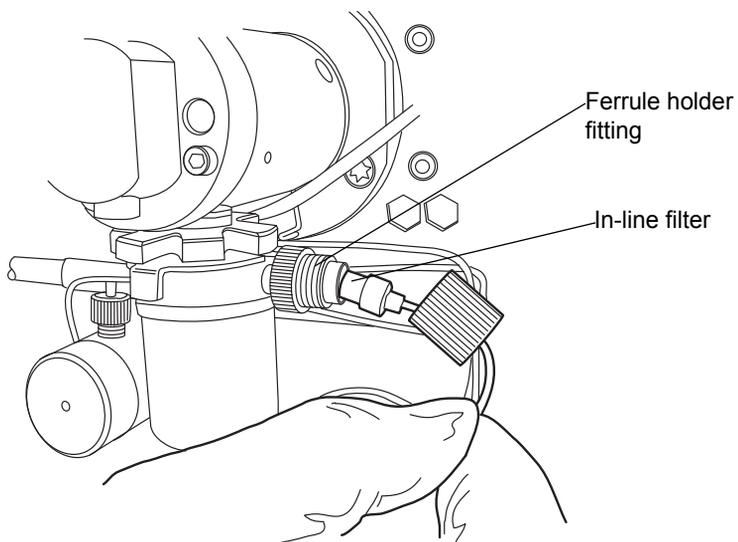
6. Connect the pressure transducer cable to the bulkhead.



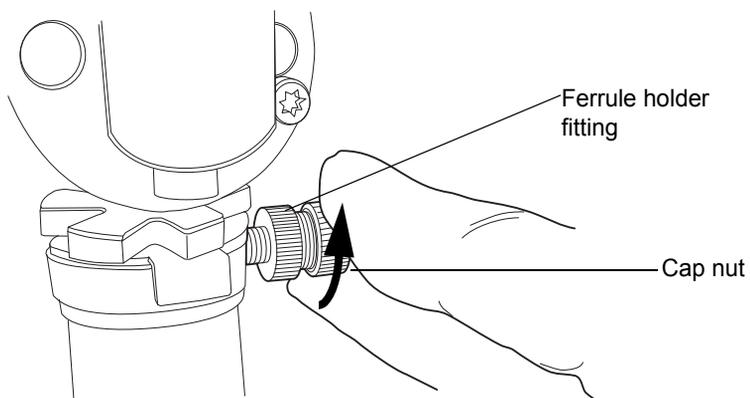
7. Orient the *i*²Valve assembly so that the cable exits from the left-hand side. Insert the *i*²Valve assembly into the bottom of the primary pump head, and route the cable behind the valve actuator.
8. Finger-tighten the shell nut to secure the valve. You should be able to rotate the shell nut approximately 5 full turns to reach the finger-tight condition. Use the 5/16-inch open-end wrench to tighten the nut an additional 1/8-turn.



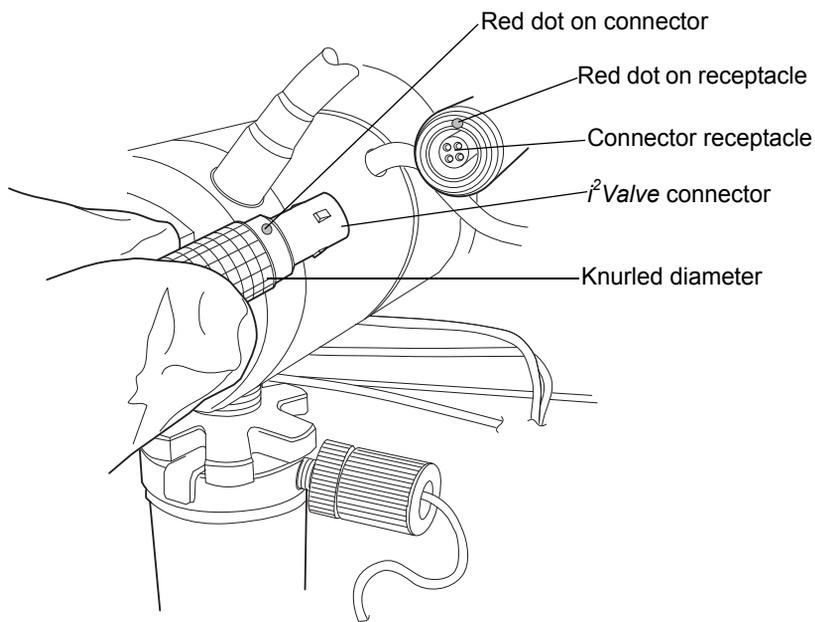
9. Reinsert the in-line filter and tube into the ferrule holder fitting.



10. Place the cap nut over the ferrule holder fitting and finger-tighten the cap nut to the extent possible.



11. Align the red dot on the *i*²Valve connector with the red dot on the receptacle, in the 12 o'clock position, and insert the connector into the receptacle.



12. Reattach all fittings and seal wash tubes.
13. Return the solvent bottles to their original location.
14. Power-on the binary solvent manager.
15. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Replacing the accumulator head plungers



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the plungers.

Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- T27 TORX driver (startup kit)
- Clean, chemical-resistant, powder-free gloves
- Head seal and seal wash spacer (recommended)
- Methanol
- Pliers
- Plunger removal tool
- PTFE O-ring (recommended)
- Replacement plunger
- Seal extraction tool (recommended)
- Seal wash seal (recommended)
- Sharp tool (recommended)

To remove the accumulator head

1. Flush the binary solvent manager with non-hazardous solvent.
2. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
3. In the binary solvent manager information window, click Maintain > Heads.

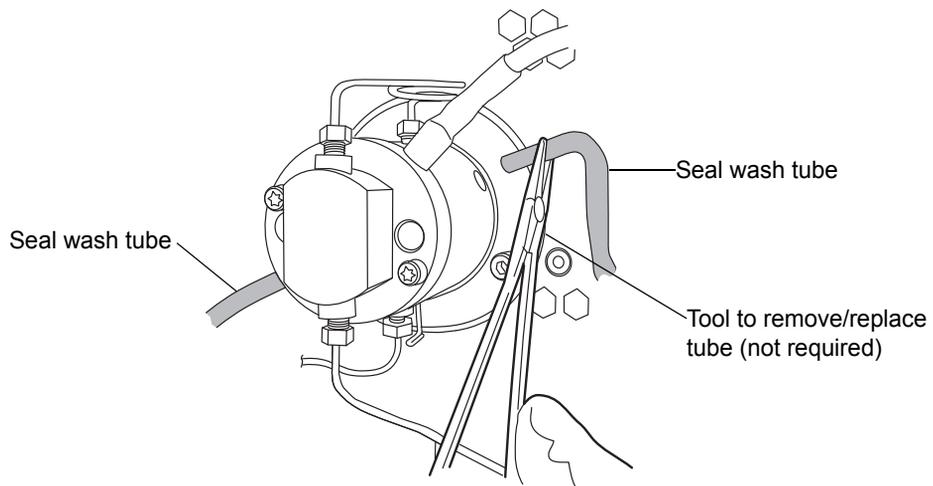
4. In the Head Maintenance dialog box, select the head (Accumulator) from the side of the solvent manager (A or B) that you plan to perform maintenance on.
5. Click Move Backward, and then wait for the plunger to stop.

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

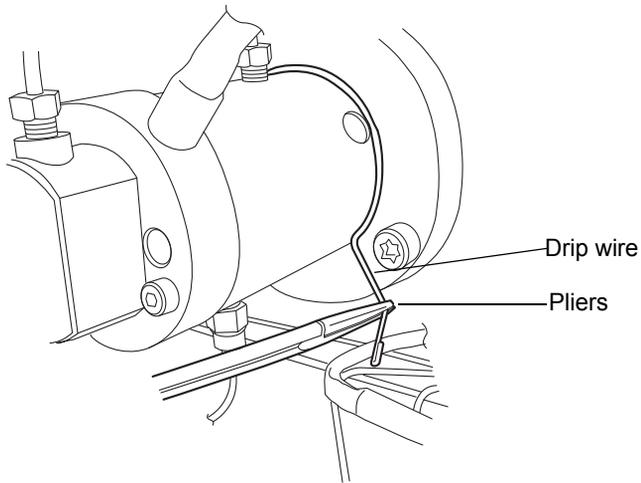
6. Power-off the binary solvent manager.

 **Warning:** To avoid solvent spills, move the solvent bottles to a location below the binary solvent manager.

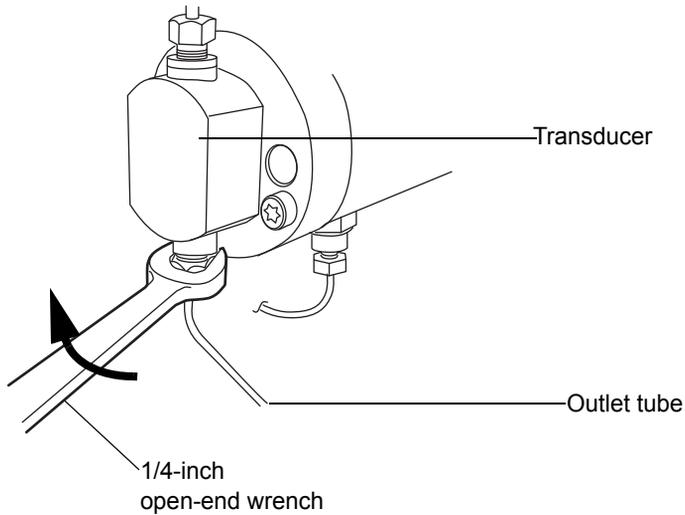
7. Move the solvent bottles to a location below the binary solvent manager.
8. Remove both seal wash tubes secured to the seal wash housing by barbed fittings, using a tool or by pulling on the tubes as close to the head as possible.



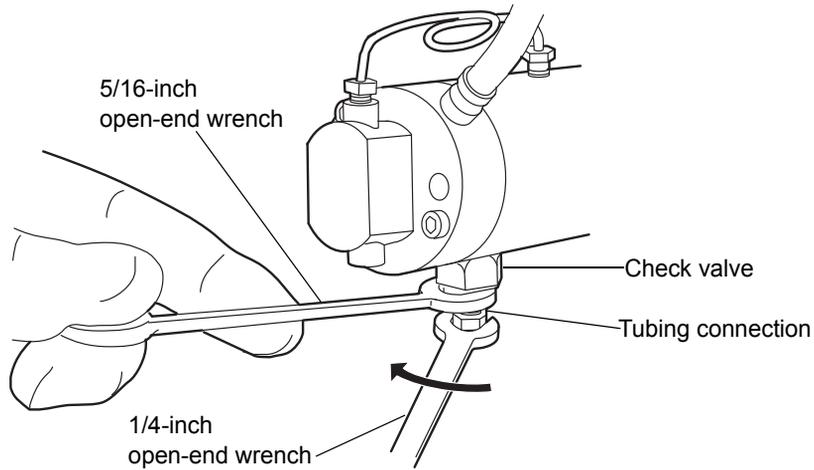
9. Using pliers, remove the drip wire from the head assembly.



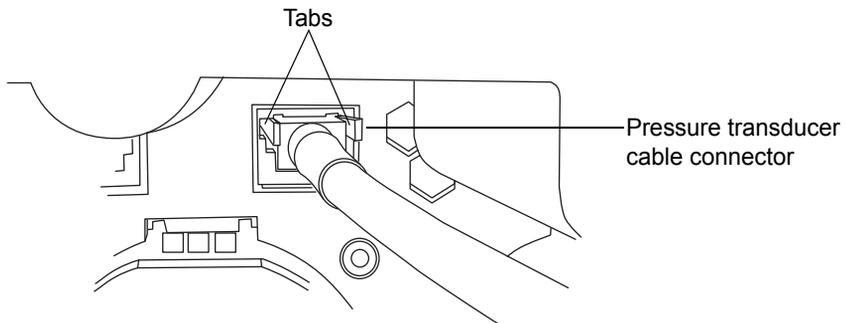
10. Use the 1/4-inch open-end wrench to disconnect the outlet tube from the transducer.



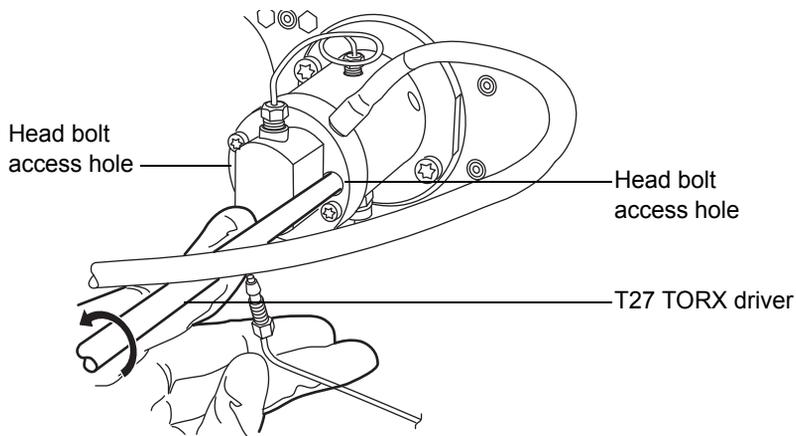
- Using the 5/16-inch open-end wrench to hold the check valve cartridge in place, disconnect the tubing connection from the check valve with the 1/4-inch open-end wrench.



- Disconnect the pressure transducer cable from the bulkhead by squeezing on the tabs and pulling gently.



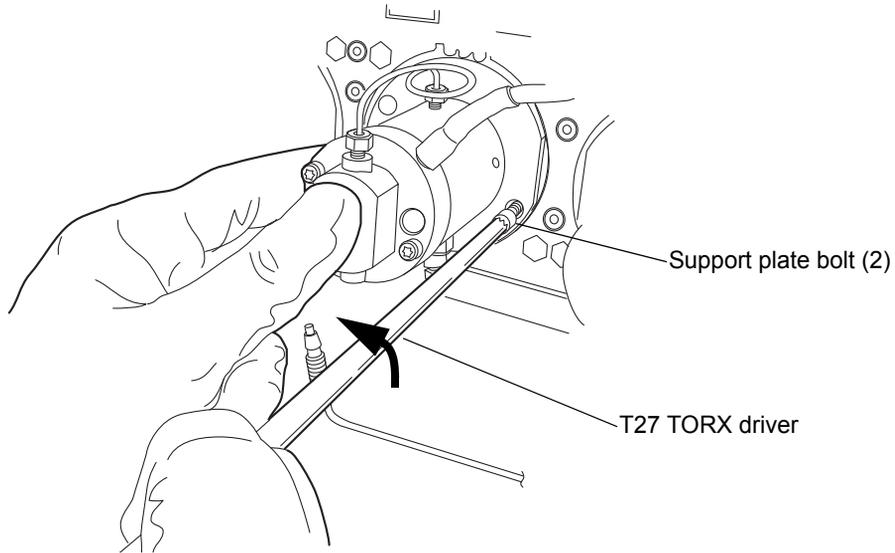
- Using the T27 TORX driver, loosen the 2 head bolts 1/2-turn. The bolts are accessible from the front of the pressure transducer.



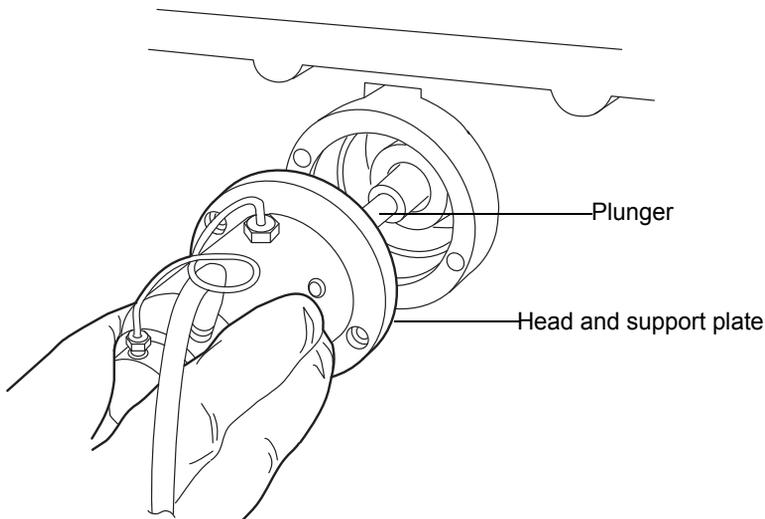
 **Caution:** To avoid damaging the plunger, support the head from below as you remove it.

- Using the T27 TORX driver, loosen and remove the 2 support plate bolts, and then gently pull the head and support plate off the actuator housing, making sure not to tilt the head during the extraction.

Removing support plate bolts



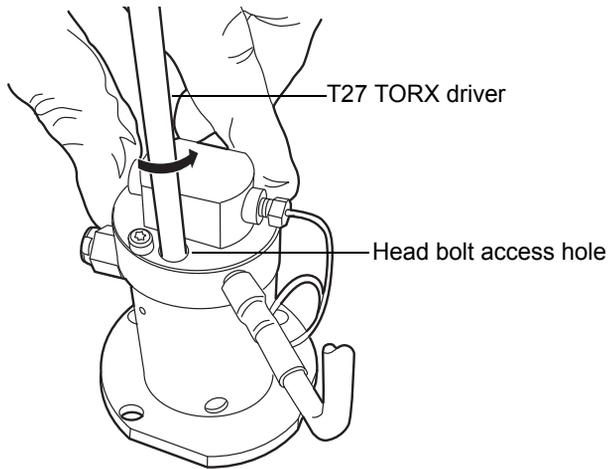
Pulling head and support plate off actuator housing



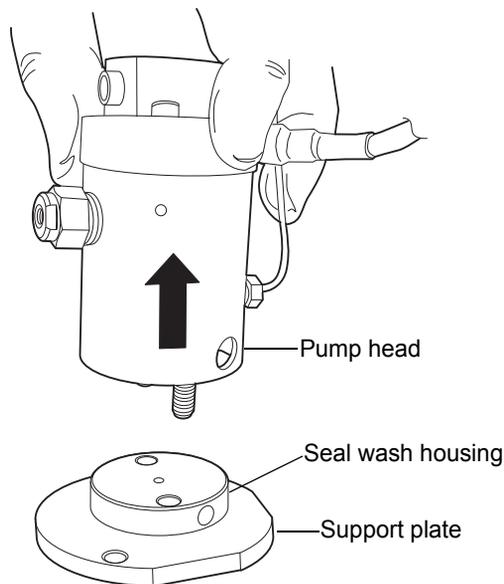
Recommendation: Waters strongly recommends that you replace the head seals when you replace the plunger. If you are not replacing any seals as part of this procedure, proceed to [“To replace the accumulator head plunger”](#) on page 6-114.

To remove the accumulator head seals

1. Stand the head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the 2 head bolts to release the support plate from the pump head.

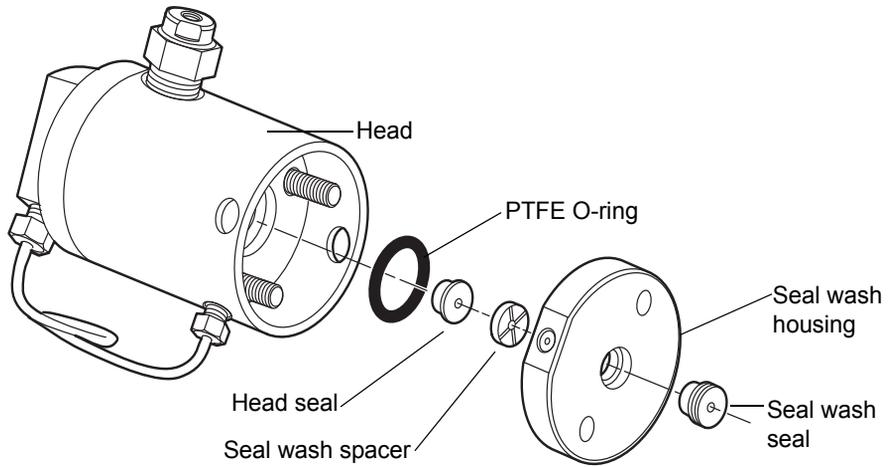


3. Lift the pump head from the support plate.

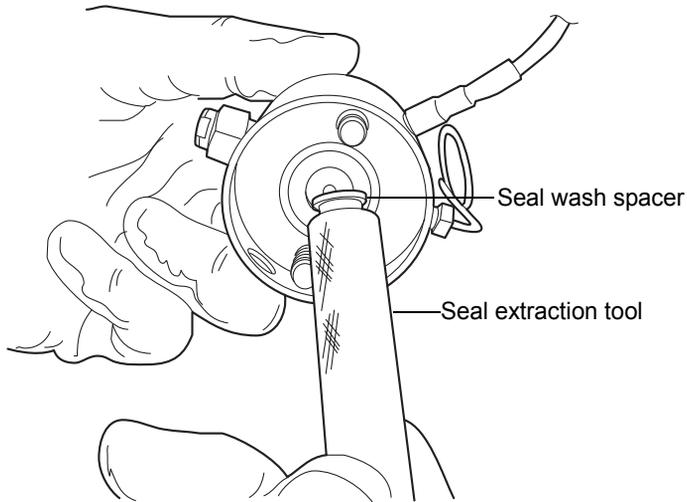


4. Remove the old seal wash seal and discard it.

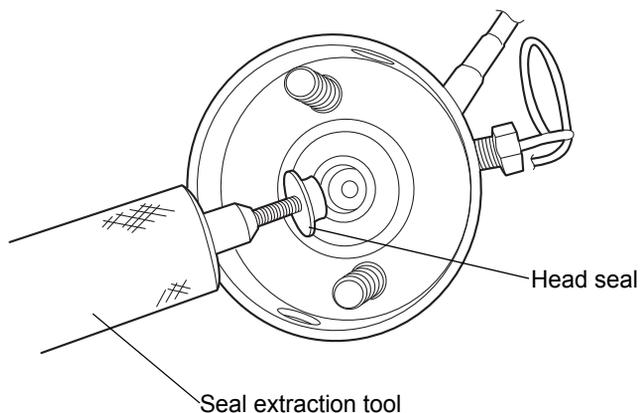
Head seals



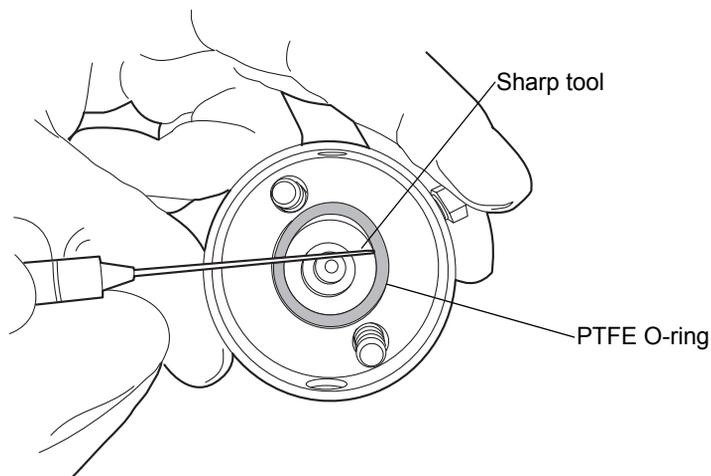
5. Using the smooth end of the seal extraction tool, pull the seal wash spacer from the head.



6. Taking care not to scratch any surfaces, screw the threaded end of the seal extraction tool into the head seal and carefully pull it out of the head.

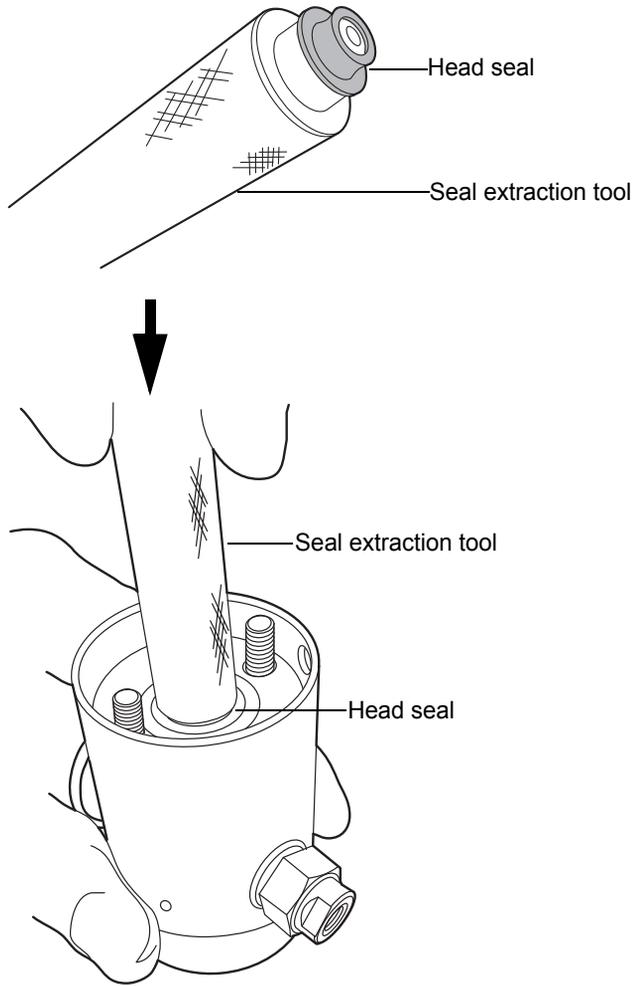


7. Taking care not to scratch any metal surfaces, use a sharp tool to remove the PTFE O-ring.

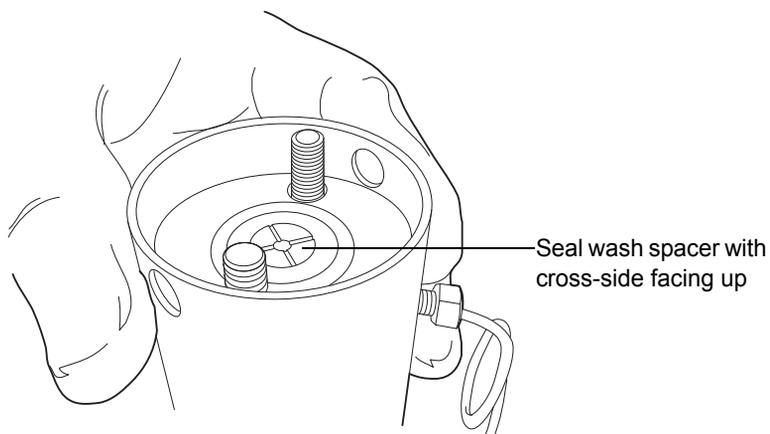


8. Lubricate the new PTFE O-ring with methanol and press the O-ring into its seat with your thumbs.

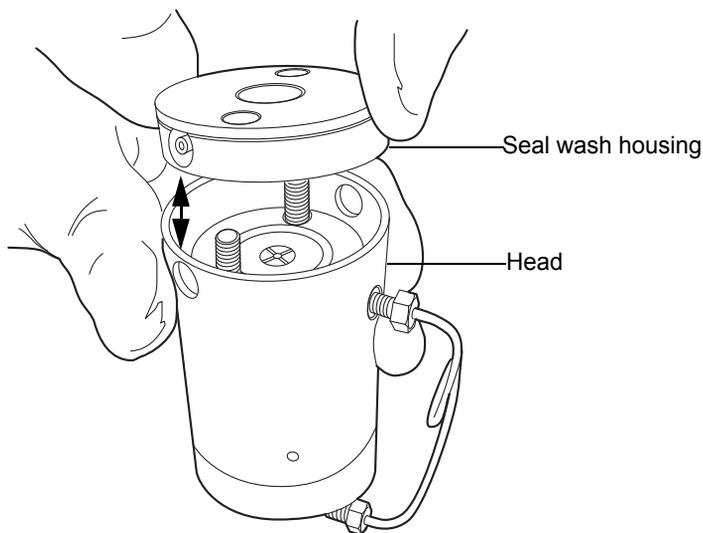
9. Lubricate the new head seal with methanol, and use the smooth end of the seal extraction tool to place it in the head.



10. Center the seal wash spacer over the head seal so that the cross-side faces upward.

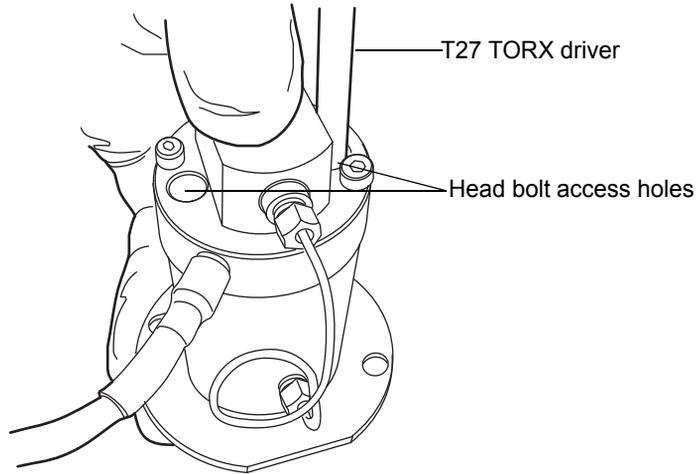


11. Orient the seal wash housing so that the holes on the side of it line up with the holes on the side of the head, and then drop it into place.



12. Install the new seal wash seal in the seal wash housing.
13. Place the support plate on top of the head.

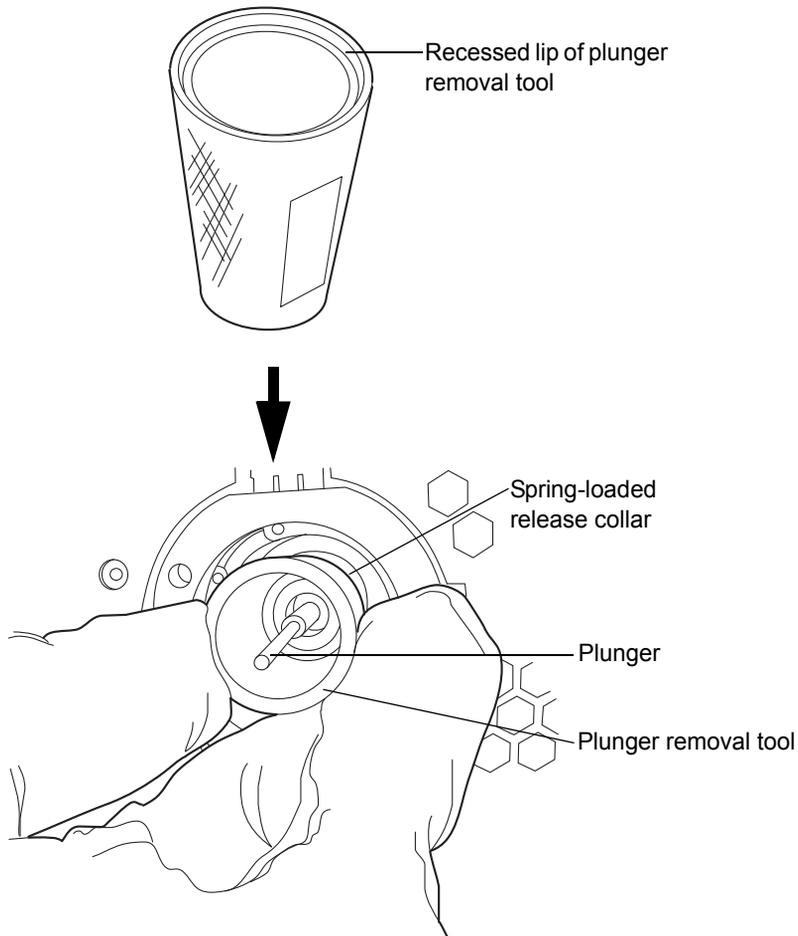
14. Holding the assembly together, use the T27 TORX driver to tighten the 2 head bolts finger-tight.



To replace the accumulator head plunger

1. Stand the head assembly upright on a clean surface, and set it aside.
Recommendation: Waters strongly recommends that you replace the head seals when you replace the plunger.

2. Use the recessed side of the plunger removal tool to apply pressure to both sides of the release collar, and then remove the old plunger.



3. While applying pressure to the release collar with the plunger removal tool, grasp the new plunger with a clean, lint-free cloth, and insert it.
4. Remove the plunger removal tool from the release collar.
5. Grasp the new plunger by the metal portion and make sure it is secure.

To reattach the accumulator head

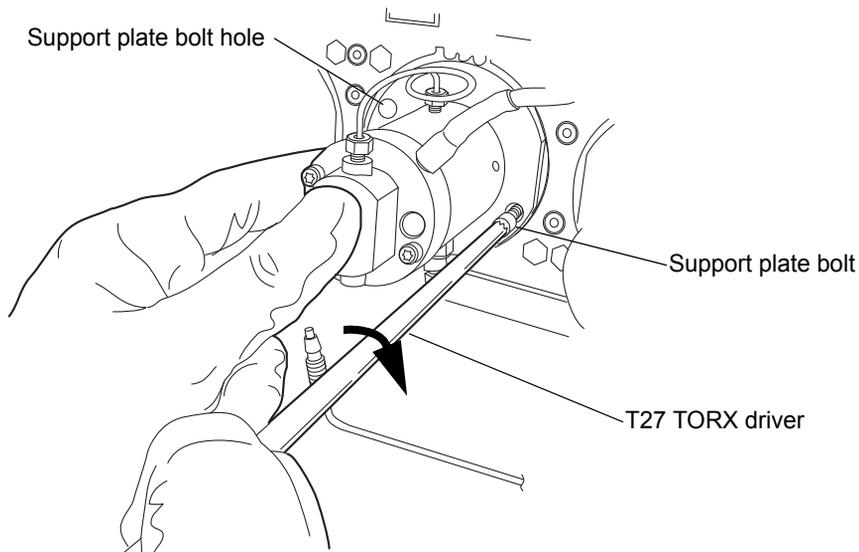
1. Lubricate the seals and plunger with methanol.

 **Caution:** To avoid damaging the plunger, ensure that the head assembly is not tilted relative to the plunger when you position it on the mechanism.

2. Carefully slide the head assembly and support plate over the sapphire plunger, making sure not to tilt the head.

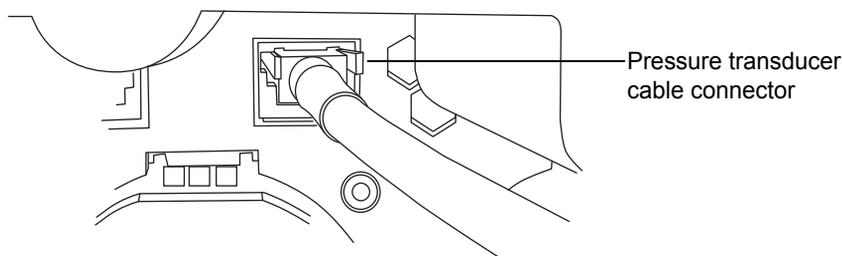
 **Caution:** To avoid damaging the plunger, when tightening the support plate screws, alternate 1/4-turn between each screw so that they are tightened evenly.

3. Hold the head assembly securely against the actuator housing, and then use the T27 TORX driver to tighten the support plate screws securely.



4. Tighten the head bolts evenly as well.
5. Reinstall the drip wire around the head assembly, ensuring that the tip is in the 6 o'clock position.

6. Connect the pressure transducer cable to the bulkhead.



7. Reattach all fittings and seal wash tubes.
8. Return the solvent bottles to their original location.
9. Power-on the binary solvent manager.
10. Prime the binary solvent manager (see [“Priming the binary solvent manager” on page 3-9](#)).

Replacing the vent valve cartridge



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the binary solvent manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the vent valve cartridge.

Required materials

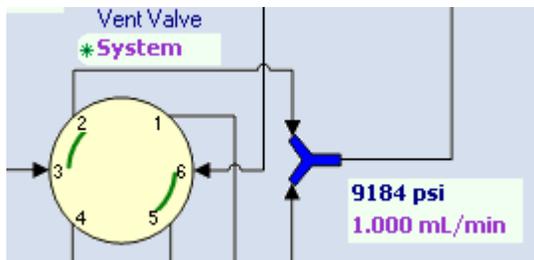
- 1/4-inch open-end wrench
- Allen wrench, 2-mm (startup kit)
- Clean, chemical-resistant, powder-free gloves
- Vent valve cartridge

To replace the vent valve cartridge

1. In the ACQUITY UPLC Console, select Binary Solvent Manager from the system tree.
2. In the binary solvent manager information window, click Interactive Display.
3. In the binary solvent manager interactive display dialog box, click Control .
4. Ensure the vent valve is set to Vent.

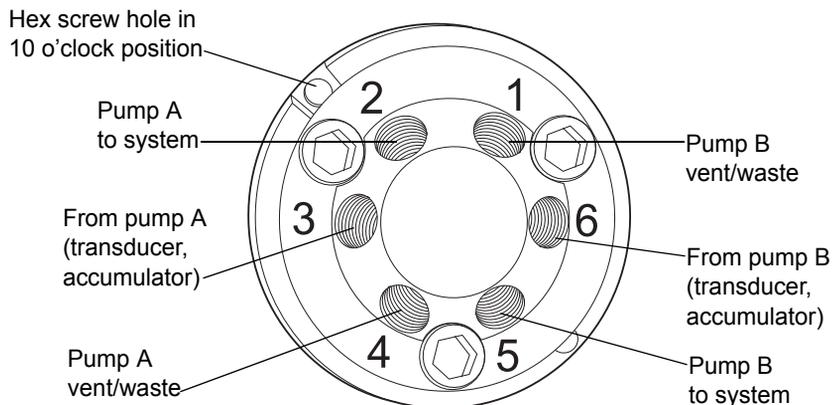
Tip: To change the setting to Vent, click System and select Vent.

Interactive display showing vent valve setting



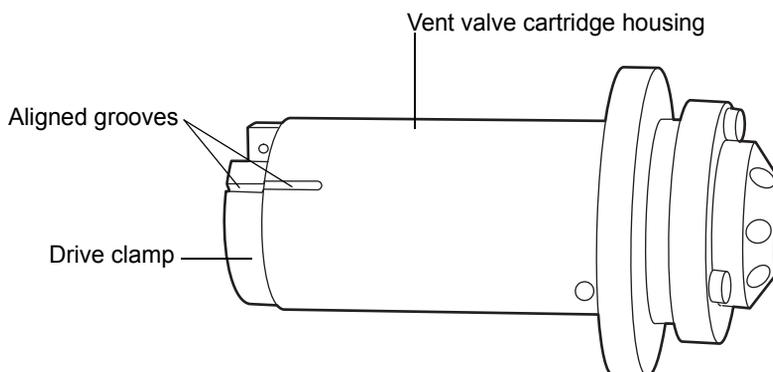
5. Use the 1/4-inch wrench to remove the fittings attached to the vent valve cartridge.

Vent valve cartridge



6. Use the 2-mm Allen wrench to remove the hex screw at the 10 o'clock position on the vent valve cartridge.
7. Remove the vent valve cartridge from the vent valve assembly by pulling straight forward.
8. Unpack the replacement vent valve cartridge.
9. Ensure that the groove in the cartridge housing aligns with the groove on the drive clamp. If it does not, turn the drive clamp until the grooves align.

Note: Be careful not to scratch the drive clamp or body.

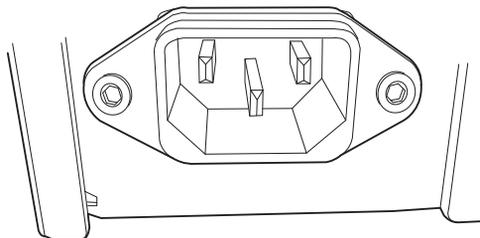


10. Insert the new vent valve cartridge into the vent valve cartridge chamber. Make sure it is in the same orientation as the old vent valve cartridge.
- Requirement:** The vent valve cartridge must slide fully into the vent valve assembly. If it does not, contact Waters Technical Service.
11. Insert the 2-mm hex screw at the 10 o'clock position on the vent valve cartridge. Use the 2-mm Allen wrench to tighten it.
 12. Use the 1/4-inch wrench to reattach all fittings and tighten them 1/4-turn beyond finger-tight for existing fittings, or 3/4-turn beyond finger-tight for new fittings.
 13. Prime the binary solvent manager. See [“Priming a dry binary solvent manager” on page 3-10](#).

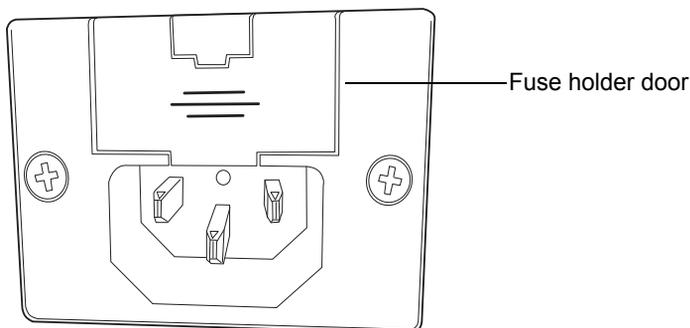
Replacing the fuses

Note: Recently manufactured instruments do not have fuses above the power entry module because the power supply is thermally protected. If your binary solvent manager has the power entry module shown below and it fails to power-on, the status LEDs are unlit, or the power supply fan does not operate, call Waters Technical Service (see [page 6-6](#)).

Power entry module on recently manufactured instruments



Power entry module with fuse holder



Warning: To avoid electric shock, power-off and unplug the binary solvent manager before examining the fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

Suspect an open or otherwise defective fuse when the

- binary solvent manager fails to power-on, and
- binary solvent manager status LEDs are unlit, and
- power supply fan does not operate.

Required materials

- 5 A, 250 V, 5 × 20 mm, slow-blow, IEC type fuse (2)
- Flat-blade screwdriver

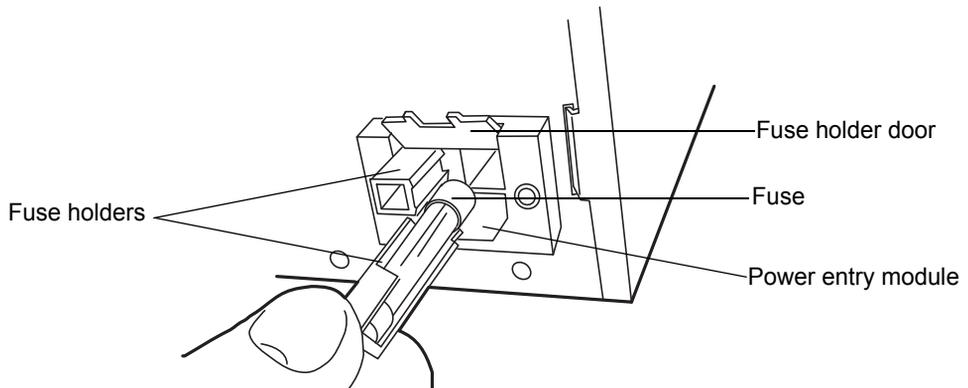
To replace the fuses

Requirement: Replace both fuses, even when only one is defective.



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

1. Power-off the binary solvent manager.
2. Disconnect the power cord from the power entry module.
3. Use a flat-blade screwdriver to open the fuse holder door, located above the power entry module on the rear panel.
4. With minimum pressure, pull on each spring-loaded fuse holder to remove it.



5. Discard the fuses.



Warning: For continued protection against fire, replace fuses with the appropriate type and rating.

6. Insert the new fuses into the holders and the holders into the power entry module.
7. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the binary solvent manager.

Maintaining the sample manager

Defrosting the sample compartment

Defrost the sample compartment when the sample manager is unable to reach a set temperature between 4 and 10 °C (39.2 to 50 °F) in a hot and humid environment.

Tips:

- If the ambient temperature is 27 °C (80.6 °F) or higher, the sample manager cannot reach a set temperature of 4 °C (39.2 °F).
- To keep the sample compartment from freezing, open its door only when necessary. (Opening the door admits humid air into the sample compartment, which causes condensation and freezing.)

To defrost the sample compartment



Caution: To prevent heavy condensation from forming inside the sample compartment and damaging it, the sample compartment door must remain open throughout the defrost procedure.

1. Open the sample compartment door, and remove any samples.

Tip: The sample organizer (if present) automatically defrosts along with the sample manager.



Caution: To prevent heavy condensation from forming inside the sample organizer and damaging it, the sample organizer door must remain open throughout the defrost procedure.

2. If your system contains a sample organizer, open the sample organizer door and remove any samples.
3. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
4. In the sample manager information window, click Maintain > Defrost.
5. In the Defrost dialog box, click Start. The sample compartment defrosts for 60 minutes.

Resolving sample manager leak sensor errors

The sample manager is the only ACQUITY UPLC instrument with 2 leak sensors, bottom and top, called the sample manager leak sensor and the column heater leak sensor, respectively.

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

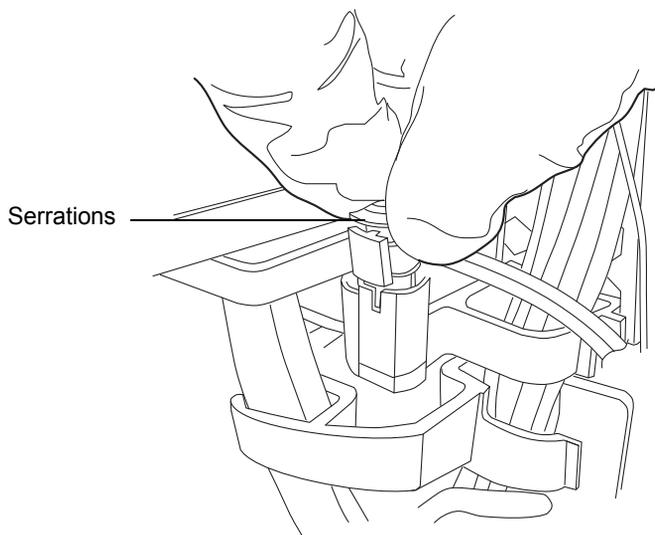
To resolve a sample manager leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to determine which of the sample manager's 2 leak sensors detected a leak.
2. If the message reads "Leak Detected", slide out the sample manager fluidics tray. Otherwise, see ["Resolving HT column heater leak sensor errors" on page 6-162](#).
3. Locate the source of the leak, and make the repairs necessary to stop the leak.



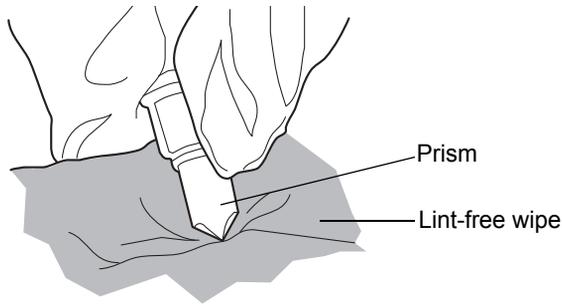
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

4. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

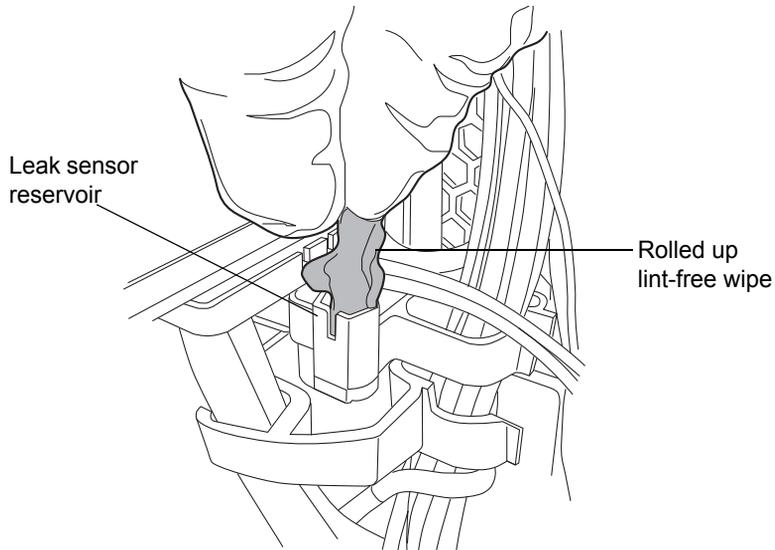


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-128](#)).

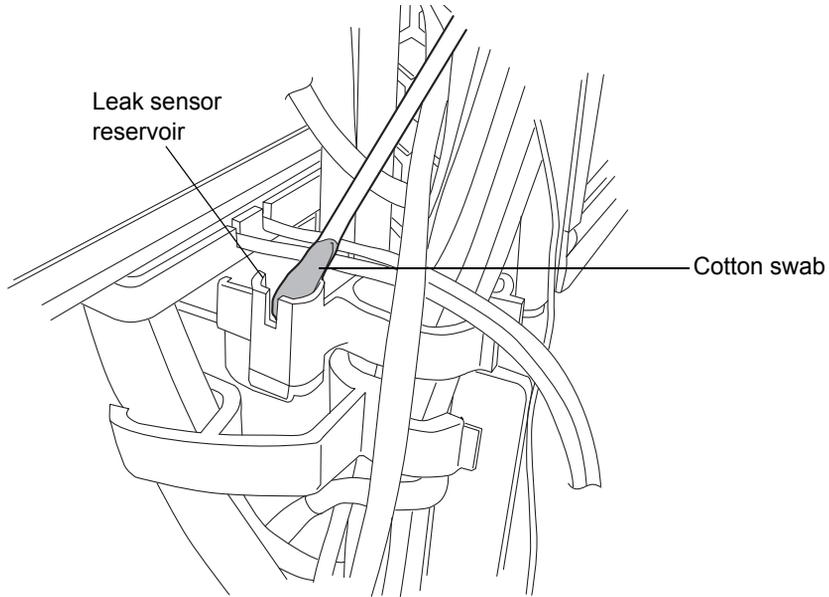
5. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



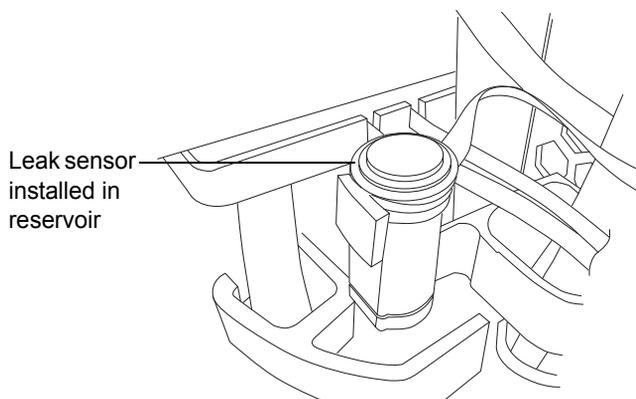
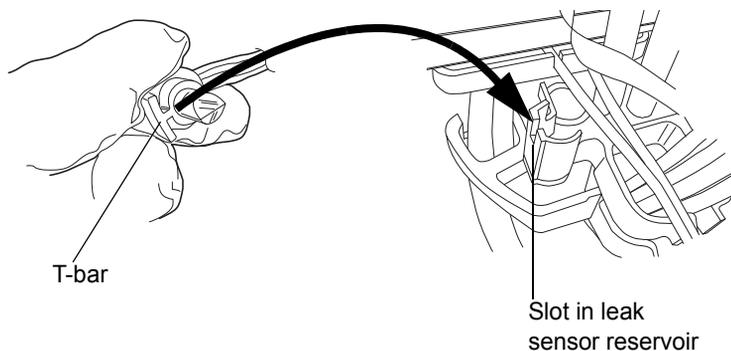
6. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



7. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



- Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



- If you detached the connector from the front of the instrument, reattach it.
- In the ACQUITY UPLC Console, select Sample Manager from the system tree.
- In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Replacing the sample manager's leak sensor



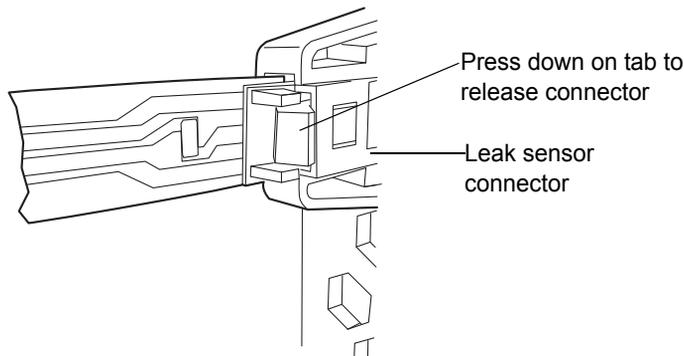
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

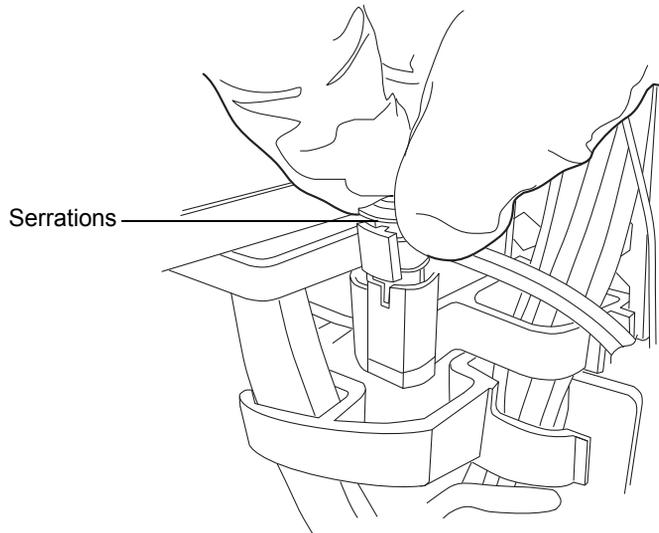
- Clean, chemical-resistant, powder-free gloves
- New leak sensor

To replace the sample manager's leak sensor

1. Open the sample manager door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

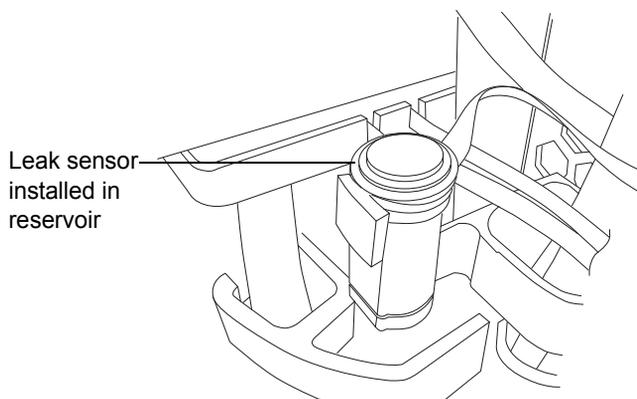
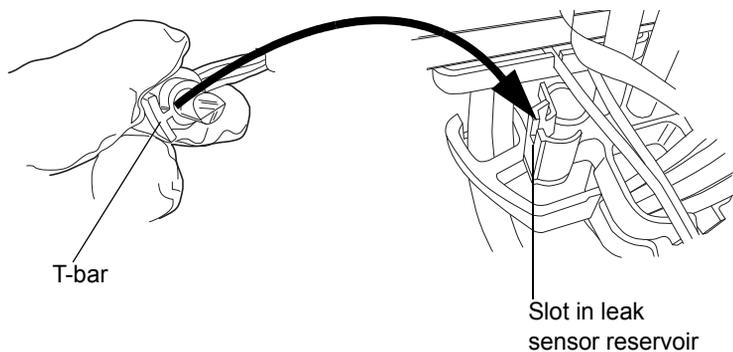


3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



4. Unpack the new leak sensor.

5. Align the leak sensor's T-bar with the slot in the leak sensor reservoir, and slide the leak sensor into place.



6. Plug the leak sensor connector into the front of the instrument.
7. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
8. In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Replacing the sample needle assembly



Warning: To avoid puncture wounds keep hands or loose clothing clear of the needle assembly mechanism while it is moving. The sample manager beeps three times whenever the door is open and the needle assembly mechanism is about to move.



Caution: To avoid operational problems, ensure the sample needle is properly installed.

Replace the needle assembly when the

- sample manager is unable to reach sample transfer pressure
- needle is bent
- needle tip is damaged
- needle is plugged

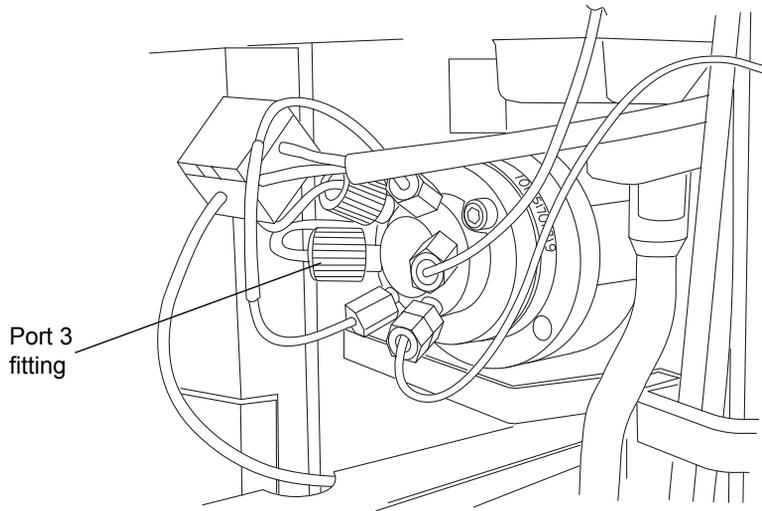
Required material

Needle assembly

To remove the needle assembly

1. In the ACQUITY UPLC Console, click Sample Manager, in the left-hand pane.
2. Select Maintain > Change needle. A message requesting that you remove the right-hand sample plate from the sample manager compartment appears.
3. Open the sample manager door. Pull out the right-hand tray, and remove the sample plate, if one is loaded.
4. After removing the sample plate from the tray, click OK on the ACQUITY UPLC Console message window.

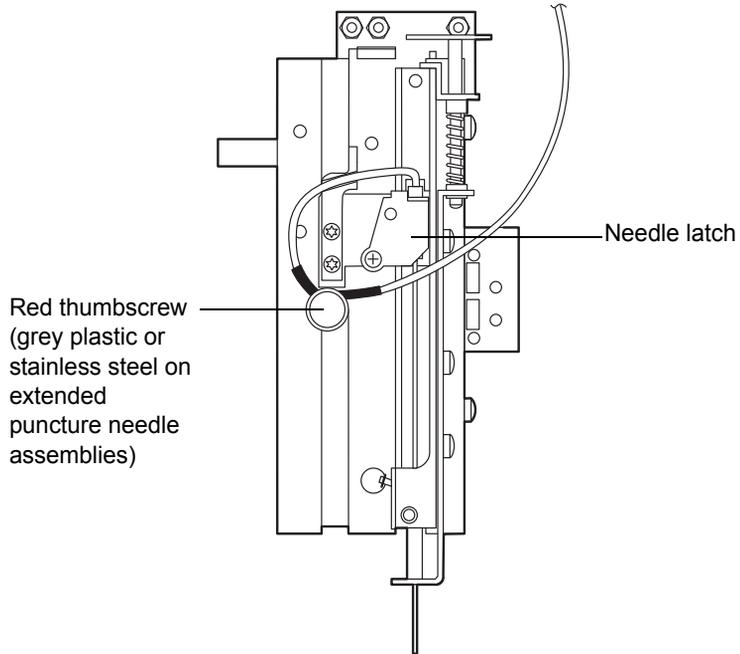
5. Unscrew the needle assembly's fitting from injector port 3.



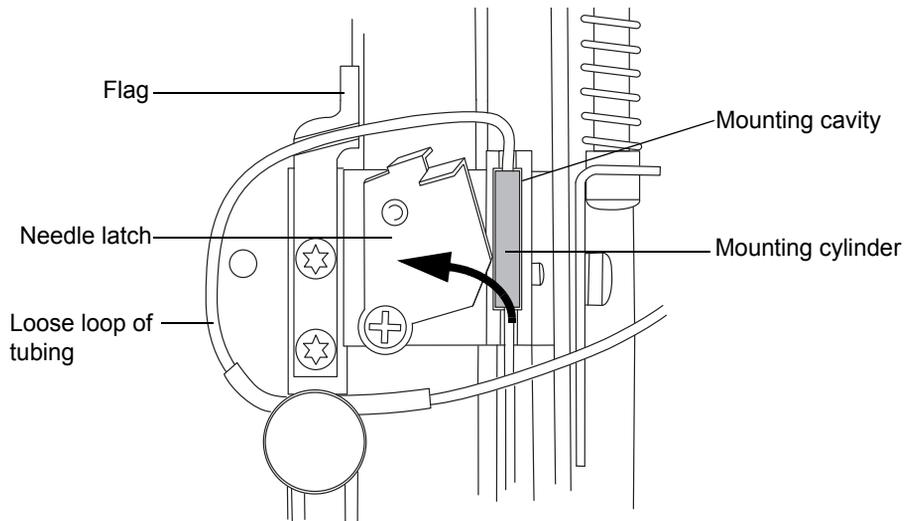
6. Remove the fitting from the needle.
7. In the sample compartment, loosen the red thumbscrew on the needle mounting bracket.

Tip: Extended puncture needles have a grey plastic or stainless steel thumbscrew on the needle mounting bracket.

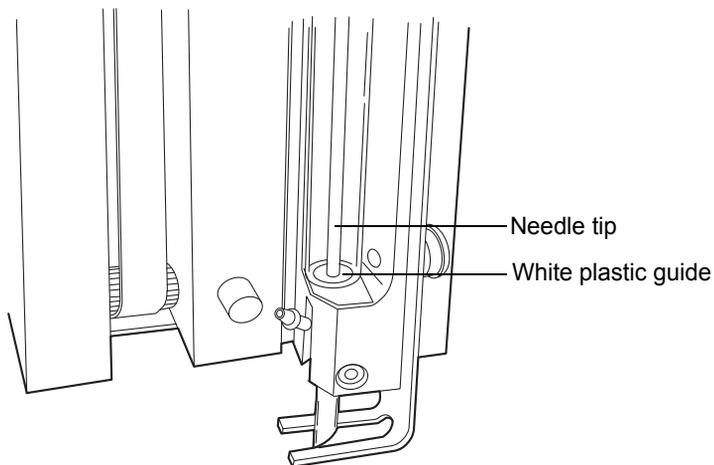
XYZ Mechanism



8. Push the needle latch back to release the needle mounting cylinder from its mounting cavity.



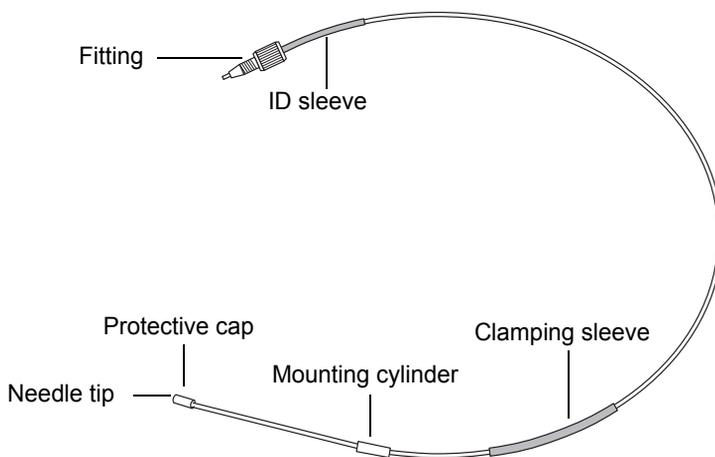
9. Lift the needle tip out of the white plastic guide at the bottom of the XYZ mechanism.



Warning: To avoid puncture wounds, or damage to the end of the needle, do not touch or press the end of the sample needle.

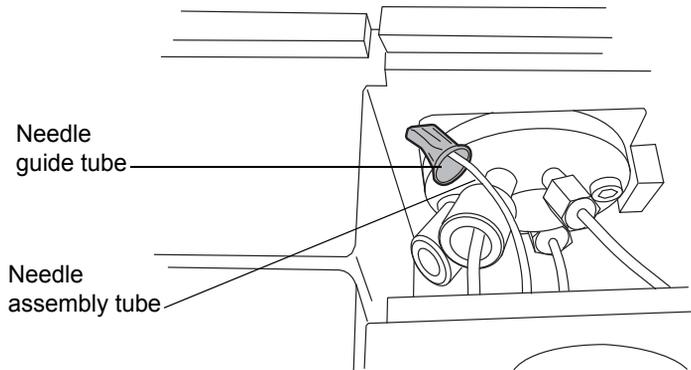
10. Remove the needle assembly from the sample compartment.

ACQUITY UPLC sample needle assembly

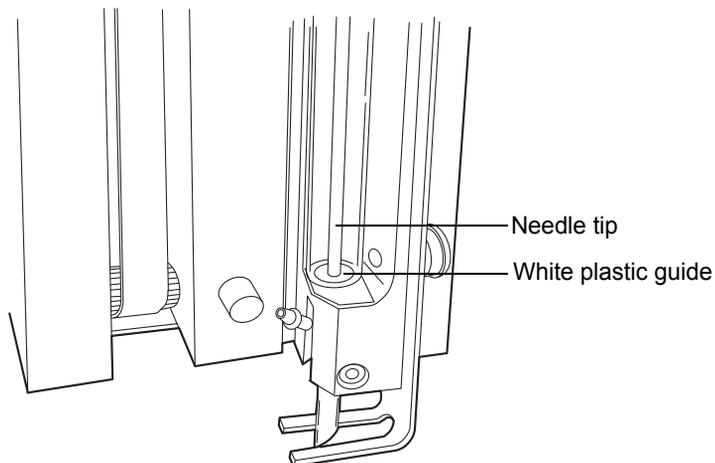


To install the sample needle assembly

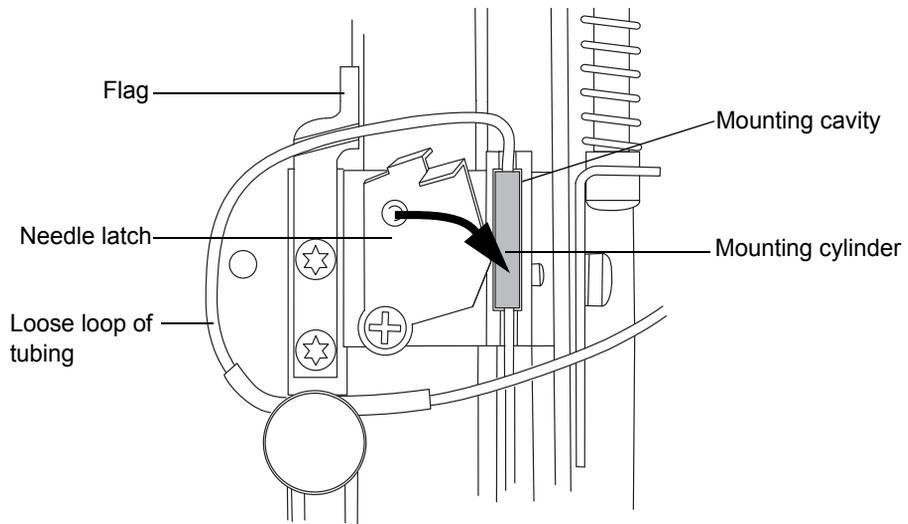
1. Insert the needle tip into the guide tube next to the injector.



2. Gently push the needle assembly into the sample compartment.
3. From inside the sample compartment, take hold of the needle assembly tubing as it enters the chamber from above.
4. Remove the protective cap from the needle tip.
5. Hold the needle by the mounting cylinder, and with the needle tip pointing downward, insert its tip into the white plastic guide at the bottom of the XYZ mechanism.



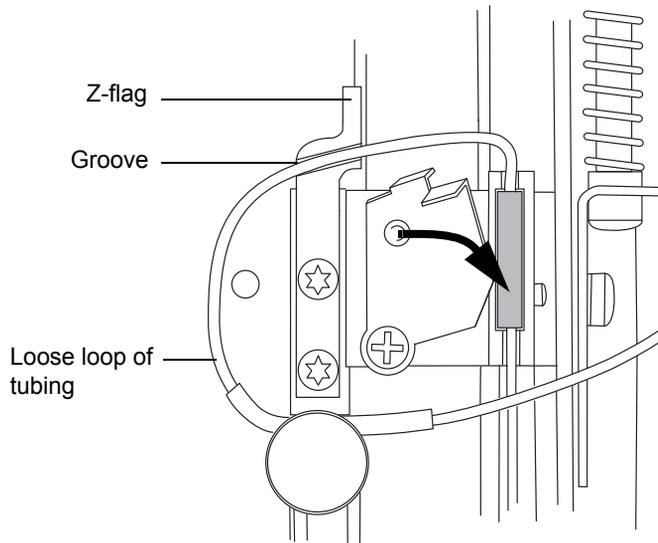
6. Insert the needle mounting cylinder into the mounting cavity.



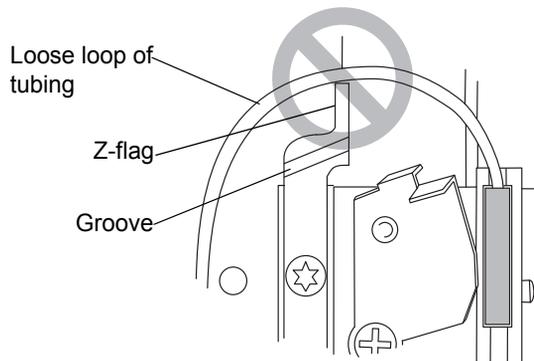
7. Pull the needle latch forward to secure the needle assembly.
8. Form a loop so that the portion of the tubing with the black clamping sleeve can be secured by the thumbscrew.

 **Caution:** To avoid damaging the instrument, do not place the loose loop of tubing above the Z-flag.

9. Route the tubing through the groove below the Z-flag, as shown in the figure below.

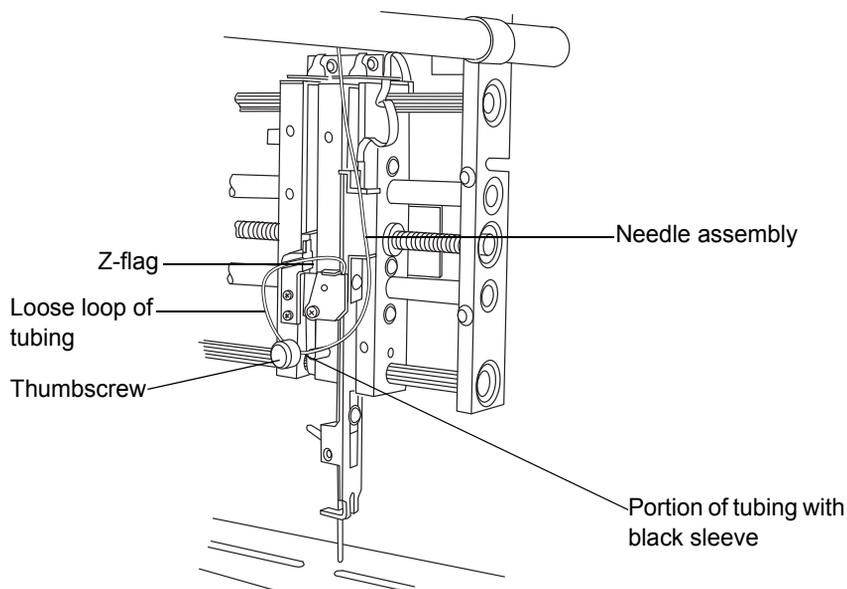


Incorrect placement of loose loop of tubing



10. Align the tubing so that the portion just to the left-hand side of the clamping sleeve fits into the notch behind the thumbscrew.

11. Tighten the thumbscrew so that it secures the portion of the tubing with the black sleeve.



12. Ensure that the needle tubing is fully inserted into port 3, on the injection valve, and then thread the fitting into the port, tightening the fitting securely.
13. Close the sample manager door.
14. On the ACQUITY UPLC Console, click Control > Prime syringe.
15. In the Prime Syringes dialog box, select Sample syringe and wash syringes.
16. Type 1 in the “Number of cycles” text box.
17. Click OK. The sample manager status displays “Priming”. When priming is complete, the status returns to “Idle”.

Recommendation: After you replace the sample needle assembly, calibrate the needle Z-axis (see [page 6-139](#)) and characterize the needle seal (see [page 6-140](#)).

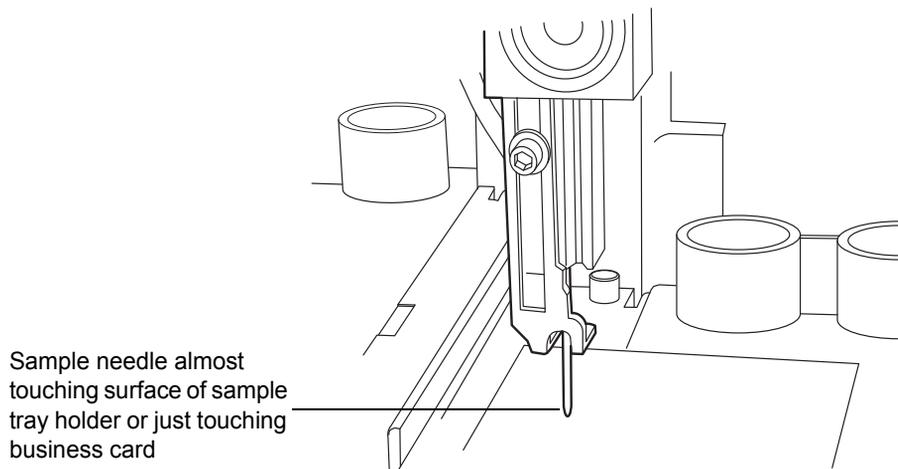
Calibrating the needle Z-axis

You must calibrate the needle Z-axis whenever you replace the sample needle.

To calibrate the needle Z-axis

1. Click Maintain > Calibrate needle Z axis.
2. In the Calibrate Needle Z Axis dialog box, click Start, and then click OK in the confirmation window.
3. Use the +Z button to drive the sample needle down to within 1 mm of the tray surface.
4. Switch the displacement increment to 0.1 mm, and lower the sample needle until it is almost touching the surface of the sample tray holder.

Tip: To easily and efficiently achieve the correct needle setting, slide a business card under the needle. Then lower the needle until it touches the card lightly but does not restrict the card's free movement.



5. Click Save.
- Result:** The confirmation window appears.
6. Click Yes.

Characterizing the needle seal

The needle seal characterization function finds the position at which the needle achieves a seal on the wash station block. Characterizing the needle seal is critical to acceptable sample manager performance.

Perform this procedure after you replace and/or adjust these items:

- Sample needle or any part of the needle assembly
- Needle (Z) or piercing needle (Zp) flags (home and top-of-plate)
- Home or top-of-plate sensor
- Needle seal
- Inject port seal
- Wash station

Requirements:

- Make sure the sample manager is primed before characterizing the needle seal.
- Perform this procedure before calibrating the needle and sample loop volumes.

To characterize the needle seal

1. Click Maintain > Characterize > Needle seal.
2. Click Start. The characterization begins, and the sample manager status displays “Characterizing seal”.

Result: When the operation ends, the Results pane appears.

3. If characterization is unsuccessful, examine the needle to ensure you installed it properly. Make any necessary adjustments, and then characterize the needle seal again. If unsuccessful, consult the ACQUITY UPLC Console online Help.
4. If the characterization is successful, click Close.

Characterizing the needle and sample loop volumes

Perform this procedure whenever you change the

- mobile phases.
- wash solvents.

- sample loop.
- needle.
- syringes.

Whenever you replace the sample loop and/or sample needle, you must characterize the volume of the replacement parts to accommodate the differently sized parts and to ensure optimal chromatographic results. Perform this procedure regardless of whether the sizes of the replacement parts equal those of the original parts or differ from them.

Changes in the composition of the weak wash solvent can affect viscosity, surface tension, and/or polarity. During sample injection, the weak wash solvent both precedes and follows the sample in the fluid-carrying lines, so the sample is directly affected by the weak wash solvent.

Requirements:

- Specify the sizes of the sample needle, loop, and syringe in the Volumes dialog box before you characterize their volumes.
- Prime the sample manager before characterizing the volumes.
- Perform a method setup using Empower or MassLynx software with any method that has the same air gap and sample draw rate that you will be using.
 - If you are running under Empower software control, in the Project window, click File > New Method > Instrument Method.
 - If you are running under MassLynx software control, in the Inlet Editor window, click Inlet Method > Inlet > Autosampler.

To characterize the needle and sample loop volumes

1. Click Maintain > Characterize > Needle and loop volumes.
2. In the Characterize Needle and Loop Volumes dialog box, click Start, and then allow approximately 10 minutes for the test to run (see the run time bar graph).
 - Characterizing the loop volume measures the volume of the loop (in μL).
 - Characterizing the needle volume measures the volume of the needle (in μL).

Tip: The system records this test in the log as a diagnostic test event.

3. To view the data, click Results. If the loop test produces a failing result, suspect that the loop is blocked or leaking. If the needle test produces a failing result, suspect that the needle is bent, broken, or blocked. Also, ensure the needle, sample loop, and sample syringe configurations are correct.
4. To print the results, click Print.
5. Click Close.

Replacing the puncture needle holder

Replace the puncture needle holder if it becomes damaged.



Warning: To avoid injury, do not remove the protective cover from the puncture needle until the replacement procedure is complete. Touching or pressing the end of the needle can cause a puncture wound.

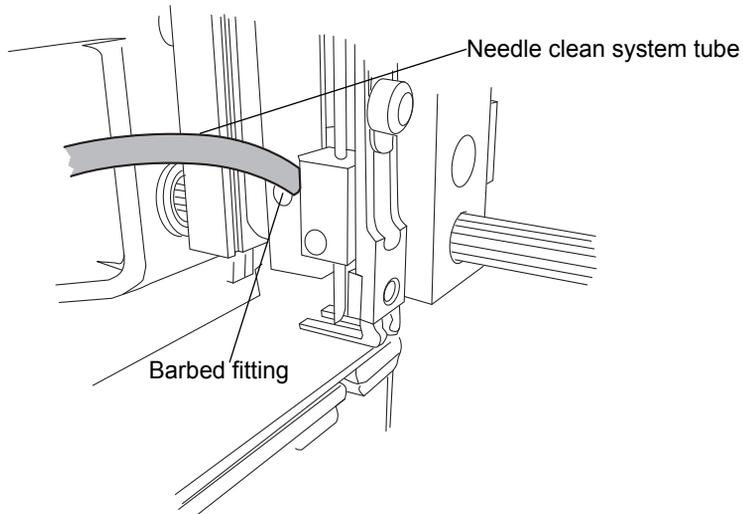
Required materials

- T6 TORX driver
- Clean sheet of paper
- Needle-nose pliers
- Puncture needle cartridge kit

To replace the puncture needle

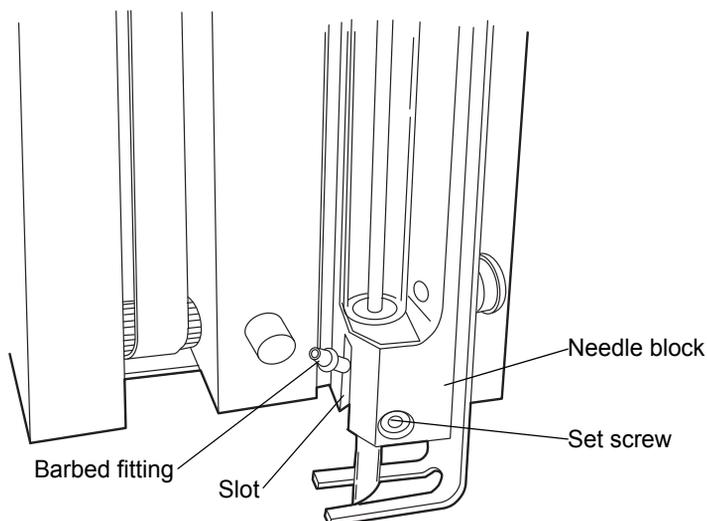
1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. Select Maintain > Replace > Needle. A message requesting that you remove the right-hand sample plate from the sample manager compartment appears.
3. Open the sample manager door. Pull out the right-hand tray, and remove the sample plate, if one is loaded. Use a small, thin coin to turn the screw on the bottom of the tray 1/4-turn counterclockwise, releasing the tray.
4. Remove the well plate tray from the sample manager compartment.
5. In the ACQUITY UPLC Console, click OK to move the needle to the maintenance position.

6. Place a sheet of paper on the floor of the sample manager compartment, beneath the puncture needle. The paper will catch the needle if it falls during replacement.
7. Remove the needle clean system tube from the barbed fitting on the existing puncture needle holder.



8. Loosen the T6 set screw that secures the puncture needle holder. If the puncture needle holder does not fall out of the housing, grasp the puncture needle holder with the needle-nose pliers, and carefully pull downward. If the puncture needle holder does not come out of the needle block, loosen the set screw until it pulls free.
9. Grasp the new puncture needle holder by the protective plastic cover on the needle tip.

- Slide the puncture needle holder over the sample needle and into the housing. Orient the needle so that the barbed fitting enters the slot on the needle block.



- Hold the puncture needle assembly in the fully up position while you tighten the set screw with the T6 TORX driver.
- Reattach the needle clean system tube to the barbed fitting.
- Carefully remove the protective cover from the puncture needle.
- Remove the paper from the sample manager compartment.
- Refit the tray, and turn the screw 1/4-turn clockwise to secure it.
- Select Control > Reset SM to end the maintenance procedure and reset the sample manager.

Replacing the sample loop

Replace the sample loop when it clogs or when its capacity is inappropriate for your chromatographic need.



Caution:

- To prevent bandspreading or excessive carryover, be sure to reinstall a previously installed sample loop in exactly the same way that it was installed before, so that the same end goes into the same port.
- To avoid leaks, remove the O-rings prior to installing the sample loop. The O-rings on the new sample loop hold the seals in place during shipping.

Required materials

- 1/4-inch open-end wrench
- ACQUITY UPLC sample loop

To replace the sample loop

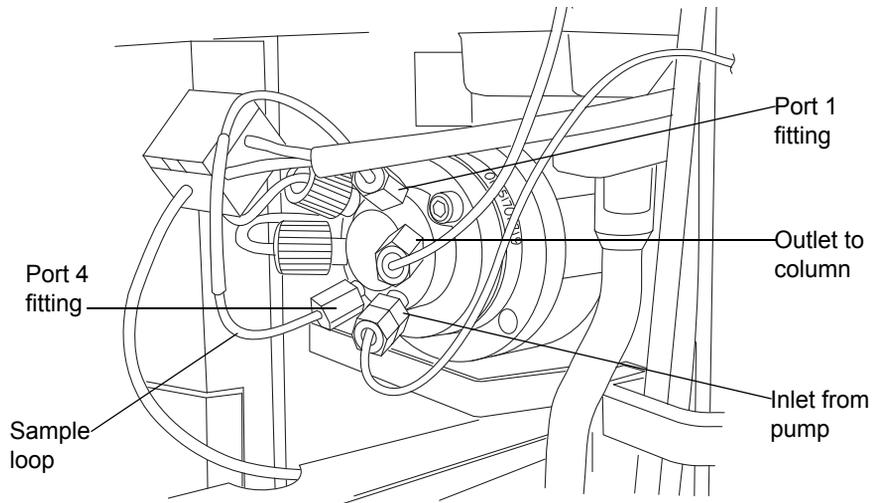
1. Slide out the sample manager fluidics tray.



Caution: To avoid kinks, do not pull on the volume detection device (port 2) or sample needle (port 3) tubing.

2. Hold the sample loop, and pull the injection valve toward you.
3. Use the wrench to remove the fittings on ports 1 and 4 of the injection valve.

Sample loop fittings on the injection valve

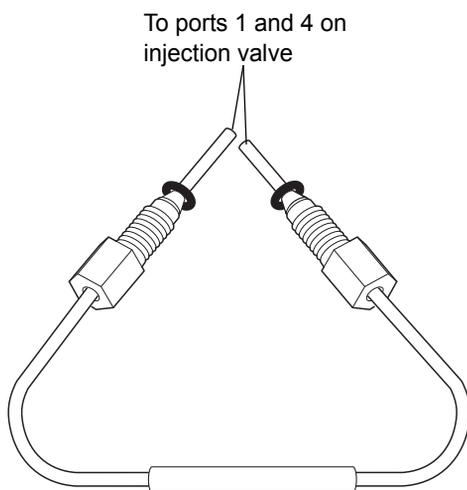


4. Remove the sample loop and its fittings.
5. If you are going to reuse the sample loop, label each end with the port it was connected to.

Tip: If you replaced the injection valve cartridge, do not install previously used sample loops on the new cartridge.

6. Place the sample loop and its fittings in a contamination-free bag.
7. Unpack the replacement sample loop and fittings.

Sample loop (assembled)



 **Caution:** To avoid leaks, remove the O-rings prior to installing the sample loop. The O-rings on the new sample loop are used to hold the seals in place during shipping.

8. Remove the O-rings from the sample loop.

 **Caution:**

- To prevent dead volume in the system, ensure that the ends of the loop are fully inserted into the ports of the injection valve before swaging the ferrules.
- Use only Waters approved ACQUITY UPLC loops with ACQUITY UPLC fittings. Each loop must be fitted to its injector.

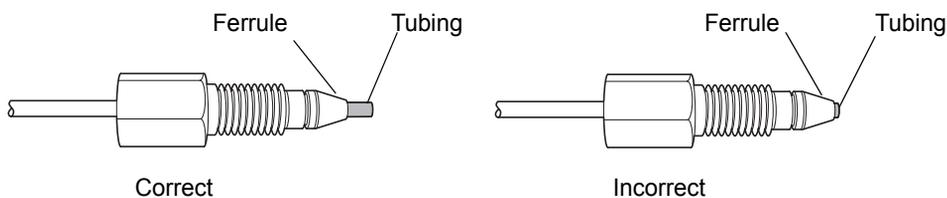
9. Ensure the 2-piece ferrule is in place on one end of the sample loop. If not, slide a 2-piece ferrule and compression screw onto one end of the sample loop, and then seat the end into injection valve port 1.

10. Finter-tighten the fitting and then add 3/4-turn with the wrench.

See also: “[Installation recommendations for fittings](#)” on page 2-21.

11. Remove the fitting and inspect the ferrule to ensure it has not moved.

Ferrule installation



12. Repeat [step 8](#) through [step 10](#) for the other end of the sample loop and for injection valve port 4.
13. Reinstall the sample loop fitting into valve ports 1 and 4.



Caution:

- To avoid kinks, do not push on the volume detection device (port 2) or sample needle (port 3) tubing.
- To prevent bandspreading or excessive carryover, be sure to reinstall the sample loop in exactly the same way that it was installed before, so that the same end goes into the same port.

14. Push the injection valve back to the closed position.



Caution: Be careful not to crush the loop or volume detection device line.

15. Slide the fluidics tray closed.
16. Ensure the sample manager compartment door is closed.
17. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
18. Click Maintain > Calibrate System Volume to characterize the new system volumes.

Replacing the sample syringe

Air bubbles in the syringe adversely affect system pressure, baseline, volume, and peak area. Remove air bubbles by gently tapping on the syringe as its plunger retracts, such as when priming the sample syringe.

Replace the sample syringe when any of these conditions arise:

- The syringe plunger tip becomes worn or discolored
- You want to change to the other syringe size
- The syringe leaks or causes air bubbles
- The syringe fails the leak test

Required materials

- Degassed, weak wash solvent
- Replacement sample syringe



Warning: To avoid injury, ensure that no injection is in progress or pending before you remove the sample syringe.

Maximum syringe draw rates for the 30- μ L needle

Solvent Type	Aspiration (Sample Loops) ^a					
	1 μ L	2 μ L	5 μ L	10 μ L ^b	20 μ L	50 μ L ^c
50:50 methanol/water	25	65	250	280	260	310
100% water	50	120	450	500	480	550
100% acetonitrile	120	300	1130	1300	1250	1400

a. Set in the method editor, on the Advanced tab.

b. The 10- μ L loop is the standard loop.

c. The 50- μ L loop must be used with a 250- μ L sample syringe.

Maximum syringe draw rates for the 15- μ L needle

Solvent Type	Aspiration (Rheodyne Sample Loops) ^a					
	1 μ L	2 μ L	5 μ L	10 μ L ^b	20 μ L	50 μ L ^c
50:50 methanol/water	12.5	32.5	125	140	130	155
100% water	25	60	225	250	240	225
100% acetonitrile	60	150	565	650	625	700

a. Set in the method editor, on the Advanced tab.

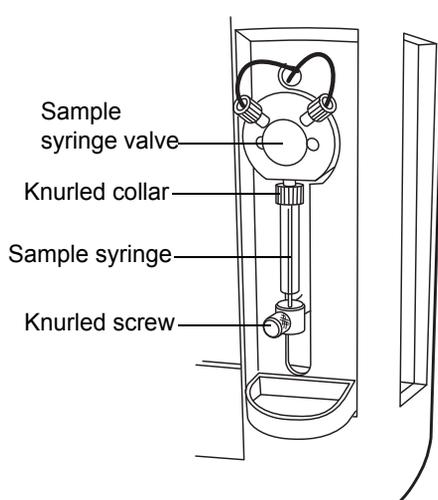
b. The 10- μ L loop is the standard loop.

c. The 50- μ L loop must be used with a 250- μ L sample syringe.

To replace the sample syringe

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. Click Maintain > Replace > Sample syringe. Doing so invokes a wizard that moves the syringe to the down position.
3. Remove the knurled screw that holds the sample syringe to the syringe mounting bracket.

Sample syringe assembly components

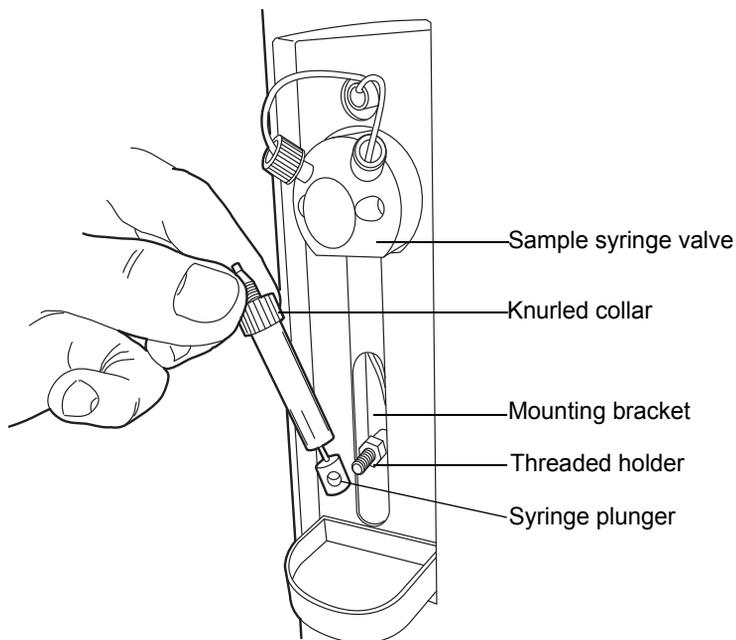


4. Unscrew the sample syringe counterclockwise until it separates from the sample syringe valve.



Caution: To avoid breaking the syringe, do not grasp it by its glass barrel. Always grasp the syringe by its knurled collar.

5. Depress the syringe barrel to clear the top mounting bracket, and remove the syringe.



6. Unpack the replacement sample syringe.
7. Partially fill the new syringe (by hand) with weak wash solvent (to help remove air bubbles).

Requirement: Make sure all air bubbles are removed.

8. Retract the syringe plunger so that the plunger end slides over the threaded post on the syringe guide mounting bracket.
9. Screw the new sample syringe partially into the sample syringe valve.
10. Finger-tighten the sample syringe.

11. Install and finger-tighten the knurled screw that holds the sample syringe plunger to the mounting bracket.
12. Run the Prime Sample Syringe Only option until there are no air bubbles in the sample syringe.

Tip: Tap the sample syringe when the plunger is depressing to remove air.

Replacing the wash syringes

The only wash syringe size available is 2.5 mL. To ensure accurate results, use only syringes that are approved by Waters for use in the ACQUITY UPLC system.

Replace the wash syringes when the syringe plunger's tip leaks, becomes worn or discolored, or fails the leak test.

Required materials

- Degassed wash solvents
- Replacement wash syringes

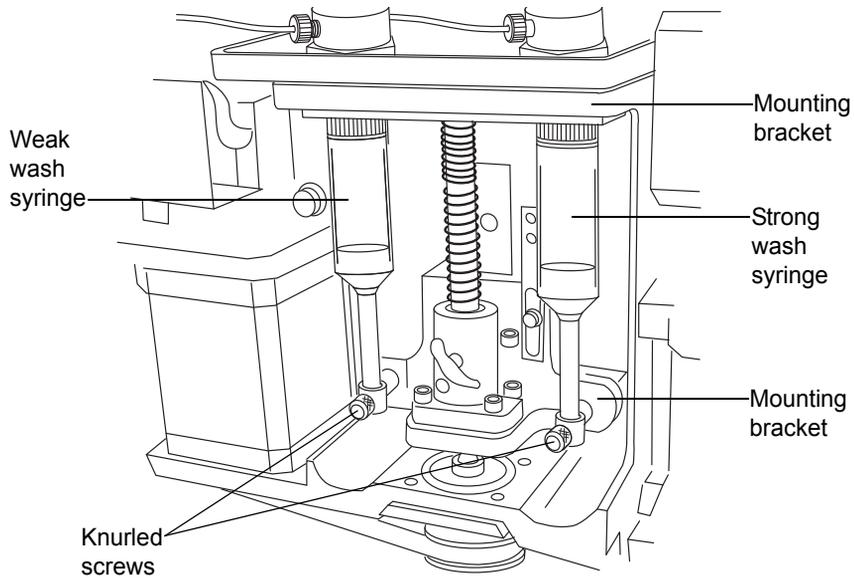


Warning: To avoid injury, ensure that no injection is in progress or pending before you remove the syringe.

To replace the wash syringes

1. Slide out the sample manager fluidics tray.
2. Remove the knurled screws that secure the wash syringe plungers to the wash syringe mounting brackets.

Wash syringe assembly components

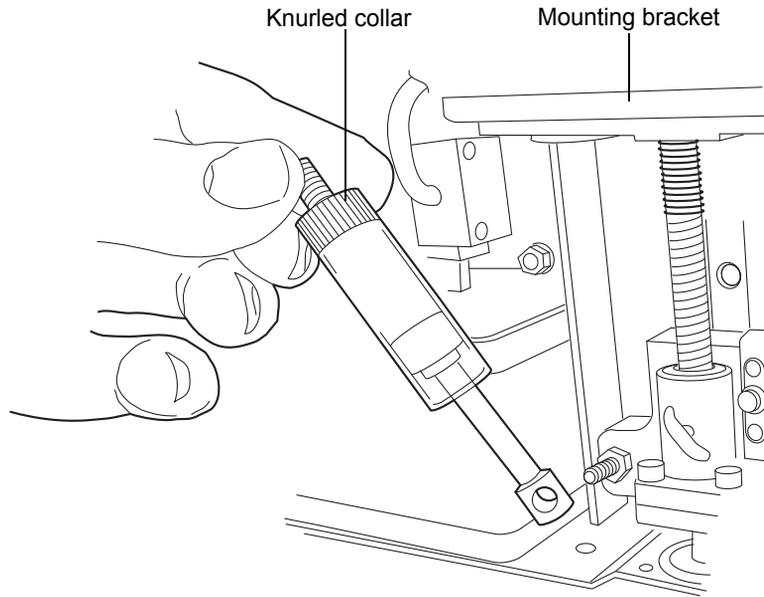


3. Looking down at the wash syringes, unscrew each syringe, clockwise, until it separates from the mounting bracket.



Caution: To avoid breaking the syringe, do not grasp it by its glass barrel. Always grasp the syringe by its knurled collar.

4. Depress the syringe barrel to clear the top mounting bracket, and remove the syringe.

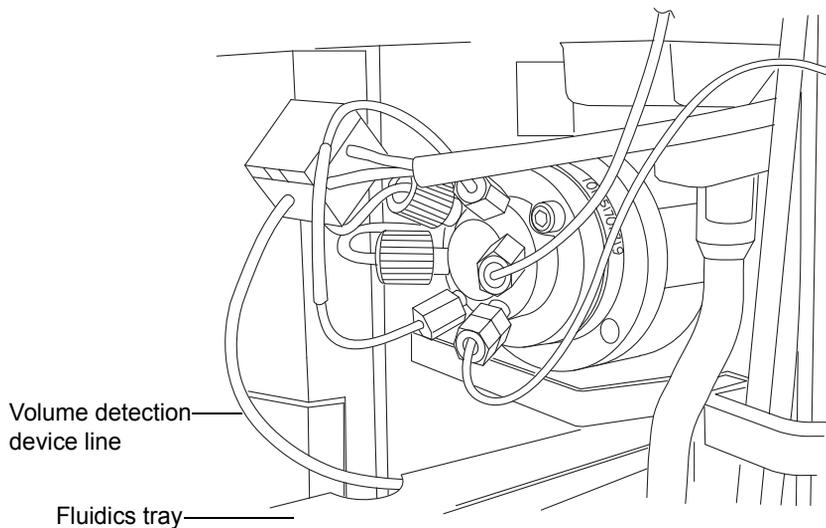


5. Unpack each replacement wash syringe.
6. Partially fill each new wash syringe (by hand) with weak or strong wash solvent (to help remove air bubbles).
7. Pull each wash syringe plunger down so that the plunger end slides over the threaded post on the syringe guide mounting bracket.
8. Screw each new wash syringe partially into the mounting bracket.
9. Finger tighten each wash syringe.
10. Install and finger-tighten the knurled screw that holds each wash syringe barrel to the mounting bracket.



Caution: Be careful not to crush the volume detection device line.

11. Slide the fluidics tray closed.



12. Run the System Prime option until there are no bubbles in the syringes.

Modifying sample syringe configuration parameters

To configure the system for a syringe size that is different from the one currently fitted

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. Select Configure > Volumes.
3. In the Volume Configuration dialog box, select the appropriate sample syringe size from the list, and then click OK.

Replacing the injection valve cartridge

Requirement: When you replace the injection valve, you must also replace the sample loop.

Required materials

- 2-mm Allen wrench
- Injection valve cartridge
- Sample loop

To replace the injection valve cartridge

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. In the sample manager information window, click Maintain > Park Inject Valve.



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

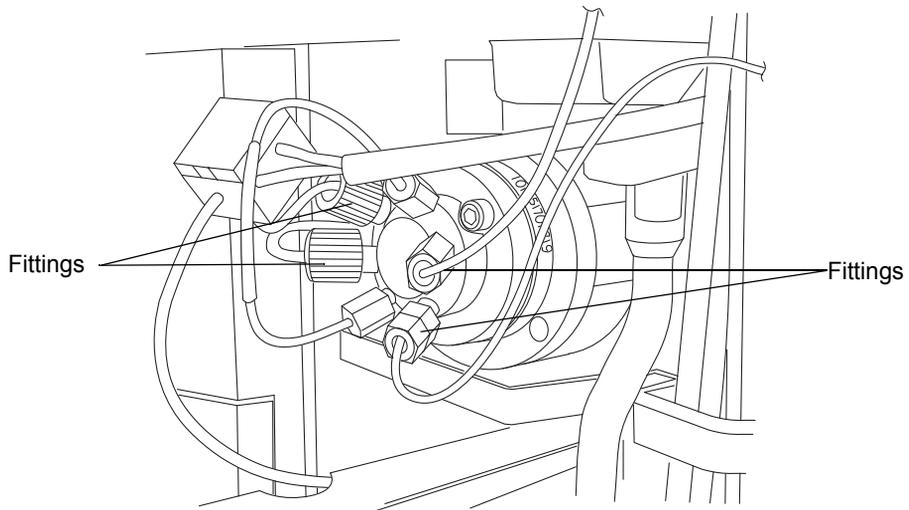
3. Power-off the sample manager.
4. Slide out the sample manager fluidics tray.



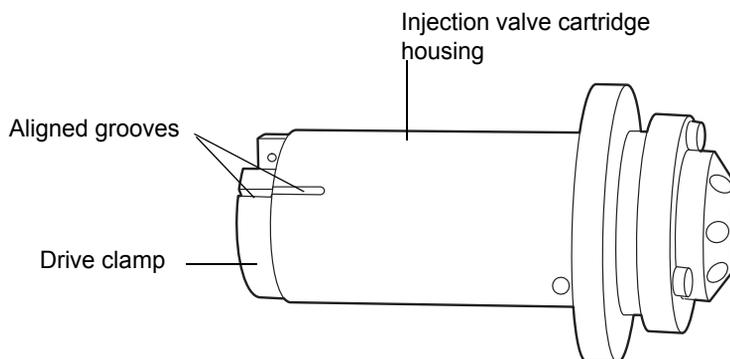
Caution: To avoid kinks, do not pull on the volume detection device (port 2) or sample needle (port 3) tubing.

5. Hold the sample loop, and pull the injection valve about 2.5 cm (1 inch) toward you.

6. Remove the fittings attached to the injection valve cartridge.



7. Use the 2-mm Allen wrench to remove the screw at the 10 o'clock position on the injection valve cartridge.
8. Remove the injection valve cartridge from the injection valve assembly.
9. Carefully unpack the replacement injection valve cartridge.
10. Ensure that the groove in the cartridge housing and the groove on the drive clamp are aligned. If they are not, turn the drive clamp until the grooves line up.



11. Insert the new injection valve cartridge into the injection valve assembly.

Requirement: If the injection valve cartridge does not slide fully into the injection valve assembly, contact your Waters service representative.

12. Insert the 2-mm hex screw at the 10 o'clock position on the injection valve cartridge, and use the 2-mm Allen wrench to tighten it.

13. Reattach all fittings and install a new sample loop (see [page 6-145](#)).

Tip: To prevent bandspreading and excessive carryover, do not install previously used sample loops on the new cartridge.



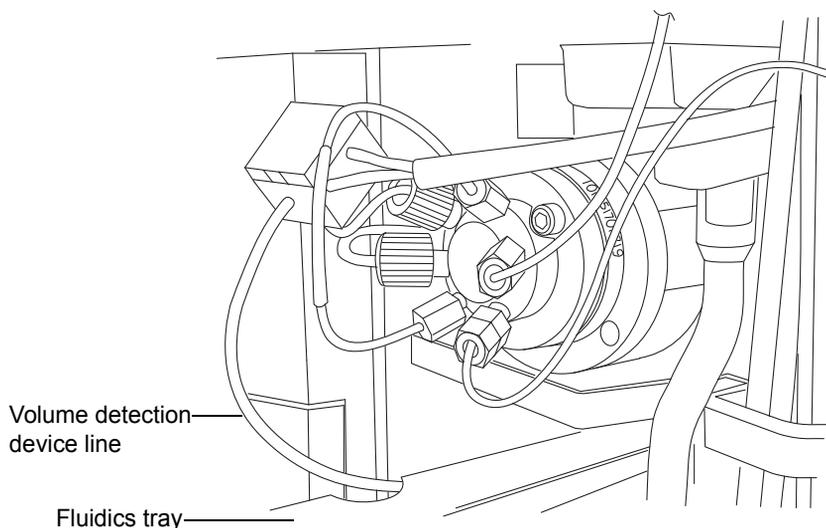
Caution: To avoid kinks, do not push on the volume detection device (port 2) or sample needle (port 3) tubing.

14. Push the injection valve back to the closed position.



Caution: Be careful not to crush the volume detection device line.

15. Slide the fluidics tray closed.



16. Ensure the sample manager compartment door is closed.

17. Power-on the sample manager.

18. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
19. In the sample manager information window, click Control > Prime syringes.
20. In the Prime Syringes dialog box
 - a. select sample syringe and wash syringes.
 - b. type 1 in the Number of Cycles text box.
 - c. click OK. The sample manager status displays “Priming”. When priming is complete, the status returns to “Idle”.
21. Click Maintain > Characterize > Needle and loop volumes.
22. In the Characterize Needle and Loop Volumes dialog box, click Start. The test results appear in the Loop and Needle areas.

Testing the sample manager backpressure regulator

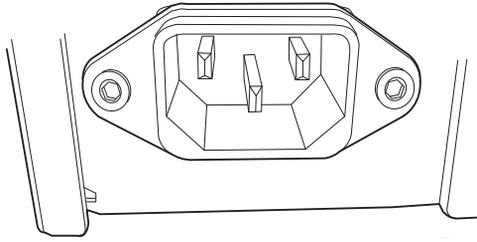
To test the sample manager backpressure regulator

1. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
2. Click Maintain > Test backpressure.
3. In the Backpressure Test dialog box, click Start. When the test is complete, the Results pane appears.

Replacing the fuses

Note: Recently manufactured instruments do not have fuses above the power entry module because the power supply is thermally protected. If your sample manager has the power entry module shown below and it fails to power-on, the status LEDs are unlit, or the power supply fan does not operate, call Waters Technical Service (see [page 6-6](#)).

Power entry module on recently manufactured instruments



Warning: To avoid electric shock, power-off and unplug the sample manager before examining fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

Suspect an open or otherwise defective fuse when any of these conditions apply:

- The sample manager or column heater fails to power-on.
- The sample manager or column heater status LEDs are unlit.
- The power supply fan does not operate.

Required materials

- 10 A fuse (2)
- Flat-blade screwdriver

To replace the fuses

Requirement: Replace both fuses, even when only one is open or defective.

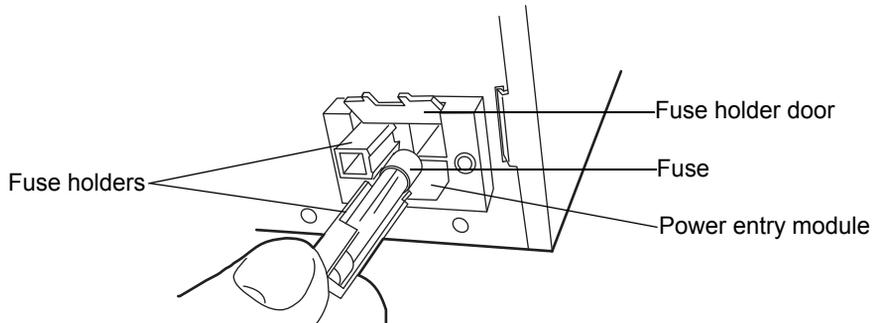


Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To completely interrupt power to an instrument, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds thereafter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

1. Power-off the sample manager.

Tip: The fans inside the sample manager run continuously, even after you power it off.

2. Disconnect the power cord from the power entry module.
3. Use a flat-blade screwdriver to open the fuse holder door, located above the power entry module on the rear panel.
4. With minimum pressure, pull on each spring-loaded fuse holder and remove it.



5. Remove and discard the fuses.



Warning: For continued protection against fire, replace fuses with the appropriate type and rating.

6. Insert the new fuses into the holders and the holders into the power entry module.
7. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the sample manager.

Maintaining the HT column heater

Under normal operating conditions, the HT column heater does not require any routine maintenance. If residue from solvent leaks accumulates in the column tray, remove the column and wipe the tray clean with a soft cloth.

The HT column heater is powered by the sample manager. If the HT column heater loses power, suspect that the fuse in the sample manager needs to be replaced. See [“Replacing the fuses” on page 6-159](#) for more information.

Resolving HT column heater leak sensor errors

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Tip: Small column leaks can be undetectable because they can evaporate before reaching the leak sensor reservoir.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

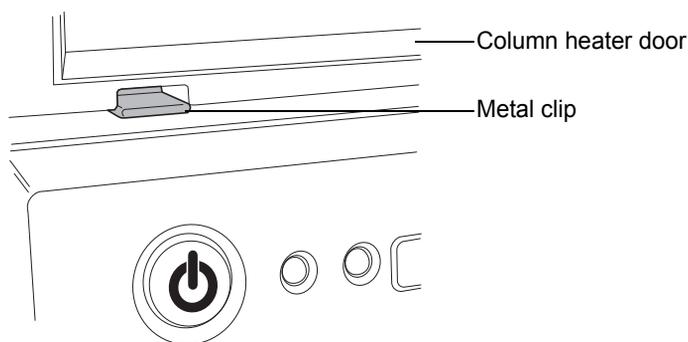
To resolve a HT column heater leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to determine which of the sample manager's 2 leak sensors detected a leak.
2. If the message reads “Leak Detected (Column)”, open the HT column heater's door. Otherwise, see [“Resolving sample manager leak sensor errors” on page 6-123](#).

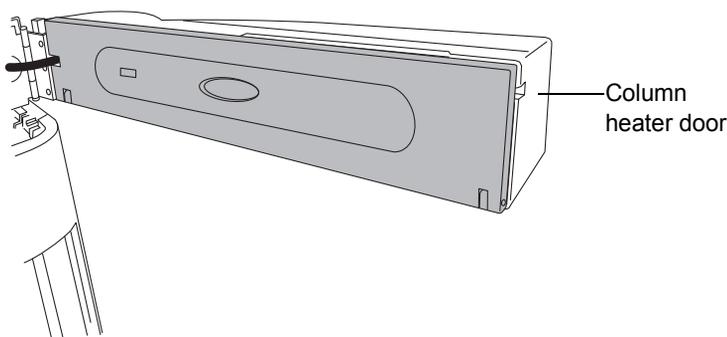
3. Locate the source of the leak, and make the repairs necessary to stop the leak.

Tip: Liquid from a detector leak can accumulate in the HT column heater leak sensor reservoir and cause the HT column heater leak sensor alarm to sound. Always inspect the detector when attempting to locate the source of a leak.

4. Remove any solvent lines routed between the HT column heater door and hinge.
5. Push down on the metal clip at the bottom, left-hand side of the HT column heater to release the door, and then pull the door toward you.

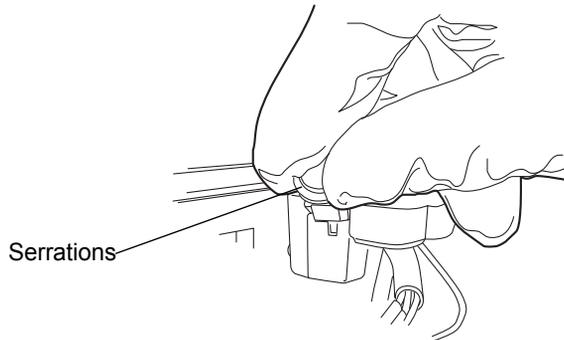


6. Swing the door fully to the right-hand side.



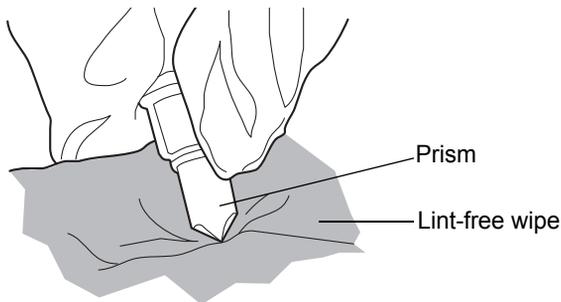
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

7. Remove the leak sensor from its reservoir by grasping it by its serrations, pulling upward on it, and tilting it to the left-hand side.

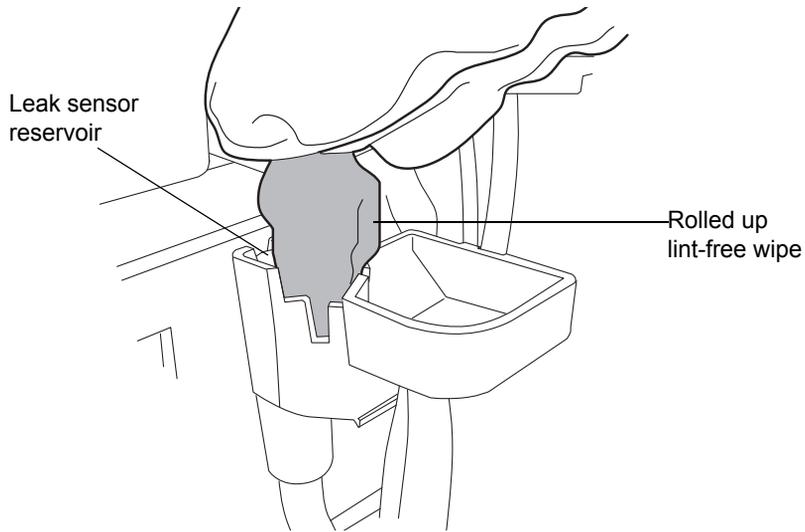


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-171](#)).

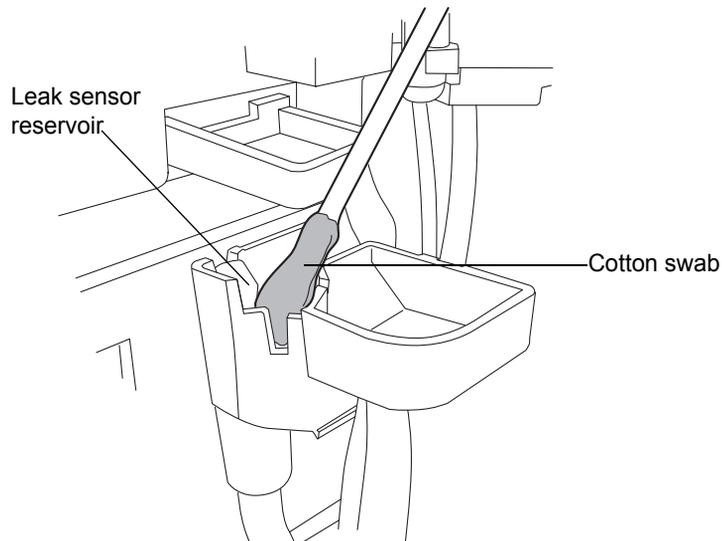
8. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



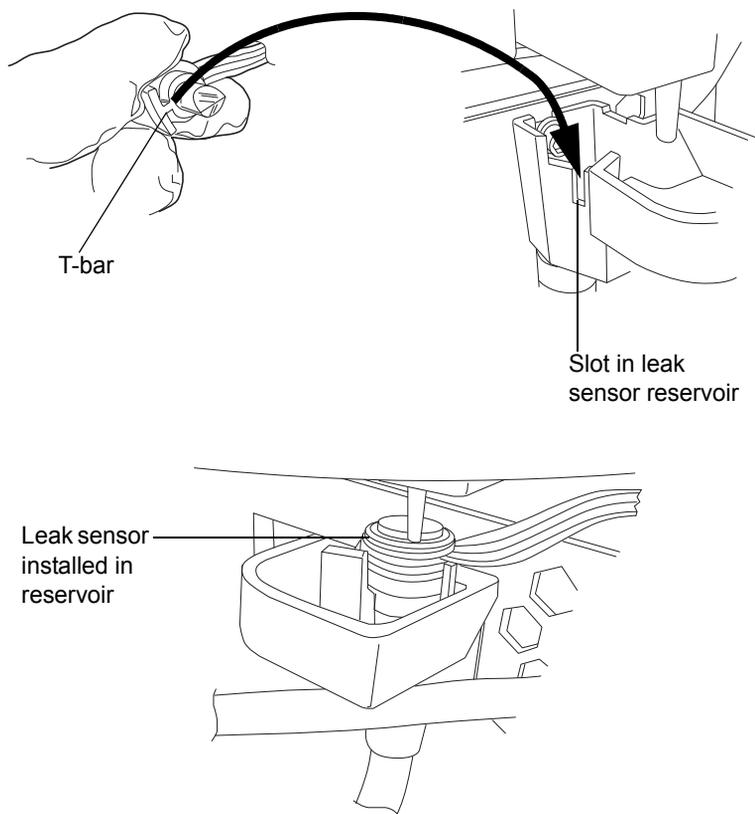
9. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



10. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



11. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



12. If you detached the connector from the front of the instrument, reattach it.
13. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
14. In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Resolving HT column heater leak sensor errors (door fully opened)

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Tip: Small column leaks can be undetectable because they can evaporate before reaching the leak sensor reservoir.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

To resolve a leak sensor error for a HT column heater with its door fully opened

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to determine which of the sample manager's 2 leak sensors detected a leak.
2. If the message reads "Leak Detected (Column)", locate the source of the leak, and make the repairs necessary to stop the leak. Otherwise, see ["Resolving sample manager leak sensor errors" on page 6-123](#).

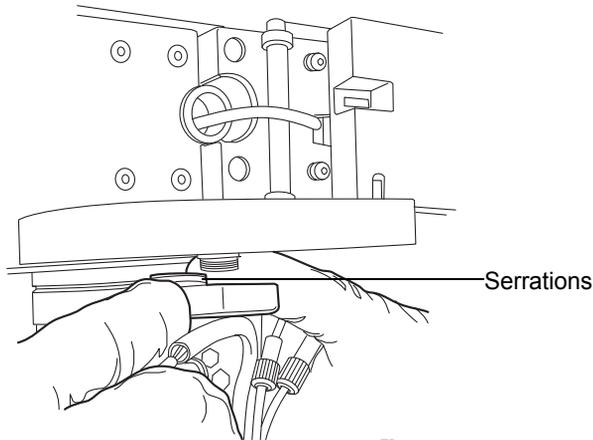
Tip: Liquid from a detector leak can accumulate in the HT column heater leak sensor reservoir and cause the HT column heater leak sensor alarm to sound. Always inspect the detector when attempting to locate the source of a leak.

- Slide out the sample manager fluidics tray.

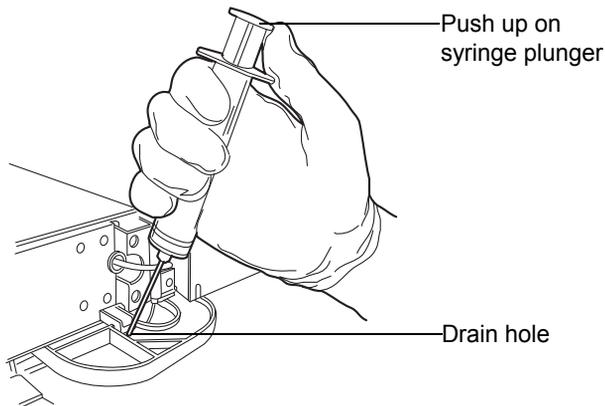


Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

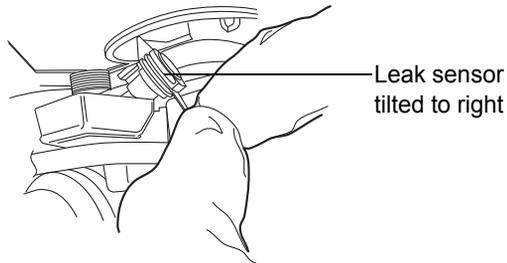
- Using one finger from each hand, remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



Alternative: Leave the leak sensor in place, use the syringe included the binary solvent manager startup kit to remove the liquid from the leak sensor reservoir, and proceed to [step 10](#).

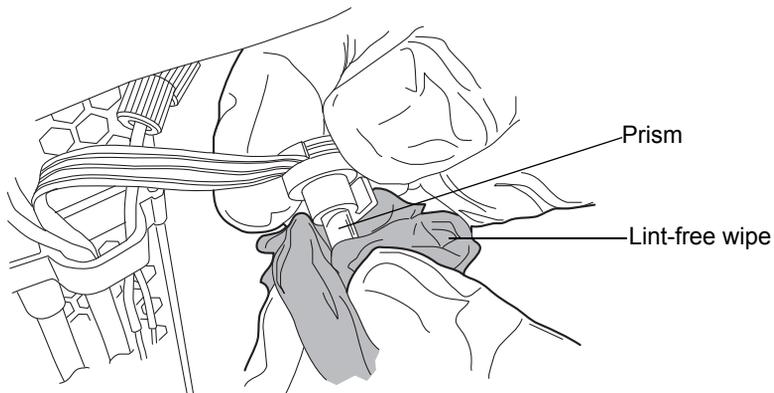


5. Tilt the leak sensor to the right-hand side and remove it from under the drip tray.

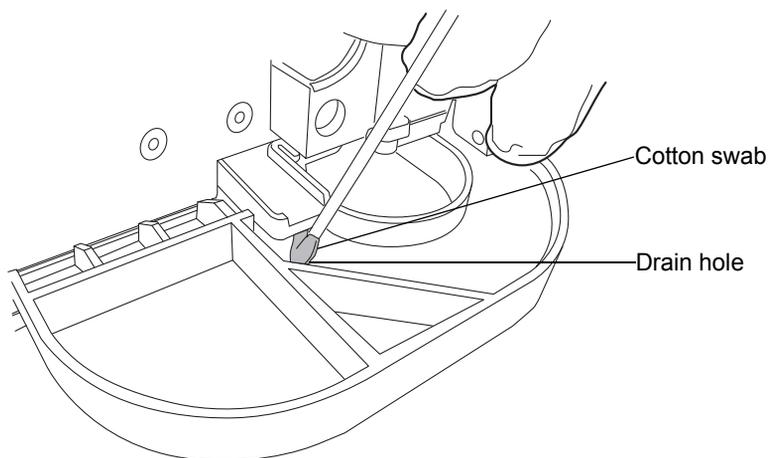


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-171](#)).

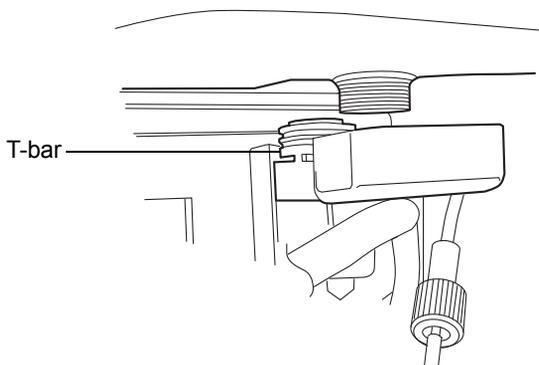
6. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



7. Insert a cotton swab through the drip tray drain hole and absorb any remaining liquid from the leak sensor reservoir and its surrounding area.



8. Using one finger from each hand, align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



9. If you detached the connector from the front of the instrument, reattach it.
10. Slide the fluidics tray closed.
11. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
12. In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Replacing the HT column heater's leak sensor



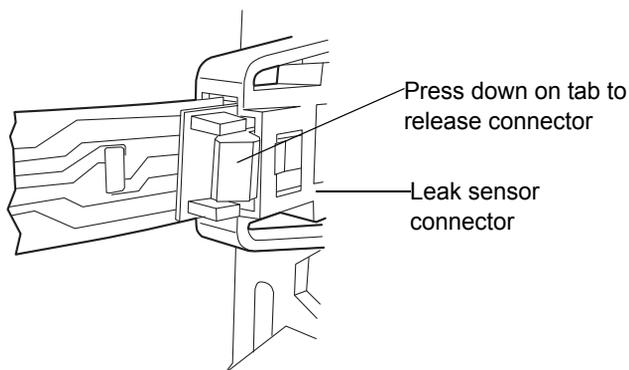
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

- Clean, chemical-resistant, powder-free gloves
- New leak sensor

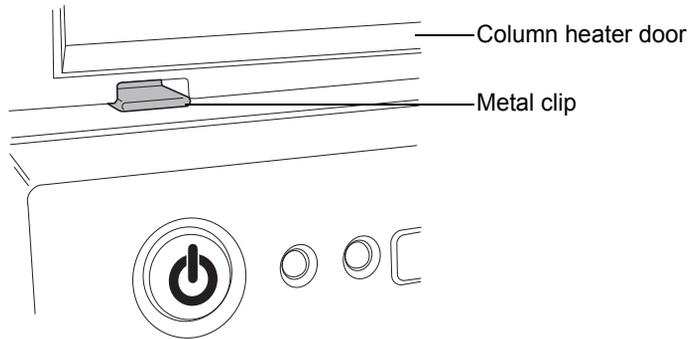
To replace the HT column heater's leak sensor

1. Open the sample manager door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

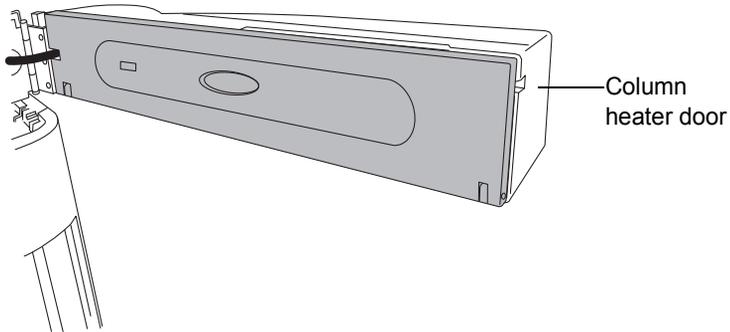


3. Open the HT column heater door.
4. Remove any solvent lines routed between the HT column heater door and hinge.

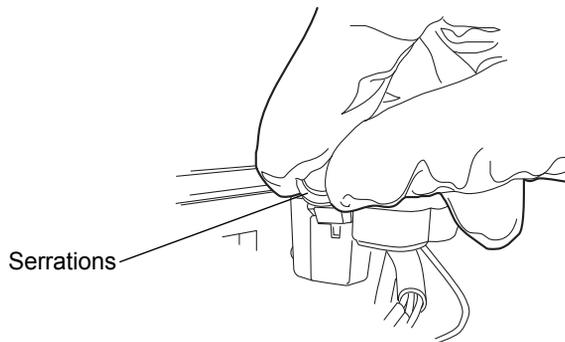
5. Push down on the metal clip at the bottom, left-hand side of the HT column heater to release the door, and then pull the door toward you.



6. Swing the door fully to the right-hand side.

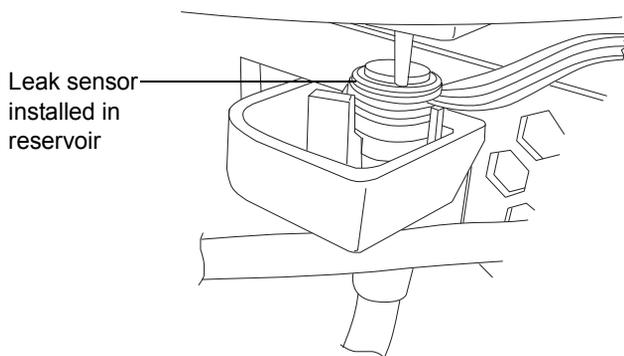
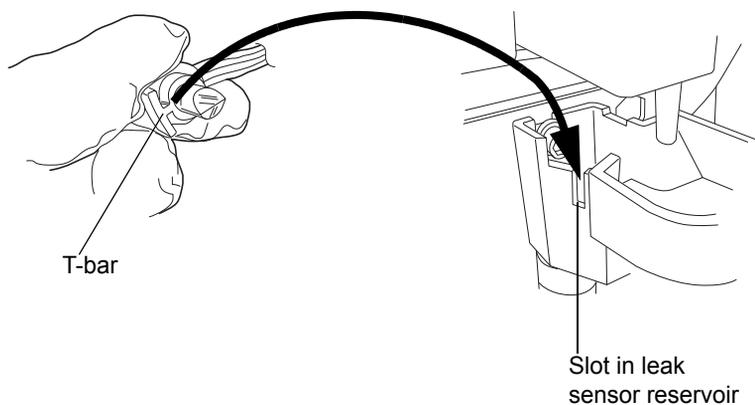


7. Remove the leak sensor from its reservoir by grasping it by its serrations, pulling upward on it, and tilting it to the left.



8. Unpack the new leak sensor.

- Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



- Plug the leak sensor connector into the front of the instrument.
- In the ACQUITY UPLC Console, select Sample Manager from the system tree.
- In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Replacing the HT column heater's leak sensor (door fully opened)



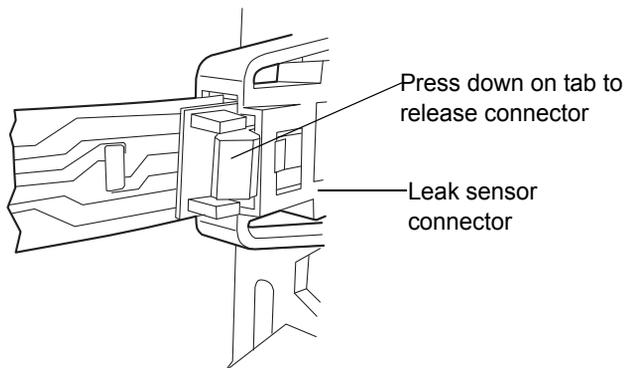
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

- Leak sensor
- Clean, chemical-resistant, powder-free gloves

To replace the leak sensor for a HT column heater with its door fully opened

1. Open the sample manager door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

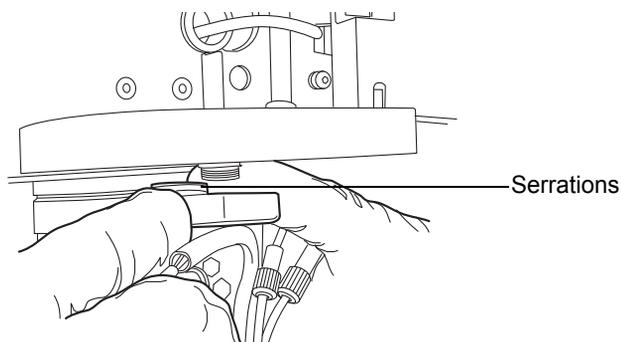


3. Slide out the sample manager fluidics tray.

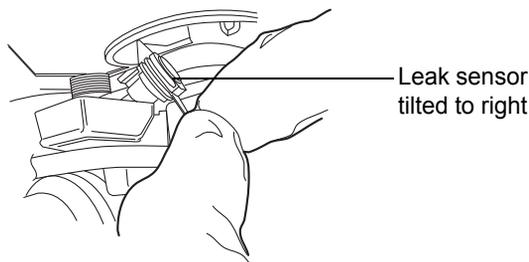


Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

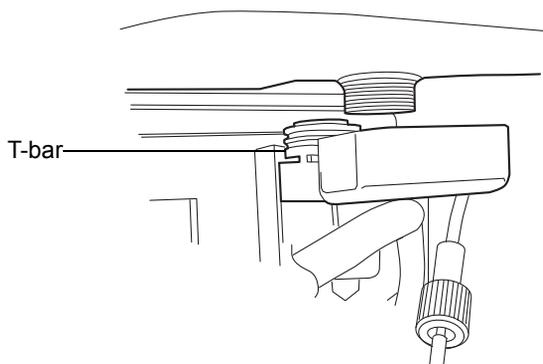
- Using one finger from each hand, remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



- Tilt the leak sensor to the right-hand side and remove it from under the drip tray.



- Unpack the new leak sensor.
- Using one finger from each hand, align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



- Plug the leak sensor connector into the front of the instrument.

9. Slide the fluidics tray closed.
10. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
11. In the sample manager information window, click Control > Reset SM, to reset the sample manager.

Replacing the column in the HT column heater



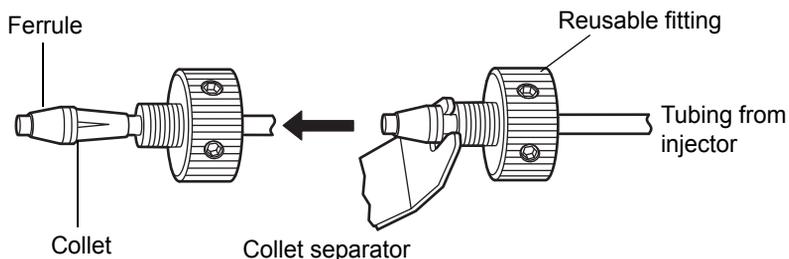
Warning: To prevent burn injuries, set the column temperature to Off, and then allow the column compartment and its components to cool for 60 minutes before touching them. Monitor the column compartment internal temperature to ensure all components are cool.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the filter in the column in-line filter unit.

To remove the column

1. Stop any currently running mobile phase before replacing the column.
2. After cooling, open the HT column heater door.
3. Disconnect the outlet of the stabilizer tube from the column or in-line filter.
4. Place the forked end of the collet separator over the collet on the column stabilizer tube, and gently wiggle it back and forth to pry the reusable fitting loose from the collet.



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

5. Replace the ferrule.
6. Continue with [“Installing the column in an HT column heater” on page 2-46.](#)

Replacing the column stabilizer assembly in the HT column heater



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 internal temperature to ensure all components are cool.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the filter in the column in-line filter unit.

Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- Clean, chemical-resistant, powder-free gloves
- Phillips[®] screwdriver
- Replacement column stabilizer assembly

To replace the column stabilizer assembly

1. Stop any currently running mobile phase before replacing the column stabilizer assembly.
2. Disconnect the inlet tube from injector port 6 of the sample manager.
3. Open the HT column heater door.
4. Using the Phillips screwdriver, loosen the 2 screws that fasten the column stabilizer assembly to the tray.
5. Remove the column stabilizer assembly from the thermal gasket.
6. Using the 1/4-inch and 5/16-inch open-end wrenches, disconnect the compression fitting that attaches the column stabilizer assembly to the optional in-line filter or to the column.
7. Continue with [“Installing the column stabilizer assembly in an HT column heater” on page 2-37.](#)

Replacing the frit in the HT column heater's in-line filter unit

If your system has the optional column in-line filter installed, follow the instructions below to replace the frit.

 **Caution:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the frit in the column in-line filter unit.

Recommendation: Because of wear on the non-replaceable compression fittings, replace the column in-line filter unit after you use the six frits that accompany the unit.

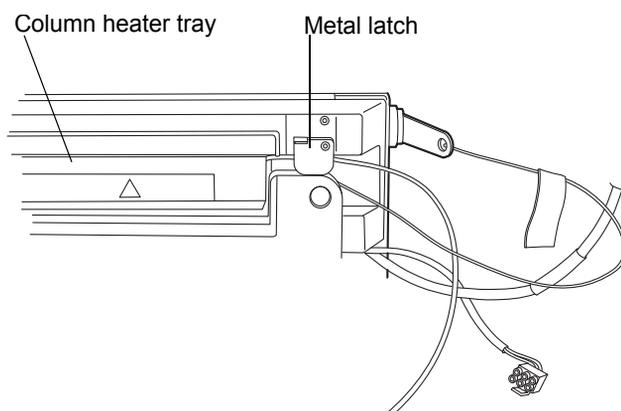
Requirement: Stop any currently running mobile phase before replacing the frit in the column in-line filter unit.

Required materials

- 5/16-inch open-end wrench (2)
- Clean, chemical-resistant, powder-free gloves
- Replacement frit

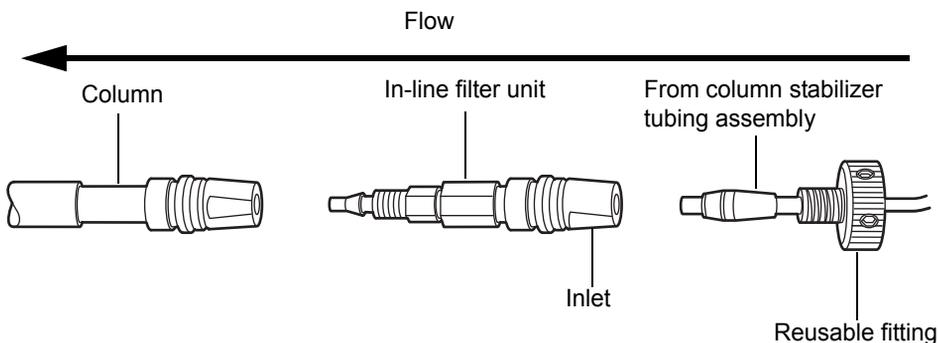
To replace the frit in the HT column heater's in-line filter unit

1. Open the HT column heater door, lift the metal latch, and tilt the HT column heater tray downward.

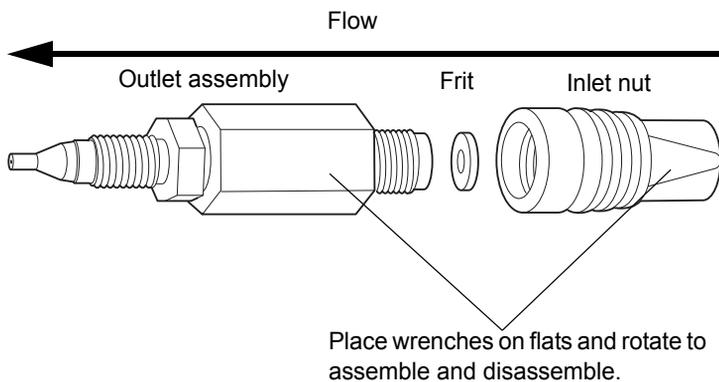


2. Remove the column components (column, in-line filter unit, and stabilizer tubing assembly).

Column components for HT column heater



3. Detach the column stabilizer tubing assembly from the column in-line filter unit. Do not detach the column in-line filter unit from the column inlet.
4. Open the column in-line filter unit by placing two 5/16-inch open-end wrenches onto the flats as shown.



5. Remove the column in-line filter unit inlet nut, which contains the used frit. Discard the frit; it cannot be reused.
Tip: Over time, the frit can expand inside the in-line filter inlet nut. If you are unable to remove the frit from the inlet nut, discard the inlet nut and replace both the inlet nut and frit.
6. Insert a new frit, and then reassemble the column in-line filter unit using the wrenches.
7. Reattach the column stabilizer tubing assembly to the in-line filter unit.

8. Start the mobile phase and examine the assembled components for leaks. Tighten fittings as needed.
9. Return the assembled components to the column tray, push down the metal latch to secure the tubing, and tilt the tray to the closed position.

Maintaining the column manager and column heater/cooler

Under normal operating conditions, the column manager and column heater/cooler do not require any routine maintenance. If residue from solvent leaks accumulates in the column slot, remove the column, and wipe the slot clean with a soft cloth.

Resolving column manager and column heater/cooler leak sensor errors

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor,

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Tip: Small column leaks can be undetectable because they can evaporate before reaching the leak sensor reservoir.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

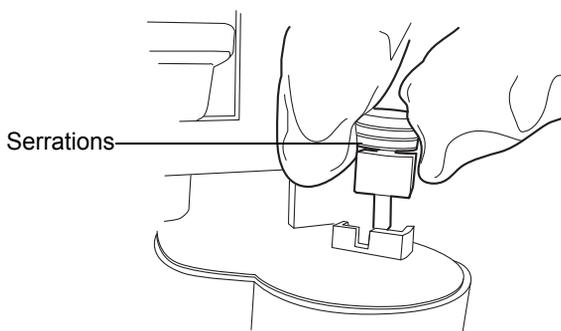
To resolve a column manager or column heater/cooler leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to confirm that the column manager leak sensor detected a leak.
2. Locate the source of the leak, and make the repairs necessary to stop the leak.



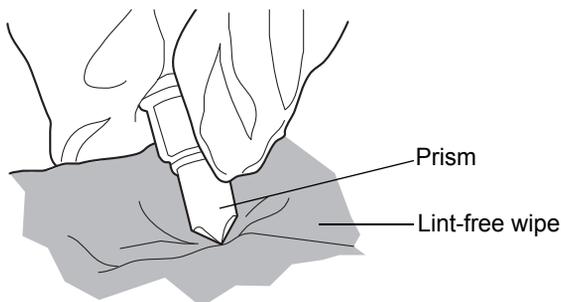
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

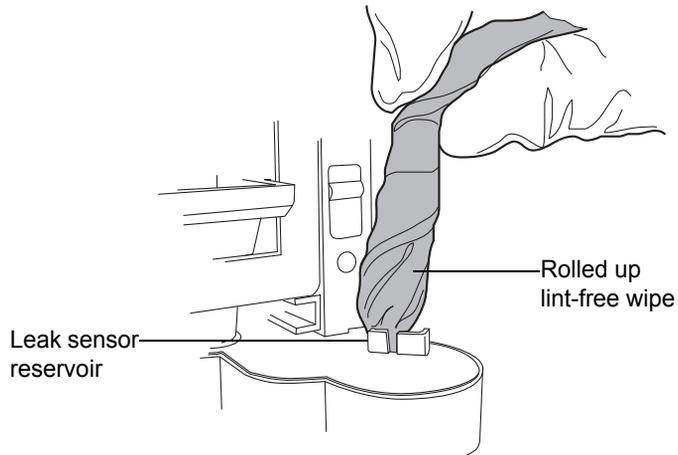


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-184](#)).

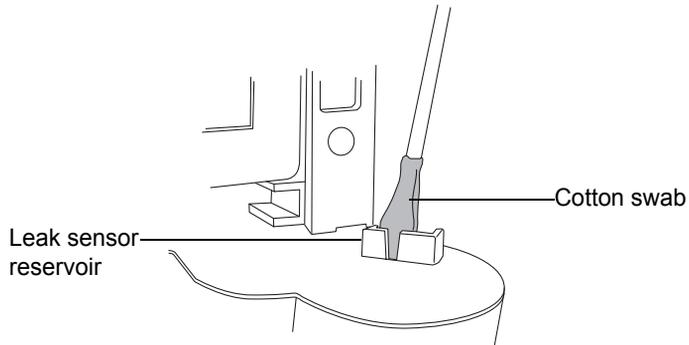
4. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



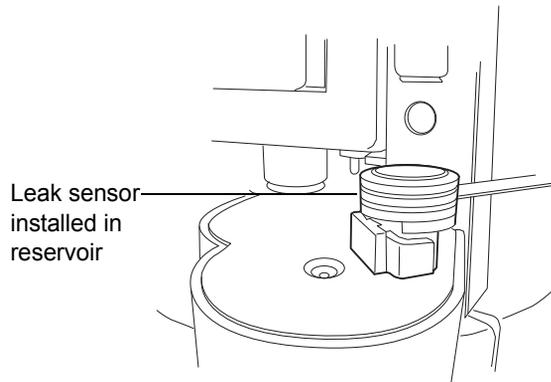
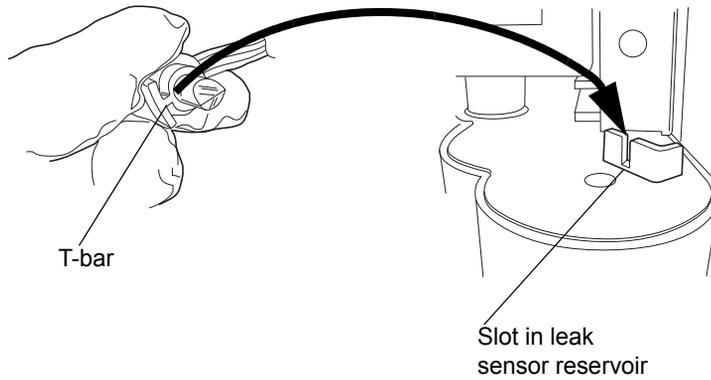
5. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



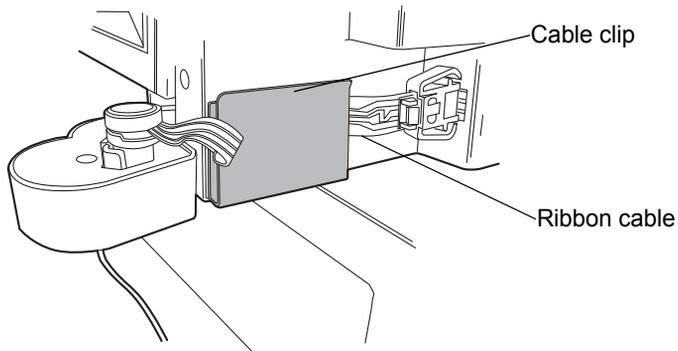
6. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



7. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



8. Ensure the ribbon cable is routed behind the cable clip.



9. If you detached the connector from the front of the instrument, reattach it.
10. In the ACQUITY UPLC Console, select Column Manager from the system tree.
11. In the column manager information window, click Control > Reset CM, to reset the column manager.

Replacing the column manager's or column heater/cooler's leak sensor



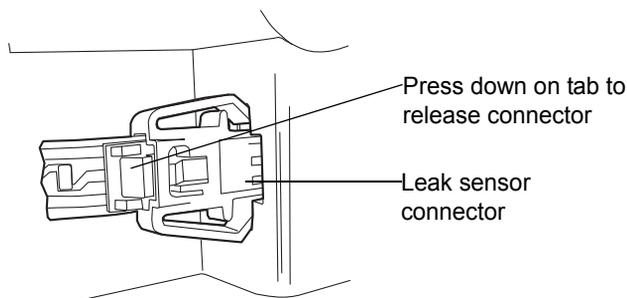
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

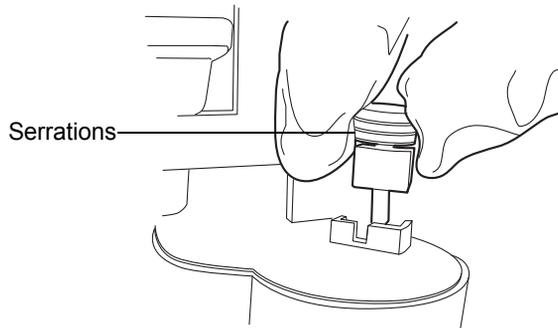
- Clean, chemical-resistant, powder-free gloves
- New leak sensor

To replace the column manager's or column heater/cooler's leak sensor

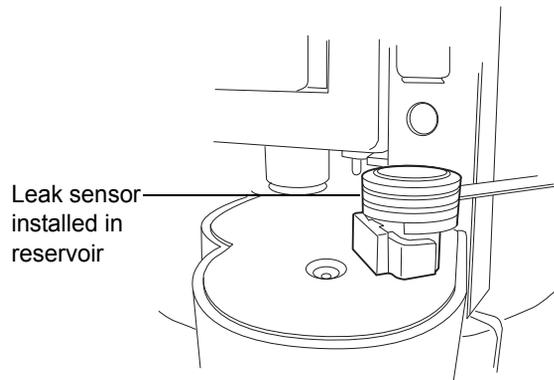
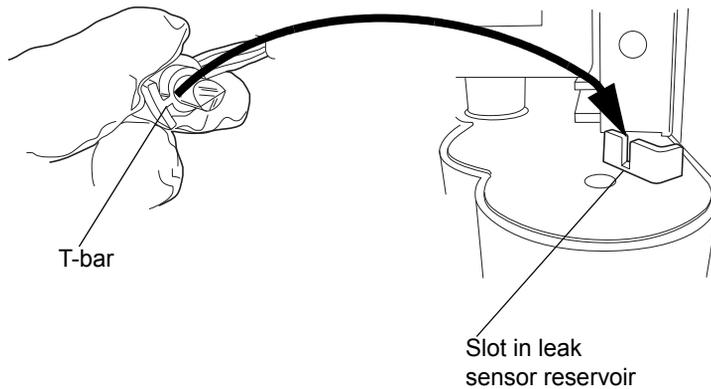
1. Press down on the tab to detach the leak sensor connector from the front of the instrument.



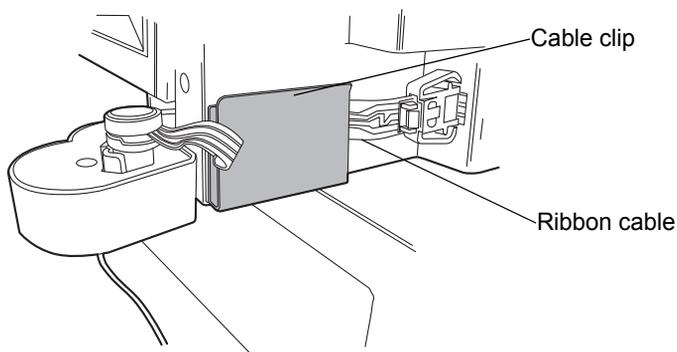
2. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



3. Unpack the new leak sensor.
4. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



5. Ensure the ribbon cable is routed behind the cable clip.



6. Plug the leak sensor connector into the front of the instrument.
7. In the ACQUITY UPLC Console, select Column Manager from the system tree.
8. In the column manager information window, click Control > Reset CM, to reset the column manager.

Replacing the column in the column manager

This procedure describes a column manager whose inlet switching valve is on the right-hand side, behind the column drawer panel. The high-pressure, stainless steel preheater tubes, which connect to a column inlet, are also on the right. The outlet of each column is plumbed through the post-cooler and through the switching valve on the left-hand side, and then to the detector or mass spectrometer.

A column heater/cooler does not have switching valves. Instead, the column stabilization tube connects the sample manager injection valve to the column inlet, and PEEK tubing connects the column outlet to the detector flow cell.

Replace a column when

- your experimental setup requires a different size or type of column.
- column performance has deteriorated and conditioning is unsuccessful, indicating the column is past its useful life.

See also: *ACQUITY UPLC Column Care and Use Instructions.*

Required materials

- 5/16-inch open-end wrench
- ACQUITY UPLC column
- Column end caps from the new column (optional; if you want to store the column that is removed)
- Finger-tight fitting wrench (for tightening the low-pressure reusable fittings at the left-hand end of the column)
- Waters collet removal tool

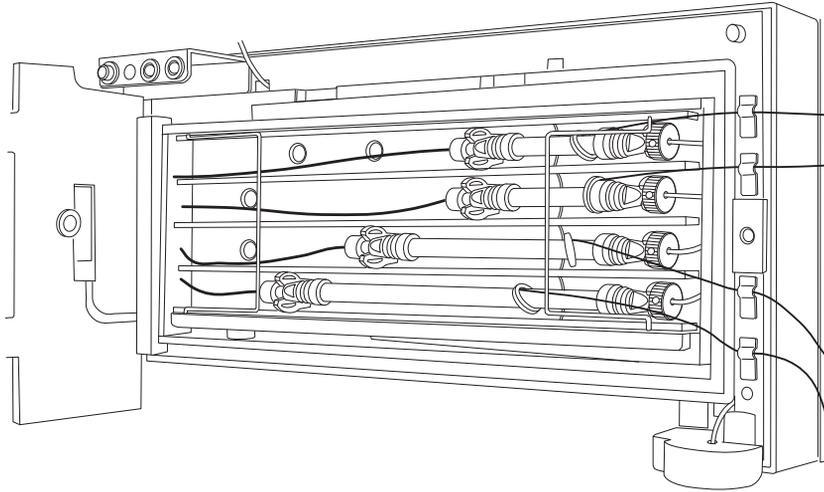
To remove a column



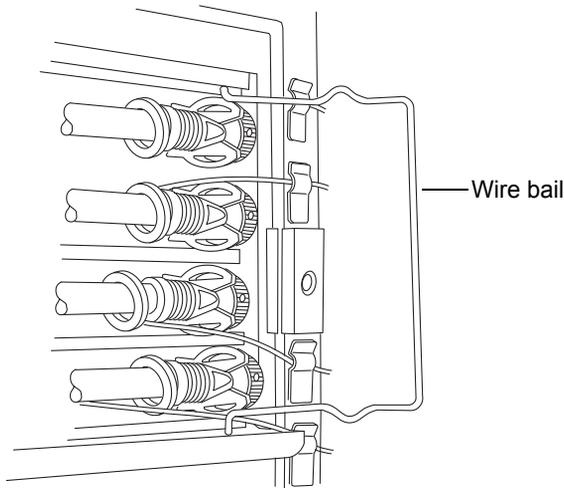
Warning: To prevent burn injuries, adjust the temperature set-point of the column manager or column heater/cooler to a safe temperature (10 to 30 °C [50 to 86 °F]) and allow the unit to attain this set-point temperature prior to handling components within the column compartment. When the column manager or column heater/cooler is set at a high temperature, all interior surfaces and components are hot.

1. Stop any currently running mobile phase before replacing the column.
2. Adjust the temperature set-point of the column manager or column heater/cooler to a safe temperature (10 to 30 °C [50 to 86 °F]) and allow the unit to attain this set-point temperature.
3. In the ACQUITY UPLC Console, click the Stop Flow button, and wait until all flow is stopped.

4. Open the door and locate the column you want to replace. Column number 1 is at the top position, and column number 4 is at the bottom.

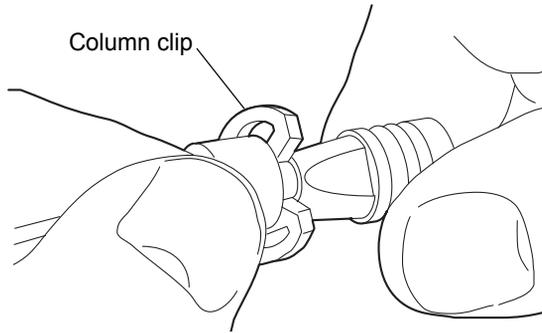


5. Swing the wire bail open.

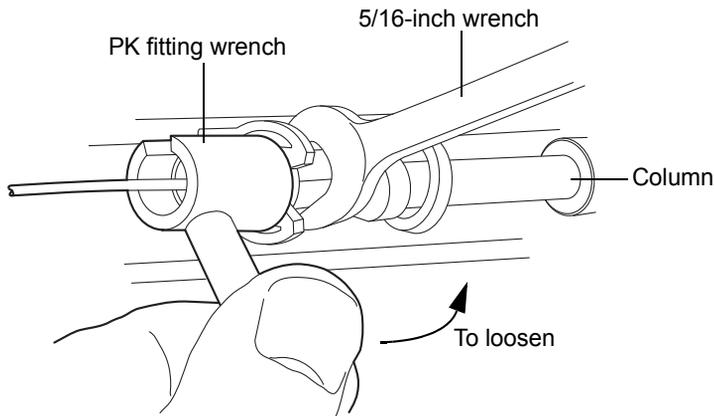


6. To disconnect the outlet side of the column from the post-cooler, pull the left-hand end of the column (outlet to detector) and the U-shaped,

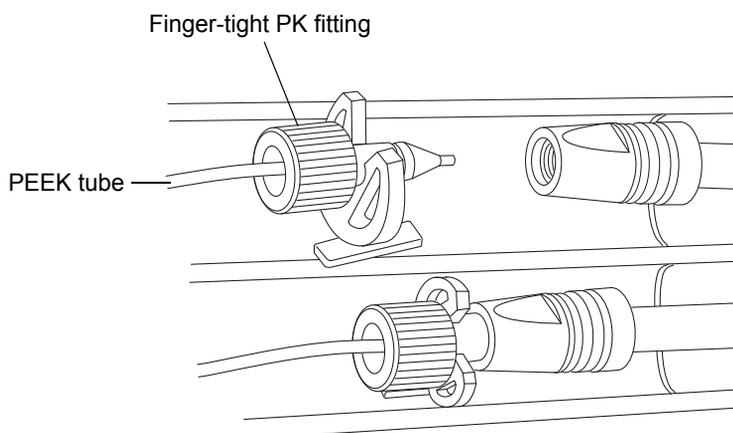
left-hand column clip away from the column slot. Unscrew the finger-tight PK fitting carefully from the column end.



If removal of the finger-tight PK fitting requires more force, place the 5/16-inch wrench on the end of the column immediately to the right-hand side of the column clip to hold the column steady, and loosen the PK fitting with the PK fitting wrench.

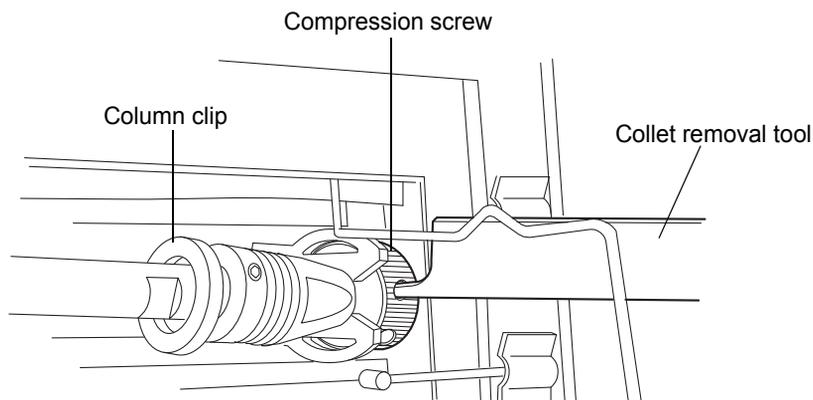


Disconnecting the outlet side of the top column



7. To disconnect the inlet side of the column from the preheater, pull the right-hand end of the column (inlet from preheater) and the right-hand column clip away from the column slot. Holding the gold compression screw steady, unscrew the right-hand end of the column to disconnect it from the preheater on the right-hand side.

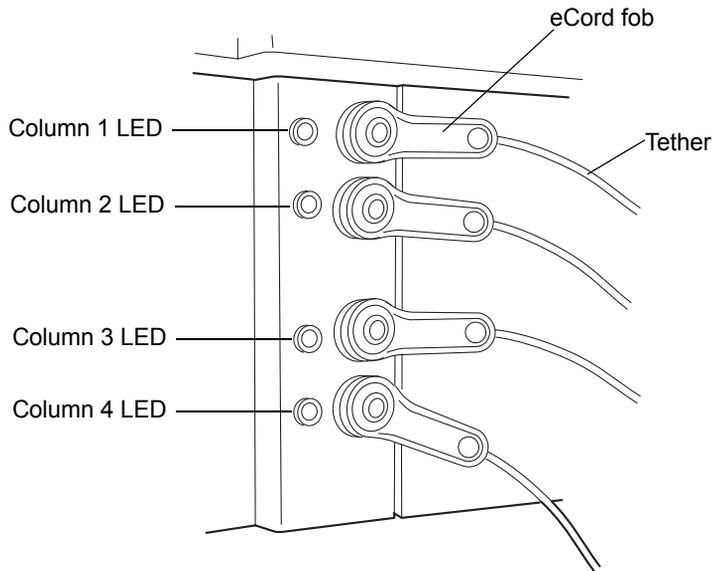
If removal of the end fitting requires more force, insert the collet removal tool into a hole in the compression screw, and hold the compression screw steady while you unscrew the column.



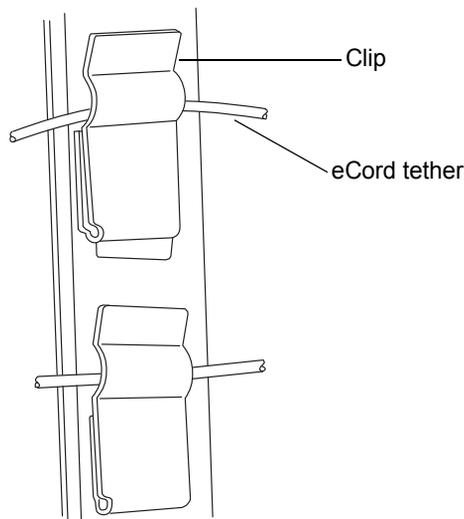
8. Dispose of the column properly, or save the column for future use by capping the ends tightly with the end caps from the new column. Store the used column horizontally, protected from light and excess heat or cold, or as discussed in the column's care and use instructions.

9. Detach the eCord from its port on the right-hand side of the column manager.

Tip: The eCord LED is constant green when the eCord is connected, and darkens when you detach the eCord from the port. Verify that the darkened LED corresponds to the column that you removed.



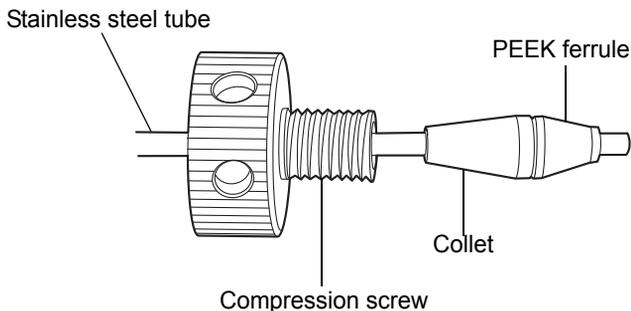
10. Remove the eCord tether from the clip on the door frame by carefully pulling it vertically out of the clip.



Tip: You can install up to four ACQUITY UPLC (or similar) columns in the column manager or column heater/cooler.

To install a column

1. Carefully connect the inlet end of the column (pressure up to 103,421 kPa [1034 bar, 15,000 psi]) to the preheater. Use a gold compression screw, PEEK ferrule, and stainless steel collet on the stainless steel tube.



To prevent bandspreading, ensure the tube is bottomed in the column end fitting before tightening the compression screw. If you are replacing a column, use the double-tine end of the collet removal tool to pry the compression screw away from the collet, so the collet is loose.

Slide the stainless steel tube through all 3 pieces until approximately 3 mm of tubing tip is visible, and then slide the tube into the inlet end of the column, holding the column and tightening the compression screw.

2. Carefully connect the outlet end of the column (pressure up to 20,684 kPa [207 bar, 3000 psi]) to the post-cooler.
3. Slide the black reusable fitting back on the PEEK tubing until approximately 3 mm of tubing extends beyond the fitting.
4. Insert the tubing into the outlet end of the column, and then tighten down the fitting.



Caution: To avoid increasing dispersion, use care when assembling and tightening the column fittings.

5. Insert the column into the slot using the high-temperature column clips.

6. Secure the columns by swinging the left-hand and right-hand wire bails to the middle, against the columns, until they latch into the column holder shelves.
7. Attach the eCord to its port on the right-hand side, and then insert the eCord tether into the clip on the door frame.
8. Close the column manager or column heater/cooler door, and power on the module. For optimal chromatography, allow 40 minutes for the column temperatures to stabilize after setting the desired temperature.

Options:

- If the column is new, condition it. Make sure to prevent the conditioning solvents from flowing downstream into the detectors.
- If the column has no eCord, you can add a comment in the ACQUITY UPLC Console to identify the column.

Replacing the column in the column heater/cooler



Warning: To prevent burn injuries, adjust the temperature set-point of the column manager or column heater/cooler to a safe temperature (10 to 30 °C [50 to 86 °F]) and allow the unit to attain this set-point temperature prior to handling components within the column compartment. When the column manager or column heater/cooler is set at a high temperature, all interior surfaces and components are hot.



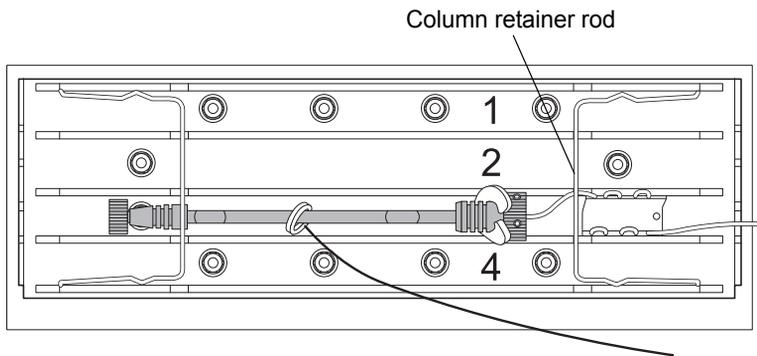
Warning: To prevent electric shock, ensure all waste line tubing is properly connected, and the drip tray is fitted and undamaged. Properly installing the tubing and drip tray keeps waste or solvent from contacting the power cord or cables.



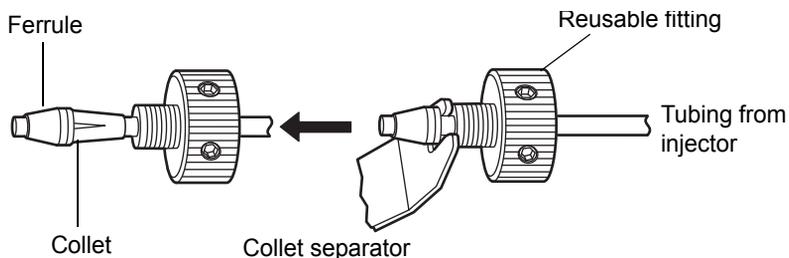
Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the filter in the column in-line filter unit.

To remove the column

1. Stop any currently running mobile phase before replacing the column.
2. After cooling, swing the column retainer rod out to the right-hand side.



3. Disconnect the stabilizer outlet tube from the column or in-line filter.
4. Place the forked end of the collet separator over the collet on the column stabilizer tube, and gently wiggle it back and forth to pry the reusable fitting loose from the collet.



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

5. Replace the ferrule.
6. Continue with [“Installing the column in a column manager or column heater/cooler”](#) on page 2-49.

Replacing the column stabilizer assembly in the column heater/cooler



Warning: To prevent burn injuries, adjust the temperature set-point of the column manager or column heater/cooler to a safe temperature (10 to 30 °C [50 to 86 °F]) and allow the unit to attain this set-point temperature prior to handling components within the column compartment. When the column manager or column heater/cooler is set at a high temperature, all interior surfaces and components are hot.



Warning: To prevent electric shock, ensure all waste line tubing is properly connected, and the drip tray is fitted and undamaged. Properly installing the tubing and drip tray keeps waste or solvent from contacting the power cord or cables.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the column stabilizer assembly.

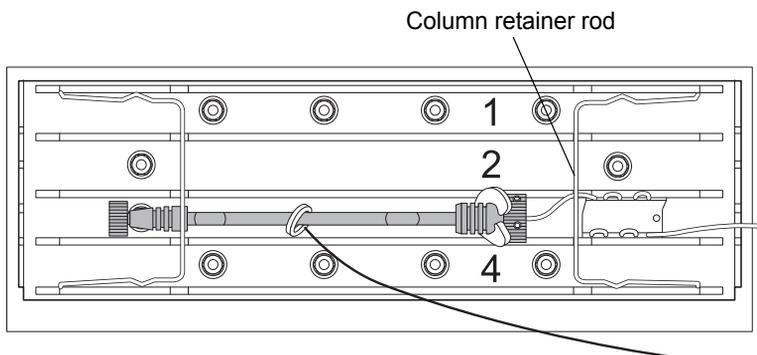
Required materials

- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- Clean, chemical-resistant, powder-free gloves
- Phillips® screwdriver
- Replacement column stabilizer assembly

To replace the column stabilizer assembly

1. Stop any currently running mobile phase before replacing the column stabilizer assembly.
2. Disconnect the inlet tube from injector port 6 of the sample manager.

3. Swing the column retainer rod out to the right-hand side.



4. Using the Phillips screwdriver, loosen the 2 screws that fasten the column stabilizer assembly to the tray.
5. Remove the column stabilizer assembly from the thermal gasket.
6. Using the 1/4-inch and 5/16-inch open-end wrenches, disconnect the compression fitting that attaches the column stabilizer assembly to the optional in-line filter or to the column.
7. Continue with [“Installing the column stabilizer assembly in a column heater/cooler” on page 2-40.](#)

Replacing the frit in the column manager’s in-line filter unit

If your system has the optional column in-line filter installed, follow the instructions below to replace the frit.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the frit in the column in-line filter unit.

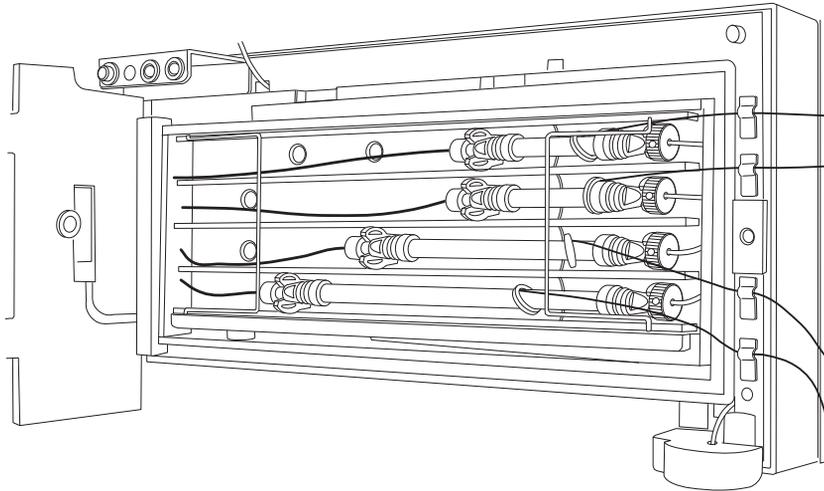
Recommendation: Because of wear on the non-replaceable compression fittings, replace the column in-line filter unit after you use the six frits that accompany the unit.

Required materials

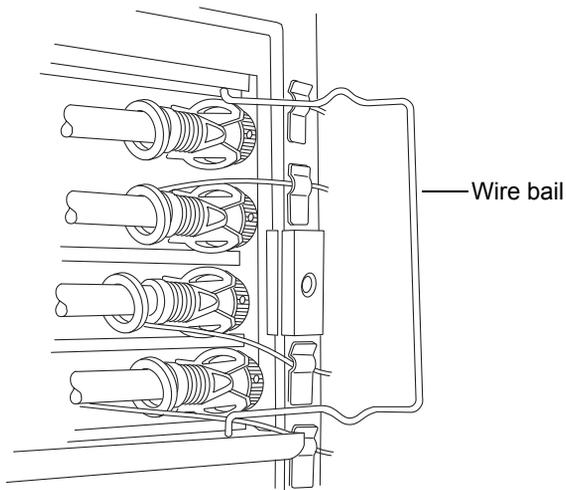
- 5/16-inch open-end wrench (2)
- Clean, chemical-resistant, powder-free gloves
- Collet and reusable fitting multi-tool (collet separator)
- Replacement frit

To replace the filter in the column in-line filter unit

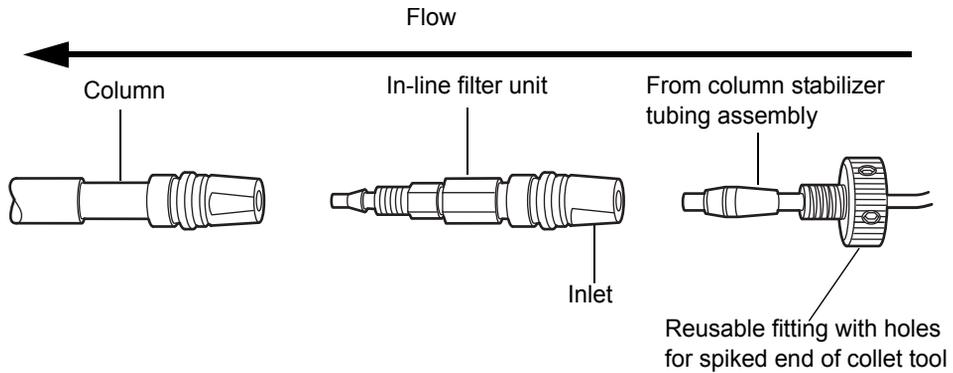
1. Stop any currently running mobile phase before replacing the column in-line filter unit.
2. Open the column manager door and locate the column in-line filter unit you want to replace. Column number 1 is at the top position, and column number 4 is at the bottom.



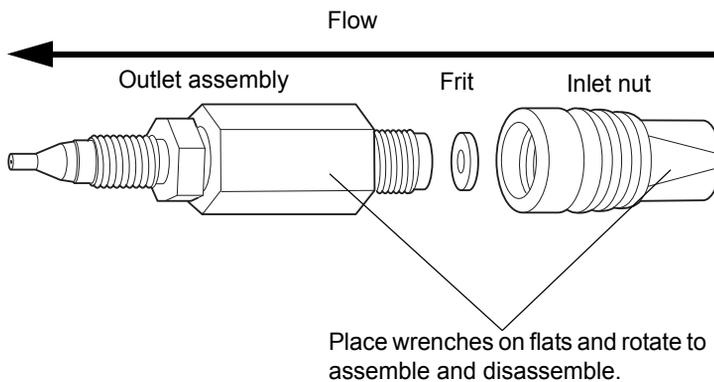
3. Swing the wire bail open.



4. Remove the column components (column, in-line filter unit, and stabilizer tubing assembly).



5. Using the 5/16-inch open-end wrench to hold the in-line filter unit, use the spiked end of the collet separator to turn the reusable fitting.
6. Detach the column stabilizer tubing assembly from the column in-line filter unit. Do not detach the column in-line filter unit from the column inlet.
7. Open the column in-line filter unit by placing two 5/16-inch open-end wrenches onto the flats as shown.



8. Remove the column in-line filter unit inlet nut, which contains the used frit. Discard the frit; it cannot be reused.

Tip: Over time, the frit can expand inside the in-line filter inlet nut. If you are unable to remove the frit from the inlet nut, discard the inlet nut and replace both the inlet nut and frit.

9. Insert a new frit, and then reassemble the column in-line filter unit using the wrenches.
10. Reattach the column stabilizer tubing assembly to the in-line filter unit.
11. Start the mobile phase and examine the assembled components for leaks. Tighten fittings as needed.
12. Return the assembled components to the column compartment sleeve.
13. Swing the column retainer rod to the left-hand side to fasten it.

Replacing the filter in the column heater/cooler's in-line filter unit

If your system has the optional column in-line filter installed, follow the instructions below to replace the filter.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when replacing the filter in the column in-line filter unit.

Recommendation: Because of the wear on the non-replaceable compression fittings, replace the column in-line filter unit after you use the six filters that accompany the unit.

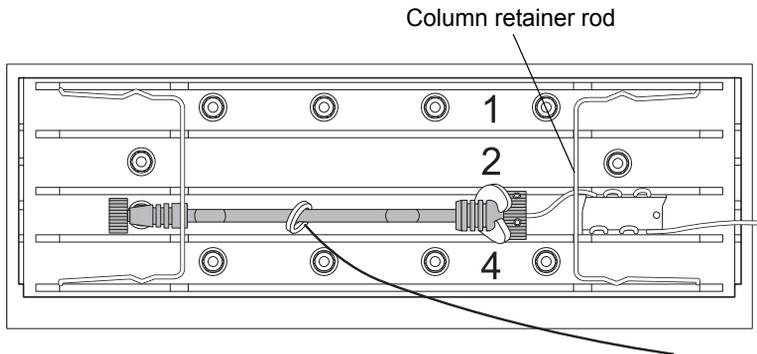
Required materials

- 5/16-inch open-end wrench (2)
- Clean, chemical-resistant, powder-free gloves
- Collet and reusable fitting multi-tool (collet separator)
- Replacement filter

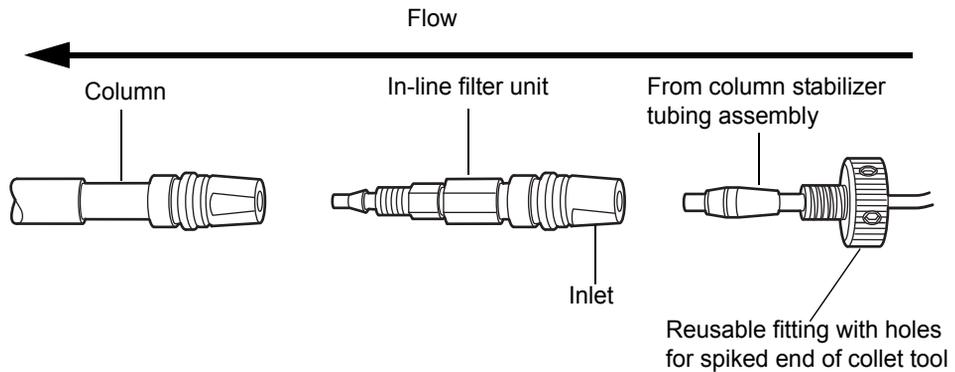
To replace the column in-line filter unit filter

1. Stop any currently running mobile phase before replacing the column in-line filter unit.

2. Swing the column retainer rod out to the right-hand side.



3. Remove the column components (column, in-line filter unit, and stabilizer tubing assembly).

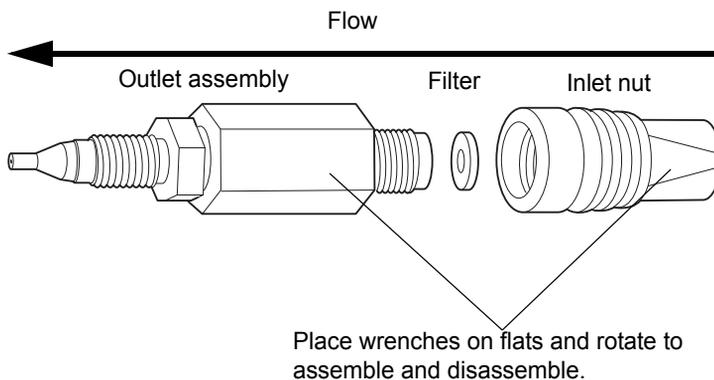


Note: Older reusable fittings do not have holes in the side of them.

4. Using the 5/16-inch open-end wrench to hold the in-line filter unit, use the spiked end of the collet separator to turn the reusable fitting.
5. Detach the column stabilizer tubing assembly from the column in-line filter unit. Do not detach the column in-line filter unit from the column inlet.
6. Place two 5/16-inch open-end wrenches onto the flats and rotate in opposite directions to open the column in-line filter unit.
7. Remove the column in-line filter unit inlet nut, which contains the used filter. Discard the filter; it cannot be reused.

Tip: Over time, the filter can expand inside the in-line filter inlet nut. If you are unable to remove the filter from the inlet nut, discard the inlet nut and replace both the inlet nut and filter.

8. Insert a new filter, and then reassemble the column in-line filter unit using the wrenches.



9. Reattach the column stabilizer tubing assembly to the in-line filter unit.
10. Start the mobile phase and examine the assembled components for leaks. Tighten fittings as needed.
11. Return the assembled components to the column compartment sleeve.
12. Swing the column retainer rod to the left-hand side to fasten it.

Cleaning the column manager and column heater/cooler

Exterior surfaces

Use a soft cloth, dampened with water, to clean the outside of the column manager.

Interior surfaces



Warning: To prevent burn injuries, adjust the temperature set-point of the column manager or column heater/cooler to a safe temperature (10 to 30 °C [50 to 86 °F]) and allow the unit to attain this set-point temperature prior to handling components within the column compartment. When the column manager or column heater/cooler is set at a high temperature, all interior surfaces and components are hot.



Caution: To prevent contamination, wear clean, chemical-resistant, powder-free gloves when cleaning interior surfaces of the column manager and column heater/cooler.

If the spills are behind the columns, remove the columns from the brackets. Wipe interior surfaces with a clean, lint-free, soft cloth, dampened with water.

Waste outlet

If the waste outlet is dirty or blocked, use cotton-tipped swabs to clean it out. Make sure the waste outlet and tubing are not constricted. If the outlet is blocked or the tubing is constricted, contact Waters Technical Service.

Drainage of leaks

One drainage cup is provided in the bottom front of the column manager and the column heater/cooler. The drainage cup collects leaks from the switching valves and the temperature compartment. You can remove the drainage cup and flush it out in the sink (this waste is not hazardous).

If an ACQUITY UPLC detector (TUV or PDA) is installed on top of the column manager or column heater/cooler, leaks and drainage from the detectors merge with the leak path of the column manager or column heater/cooler.

If fluid reaches an optional leak sensor and the leak sensor option is enabled, an alarm sounds and the pump stops. Remove and dry the leak sensor and the leak sensor reservoir, and then reinstall the leak sensor. Reset the column manager.

If residue or solvent accumulates, call Waters Technical Service.

Maintaining the TUV detector



Warning: To avoid electric shock, do not remove the detector's top cover. The components within are not user-serviceable.

Tip: Do not remove the TUV detector's top cover. No user-serviceable parts are inside. If you open the detector door, close it firmly before resuming normal operation.

The TUV detector requires minimal routine maintenance. To achieve optimal performance, follow these recommendations:

- Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- To conserve lamp life, extinguish the lamp while leaving the detector running but idle for more than 4 hours.
- Invoke the lamp optimization software routine at least once a week by powering-off the detector, waiting 10 seconds, and then powering-on the detector.

Alternative: In the ACQUITY UPLC Console, select TUV Detector > Maintain > Calibrate wavelength. This function will correct errors in wavelength and update and save the new calibration values.

- If you use buffered mobile phase, flush it from the detector before powering-off to prevent
 - precipitation of salts in the flow cell and associated tubing
 - damaged instrument components
 - microbial growth



Caution: To avoid damaging the column, remove it before you flush the system.

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

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

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

Resolving detector leak sensor errors

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

To resolve a detector leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to confirm that the detector leak sensor detected a leak.

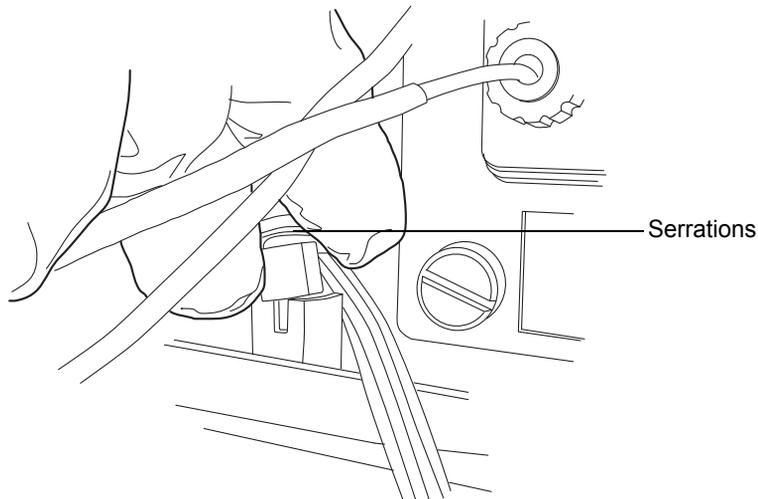
Tip: If a leak is detected, a “Leak Detected” error message appears.

2. Open the detector door, gently pulling its right-hand edge toward you.
3. Locate the source of the leak, and make the repairs necessary to stop the leak.



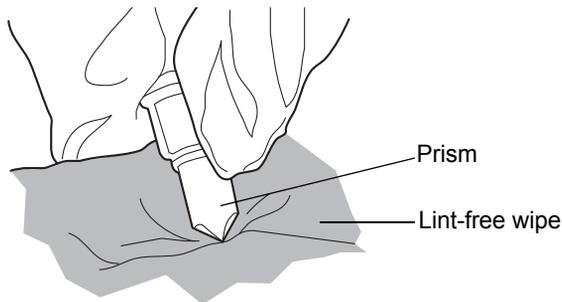
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

4. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

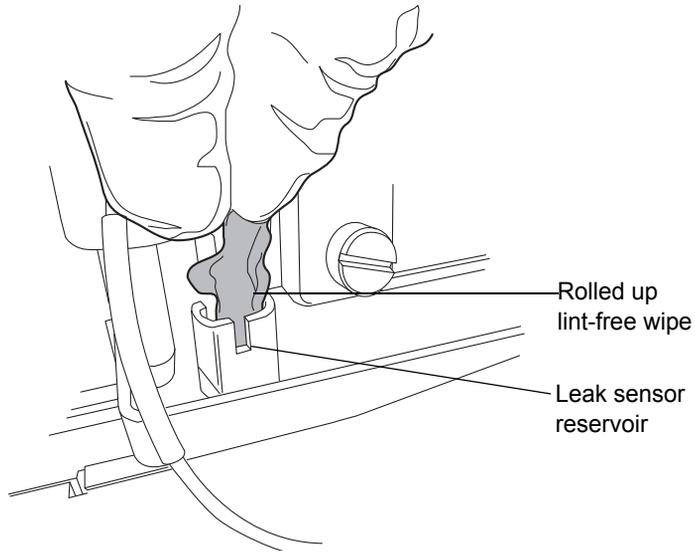


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 6-208](#)).

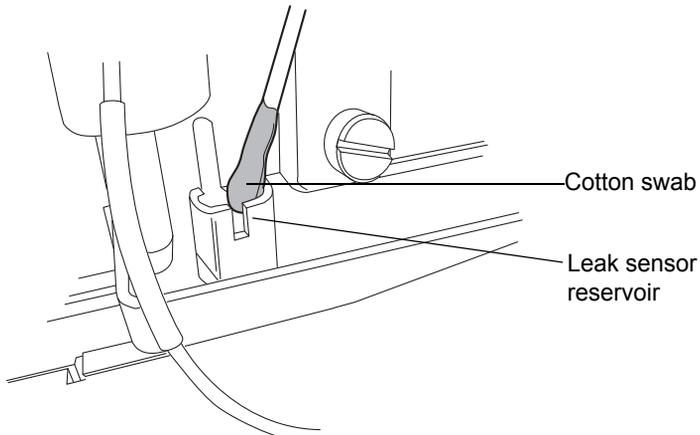
5. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



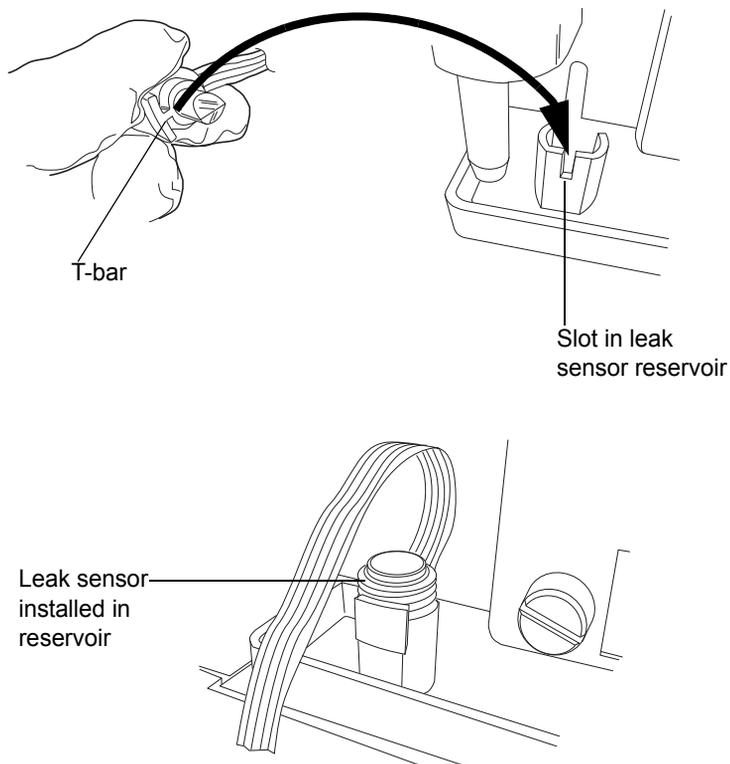
6. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



7. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



- Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



- If you detached the connector from the front of the instrument, reattach it.
- In the ACQUITY UPLC Console, select your detector from the system tree.
- In the detector information window, click Control > Reset, to reset the detector.

Replacing the detector's leak sensor



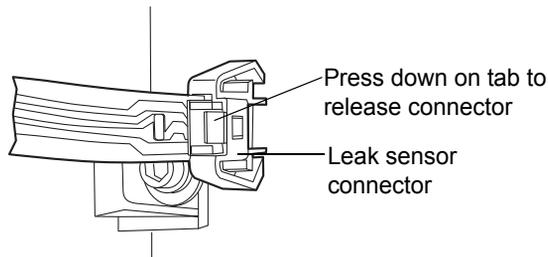
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

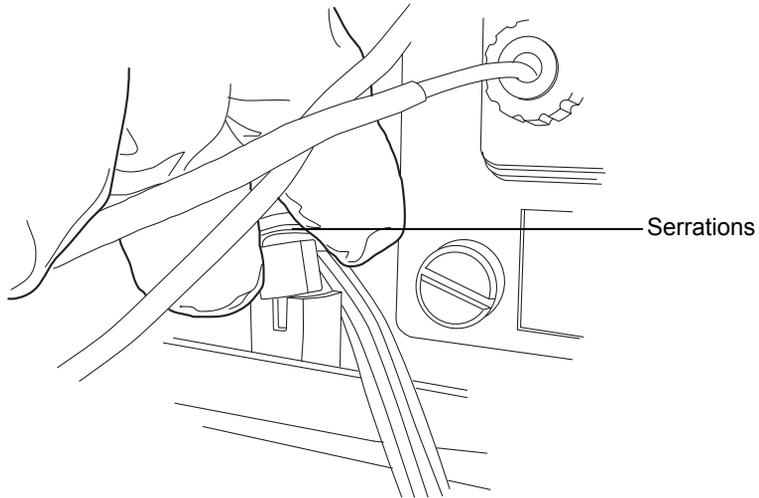
- Clean, chemical-resistant, powder-free gloves
- Leak sensor

To replace the detector leak sensor

1. Open the detector door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

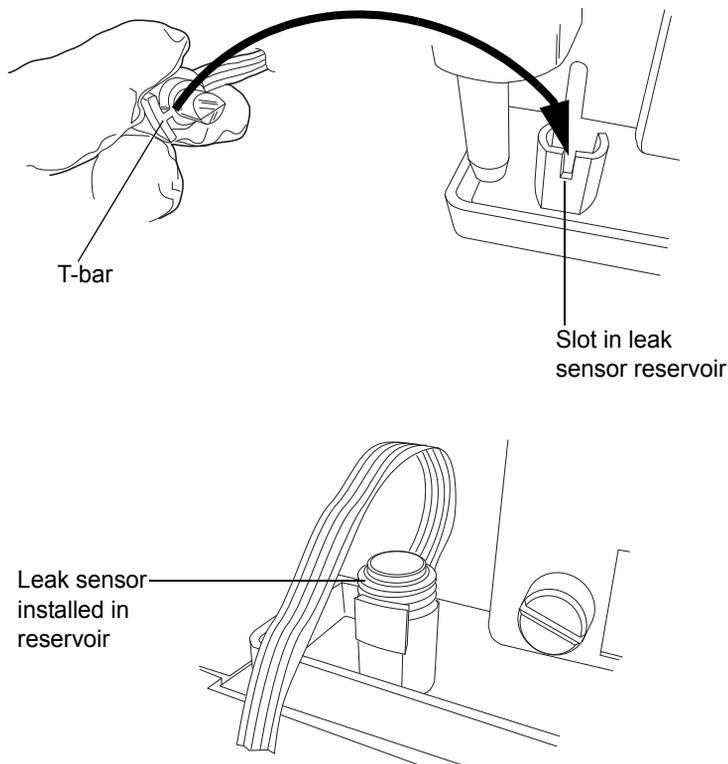


3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



4. Unpack the new leak sensor.

5. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



6. Plug the leak sensor connector into the front of the instrument.
7. In the ACQUITY UPLC Console, select your detector from the system tree.
8. In the detector information window, click Control > Reset, to reset the detector.

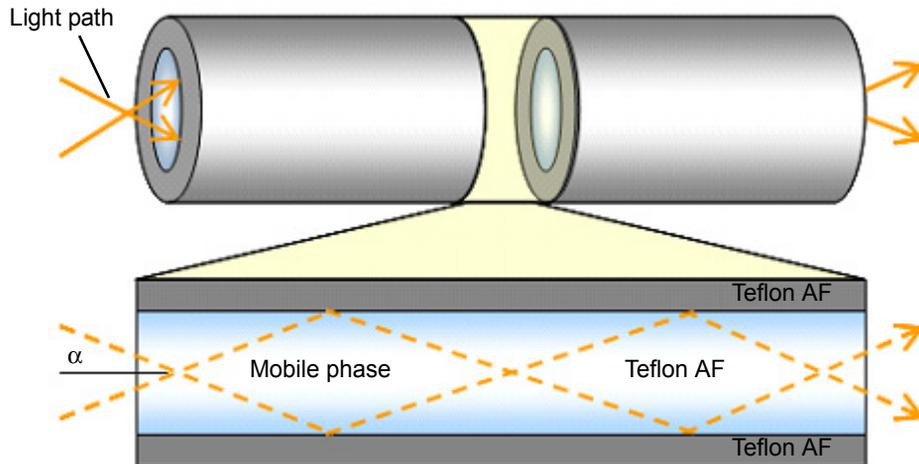
Maintaining the flow cell

Waters light-guiding flow cell overview

Waters light-guiding flow cells transport light and sample via Teflon AF tubing. The tubing transmits energy through low-volume flow cells, resulting in heightened analytical sensitivity. The mechanism by which light is efficiently transmitted through the sample tube is known as total internal reflection (TIR); light is confined within the fluid stream because the

refractive index of the fluid is greater than that of the tubing material (Teflon AF).

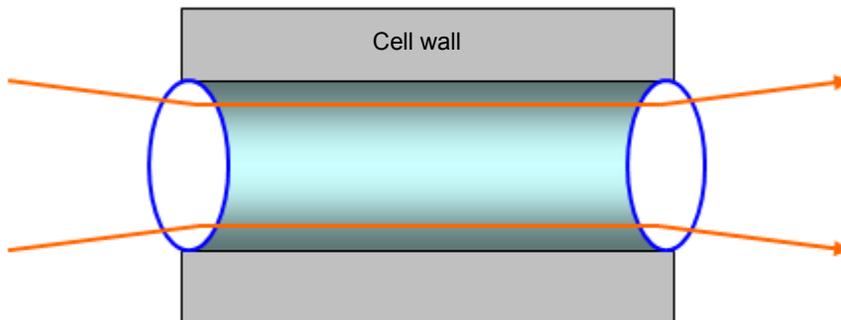
Light transmission through light guiding flow cell



In the figure, above, the light path through the cell is depicted by a pair of rays (dashed lines) that bounce off the cell wall. The energy carried by each ray is conserved after each bounce. One hundred percent of the light is reflected, hence the term “total internal reflection”. The Teflon AF tubing is an active component in the flow cell light path.

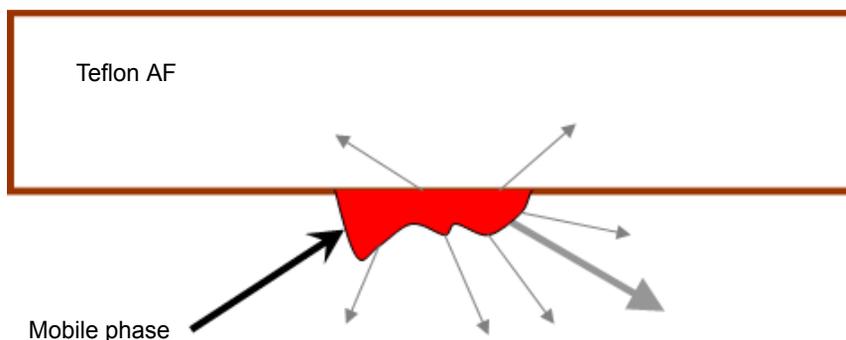
In contrast to the light-guiding flow cell, a conventional cell is typically an all-metal body with lenses at each end.

Light transmission through a conventional flow cell



The light path through a conventional flow cell is designed to avoid contact with the cell walls, primarily to prevent rays that encounter the walls from contributing to the measured signal. The energy associated with errant rays is highly variable depending on the mobile phase composition, quality of wall finish (which is never 100% reflective) or the slow build-up of contaminants. The Teflon AF surface, however, is mirror-like, and the relatively slight RI-dependence associated with a well-maintained cell is negligible. Surface contamination, depicted by the red, irregularly shaped object in the next figure can lead to undesirable beam effects like scattering (thin, gray arrows) or absorption (grey, thick arrow), both of which decrease reference energy.

Unwanted beam effects from a light guide flow cell



The operational differences between the light-guiding method, where light is transmitted through interactions with the cell wall, and the conventional method, which avoids such interactions, underscores these practical measures for maintaining the liquid core flow cell:

- Periodically determine flow cell transmission under conditions similar to those used to characterize a new cell. (Doing this typically means determining cell transmission with clean mobile phase.)
- Avoid fouling the flow cell by changing or disturbing upstream system components, as in the case when a new column is brought online.

Cleaning the flow cell

Clean the flow cell when it becomes contaminated with the residues of previous runs and also after each detector shutdown. A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems.

- Always flush the flow cell with mobile phase as your initial attempt to correct these problems.
- If there is still no improvement, flush the flow cell with 100% pure organic solution.

Recommendation: 100% acetonitrile.

- If the problems persist, flush the flow cell with 1% formic acid for 30 minutes, then flush with water until the formic acid is removed or until the pH is neutral.
- If flushing with the 1% formic acid solution also fails, perform a system acid cleansing flush (see [“Performing a system acid cleansing flush” on page 6-215](#)).
- If there is still no improvement, call Waters Technical Service.



Caution:

- To prevent flow cell failure, do not connect any tubing or device that can create backpressure exceeding the flow cell’s maximum rating of 6895 kPa (69 bar, 1000 psi).
- The pressure through the flow cell must not exceed 6895 kPa (69 bar, 1000 psi). Increasing the flow rate usually increases the pressure. High-viscosity fluids generally increase the pressure through the flow cell and therefore require a lower flow rate. Allowable flow rates are based on the limit of pressure that each flow cell can withstand.



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

Rule: Always use clean, well-degassed eluents.

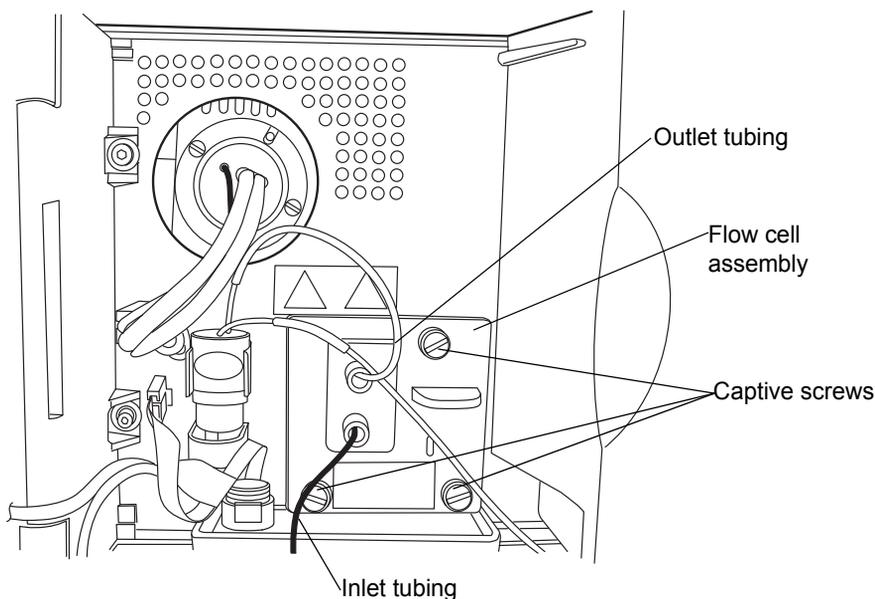
Required materials

- 1% formic acid
- Clean, chemical-resistant, powder-free gloves
- Water (for flushing buffers)
- Intermediate solvent that is miscible in both the mobile phase and water
- Stainless steel unions (to replace the column during flushing)
- Wrench suitable for removing and replacing the column

To clean the flow cell

1. In the detector control panel, click  (Lamp Off).
2. Stop the solvent flow and remove the column.
3. Replace the column with a union or piece of tubing.
4. If another instrument is downstream of the flow cell outlet, break the connection at the other instrument and route the outlet tubing to waste while flushing.

Rule: Do not flush while connected to a mass spectrometer.



5. Flush the detector with HPLC-grade water. If the mobile phase is not compatible with water, flush with an intermediate solvent first.
6. Pump an acid wash composition of 1.0% formic in water or 90% water/10% organic mixture. Flush the flow cell for minimum of 4 hours at a low flow of 0.05 to 0.1 ml/min. Do not exceed 6895 kPa (69 bar, 1000 psi).
7. Flush the detector with HPLC-grade water until the pH is neutral. If the mobile phase is not compatible with water, flush with an intermediate solvent first.

Tip: Remove any other active detectors of instruments from the system.

8. Reattach the column.
9. Resume pumping mobile phase. If the mobile phase is not miscible in water, first flush with an intermediary solvent.

Performing a system acid cleansing flush

System contamination can cause the flow cell to become contaminated. If the system becomes contaminated, perform a system acid cleansing flush. This procedure cleans the binary solvent manager, sample manager, and flow cell.



Caution: If you are running an MS detector, do not perform the system acid cleansing flush. Instead, call Waters Technical Service.

To prepare the solvent

1. Prepare a mixture of 50:50 (v/v) methanol:water as follows:
 - a. Measure 500 mL of water in a graduated cylinder.
 - b. In a separate graduated cylinder, measure 500 mL of methanol.
 - c. Add methanol to water and mix for 5 minutes.
2. Prepare a mixture of 30:70 (v/v) phosphoric acid:water as follows:
 - a. Measure 700 mL of water in a graduated cylinder.
 - b. In a separate graduated cylinder, measure 300 mL of phosphoric acid.
 - c. Add phosphoric acid to water and mix for 5 minutes.
3. Fill a 1L mobile phase reservoir with 100% water.
4. Fill a 1L mobile phase reservoir with 100% isopropanol.

Note: The cleaning procedure takes approximately 6 hours once the solvents are prepared to remove flow cell contaminants.

To perform the system acid cleansing flush



Caution: Failure to remove the Hastalloy bottle filters will contaminate the flow path.

1. Remove the sample and solvent manager bottle filters.

2. Place all lines A1, A2, B1, B2 seal wash, weak needle wash and strong needles in 50:50 methanol:water.
3. Prime the solvent lines for 5 minutes each.
4. Prime the seal wash.
5. Prime the wash syringes and sample syringe for 4 cycles.
6. Connect a pressure restrictor in the fluid path after the injector, to create 13,790 kPa (138 bar, 2000 psi) backpressure in the system.
7. Transfer 1 mL of mobile phase to an autosampler vial and place in position 1:A,1.
8. Create an instrument method with the following parameters:
 - a. Flow rate = 0.5 mL/min
 - b. Gradient composition 50% A1:50% B1
 - c. Full loop injection
9. Make 30 full loop injections from the vial containing the mobile phase. Set the run time to 0.5 minutes.

Notes: This step should take approximately 30 minutes.
10. Repeat steps 1 through 8 using 100% isopropanol as the solvent. Do not pass effluent through optical detector for this wash step. Restrictor should be routed to waste.
11. Repeat steps 1 through 8 using 100% water as the solvent.

Note: Remove the Seal wash line from the mobile phase bottle prior to performing the Phosphoric Acid Wash.
12. Repeat steps 1 through 8 using 30:70 (v/v) phosphoric acid:water as the solvent.
13. To completely remove all contaminants from the flow cell, continue pumping the phosphoric acid mixture for an additional 3 hours.
14. Repeat steps 1 through 8 using 100% water as the solvent.
15. Repeat steps 1 through 8 using 50:50 (v/v) methanol:water as the solvent.
16. Replace the sample and solvent manager bottle filters.

Replacing the flow cell



Caution:

- To avoid contaminating the flow cell, wear clean, chemical-resistant, powder-free gloves when handling, removing, or replacing it.
- To avoid damaging the flow cell, handle it with care. Do not disassemble the flow cell.

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

Required materials

- 1/4-inch, flat-blade screwdriver
- Clean, chemical-resistant, powder-free gloves
- Flow cell

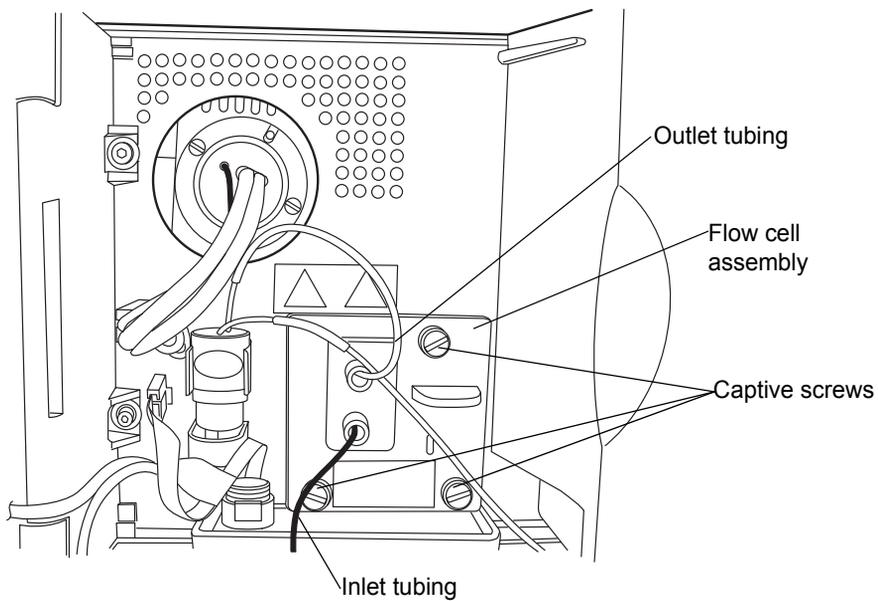
To replace the flow cell



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

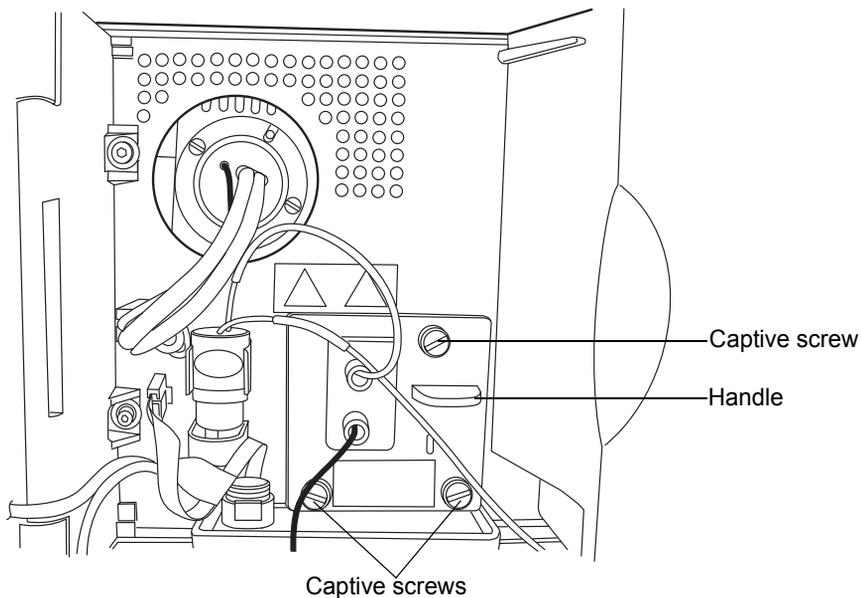
1. Power-off the detector.
2. Stop the solvent flow.
3. Open the detector door, gently pulling its right-hand edge toward you.

4. Disconnect the detector's inlet and outlet tubing from the main column connection.



5. Remove the flow cell:

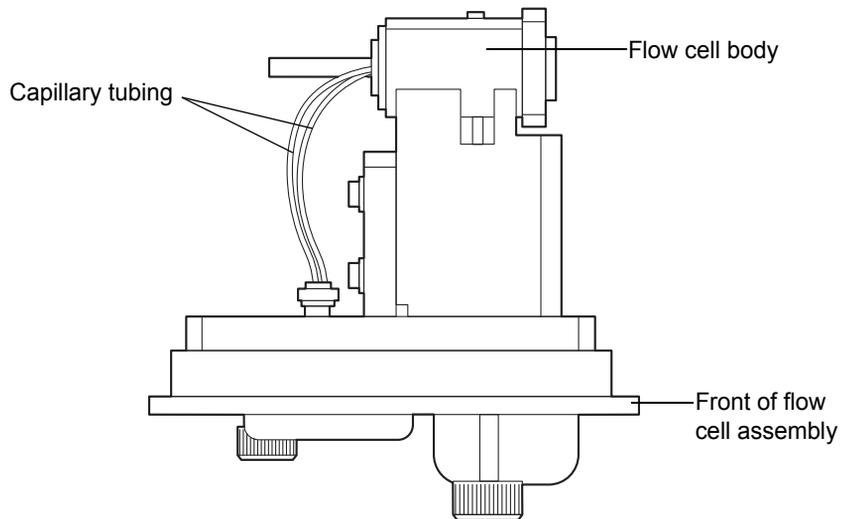
- Using a 1/4-inch, flat-blade screwdriver, loosen the 3 captive screws on the flow cell assembly's front plate.
- Grasp the handle and gently pull the assembly toward you.



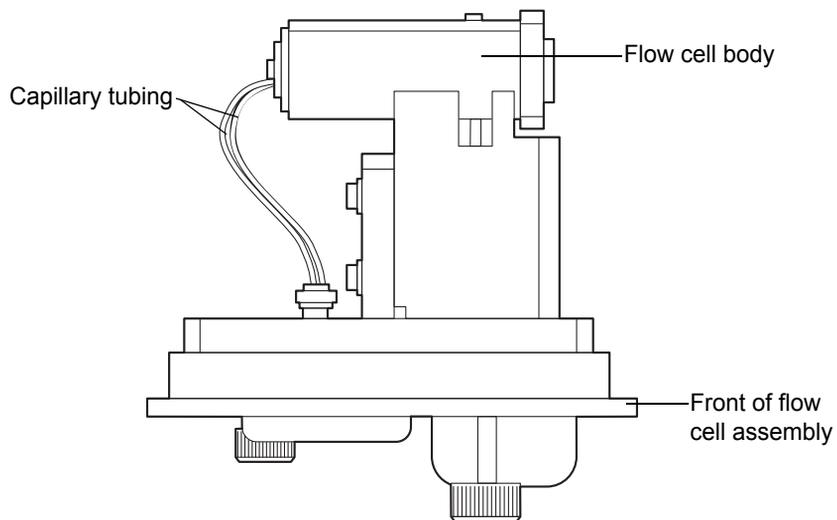
Caution: To avoid damaging the capillary tubing, do not touch it.

6. Unpack and inspect the new flow cell. Ensure that the flow cell type (analytical or high sensitivity) suits your application.

Top view of the analytical flow cell assembly



Top view of the high sensitivity flow cell assembly



7. Insert the new flow cell assembly into the detector, and then tighten the captive screws.
8. Connect the inlet tubing to the main column connection and flow cell inlet, and connect the outlet tubing to the flow cell outlet.

9. Close the detector door.
10. Before you power-on the detector, ensure the flow cell is filled with degassed, transparent solvent (acetonitrile or water) and free of air bubbles. The detector will not initialize correctly if the cell contains air.

Replacing the lamp

Change the lamp when it repeatedly fails to ignite or when the detector fails to calibrate.

Tip: Record the new lamp's serial number to properly track lamp life. If you do not record a new lamp's serial number in the ACQUITY UPLC Console, the date of the previous lamp installation remains in the detector's memory.



Warning: To prevent burn injuries, allow the lamp to cool for 30 minutes before removing it. The lamp housing becomes extremely hot during lamp operation.



Warning: To avoid eye injury from ultraviolet radiation exposure,

- power-off the detector before changing the lamp.
- wear eye protection that filters ultraviolet light.
- keep the lamp in the housing during operation.

Required material

Performance maintenance kit

To remove the lamp

1. Power-off the lamp.



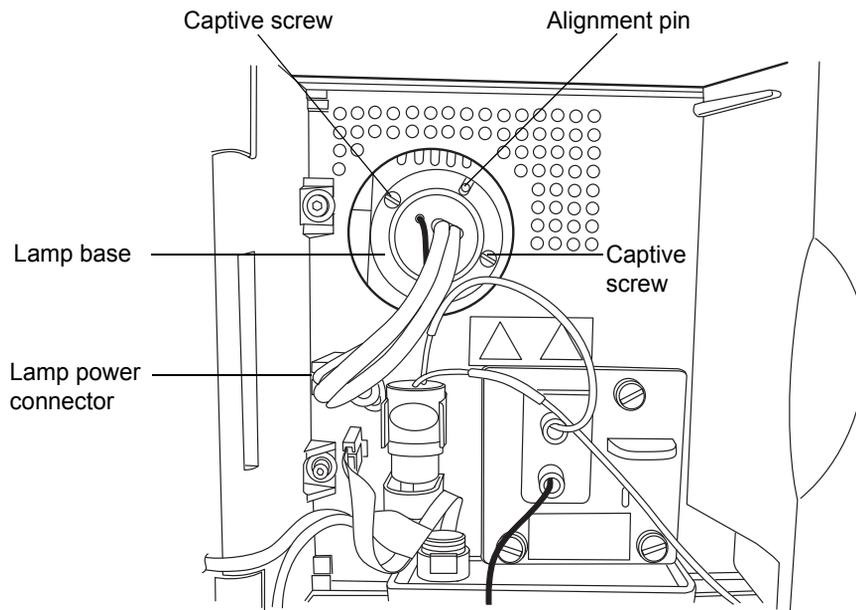
Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To completely interrupt power to an instrument, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds thereafter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

2. Power-off the detector and disconnect the power cable from the rear panel.



Warning: The lamp and lamp housing can be hot. Wait 30 minutes after powering off the detector for these components to cool before touching them.

3. Allow the lamp to cool for 30 minutes, and then open the door, gently pulling its right-hand edge toward you.
4. Detach the lamp's power connector from the detector.



Warning: Lamp gas is under slight negative pressure. To prevent shattering the glass, use care when disposing of the lamp.



Caution: Do not touch the glass bulb of the new lamp. Dirt or skin oils on the bulb adversely affect detector operation. If the bulb needs cleaning, gently rub it with ethanol and lens tissue. Do not use abrasive tissue. Do not apply excessive pressure.

5. Loosen the 2 captive screws in the lamp base. Pull the lamp assembly out of the lamp housing, and then gently withdraw the lamp.

To install the lamp

1. Without touching the bulb, unpack the new lamp from its packing material.
2. Inspect the new lamp and lamp housing for cleanliness.
3. Position the lamp so that the cut-out on its base plate is at the 1 o'clock position, in line with the alignment pin on the lamp housing, and then gently push the lamp forward until it bottoms into position. Ensure that it seats flush against the optics bench.
4. Tighten the 2 captive screws, and then reconnect the power connector.
5. Power-on the detector, and then wait about 30 minutes for the lamp to warm before resuming operations.

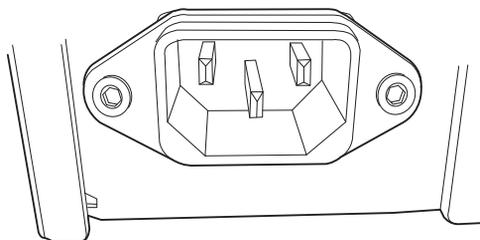
Tip: Cycling power to the detector (that is, powering-off and then powering-on the instrument) initiates the verification procedures.

6. In the ACQUITY UPLC Console, select Maintain > Change Lamp.
7. In the Change Lamp dialog box, click New Lamp.
8. Type the serial number for the new lamp (see the label attached to the lamp's connector wire), and then click OK.

Replacing the fuses

Note: Recently manufactured instruments do not have fuses above the power entry module because the power supply is thermally protected. If your detector has the power entry module shown below and it fails to power-on, the status LEDs are unlit, or the power supply fan does not operate, call Waters Technical Service (see [page 6-6](#)).

Power entry module on recently manufactured instruments





Warning: To avoid electric shock, power-off and unplug the TUV detector before examining the fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

Required material

100 to 240 VAC, 50 to 60-Hz, F 3.15-A, 250-V (fast-blow), 5 × 20 mm (IEC) fuses (2)

Suspect a fuse is open or otherwise defective when

- the detector fails to power-on.
- the power supply fan does not operate.

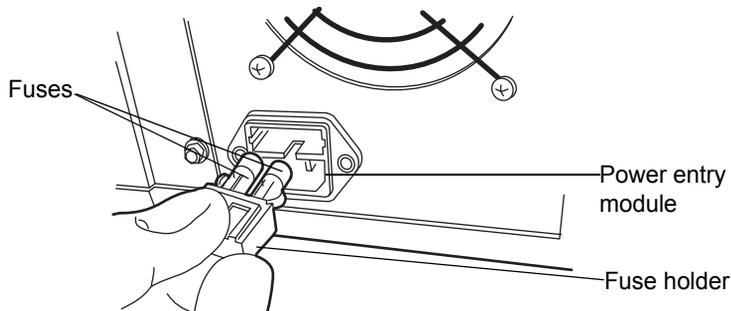
To replace the fuses

Requirement: Replace both fuses, even when only one is open or defective.



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

1. Power-off the detector and disconnect the power cord from the power entry module.
2. Pinch the sides of the spring-loaded fuse holder, located above the power entry module on the rear panel of the detector.
3. With minimum pressure, withdraw the spring-loaded fuse holder.



4. Remove and discard the fuses.
5. Make sure that the new fuses are properly rated for your requirements, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
6. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the TUV detector.

Maintaining the sample organizer



Caution: Never lubricate sample organizer components. Doing so will damage the instrument.

Maintaining the sample organizer consists of defrosting, cleaning its air filter, replacing fuses, and cleaning the outside of the instrument.

Defrosting the sample organizer

For information on defrosting the sample organizer, refer to [“Defrosting the sample compartment” on page 6-122](#).

Cleaning the air filter

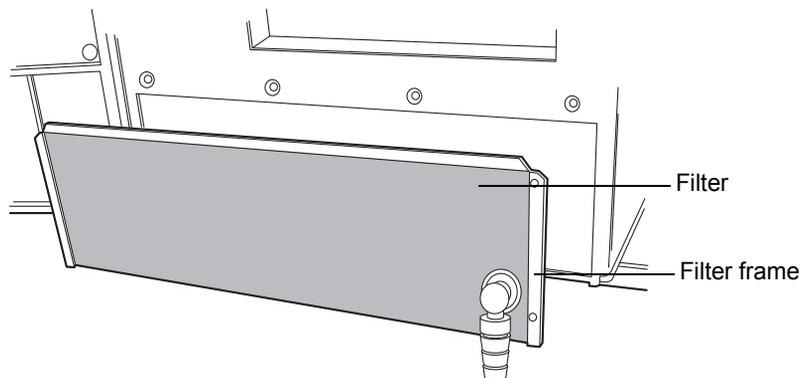
To clean the air filter



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

1. Power-off the sample organizer.
2. Remove the drain tube from the filter frame.

3. Pull the filter frame toward you, and remove it from the sample organizer.



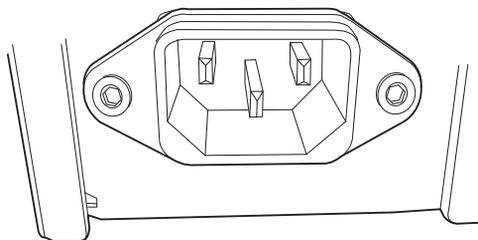
Caution: Do not remove the air filter from the filter frame.

4. With the filter still attached to the filter frame, rinse the filter in a sink or vacuum it.
5. Refit the filter frame.

Replacing the fuses

Note: Recently manufactured instruments do not have fuses above the power entry module because the power supply is thermally protected. If your sample organizer has the power entry module shown below and it fails to power-on, the status LEDs are unlit, or the power supply fan does not operate, call Waters Technical Service (see [page 6-6](#)).

Power entry module on recently manufactured instruments





Warning: To avoid electric shock, power-off and unplug the sample organizer before examining the fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

Suspect a fuse is open or otherwise defective when

- the sample organizer fails to power on.
- the power supply fan does not operate.

Required materials

- 10 A fuse (2)
- Flat-blade screwdriver

To replace the fuses

Requirement: Replace both fuses, even when only one is open or defective.



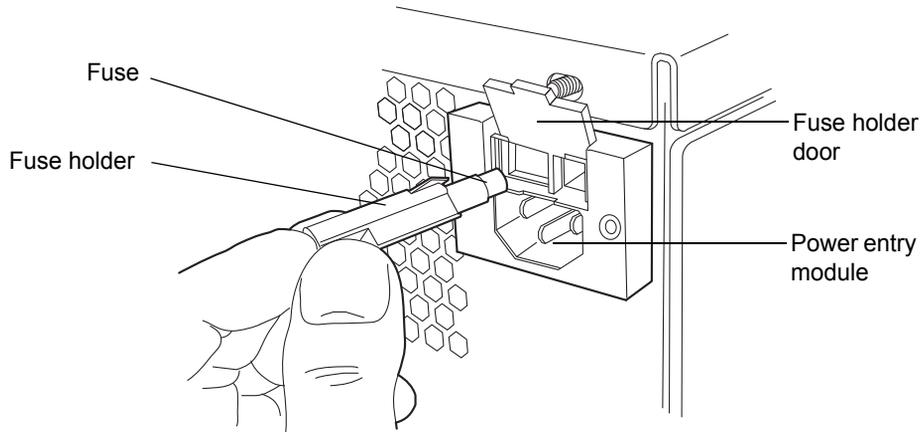
Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To completely interrupt power to an instrument, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds thereafter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

1. Power-off the sample organizer.

Tip: The fans inside the sample organizer run continuously, even after you power it off. However, if the fuse is open or defective, the fans do not run.

2. Disconnect the power cord from the power entry module.
3. Use a flat-blade screwdriver to open the fuse holder door, located above the power entry module on the rear panel of the sample organizer.

4. With minimum pressure, pull on each spring-loaded fuse holder to remove it.



5. Remove and discard the fuses.



Warning: For continued protection against fire, replace fuses with the appropriate type and rating.

6. Make sure that the new fuses are properly rated for your requirements, and then insert the new fuses into the holders and the holders into the power entry module.
7. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the sample organizer.

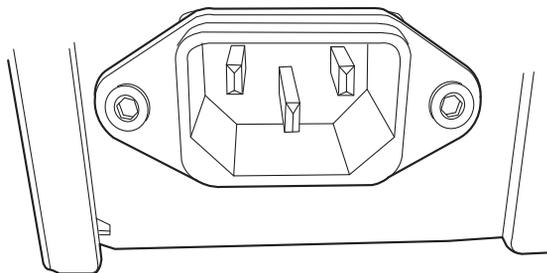
Maintaining the 30-cm column heater/cooler

Maintaining the 30-cm column heater/cooler consists of replacing fuses and cleaning the outside of the instrument.

Replacing the fuses

Note: Recently manufactured instruments do not have fuses above the power entry module because the power supply is thermally protected. If your 30-cm column heater/cooler has the power entry module shown below and it fails to power-on, the status LEDs are unlit, or the power supply fan does not operate, call Waters Technical Service (see [page 6-6](#)).

Power entry module on recently manufactured instruments



Warning: To avoid electric shock, power-off and unplug the sample organizer before examining the fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

Suspect a fuse is open or otherwise defective when

- the 30-cm column heater/cooler fails to power on.
- the power supply fan does not operate.

Required materials

- 3.15 A, 250 V fuse (2)
- Flat-blade screwdriver

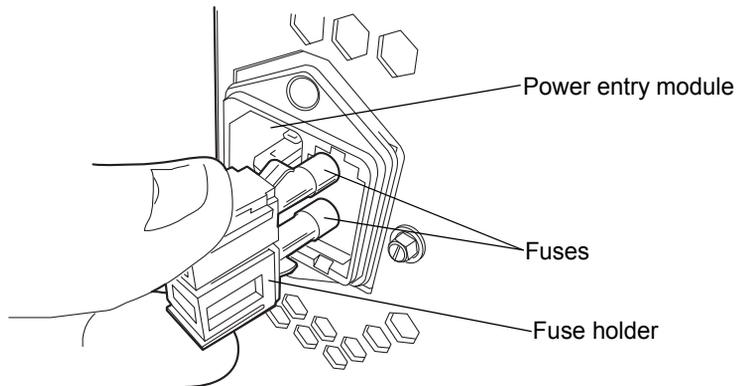
To replace the fuses

Requirement: Replace both fuses, even when only one is open or defective.



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

1. Power-off the 30-cm column heater/cooler.
2. Disconnect the power cord from the power entry module.
3. Pinch the sides of the spring-loaded fuse holder, located to the right of the power entry module on the rear panel of the column heater.
4. With minimum pressure, withdraw the spring-loaded fuse holder.



5. Remove and discard the fuses.



Warning: For continued protection against fire, replace fuses with the appropriate type and rating.

6. Make sure that the new fuses are properly rated for your requirements, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
7. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the 30-cm column heater/cooler.

7 Troubleshooting

This chapter describes how to find the cause of problems that can affect performance of the ACQUITY UPLC[®] system and how to correct them.

Contents

Topic	Page
Troubleshooting with Connections Insight	7-2
Proper operating procedures	7-3
Solving baseline noise and drift problems	7-5
Binary solvent manager troubleshooting	7-10
Sample manager troubleshooting	7-14
Sample organizer troubleshooting	7-17
HT column heater troubleshooting	7-21
Column manager and column heater/cooler troubleshooting	7-21
TUV detector troubleshooting	7-24
PDA detector troubleshooting	7-27
ELS detector troubleshooting	7-32
FLR detector troubleshooting	7-33
Chromatography troubleshooting	7-35

Troubleshooting with Connections Insight

Connections Insight™ is an intelligent device management (IDM) Web service that enables Waters to provide proactive service and support for the ACQUITY UPLC system. To use Connections Insight, a Waters technician must install the Connections Insight service agent software on the system's Empower or MassLynx workstation. In a client/server system, the service agent must also be installed on the computer from which you control the system. The service agent software automatically and securely captures and sends information about the support needs of your system directly to Waters.

If you encounter a performance issue as you are using the ACQUITY UPLC Console, you can also manually submit a Connections Insight request to Waters customer support.

Consult these sources for more information about Connections Insight:

- Connections Insight Installation Guide
- Connections Insight User Guide
- Connections Insight FAQ Document
- <http://www.waters.com>
- Your sales representative
- Your local Waters subsidiary
- Waters Customer Support, at 800 252-4752 (in the USA or Canada)

To submit a Connections Insight request

1. In the ACQUITY UPLC Console, select Troubleshoot > Submit Connections Insight request.
2. In the Connections Insight Request dialog box, type your name, telephone number, email address, and a description of the problem.

Connections Insight request dialog box



The screenshot shows a dialog box titled "Connections Insight™ Request". It features three input fields for contact information: "Name:" (Bob Smith), "Telephone:" (978-555-1212), and "Email:" (Bob_Smith@YourCompany.com). Below these is a "Description:" label and a text area containing the text "My system pressure is fluctuating.". At the bottom of the dialog are three buttons: "Settings...", "Submit", and "Cancel".

3. Click Submit. Allow approximately 5 minutes to save the service profile.

Result: A .ZIP file containing your Connections Insight profile is forwarded to Waters customer support for review. Saving a service profile or plot file from the ACQUITY UPLC Console can require up to 150 MB of file space.

Proper operating procedures

So that your system always runs optimally, follow the operating procedures and guidelines in Chapters 2 through 6.

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). Otherwise phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Our Web site includes phone numbers and

e-mail addresses for Waters locations worldwide. Visit www.waters.com, and click Waters Division > Regional/Global Contacts.

Support Center dialog box

The screenshot shows the Waters website's Support Center interface. At the top, there is a dark blue header with the 'Waters' logo on the left and 'My Waters :: Shop :: Worldwide' on the right, with a 'Welcome to Waters' message and a 'Login' link. Below the header is a navigation bar with tabs for 'About Waters', 'Products', 'Library', 'News & Events', and 'Services & Support'. A search box with a 'Go' button is located on the right side of the navigation bar. The main content area is titled 'Connections Elite' and contains the following text:

You are here > [Services & Support](#) > [Support Center](#) > [Connections Elite](#)

Connections Elite Support Center - your link to valuable information relating to your Waters instruments and software.

Through the Connections Elite Support Center you can:

- Access technical notes, release notes, user guides, self-service procedures and troubleshooting tips.
- Learn the basics of how to operate Waters software using an on-line tutorial or download the file for future reference.
- Use the Waters Quality Part Locator to locate, view and order instrument components. Drill-down capability lets you find parts within subsystems.

Access to the Millennium³², Empower™ and MassLynx™ Software advanced technical information is reserved for our Connections Software Plan customers.

Contact Waters to purchase a plan or request additional information. In the US and Canada call 1-800-252-4752. Outside of North America contact your local Waters office.

We are continuously improving the content and usefulness of our site thanks to your suggestions. If you have any recommendations, please let us know.

On the right side of the dialog box, there are two sections: 'Take Action' with a 'Contact Waters' link, and 'Library' with a 'Connections Software Plan Information' link.

Locating system serial numbers

Each system instrument's unique serial number facilitates service and support. Serial numbers also provide a way to create single log entries for each instrument so that you can review the usage history of only that instrument.

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

To view instrument information

1. In the ACQUITY UPLC Console, select an instrument from the system tree.

2. Click Configure > View module information. The Module Information dialog box displays this information:
 - Serial number
 - Firmware version
 - Firmware checksum
 - Component software version

Alternatives:

- From the main window, place the pointer over the visual representation of the system instrument you want information for.
- Obtain the serial number from the printed labels on the rear panels of instruments or inside their front doors.

Tip: Column part numbers and serial numbers appear on the column page. To see column information, click Column on the navigation tree.

Solving baseline noise and drift problems

The following table lists possible causes for baseline noise, drift, and spikes in the system and suggests possible corrective actions.

Corrective actions for baseline noise and drift problems

Symptom	Possible cause	Corrective action
Baseline drift during gradient	There is an absorbance difference between the A and B mobile phases.	Balance the absorbance of the two mobile phases by reducing the concentration of the additive in the higher-absorbing mobile phase. Note: This makes the mobile phase slightly different with respect to the additive.

Corrective actions for baseline noise and drift problems (Continued)

Symptom	Possible cause	Corrective action
Flat baseline with no peaks	No solvent flow	<ol style="list-style-type: none"> 1. Check the solvent reservoirs and ensure they contain solvent. 2. Make sure the flow rate is > 0.
	Detector lamp not ignited	<ul style="list-style-type: none"> • Use detector diagnostic tests to determine reference and sample energy. Zero energy indicates the lamp is extinguished. • Power-on the lamp. If this does not resolve the problem, replace the lamp.
	Detector lamp burned out	Replace the lamp.
	Incorrect detector wavelength	Check wavelength setting.
	Leak in solvent path	Check fittings.

Corrective actions for baseline noise and drift problems (Continued)

Symptom	Possible cause	Corrective action
Flat baseline with no peaks (continued)	The mobile phase is absorbing too much UV at the wavelengths selected	<ul style="list-style-type: none"> Do not monitor at such low wavelengths. Lower the concentration of the mobile phase additive. Use a different additive or solvent, one with less absorbance at the chosen wavelength.
	Incorrect vial position	Place vial in correct position.
	Incorrect vial	Replace vial with correct one.
	Injector not injecting	<ol style="list-style-type: none"> Prime the sample syringes (see page 3-17). Run the sample syringe leak test (see the ACQUITY UPLC Console online Help). If the problem persists, call Waters Technical Service (see page 7-3).
	Method parameters incorrect	Set correct method parameters.
Large, unexpected spikes on baseline	Air bubbles due to outgassing	<ul style="list-style-type: none"> Install the backpressure regulator. Repair the degasser.
	System improperly grounded	<ul style="list-style-type: none"> Plug into an outlet on a different electrical circuit. Use a power conditioner.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Long-term baseline noise cycling (approximately 10 minutes to 1 hour)	Ambient temperature fluctuations	Stabilize ambient temperature.

Corrective actions for baseline noise and drift problems (Continued)

Symptom	Possible cause	Corrective action
Random baseline noise	Air in detector flow cell	<ol style="list-style-type: none"> 1. Flush the detector flow cell to remove air. 2. Check the backpressure regulator and ensure it is properly installed.
	Bubble detected	Prime the solvent management system.
	Solvents contaminated	Use fresh solvent.
	Column contaminated	Clean or replace the column.
	Dirty flow cell	Clean the flow cell.
	System improperly grounded	<ul style="list-style-type: none"> • Plug into an outlet on a different electrical circuit. • Use a power conditioner.
	Unit not cooling properly	<ul style="list-style-type: none"> • Operate the system with all covers in place. • Check the back panels for proper clearance.
	Radio frequency noise	Eliminate interference.
	The mobile phase is absorbing too much at the wavelengths selected	<ul style="list-style-type: none"> • Do not monitor at such low wavelengths. • Lower the concentration of the additive. • Use a different additive or solvent with less absorbance at the chosen wavelength.
Old lamp	Replace the lamp (see page 6-221).	

Corrective actions for baseline noise and drift problems (Continued)

Symptom	Possible cause	Corrective action
Random baseline noise (continued)	Low lamp energy	<ol style="list-style-type: none"> 1. Check the lamp energy (see the ACQUITY UPLC Console online Help). 2. Replace the lamp, if necessary (see page 6-221).
Rapid baseline shift	Column not equilibrated	Equilibrate the column.
	Detector not allowed to warm up	Allow the detector to warm up until baseline is stable. Time varies with wavelength and sensitivity.
	Solvent contaminated	Use fresh solvent.
	Flow fluctuations (rapid or slow drift)	Prime the pump, replace pump seals, check valves. See “Erratic retention times” in the table titled “Chromatography troubleshooting” on page 7-36 .
	Incorrect wavelength for solvent	Ensure solvent does not absorb at the wavelength used.
Short term baseline noise cycling (30 to 60 seconds)	Flow fluctuating	Look for leaks. See “Erratic retention times” in the table titled “Chromatography troubleshooting” on page 7-36 .
	Radio frequency noise (short- term or long-term cycling)	Eliminate interference.
Slow baseline drift	Solvent contaminated	Use fresh solvent.
		Check or repair solvent filters.
	Decreased UV lamp energy	Determine lamp energy at the ACQUITY UPLC Console.

Corrective actions for baseline noise and drift problems (Continued)

Symptom	Possible cause	Corrective action
Slow baseline drift (continued)	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration.
	UV detector flow cell leaking	Inspect flow cell, tighten connections. If flow cell is leaking, call Waters Technical Service (see page 7-3).
	Dirty flow cell	Clean the flow cell.
	There is an absorbance difference between the A and B mobile phases.	Balance the absorbance of the two mobile phases by reducing the concentration of the additive in the higher-absorbing mobile phase. Note: This makes the mobile phase slightly different with respect to the additive.
	Leaking connections at flow cell inlet and outlet	Tighten fittings.

Binary solvent manager troubleshooting

Power LED

The power LED, on the left-hand side of the binary solvent manager's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Flow LED

The flow LED, on the right-hand side of the power LED, indicates the flow status.

Binary solvent manager flow LED indications

LED mode and color	Description
Unlit	Indicates the binary solvent manager is idle.
Steady green	Indicates the binary solvent manager is operating normally.
Flashing red	Indicates a serious error that has stopped pump operation. Information about the error can be found in the ACQUITY UPLC Console.
Steady red	Indicates a serious failure within the binary solvent manager that prevents further operation. Call Waters Technical Service (see page 7-3).

Binary solvent manager troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see page 6-120).
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Erratic flow or pressures	Air in system	Prime binary solvent manager (see page 3-9).

Binary solvent manager troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Erratic flow or pressures (continued)	Faulty check valve	<ol style="list-style-type: none"> 1. Prime binary solvent manager (see page 3-9). 2. Perform the static decay test to identify faulty check valve (see ACQUITY UPLC Console online Help). 3. Replace faulty check valve (see page 6-46).
	High-pressure leak in the system	Locate and repair the leak (see ACQUITY UPLC Console online Help).
	Plugged solvent reservoir filter	Clean or replace filter (see page 6-49).
	High pressure seal leaking	Replace high pressure seal (see page 6-52).
Fans do not run	Open (spent) or defective fuse	Replace fuse (see page 6-120).
	Power supply fan wiring or motor problem	<ol style="list-style-type: none"> 1. Reset the binary solvent manager via the control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).

Binary solvent manager troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Fans do not run (continued)	Power supply not working	<ol style="list-style-type: none"> 1. Reset the binary solvent manager via the control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
Leak detected	Leak in binary solvent manager.	Follow the procedure to resolve a binary solvent manager leak sensor error on page 6-10 .
Pump head leaks solvent	Loose head	Tighten head bolts through pressure transducer.
	Loose check valve	Tighten the check valve.
	Seal wash tubes not properly installed	Install tubes properly.
	Worn high pressure seal	Replace high pressure seal (see page 6-52).
Priming problems	Solvent inlet tubes to the solvent manager head are leaking	Inspect solvent connect tubes.
	Pump heads not adequately primed	Prime pump heads (see page 3-9).
	Defective solvent tube	Replace solvent tube.
	Defective check valve	Replace check valve (see page 6-46).

Binary solvent manager troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Solvent leaks from seal wash leak tube (see page 6-9)	Worn seal wash seals	Replace seal wash seals (see page 6-52).
Startup diagnostic tests failure	Internal problem with controller board, solvent management system, or sample management system	<ol style="list-style-type: none">1. Reset the binary solvent manager via the control panel or ACQUITY UPLC Console.2. If the problem persists, power-off and power-on again.3. If the problem persists, call Waters Technical Service (see page 7-3).

Sample manager troubleshooting

Power LED

The power LED, on the left-hand side of the sample manager's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Run LED

The run LED, on the right-hand side of the power LED, indicates the run status.

Sample manager run LED indications

LED mode and color	Description
Unlit	Indicates the sample manager is currently idle.
Steady green	Indicates the sample manager is operating normally, attempting to complete any outstanding samples or diagnostic function requests. When sample and diagnostic function requests are finished, the LED returns to the unlit state.
Flashing green	Indicates the system is waiting for an instrument to reach operational condition (for example, waiting for the column heater to reach its set point).
Flashing red	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Steady red	Indicates a serious failure within the sample manager that prevents further operation. Call Waters Technical Service (see page 7-3).

Sample manager troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see page 6-159).
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.

Sample manager troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Fans do not run	Sample manager not plugged in	Ensure sample manager is plugged in.
	Power supply fan wiring or motor problem	<ol style="list-style-type: none"> 1. Reset the sample manager via control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
	Power supply not working	<ol style="list-style-type: none"> 1. Reset the sample manager via the control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).

Sample manager troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Leak detected	Leak in sample manager.	Follow the procedure to resolve a sample manager leak sensor error on page 6-123 .
Sample compartment freezes	Sample compartment door opened too frequently	<ol style="list-style-type: none">1. Defrost sample manager, and then choose a sample compartment temperature 4 °C (39.2 °F) or greater (see page 6-122).2. Minimize sample door access.

Sample organizer troubleshooting

Power LED

The power LED, on the left-hand side of the sample organizer's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Run LED

The run LED, on the right-hand side of the power LED, indicates the run status.

Sample organizer run LED indications

LED mode and color	Description
Unlit	Indicates the sample organizer is currently idle.
Steady green	Indicates the sample organizer is operating normally, attempting to complete any outstanding samples or diagnostic function requests. When sample and diagnostic function requests are finished, the LED returns to the unlit state.
Flashing green	Indicates the system is waiting for an instrument to reach operational condition.
Flashing red	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Steady red	Indicates a serious failure within the sample organizer that prevents further operation. Call Waters Technical Service (see page 7-3).

Sample organizer troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see page 6-226).
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Fans do not run	Sample organizer not plugged in	Ensure sample organizer is plugged in.

Sample organizer troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Fans do not run (continued)	Power supply fan wiring or motor problem	<ol style="list-style-type: none"> 1. Reset the sample organizer via the sample manager control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
	Power supply not working	<ol style="list-style-type: none"> 1. Reset the sample organizer via the sample manager control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).

Sample organizer troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Sample plates jam	Nonconforming vial or microtiter plates	Ensure that all plates conform to ANSI standards.
	Bad plate sensor fiber optic	Call Waters Technical Service (see page 7-3).
	Incorrect alignment of X and Y stops	Call Waters Technical Service (see page 7-3).
Startup diagnostic tests failure	Internal problem with controller board, solvent management system, or sample management system	<ol style="list-style-type: none"> 1. Reset the sample organizer via the sample manager control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).

HT column heater troubleshooting

HT column heater troubleshooting

Symptom	Possible cause	Corrective action
Leak detected	Leak in HT column heater.	Follow the procedure to resolve a HT column heater leak sensor error on page 6-162 and page 6-167 .

Column manager and column heater/cooler troubleshooting

Power LED

The power LED, on the left-hand side of the sample manager's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Run LED

The run LED, on the right-hand side of the power LED, indicates the run status.

Column manager and column heater/cooler run LED indications

LED mode and color	Description
Unlit	Indicates the column manager or column heater/cooler is currently idle.
Steady green	Indicates the column manager or column heater/cooler is operating normally, attempting to complete any outstanding samples or diagnostic function requests. When sample and diagnostic function requests are finished, the LED returns to the unlit state.

Column manager and column heater/cooler run LED indications (Continued)

LED mode and color	Description
Flashing green	Indicates the system is waiting for an instrument to reach operational condition (for example, waiting for the column heater to reach its set point).
Flashing red	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Steady red	Indicates a serious failure within the column manager or column heater/cooler that prevents further operation. Call Waters Technical Service (see page 7-3).

Column manager and column heater/cooler troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see page 6-159).
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.

Column manager and column heater/cooler troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Fans do not run	Column manager or column heater/cooler not plugged in	Ensure column manager or column heater/cooler is plugged in.
	Power supply fan wiring or motor problem	<ol style="list-style-type: none"> 1. Reset the column manager or column heater/cooler via control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
	Power supply not working	<ol style="list-style-type: none"> 1. Reset the column manager or column heater/cooler via the control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
Leak detected	Leak in column manager or column heater/cooler.	Follow the procedure to resolve a column manager or column heater/cooler leak sensor error on page 6-180 .

TUV detector troubleshooting

Power LED

The power LED, on the left-hand side of the detector's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Lamp LED

The lamp LED, on the right-hand side of the power LED, indicates the lamp status.

TUV detector lamp LED indications

LED state	Description
Unlit	Indicates the detector lamp is extinguished.
Steady green color	Indicates the detector is operating normally.
Flashing green color	Indicates the detector is initializing or calibrating.
Steady red color	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Flashing red color	Indicates a serious failure within the detector that prevents further operation. Call Waters Technical Service (see page 7-3).

Clearing bubbles from the flow cell

To clear bubbles from the flow cell

1. Ensure the pressure regulator at the outlet of the flow cell is in place. If the pressure regulator has been bypassed, ensure that a device that develops a minimum of 1724 kPa (17 bar, 250 psi), but not greater than 6895 kPa (69 bar, 1000 psi), for the given flow rate and mobile phase is placed downstream from the detector.

2. Establish liquid flow through the detector flow cell with degassed acetonitrile or methanol at a flow rate that is anticipated to be used for subsequent analysis.

TUV detector troubleshooting

Symptom	Possible cause	Corrective action
Analog output incorrect	AUFS setting changed	Reset the AUFS setting.
Both LEDs unlit	No power	<ol style="list-style-type: none"> 1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open or defective fuse	Replace fuse (see page 6-223).
Calibration or energy error on startup	No solvent flowing through flow cell UV absorber in flow cell	<ol style="list-style-type: none"> 1. Make sure that solvent is flowing through the flow cell. 2. Ensure the mobile phase is transparent (such as water). 3. Clean the flow cell (see page 6-212). 4. Perform manual calibration.
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.

TUV detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Deuterium lamp does not light; lamp LED is unlit, solid red, or flashing red	Faulty lamp	Replace the lamp (see page 6-221).
	Lamp not plugged in	Plug in the lamp connector (see page 6-221).
	Lamp input shorted in “off” position	Inspect the rear panel connections (see ACQUITY UPLC Console online Help) or look for a timed event within a method (possibly programmed to extinguish the lamp).
	Bad lamp power supply board	<ol style="list-style-type: none"> 1. Reset the TUV detector via the control panel or ACQUITY UPLC Console. 2. If the problem persists, power-off and power-on again. 3. If the problem persists, call Waters Technical Service (see page 7-3).
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 6-204 .

TUV detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
No sample and reference energy; lamp LED is off, solid red, or flashing red	Lamp burned out	Attempt to reignite the lamp. If it fails to ignite, replace the lamp.
	Lamp extinguished	<ol style="list-style-type: none">1. Ensure the lamp switch is in the “on” position.2. Run the Sample and Reference Energy diagnostic function (see page 3-38).
Peak out of range error at start up	Mobile phase absorbance too high; or air bubbles in the flow cell	<ol style="list-style-type: none">1. Ensure the mobile phase in the flow cell does not absorb above 250 nm.2. Ensure no air bubbles are in the flow cell (see page 7-24).3. Recalibrate the detector.
	Dirty flow cell	Clean the flow cell (see page 6-212).

PDA detector troubleshooting

Power LED

The power LED, on the left-hand side of the detector’s front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Lamp LED

The lamp LED, on the right-hand side of the power LED, indicates the lamp status.

PDA detector lamp LED indications

LED state	Description
Unlit	Indicates the detector lamp is extinguished.
Steady green color	Indicates the detector is operating normally.
Flashing green color	Indicates the detector is initializing or calibrating.
Steady red color	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Flashing red color	Indicates a serious failure within the detector that prevents further operation. Call Waters Technical Service (see page 7-3).

PDA detector troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>).

PDA detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Change in reference spectrum	Mobile phase contains gas or is contaminated	Prepare fresh mobile phase and degas thoroughly.
	Air bubbles trapped in flow cell	<ul style="list-style-type: none"> • Reseat and check alignment of flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>). • Flush the flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>), or apply slight backpressure on the detector waste outlet. • Ensure the backpressure regulator is connected to the detector waste outlet.
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.

PDA detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Detector not responding to ACQUITY UPLC Console	Bad or disconnected cable	Inspect cable connections, tighten connectors, or replace cable.
	Configuration problem	Check Ethernet configuration. For details, see Empower online Help.
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 6-204 .
Shutter failure message	Shutter failed	<ol style="list-style-type: none"> 1. Clear bubbles from the flow cell (see page 7-24). 2. Power-off and then power-on the PDA detector.
Solvent in drain line	Leak from flow cell gasket	Replace the flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>).
	Leak from flow cell inlet and outlet fittings	Check fittings for overtightening or undertightening, and replace fittings if necessary.
Status light blinks and lamp light is off	The detector is running confidence tests	No corrective action required. Wait until tests finish.

PDA detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Status light blinks and lamp light is on	Failed startup diagnostic tests	<ul style="list-style-type: none"> • Reseat and check alignment of flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>). • Flush the flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>).
	Dirty flow cell causing shutter diagnostic test to fail	Flush the flow cell (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>).
	Insufficient energy reaching photodiode array because of air bubble	To prevent air bubbles from forming, check that there is a 30- to 60-cm (1- to 2-foot) length of 0.009-inch (0.23-mm) ID tubing connected to the detector waste outlet.
	Weak lamp	Replace the lamp (see the <i>ACQUITY UPLC Photodiode Array Detector Getting Started Guide</i>).

ELS detector troubleshooting

Power LED

The power LED, on the left-hand side of the detector's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Lamp LED

The lamp LED, on the right-hand side of the power LED, indicates the lamp status.

ELS detector lamp LED indications

LED state	Description
Unlit	Indicates the detector lamp is extinguished.
Steady green color	Indicates the detector is operating normally.
Flashing green color	Indicates the detector is initializing or calibrating.
Steady red color	Indicates a serious error that prevents further operation. You can find information about the error in the ACQUITY UPLC Console.
Flashing red color	Indicates a serious failure within the detector that prevents further operation. Call Waters Technical Service (see page 7-3).

ELS detector troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Cable loose or defective	Inspect line cord connections.
	Open (spent) or defective fuse	Replace fuse (see the <i>ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide</i>).
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 6-204 .

FLR detector troubleshooting

Power LED

The power LED, on the left-hand side of the detector's front panel, indicates when the instrument is powered-on or powered-off. It is steady green when the instrument is working properly.

Lamp LED

The lamp LED, on the right-hand side of the power LED, indicates the lamp status.

Lamp LED indications

LED mode and color	Description
Unlit	Indicates the detector lamp is extinguished.
Constant green	Indicates the detector lamp is ignited.
Flashing green	Indicates the detector is initializing or calibrating.
Flashing red	Indicates that an error stopped the detector. Refer to the console for information regarding the error.
Constant red	Indicates a detector failure that prevents further operation. Power-off the detector, and then power-on. If the LED is still steady red, contact your Waters service representative.

FLR detector troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	1. Inspect line cord connections. 2. Test electrical outlet for power.
	Cable loose or defective	Inspect line cord connections.
	Open (spent) or defective fuse	Replace fuse (see the <i>ACQUITY UPLC Fluorescence Detector Getting Started Guide</i>).

FLR detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Communication problems	Configuration problem	Check Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 6-204 .

Chromatography troubleshooting



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the ACQUITY UPLC system. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.

The table below lists chromatographic symptoms, possible causes, and suggested corrective actions for the ACQUITY UPLC system.

Before you use the table, read the General Troubleshooting section, and follow the basic troubleshooting steps to isolate the cause of the chromatographic symptom.

Chromatography troubleshooting

Symptom	Possible cause	Corrective action
Bandspreading, poor peak shape, or loss of column resolution	Wrong fittings to ACQUITY UPLC column or anywhere in the system.	Use ACQUITY UPLC fittings (Parker-style).
	One or more connections is defective.	Check connections. All tubes must be bottomed out into female connectors.
	The length or diameter of one or more connection tubes is incorrect.	Connecting tube between the sample manager injection valve and column must be ≤ 0.005 inches for UPLC systems. Most connecting tubes are pre-cut with specified diameters and lengths.
	Defective column.	Replace column.
	System not stabilized or chemically equilibrated.	<p>Equilibrate column with a minimum of 10 column volumes of the mobile phase to be used. If running an automated gradient method, ensure you are using sufficient and reproducible equilibration times between injections.</p> <p>Example: For a 2.1×100 mm ACQUITY UPLC BEH column, run about 2.5 mL of solution at 0.5 mL/min for about 5 minutes.</p> <p>See also: <i>ACQUITY UPLC BEH Column Care and Use Instructions.</i></p>

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Bandspreading, poor peak shape, or loss of column resolution (continued)	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes. See “Solvent recommendations” on page C-4.
	Temperature fluctuations.	Set an appropriate column temperature.
	Column is dirty, defective, or contaminated.	<ol style="list-style-type: none"> 1. Flush the column with a neat organic solvent, taking care not to precipitate buffers. 2. If symptom persists, clean the column using the cleaning and regeneration procedures described in <i>Waters ACQUITY UPLC BEH Column Care and Use Instructions</i>. 3. If system persists, replace the column.
Decreased retention times	Incorrect flow rate.	Change flow rate.
	Incorrect solvent composition.	Change composition.
	High column temperature.	Reduce column temperature.
	Incorrect mobile phase.	Use correct mobile phase.
	Column contaminated.	Clean or replace column.
	Incorrect column.	Use correct column.
	Sample diluent is be stronger than the initial mobile phase.	<ul style="list-style-type: none"> • Dilute sample in a weaker solution. • Inject less.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Erratic retention times	Air bubble in pump head.	Prime binary solvent manager.
	Malfunctioning check valves.	Clean or replace inlet check valve cartridges.
	Leaking plunger seals.	Replace seals (see page 6-52).
	Separation chemistry.	Determine the suitability of mobile phase and column.
	Clogged solvent filters.	Replace filters.
	Equilibration time after a gradient is insufficient.	Specify a longer equilibration time.
	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes.
	Temperature fluctuations.	Set an appropriate temperature.
Flat-topped peaks	Sample concentration or injection volume exceeds voltage output of detector.	Decrease sample concentration or injection volume.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Increased retention times	Incorrect flow rate.	Change flow rate.
	Incorrect solvent composition.	Change solvent composition.
	Incorrect mobile phase.	Use correct mobile phase.
	Column contaminated.	Clean or replace column.
	Incorrect column.	Use correct column.
	Fluid leak (causes lower flow rate).	<ul style="list-style-type: none"> • Inspect fittings for leaks. • Perform static decay test.
Increase in system pressure	Column in-line filter is plugged.	Replace the filter in the in-line column filter unit (see page 6-178 and page 6-199).
	Column is plugged.	<ol style="list-style-type: none"> 1. Flush the column with a neat organic solvent, taking care not to precipitate buffers. 2. If symptom persists, clean the column using the cleaning and regeneration procedures described in <i>Waters ACQUITY UPLC BEH Column Care and Use Instructions</i>. 3. If symptom persists, replace the column.
	Tubing is plugged.	Systematically check tubing by making and breaking connections.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Loss of column efficiency	System not stabilized or chemically equilibrated.	<p>Equilibrate column with a minimum of 10 column volumes of the mobile phase to be used. If running an automated gradient method, ensure you are using sufficient and reproducible equilibration times between injections.</p> <p>Example: For a 2.1 × 100 mm ACQUITY UPLC BEH column, run about 2.5 mL of solution at 0.5 mL/min for about 5 minutes.</p> <p>See also: <i>ACQUITY UPLC BEH Column Care and Use Instructions</i>.</p>
	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes.
	Temperature fluctuations.	Set an appropriate temperature.
	Column is dirty, defective, or contaminated.	<ol style="list-style-type: none"> 1. Flush the column with a neat organic solvent, taking care not to precipitate buffers. 2. If symptom persists, clean the column using the cleaning and regeneration procedures described in <i>Waters ACQUITY UPLC BEH Column Care and Use Instructions</i>. 3. If symptom persists, replace the column.
Reproducibility errors	Incorrect chemistry/integration.	Review chemistry and integration.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Reproducibility errors (continued)	Sample management system problem.	Troubleshoot sample management system.
	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes.
	Incorrect volume range for injection method or type.	Change volume range.
Sensitivity loss	Leak in binary solvent manager.	Troubleshoot solvent management system.
	Degraded, contaminated, or improperly prepared sample.	Use fresh sample.
	Column contaminated.	Clean or replace column.
	Loss of column efficiency.	Clean or replace column.

A Safety Advisories

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

Contents

Topic	Page
Warning symbols	A-2
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-12

Warning symbols

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

Task-specific hazard warnings

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

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



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



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



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of needle puncture.)



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.)

Warnings that apply to particular instruments, instrument components, and sample types

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

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



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

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

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



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

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

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



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

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



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

Biohazard warning

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



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

Chemical hazard warning

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



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

Caution symbol

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



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

Warnings that apply to all Waters instruments

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



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

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

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

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

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

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

注意: 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。

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

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



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

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

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

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

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

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



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

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

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

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

警告: 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

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



警告: 当有压力的情况下使用管线时, 小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。

要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀, 大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.

염화 메틸렌(Methylene chloride) 및 디메틸설폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 과열 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ます、その場合、チューブは極めて低い圧力で破裂します。



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

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

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

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

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

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

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

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

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



Warning: To protect against fire, replace fuses with those of the type and rating printed on the panel adjacent to the instrument's fuse cover.



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



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



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



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



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



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



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

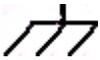
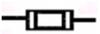


警告: 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols

Electrical symbols

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

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

Handling symbols

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

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

B Specifications

The system specifications outlined in this appendix depend on the conditions in your laboratory. Refer to the *ACQUITY UPLC Site Preparation Guide* or contact Waters Technical Service for more information on specifications.

Contents:

Topic	Page
Binary solvent manager specifications	B-2
Sample manager specifications	B-4
Sample organizer specifications	B-7
HT column heater specifications	B-11
Column manager and column heater/cooler specifications	B-13
30-cm column heater/cooler specifications	B-15
TUV detector specifications	B-17

If your system includes an ELS detector, see the *ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide* for physical and environmental specifications.

If your system includes a PDA detector, see the *ACQUITY UPLC Photodiode Array Detector Getting Started Guide* for physical and environmental specifications.

If your system includes an FLR detector, see the *ACQUITY UPLC Fluorescence Detector Getting Started Guide* for physical and environmental specifications.

If your system includes a mass spectrometer, see the documentation included with it for specifications.

Binary solvent manager specifications

Binary solvent manager environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

Binary solvent manager electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overtoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

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

Binary solvent manager performance specifications

Attribute	Specification
Number of solvents	Up to four, in any combination of two: A1 or A2 and B1 or B2.
Solvent storage	Solvent tray accommodates up to four chromatographic solvents, two sample manager wash solvents, and one binary solvent manager seal wash solvent.
Solvent conditioning	Vacuum degassing (six-channel): one channel per solvent, and two channels for sample manager wash solvents.
Settable flow rate range	0.001 to 2.000 mL/min, in 0.001 mL increments
Compressibility compensation	Automatic and continuous
Effective system delay volume	Less than 120 μ L, independent of system backpressure (with standard 50 μ L mixer installed)
Plunger seal wash	Integral, active, programmable
Gradient profiles	Eleven gradient curves [including linear, step (2), concave (4), and convex (4)]
Wet prime	Automatic
Maximum operating pressure	103,421 kPa (1034 bar, 15,000 psi) up to 1 mL/min, 62,053 kPa (621 bar, 9000 psi) up to 2 mL/min
Composition accuracy	\pm 0.5% absolute (full scale) from 5 to 95% from 0.50 to 2.00 mL/min ^a
Composition precision	0.15% RSD or \pm 0.04 min SD, whichever is greater, based on retention time

Binary solvent manager performance specifications (Continued)

Attribute	Specification
Flow precision	0.075% RSD or ± 0.02 min SD, six replicates, based on retention time or volumetric measures (0.5 to 2.0 mL/min)
Flow accuracy	$\pm 1.0\%$ at 0.5 mL/min, per Waters ACQUITY UPLC AQT/SystemsQT protocol
Primary wetted materials	316 stainless steel, UHMWPE, sapphire, ruby, FEP, PTFE, ETFE, diamond-like coating, PEEK and PEEK alloys, titanium alloys.
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through ACQUITY UPLC console software

a. Contact Waters for the conditions used to derive this attribute.

Sample manager specifications

Sample manager environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

Sample manager electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overvoltage category ^b	II

Sample manager electrical specifications (Continued)

Attribute	Specification
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

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

Sample manager performance specifications

Attribute	Specification
Number of SBS/ANSI sample plates See also: <ul style="list-style-type: none"> • <i>Waters Vials for ACQUITY UPLC Systems</i> (part number 720002449EN) • <i>Sample Vials and Accessories for all ACQUITY UPLC, HPLC, and GC Systems</i> (part number 720001818EN) 	Total of two plates, expandable to up to 22 plates with optional sample organizer. Maximum of 22 microtiter plates, 11 intermediate depth plates, or 8 deep-well (or 2 mL vial) plates: <ul style="list-style-type: none"> • 96 and 384 microtiter plates • 48-position 2 mL vial plates • 48-position 0.65 mL micro-centrifuge tube plates • 24-position 1.5 mL micro-centrifuge tube plates
Maximum sample capacity	768 in two 384-well plates; expandable to up to 8,448 samples with optional sample organizer

Sample manager performance specifications (Continued)

Attribute	Specification
Injection volume range	0.1 to 50 μ L, in 0.1 μ L increments, partial or full loop mode, 10 μ L loop is standard; 1, 2, 5, 20, and 50 μ L loops also available
Sample delivery precision (full loop injection mode)	<0.3% RSD, full loop, standard 10 μ L (default wash/purge conditions) per Waters ACQUITY UPLC AQT/SystemsQT protocol, UV detection
Sample delivery precision (PLNO injection mode)	Less than 1% RSD within 20 to 75% of the loop volume for 1, 2, 5, 10, 20, and 50 μ L loops, UV detection.
Injector linearity	>0.999 coefficient of deviation (from 20 to 75% of the loop volume, partial loop overflow mode (PLNO injection mode) per Waters ACQUITY UPLC AQT/SystemsQT protocol
Sample temperature control	4 to 40 $^{\circ}$ C, programmable in 0.1 $^{\circ}$ C increments [assumes ambient temperature of 21 $^{\circ}$ C (69.8 $^{\circ}$ F)] At an ambient temperature of 21 $^{\circ}$ C (69.8 $^{\circ}$ F) or lower, the sample manager will maintain the temperature of the sample compartment down to 4 $^{\circ}$ C (39.2 $^{\circ}$ F) with a tolerance of $-2/+6$ $^{\circ}$ C ($-3.6/+10.8$ $^{\circ}$ F), when configured with the maximum number of vials and/or plates.
Injection cycle time	<15 sec between multiple injections with "load ahead" enabled, 30 sec with single weak wash, 10 μ L loop, pressure assist mode
Sample probe	XYZZ based needle-in-needle design

Sample manager performance specifications (Continued)

Attribute	Specification
Minimum sample required	5 μ L residual volume at a needle offset of 0, or 22 μ L at the preset 2 mm needle offset
Wash solvents	Two degassed solvents: strong solvent and weak solvent, programmable wash volumes to suit application
Sample carryover	<0.005% or <2.0 nL, whichever is greater
Advanced operations	Loop off-line mode, load ahead
Wetted materials	Titanium Alloy, 316 Stainless Steel, Fluoropolymer, Fluoroelastomer, PPS Alloy, PEEK Alloy, PPS, PEEK, DLC Coating, Gold
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through ACQUITY UPLC console software

Sample organizer specifications

Sample organizer environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

Sample organizer electrical specifications

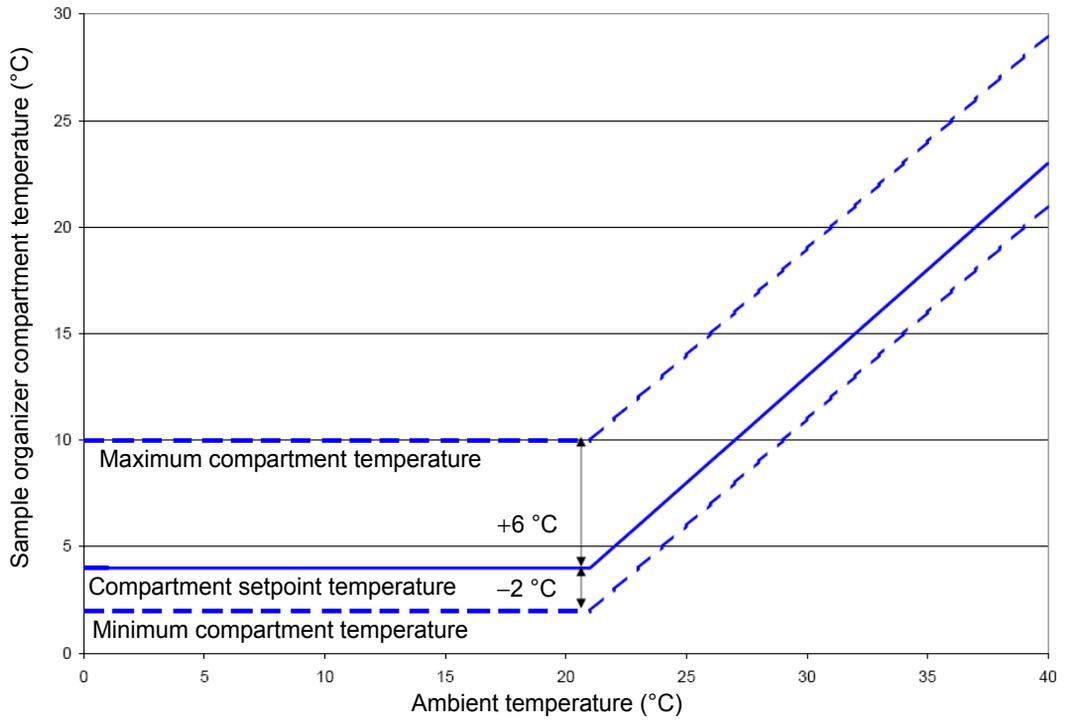
Attribute	Specification
Protection class ^a	Class I
Overtoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

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

Sample organizer performance specifications

Attribute	Specification
Heater / cooler	<p>4 to 40 °C</p> <p>Programmable in 1 °C (1.8 °F) increments.</p> <p>At an ambient temperature of 21 °C (69.8 °F) or lower, the sample organizer will maintain the temperature of the compartment down to 4 °C (39.2 °F) with a tolerance of -2/+6 °C (-3.6/+10.8 °F), as represented in the figure “Sample organizer thermal specification” on page B-10. This performance is independent of the number of samples and shelves in place. For ambient temperatures above 21 °C (69.8 °F), there is a delta of 17 °C (30.6 °F) from actual ambient temperature, as the figure “Sample organizer thermal specification” on page B-10 represents.</p>
<p>Number of SBS/ANSI compliant plates</p> <p>See also:</p> <ul style="list-style-type: none"> • <i>Waters Vials for ACQUITY UPLC Systems</i> (part number 720002449EN) • <i>Sample Vials and Accessories for all ACQUITY UPLC, HPLC, and GC Systems</i> (part number 720001818EN) 	<p>Total of up to 21 standard microtiter plates, or 10 intermediate height plates, or 7 deep-well (or 2-mL vial) plates, and combinations thereof.</p>
Maximum sample capacity	<p>Total of up to 8,064 samples: in twenty one 384-well plates.</p>

Sample organizer thermal specification



HT column heater specifications

HT column heater environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

HT column heater electrical specifications

Attribute	Specification
Protection class ^a	Class I
Oversvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

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

HT column heater performance specifications

Attribute	Specification
Set point temperature range	25° C (77° F) to 90° C (149° F)
Compartment operational temperature range	5° C (9° F) above ambient to 90° C (149° F)
Compartment temperature measurement accuracy	±1.0 °C Temperature measured by an NIST traceable probe located next to the measurement sensor will be within the specification.
Column compartment	Accommodates one column, up to 150 mm length × 4.6 mm ID, pivots out for use with optional mass spectrometer
Column tracking	Usage information tracked via the eCord column chip
Over temperature	Thermal switch shuts (switch opens) off heater at temperatures greater than 115 °C ±4.4 °C. Switch recloses at 100 °C ±3.3 °C
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through ACQUITY UPLC console software

Column manager and column heater/cooler specifications

Column manager and column heater/cooler environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

Column manager and column heater/cooler electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

a. **Protection Class I** – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.

b. **Overvoltage Category II** – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.

c. **Pollution Degree 2** – A measure of pollution on electrical circuits, which can produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.

d. **Moisture Protection** – Normal (IPXO) – IPXO means that *no* Ingress Protection against any type of dripping or sprayed water exists. The X is a placeholder that identifies protection against dust, if applicable.

Column manager and column heater/cooler performance specifications

Attribute	Specification
Set point temperature range	10 to 90 °C
Compartment operational temperature range	15 °C below ambient to 90 °C; to a minimum of 10 °C
Compartment temperature measurement accuracy	±1.0 °C Temperature measured by an NIST traceable probe located next to the measurement sensor will be within the specification.
Column compartment	Column manager: Accommodates up to four columns in parallel, 20 to 150 mm length × 2.1 to 4.6 mm ID, plus either a guard column or an in-line filter (30 mm maximum)
	Column heater/cooler: Accommodates one column; serially connected columns can also be supported (up to four), 20 to 150 mm length × 1.0 to 4.6 mm ID, plus either a guard column or an in-line filter (30 mm maximum)
Valve management (std) ^a	Two injector-style valves; 7 port, 6 position
Over temperature	Thermal switch shuts (switch opens) off heater at temperatures greater than 115 °C ±4.4 °C. Switch recloses at 100 °C ±3.3 °C.
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through ACQUITY UPLC console software

a. Column manager only.

30-cm column heater/cooler specifications

Column manager environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

Column manager electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overtoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
⚠ Line voltages, nominal	Grounded AC

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

30-cm column heater/cooler performance specifications

Attribute	Specification
Set point temperature range	4 to 65 °C
Compartment operational temperature range	The greater of 15 °C below ambient or 4 to 65 °C Column heater: 5 °C above ambient to 65 °C
Column compartment	Accommodates two 300 mm columns with guards, four 150 mm columns without guards, or six 50 mm columns without guards
Compartment temperature measurement accuracy	±0.8 °C (±1.4 °F) between 20 and 50 °C (68 and 122 °F) Temperature measured by an NIST traceable probe located next to the measurement sensor will be within the specification, per Waters ACQUITY UPLC AQT/SystemsQT protocol.
Over temperature	Thermal switch shuts off heater at temperatures greater than 80.0 °C (176 °F) ±10% (temperature measured at thermal switch).

TUV detector specifications

TUV detector environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<50%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing

TUV detector electrical specifications

Attribute	Specification
Protection class ^a	Class I
Oversvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC

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

TUV detector operational specifications^a

Attribute	Specification
Wavelength range	190 to 700 nm
Bandwidth	5 nm
Wavelength accuracy	±1 nm
Wavelength repeatability	±0.25 nm
Baseline noise, single wavelength ^b	7×10^{-6} AU, shunt cell in place, 230 nm, 1.0 second filter time constant, 2 Hz
Baseline noise, dual wavelength ^b	$< 10.0 \times 10^{-5}$ AU, shunt cell in place, 230 nm and 280 nm, 2.0 second filter time constant, 1 Hz
Linearity ^b	≤5% at 2.5 AU, propyl paraben, 257 nm
Drift	5.0×10^{-4} AU/h, shunt cell in place, at 230 nm (after 1-h warm-up) $\Delta T = \pm 2$ °C/h (± 3.6 °F/h)
Maximum data rate	80 Hz
Sensitivity setting range	0.0001 to 4.0000 AUFS
Filter setting range	Single wavelength: 0.0125 to 5.0 seconds, Hamming (sampling rate dependent) Dual wavelength: 0.5 to 5.0 seconds, Hamming (sampling rate dependent)
Unattended operation	Leak sensors, full 96-hour diagnostic data displayed through ACQUITY UPLC console software
Optical Component Specifications	
Lamp source	Deuterium arc lamp
Lamp warranty	2000 hours

TUV detector operational specifications^a (Continued)

Attribute	Specification
Flow cell	10 mm light-guiding flow cell (Analytical)
Wetted materials	316 stainless steel, fused silica, PEEK, PTFE AF2400

a. All specifications derive from instrument performance after a 1-hour warm-up time.

b. ASTM Standard E1657-94.

TUV detector flow cell specifications

Type	Flow range (mL/min)	Volume (nL)	Path length (mm)	Sample tubing internal diameter (µm)		Pressure rating (bar/psi)
				In	Out	
Analytical	0 to 2	500	10	125	125	69/1000
High-sensitivity	0 to 2	2400	25	125	125	69/1000

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

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

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

If your system includes a mass spectrometer, see the documentation included with it for specifications.

C Solvent Considerations



Warning: To avoid chemical hazards, always observe Good Laboratory Practices when operating your system.

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

- ACQUITY UPLC[®] system modules
- ACQUITY UPLC PDA detector
- ACQUITY UPLC PDA e λ detector
- ACQUITY UPLC TUV detector
- ACQUITY UPLC ELS detector
- ACQUITY UPLC FLR detector

Contents

Topic	Page
Introduction	C-2
Solvent recommendations	C-4
Solvent miscibility	C-8
Solvent stabilizers	C-10
Solvent viscosity	C-10
Wavelength selection	C-11

Introduction

Preventing contamination

For information on preventing contamination, refer to *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD, or visit <http://www.waters.com> and click Waters Division > Services & Support.

Clean solvents

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

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

Solvent quality

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

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

Solvent preparation

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

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

Water

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



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

Using buffers

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

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



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

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

Buffered solvents

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

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

See also: For information on preventing contamination, refer to *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD, or visit <http://www.waters.com> and click Waters Division > Services & Support.

Solvent recommendations

General solvent guidelines

Always observe the following general solvent guidelines:

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

Solvents to use

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

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

See [“Contacting Waters technical service”](#) on page 6-6.

Recommended solvents

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

Other solvents

You can use the following solvents. Note, however, that these solvents can shorten instrument life.

- Tetrahydrofuran (THF)
- Hexane

- Hexafluoroisopropanol (HFIP)

Notes:

- 1-4% aqueous solutions of HFIP for oligonucleotide applications.
- HFIP should never be used in weak or strong washes or in seal wash.

See “[ACQUITY UPLC system recommendations](#)” on page C-6.

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

Hexane/THF Compatibility Kit

The ACQUITY UPLC System Hexane/THF Compatibility Kit (part number 205000464) can be installed in ACQUITY UPLC systems with closed waste management. It is designed for users that need to run their systems with hexane or THF at high concentrations and high pressure and is recommended for many ELS detector-based applications where THF is used in the mobile phase, at high concentrations.

Additives/Modifiers

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

Sample diluents

- Acetonitrile
- Acetonitrile/water mixtures
- Dimethylformamide (DMF)

- Dimethyl sulfoxide (DMSO)
- Isopropanol
- Methanol
- Methanol/water mixtures
- Water

Recommendation: Do not use buffers as needle wash.

Cleaning agents

Recommendation: See the cleaning procedures in *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307) on the ACQUITY UPLC System Bookshelf CD, or visit <http://www.waters.com> and Waters Division > Services & Support.

- Phosphoric acid ($\leq 30\%$)
- Sodium hydroxide ($\leq 1M$)

Solvents to avoid

Avoid these solvents:

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

ACQUITY UPLC system recommendations

Contact Waters for recommended system cleaning and flushing procedures. See “[Contacting Waters technical service](#)” on page 6-6.

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

System Bookshelf CD, or visit <http://www.waters.com> and click Waters Division > Services & Support.



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



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

- THF and hexane can be used as the mobile-phase in ACQUITY UPLC systems. However, as with many nonaqueous solvents, they can shorten system and instrument life compared with equipment running typical reversed-phase solvents.
- When using unstabilized THF, ensure that your solvent is freshly prepared. Previously opened bottles contain peroxide contaminants, which cause baseline drift.
- Chloroform, methylene chloride, ethyl acetate, and toluene are generally not recommended for use in ACQUITY UPLC systems. Nevertheless, you can use these solvents in weak dilutions (<10%) as additives, sample diluents, or modifiers.
- Contact your Waters sales representative or local technical support organization to determine whether a specific method is suitable to use with the ACQUITY UPLC system instruments and components.
- When using THF or hexane, install stainless steel tubing, and minimize the use of PEEK components.
- Aqueous solvents must not remain in a shut-down system because they serve as a substrate for microbial colonies. Microbes can clog system filters and capillary lines. To prevent their proliferation, add a small amount (~10%) of an organic solvent such as acetonitrile or methanol.
- Methanesulfonic acid is not recommended for use in ACQUITY UPLC systems.

Binary solvent manager recommendations

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

Sample manager recommendations

- Install the all-stainless-steel needle (part number 205000362) on the sample manager when using THF or hexane.
- Do not use concentrations of THF or hexane greater than 10% as weak-wash solvent.
- Typical organic sample diluents such as dimethylsulfoxide (DMSO) and dimethylformamide (DMF) are supported.

Detector recommendations

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

Solvent miscibility

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

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

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

Solvent miscibility

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

Using miscibility numbers (M-numbers)

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

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

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

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

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

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

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

Solvent stabilizers

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

Solvent viscosity

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

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

Wavelength selection

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

- Common solvents
- Common mixed mobile phases
- Chromophores

UV cutoffs for common solvents

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

UV cutoff wavelengths for common chromatographic solvents

Solvent	UV cutoff (nm)
Acetone	330
Acetonitrile	190
Diethyl amine	275
Ethanol	210
Isopropanol	205
Isopropyl ether	220
Methanol	205
<i>n</i> -Propanol	210
Tetrahydrofuran (THF)	230

Mixed mobile phases

The following table provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different

concentration, you can determine approximate absorbance using Beer's law, because absorbance is proportional to concentration.

Wavelength cutoffs for different mobile phases

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

Mobile phase absorbance

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

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

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

Mobile phase absorbance measured against air or water

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Solvents										
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<0.01	—	—	—	—
Methanol (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	—
Methanol (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	—	—
Isopropanol	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02
Unstablized tetrahydrofuran (THF, fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstablized tetrahydrofuran (THF, old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45
Acids and bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	—
Hydrochloric acid, 0.1%	0.11	0.02	<0.01	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Trifluoroacetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Buffers and salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	—	—	—
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	—	—	—	—	—	—
Ethylenedinitrilo) tetraacetic acid disodium salt (disodium EDTA), 1 mM	0.11	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Morpho- linoethanes ulfonic acid (MES), 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	—	—	—
Potassium phosphate, monobasic (KH ₂ PO ₄), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K ₂ HPO ₄), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	—	—	—
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	—	—	—	—	—
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	—	—	—
Sodium phosphate, 100 mM, pH 6.8	1.99	0.75	0.19	0.06	0.02	0.01	0.01	0.01	0.01	<0.01

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Tris HCl, 20 mM, pH 7.0	1.40	0.77	0.28	0.10	0.04	<0.01	—	—	—	—
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	—	—	—	—
Waters PIC[®] reagents										
PIC A, 1 vial/L	0.67	0.29	0.13	0.05	0.03	0.02	0.02	0.02	0.02	<0.01
PIC B6, 1 vial/L	2.46	2.50	2.42	2.25	1.83	0.63	0.07	<0.01	—	—
PIC B6, low UV, 1 vial/L	0.01	<0.01	—	—	—	—	—	—	—	—
PIC D4, 1 vial/L	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Detergents										
BRIJ 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	—	—	—
3-[(3-chol- amidopropyl)-dimethyl- ammonio]-1- propanesul- fonate) (CHAPS), 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
Sodium dodecyl sulfate (SDS), 0.1%	0.02	0.01	<0.01	—	—	—	—	—	—	—
4-octyl- phen-ol polyethox- ylate (Triton [®] X-100), 0.1%	2.48	2.50	2.43	2.42	2.37	2.37	0.50	0.25	0.67	1.42
Polyoxyeth- ylene sorbitan monolau- rate (Tween [™] 20), 0.1%	0.21	0.14	0.11	0.10	0.09	0.06	0.05	0.04	0.04	0.03

Index

Numerics

- 30-cm column heater/cooler
 - fuses, replacing [6-229](#)
 - overview [1-9](#)
 - plumbing [2-35](#)
 - specifications
 - electrical [B-15](#)
 - environmental [B-15](#)
 - performance [B-16](#)

A

- absorbance values, display of [3-37](#), [4-9](#)
- accumulator check valve, replacing [6-46](#)
- accumulator head
 - removing [6-103](#)
- accumulator head, binary solvent manager
 - reattaching [6-116](#)
- acids [C-13](#)
- acquisition speed [1-11](#)
- ACQUITY UPLC system
 - columns calculator [1-5](#)
 - Console
 - starting from Empower software [4-12](#)
 - starting from MassLynx software [4-14](#)
 - plumbing [2-20](#)
 - refreshing [3-12](#), [4-6](#), [4-8](#)
 - starting up [3-13](#)
 - startup [4-6](#)
- additives [C-5](#)
- air filter
 - binary solvent manager
 - cleaning [6-51](#)
 - replacing [6-52](#)

- sample organizer, cleaning [6-225](#)

- ANSI/SBS [3-24](#), [3-31](#)

- audience and purpose [v](#)

- autozero control [4-10](#)

B

- backpressure regulator [2-23](#)
 - explanation [2-22](#)
 - pictured [2-24](#)
- backpressure test, sample manager [6-159](#)
- bandspreading, preventing [2-21](#)
- baseline noise, troubleshooting [7-5–7-10](#)
- baseline spikes [7-7](#)
- bases [C-13](#)
- binary solvent manager [3-9](#)
 - accumulator check valve, replacing [6-46](#)
 - accumulator head
 - reattaching [6-116](#)
 - removing [6-103](#)
 - accumulator head plunger, replacing [6-114](#)
- air filters
 - cleaning [6-51](#)
 - replacing [6-52](#)
- check valve, replacing [6-19](#)
- cleaning, air filters [6-51](#)
- control panel, using [4-4](#)
- dry prime [3-10](#)
- flow LED [3-4](#), [4-5](#), [7-11](#)
- fuses, replacing [6-121](#)
- head seals
 - reattaching [6-66](#), [6-81](#)
 - removing [6-61](#), [6-76](#), [6-92](#), [6-109](#)

- I/O signal connectors [2-59](#)
- installing [2-10](#)
- maintaining [6-9](#)
- overview [1-5](#)
- plumbing [2-26](#)
- power LED [3-4](#), [7-10](#), [7-17](#)
- preparing [3-7](#)
- primary head
 - reattaching [6-99](#)
 - removing [6-83](#)
- primary head plunger, replacing [6-97](#)
- priming [3-9](#)
- pumped volume warning [6-8](#)
- signal connectors [2-59](#)
- solvent filters, replacing [6-49](#)
- solvent manager head
 - removing [6-53](#), [6-70](#)
- solvent recommendations [C-8](#)
- specifications
 - electrical [B-2](#)
 - environmental [B-2](#)
 - performance [B-3](#)
- troubleshooting [7-10–7-14](#)
- vent valve cartridge, replacing [6-118](#)
- wetted, prime [3-12](#)
- biohazard warning [A-4](#)
- brake, FlexCart [2-7](#), [2-8](#)
- buffered solvents [C-3](#)
- burst warning [A-3](#)

C

- calculator, columns [1-5](#)
- calibrating
 - needle Z-axis [2-71](#), [6-139](#)
 - XYZ mechanism [2-67](#)
- cartridge
 - injection valve, replacing [6-156](#)

- vent valve, replacing [6-118](#)
- caution symbol [A-5](#)
- characterizing
 - needle seal [3-21](#), [3-22](#), [6-140](#)
 - needle volume [3-22](#), [6-141](#)
 - sample loop volume [3-22](#), [6-141](#)
- chart-out parameters [2-61](#)
- check valve, replacing [6-19](#)
- checksum, viewing [6-7](#), [7-5](#)
- chemical hazard warning [A-5](#)
- chemistry technologies [1-12](#)
- chromatogram, gradient performance test [5-11](#)
- chromatography troubleshooting [7-35–7-41](#)
- cleaning
 - air filters
 - binary solvent manager [6-51](#)
 - sample organizer [6-225](#)
 - flow cell [6-212](#)
- cleaning agents [C-6](#)
- clearing bubbles, from flow cell [7-24](#)
- column
 - calculator [1-5](#)
 - compatibility [1-12](#)
 - conditioning [3-39](#)
 - guard, installing [2-55](#)
 - information, see eCord column chip
 - information, viewing [6-7](#)
 - in-line filter, installing [2-43](#), [2-45](#)
 - installing [2-47](#), [2-49](#)
 - installing, mass spectrometer [2-53](#)
 - overview [1-12](#)
 - replacing
 - column in-line unit filter [6-197](#), [6-199](#)
 - filter in column in-line unit [6-178](#)

- stabilizer assembly, replacing
 - 6-177, 6-195
 - stabilizer, installing 2-38, 2-41
 - usage threshold warning 6-9
 - column heater/cooler
 - installing 2-13
 - maintaining 6-180
 - overview 1-9
 - power LED 7-21
 - run LED 7-21
 - specifications
 - electrical B-13
 - environmental B-13
 - performance B-14
 - troubleshooting 7-21–7-23
 - see eCord column chip
 - column manager
 - control panel, using 4-11
 - current temperature 4-11
 - installing 2-12
 - maintaining 6-180
 - overview 1-8
 - plumbing 2-33
 - power LED 7-21
 - run LED 7-21
 - specifications
 - electrical B-13
 - environmental B-13
 - performance B-14
 - switch valve cycles warning 6-9
 - troubleshooting 7-21–7-23
 - see eCord column chip
 - column stabilizer, function 1-8
 - compatibility kit
 - hexane C-5
 - tetrahydrofuran (THF) C-5
 - composition, solvent 4-5
 - conditioning column 3-39
 - configuring
 - Empower software 4-1
 - maintenance warnings 6-8
 - MassLynx software 4-13
 - syringe parameters 6-155
 - connecting
 - eCord column chip 2-55
 - solvent supply 2-55
 - connections
 - electricity source 2-65
 - Ethernet, making 2-57
 - signal, making 2-65
 - Connections Insight request,
 - submitting 7-2
 - contacting Waters Technical Service
 - 6-6
 - contamination, preventing C-2
 - control panel 4-3
 - binary solvent manager 4-4
 - column manager 4-11
 - ELS detector 4-9
 - FLR detector 4-9
 - PDA detector 4-9
 - sample manager 4-6
 - TUV detector 4-9
 - creating
 - instrument method 5-5
 - sample set method 5-9
 - system in Empower software 4-2
 - test methods 5-5
- ## D
- damage, reporting 6-6
 - data systems 1-12
 - defrosting, sample compartment 6-122
 - degasser vent line positioning 2-32
 - detector
 - ELS, overview 1-10
 - FLR, overview 1-11
 - installing 2-13

- lamp life threshold warning [6-8](#)
- PDA, overview [1-10](#)
- solvent recommendations [C-8](#)
- SQ, overview [1-11](#)
- TQ, overview [1-11](#)
- TUV, overview [1-10](#)
- see ELS detector, FLR detector, PDA detector, and TUV detector
- detergents [C-17](#)
- diagnostic tests, startup [3-37](#)
- diluents, sample [C-5](#)
- dirty flow cell [6-212](#)
- drain routing hole [2-14](#)
- draw rates, maximum [6-149](#), [6-150](#)
- drift problems, troubleshooting [7-5–7-10](#)
- dry prime, performing [3-10](#)
- E**
- EC Authorized Representative [vi](#)
- eCord column chip
 - connecting [2-55](#)
 - overview [1-13](#)
- electrical specifications
 - 30-cm column heater/cooler [B-15](#)
 - binary solvent manager [B-2](#)
 - column heater/cooler [B-13](#)
 - column manager [B-13](#)
 - HT column heater [B-11](#)
 - sample manager [B-4](#)
 - sample organizer [B-8](#)
 - TUV detector [B-17](#)
- electrical symbols [A-12](#)
- electricity source, connections [2-65](#)
- ELS detector
 - control panel, using [4-9](#)
 - I/O signal connectors [2-64](#)
 - lamp LED [7-32](#)
 - maintaining [6-1](#), [6-203](#)
 - overview [1-10](#)
 - performing a run [5-8](#)
 - plumbing [2-25](#)
 - power LED [7-32](#)
 - preparing [3-36](#)
 - signal connectors [2-64](#)
 - specifications [B-1](#), [B-19](#)
 - troubleshooting [7-32–7-33](#)
- Empower
 - software
 - logging in [4-1](#)
 - overview [1-12](#)
 - starting [4-1](#)
 - starting ACQUITY UPLC Console from [4-12](#)
 - system test mix, preparing [5-3](#)
 - system test mix
 - running [5-10](#)
- environmental specifications
 - binary solvent manager [B-2](#)
 - column 30-cm column heater/cooler [B-15](#)
 - column heater/cooler [B-13](#)
 - column manager [B-13](#)
 - HT column heater [B-11](#)
 - sample manager [B-4](#)
 - sample organizer [B-7](#)
 - TUV detector [B-17](#)
- equipment guidelines [v](#), [5](#)
- Ethernet connections, making [2-57](#)
- exhaust hood [2-31](#)
- extended puncture needle [3-23](#), [6-132](#)

F

ferrule installation [6-148](#)

filter

binary solvent manager

cleaning [6-51](#)

replacing [6-52](#)

column in-line, replacing [6-178](#),
[6-197](#), [6-199](#)

sample organizer, cleaning [6-225](#)

firmware version, viewing [6-7](#), [7-5](#)

fittings, tightening recommendations
[2-21](#)

flammable solvents [A-3](#)

FlexCart

adjusting height [2-6](#)

brake

locking [2-7](#)

releasing [2-8](#)

components [2-4](#)

height, adjusting [2-6](#)

moving [2-9](#)

overview [1-13](#)

power connections [2-66](#)

flow cell

cleaning [6-212](#)

dirty [6-212](#)

light-guiding, overview [6-210](#)

maintaining [6-210](#)

TUV detector

clearing bubbles [7-24](#)

pictured [2-23](#)

replacing [6-217](#)

specifications [B-19](#)

flow LED [4-5](#)

flow rate

during priming [3-9](#)

total [4-5](#)

FLR detector

control panel, using [4-9](#)

I/O signal connectors [2-64](#)

lamp LED [7-34](#)

maintaining [6-1](#), [6-203](#), [B-1](#)

overview [1-11](#)

plumbing [2-25](#)

power LED [7-33](#)

preparing [3-36](#)

signal connectors [2-64](#)

specifications [B-19](#)

troubleshooting [7-33–7-35](#)

verifying [5-9](#)

flushing, TUV detector flow cell [3-38](#)

full loop mode [3-26](#)

fume hood [2-31](#)

fuses, replacing

30-cm column heater/cooler [6-229](#)

binary solvent manager [6-121](#)

sample manager [6-160](#)

sample organizer [6-227](#)

TUV detector [6-224](#)

G

gradient performance test,

chromatogram [5-1](#), [5-11](#)

H

handling symbols [A-13](#)

hardware, preparing [3-1](#)

head seals, binary solvent manager

reattaching [6-66](#), [6-81](#)

removing [6-61](#), [6-76](#), [6-92](#), [6-109](#)

hexane compatibility kit [C-5](#)

HPLC methods, system considerations
[3-42](#)

HT column heater

current temperature [4-7](#)

installing [2-11](#)

maintaining [6-161](#)

- overview 1-8
- set point 4-7
- specifications
 - electrical B-11
 - environmental B-11
 - performance B-12
- troubleshooting 7-21
- see eCord column chip

I

- I/O signal connectors
 - binary solvent manager 2-59
 - ELS detector 2-64
 - FLR detector 2-64
 - PDA detector 2-64
 - sample manager 2-61
 - TUV detector 2-63
- injection mode
 - full loop 3-26
 - partial loop 3-25
 - partial loop needle overfill 3-25
- injection valve cartridge
 - connecting 2-30
 - replacing 6-156
- injections threshold warning 6-8
- in-line filter, column 2-43, 2-45
- installing
 - binary solvent manager 2-10
 - column 2-47, 2-49
 - mass spectrometer 2-53
 - column heater/cooler 2-13
 - column in-line filter 2-43, 2-45
 - column manager 2-12
 - column stabilizer 2-38, 2-41
 - detector 2-13
 - guard column 2-55
 - HT column heater 2-11
 - lamp, TUV detector 6-223
 - needle assembly 6-135

- sample manager 2-10
- sample organizer 2-9
- shade, sample manager 3-28
- instrument
 - cleaning 6-5
 - information, viewing 6-6, 6-7, 7-4
 - method, creating 5-5
 - selecting 4-2
- intended use v
- ISM classification vi

L

- lamp
 - life threshold warning 6-8
 - turn on/turn off control 4-9
 - TUV detector
 - installing 6-223
 - removing 6-221
 - replacing 6-221
- lamp LED 4-10
- LED
 - binary solvent manager
 - flow 3-4, 7-11
 - power 3-4
 - binary solvent manager, flow 4-5
 - column heater/cooler
 - power 7-21
 - run 7-21
 - column manager
 - power 7-21
 - run 7-21
 - ELS detector
 - lamp 7-32
 - power 7-32
 - FLR detector
 - lamp 7-34
 - power 7-33
 - monitoring 3-3
 - PDA detector

- lamp 7-28
- power 7-27
- sample manager
 - power 7-14
 - run 3-4, 4-7, 4-11, 7-14, 7-17
- TUV detector
 - lamp 3-4, 4-10, 7-24
 - power 7-24
- lights, turning off 4-8
- load ahead mode overview 3-26
- loading sample plates 3-24, 3-31
- logging in
 - Empower software 4-1
 - MassLynx software 4-13

M

- maintaining
 - binary solvent manager 6-9
 - column heater/cooler 6-180
 - column manager 6-180
 - ELS detector 6-1, 6-203
 - flow cell 6-210
 - FLR detector 6-1, 6-203, B-1
 - HT column heater 6-161
 - PDA detector 6-1, 6-203
 - sample manager 6-122
 - system 6-2
 - TUV detector 6-203
- maintenance
 - column manager switching valve
 - cycles warning 6-9
 - column usage threshold warning 6-9
 - considerations 6-7
 - injections threshold warning 6-8
 - lamp life threshold warning 6-8
 - pumped volume warning 6-8
 - safety considerations 6-7
 - schedule 6-2
 - warnings, configuring 6-8

- mass spectrometer
 - preparing 3-36
 - specifications B-1, B-19
- mass spectrometer shock hazard A-4
- MassLynx software
 - logging in 4-13
 - overview 1-12
 - starting 4-13
 - starting ACQUITY UPLC Console
 - from 4-14
- median baseline filter 1-11
- miscibility
 - numbers C-9
 - solvents C-8
- mixed mobile phases, UV cutoff C-11
- mixer outlet, pictured 2-27
- M-numbers C-9
- mobile phase
 - preparing for system verification 5-3
 - viscosity considerations C-10
 - wavelengths C-12
- modifiers C-5
- monitor arm assembly 2-5
- monitoring system instrument LEDs 3-3

N

- needle
 - guide tube, pictured 6-135
 - holder, replacing 6-142
 - installing 6-135
 - removing 6-131
 - seal, characterizing 3-21, 3-22, 6-140
 - volume, characterizing 3-22, 6-141
 - wash, stopping 3-21
- needle wash connections 2-27
- needle Z-axis, calibrating 2-71, 6-139

O

- operational specifications, TUV
 - detector [B-18](#)
- organizer, see [sample organizer](#)
- overview
 - 30-cm column heater/cooler [1-9](#)
 - binary solvent manager [1-5](#)
 - column heater/cooler [1-9](#)
 - column manager [1-8](#)
 - eCord column chip [1-13](#)
 - ELS detector [1-10](#)
 - Empower software [1-12](#)
 - FLR detector [1-11](#)
 - HT column heater [1-8](#)
 - light-guiding flow cell [6-210](#)
 - MassLynx software [1-12](#)
 - PDA detector [1-10](#)
 - sample manager [1-7](#)
 - sample organizer [1-9](#)
 - solvent considerations [C-2](#)
 - SQ detector [1-11](#)
 - TQ detector [1-11](#)
 - TUV detector [1-10](#)

P

- partial loop mode [3-25](#)
- partial loop needle overfill mode [3-25](#)
 - overview [3-26](#)
- PDA detector
 - control panel, using [4-9](#)
 - I/O signal connectors [2-64](#)
 - lamp LED [7-28](#)
 - maintaining [6-1](#), [6-203](#)
 - overview [1-10](#)
 - plumbing [2-25](#)
 - power LED [7-27](#)
 - preparing [3-36](#)
 - signal connectors [2-64](#)
 - specifications [B-1](#), [B-19](#)

- troubleshooting [7-27–7-31](#)
- verifying [5-8](#)
- performance specifications [B-3](#)
 - binary solvent manager [B-3](#)
 - column 30-cm column heater/cooler
 - [B-16](#)
 - column heater/cooler [B-14](#)
 - column manager [B-14](#)
 - HT column heater [B-12](#)
 - sample manager [B-5](#)
 - sample organizer [B-9](#)
- performing a run, ELS detector [5-8](#)
- plates
 - ANSI/SBS [3-24](#), [3-31](#)
 - information, displaying [3-35](#)
- plumbing
 - 30-cm column heater/cooler [2-35](#)
 - binary solvent manager [2-26](#)
 - column manager [2-33](#)
 - ELS detector [2-25](#)
 - FLR detector [2-25](#)
 - PDA detector [2-25](#)
 - sample manager [2-26](#)
 - TUV detector [2-22](#)
- plunger
 - accumulator head, replacing [6-114](#)
 - primary head, replacing [6-97](#)
- positioning sample plate [3-31](#)
- power, removing completely [3-41](#)
- powering on [3-1](#)
- precipitation, preventing [3-40](#), [5-4](#)
- preparing, system test mix
 - Empower software [5-3](#)
- pressure, system [4-5](#)
- primary head
 - removing [6-83](#)
- primary head, binary solvent manager
 - reattaching [6-99](#)
- prime

- A/B solvents control 4-6
- seal wash control 4-6
- syringes control 4-8
- wetted, performing 3-12
- priming
 - binary solvent manager 3-9, 3-10, 3-12
 - sample manager 3-17
 - seal wash 3-7
- pumped volume maintenance warning 6-8
- puncture needle holder, replacing 6-142
- puncture needle, extended 3-23, 6-132
- purpose and audience v

R

- reagents C-16
- reattaching
 - accumulator head, binary solvent manager 6-116
 - head seals, binary solvent manager 6-66, 6-81
 - primary head, binary solvent manager 6-99
- recommendations, system C-6
- recording
 - reference energies 3-38
 - sample energies 3-38
- reference energies, recording 3-38
- refresh system control 4-6, 4-8
- refreshing, ACQUITY UPLC system 3-12, 4-6, 4-8
- regulator, backpressure 2-23
- removing
 - accumulator head 6-103
 - flow cell, TUV detector 6-218
 - head seals, binary solvent manager 6-61, 6-76, 6-92, 6-109
 - lamp, TUV detector 6-221

- needle assembly 6-131
- primary head 6-83
- solvent manager head 6-53, 6-70
- replacement parts 6-5
- replacing
 - accumulator check valve, binary solvent manager 6-46
 - accumulator head plunger, binary solvent manager 6-114
 - air filters, binary solvent manager 6-52
 - check valve, binary solvent manager 6-19
 - column in-line filter unit filter 6-197, 6-199
 - column stabilizer assembly 6-177, 6-195
 - filter in column in-line filter unit 6-178
 - flow cell, TUV detector 6-217
- fuses
 - 30-cm column heater/cooler 6-229
 - binary solvent manager 6-121
 - sample manager 6-160
 - sample organizer 6-227
 - TUV detector 6-224
- injection valve cartridge 6-156
- lamp, TUV detector 6-221
- primary head plunger, binary solvent manager 6-97
- puncture needle holder 6-142
- sample loop 6-145
- sample syringe 6-149
- solvent filters 6-49
- vent valve cartridge 6-118
- wash syringes 6-152

- reset control
 - BSM 4-6

- column manager 4-12
 - SM 4-9
 - TUV detector 4-10
- restrictors 3-32
- routine maintenance 6-2
- run LED 4-7, 4-11, 7-14, 7-17, 7-21
- running
 - HPLC methods 3-42
 - system test mix, Empower 5-10
- S**
- safety
 - advisories A-1
 - considerations, maintenance 6-7
- salts, preventing precipitation 3-40, 5-4
- sample
 - compartment, defrosting 6-122
 - energies, recording 3-38
 - loop, replacing 6-145
 - plates, loading 3-24, 3-31
 - remaining, display 3-36
- sample diluents C-5
- sample loop volume, characterizing 3-22, 6-141
- sample manager
 - backpressure testing 6-159
 - control panel, using 4-6
 - flow explanation 1-7
 - fuses, replacing 6-160
 - I/O signal connectors 2-61
 - information window 3-18
 - injection valve cartridge, replacing 6-156
 - injections threshold warning 6-8
 - installing 2-10
 - lights, turning off 4-8
 - loading sample plates 3-24
 - maintaining 6-122

- needle
 - assembly
 - installing 6-135
 - removing 6-131
 - seal, characterizing 3-21, 3-22, 6-140
 - volume, characterizing 3-22, 6-141
 - washing 3-20
 - Z-axis, calibrating 2-71, 6-139
- overview 1-7
- plumbing 2-26
- power LED 7-14
- preparing 3-15
- priming 3-17
- puncture needle holder, replacing 6-142
- resetting 4-9
- run LED 3-4, 4-7, 4-11, 7-14, 7-17
- sample compartment
 - current temperature 4-7
 - defrosting 6-122
 - set point 4-7
- sample loop volume, characterizing 3-22, 6-141
- sample loop, replacing 6-145
- sample syringe, replacing 6-149
- shade, installing 3-28
- signal connectors 2-61
- solvent recommendations C-8
- specifications
 - electrical B-4
 - environmental B-4
 - performance B-5
- status 4-7
- syringe configuration parameters, modifying 6-155
- troubleshooting 7-14–7-17
- turning lights off 4-8

wash syringes, replacing 6-152
 washing needle 3-19
 XYZ mechanism, calibrating 2-67
 sample organizer
 air filters, cleaning 6-225
 displaying plate information 3-35
 fuses, replacing 6-227
 information window 3-36
 initiating communications to 3-30
 installing 2-9
 loading sample plates 3-31
 overview 1-9
 preparing 3-30
 specifications
 electrical B-8
 environmental B-7
 performance B-9
 troubleshooting 7-17–7-20
 sample plates, loading 3-24, 3-31
 sample set method, creating 5-9
 sample syringe, replacing 6-149
 sampling rate recommendation 1-4
 scan speed 1-11
 seal wash prime, performing 3-7
 selecting, system instruments 4-2, 4-13
 serial numbers
 instrument 7-4
 serial numbers, column 6-7
 serial numbers, instrument 6-6, 6-7
 shelf spacing 3-32
 shutting down
 for more than 24 hours 3-41
 less than 24 hours 3-40
 signal cable, attaching 2-65
 signal connections, making 2-65
 solvent
 filters, replacing 6-49
 supply, connecting 2-55
 tray 2-55
 solvent manager head
 removing 6-53, 6-70
 solvent manager, see binary solvent manager
 solvents
 buffered C-3
 changing 5-4
 clean C-2
 composition 4-5
 miscibility C-8
 other C-4
 preparation C-2
 quality guidelines C-2
 recommendations
 binary solvent manager C-8
 detector C-8
 sample manager C-8
 recommended C-4
 stabilizers C-10
 strong wash 3-15
 tetrahydrofuran C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13
 THF C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13
 to avoid C-6
 UV cutoff C-11
 viscosity considerations C-10
 water C-3
 weak wash 3-15
 spare parts 6-5
 specifications
 electrical B-2, B-4, B-8, B-11, B-13, B-15, B-17
 ELS detector B-1, B-19
 environmental B-2, B-4, B-7, B-11, B-13, B-15, B-17
 FLR detector B-19
 mass spectrometer B-1, B-19
 operational B-18

- PDA detector [B-1](#), [B-19](#)
- performance [B-3](#), [B-5](#), [B-9](#), [B-12](#), [B-14](#), [B-16](#)
- speed, acquisition [1-11](#)
- SQ detector
 - overview [1-11](#)
 - verifying [5-9](#)
- stabilizer function [1-8](#)
- stabilizer, column [2-38](#), [2-41](#)
- stabilizers, solvent [C-10](#)
- start up [3-13](#)
- starting
 - ACQUITY UPLC Console
 - from Empower software [4-12](#)
 - from MassLynx software [4-14](#)
 - Empower software [4-1](#)
 - MassLynx software [4-13](#)
 - TUV detector [3-37](#)
- starting up, ACQUITY UPLC system [3-13](#)
- startup control [4-6](#)
- startup diagnostic tests [3-37](#)
- startup tests [3-3](#)
- startup, ACQUITY UPLC system [4-6](#)
- stop flow [4-5](#)
- stopping, needle wash [3-21](#)
- strong needle wash connection [2-27](#)
- symbols
 - caution [A-5](#)
 - electrical [A-12](#)
 - handling [A-13](#)
 - warning [A-2](#)
- syringe
 - configuration parameters,
 - modifying [6-155](#)
 - draw rates, maximum [6-149](#), [6-150](#)
 - priming binary solvent manager
 - with [3-10](#)
 - sample, replacing [6-149](#)
 - wash, replacing [6-152](#)
- Sys Prep [4-6](#), [4-8](#)
- system
 - powering on [3-1](#)
 - recommendations [C-6](#)
 - setup [2-2](#)
 - shutting down [3-40](#), [3-41](#)
 - test mix, Empower [5-10](#)
- system acid cleansing flush,
 - performing [6-215](#)
- system instruments
 - selecting
 - from Empower software [4-2](#)
 - from MassLynx software [4-13](#)
- system pressure [4-5](#)
- system verification [5-2](#)

T

- teach block [2-67](#)
- test methods, creating [5-5](#)
- tests, startup [3-3](#)
- tetrahydrofuran [C-4](#), [C-5](#), [C-6](#), [C-7](#), [C-8](#), [C-9](#), [C-10](#), [C-11](#), [C-13](#)
 - compatibility kit [C-5](#)
- THF [C-4](#), [C-5](#), [C-6](#), [C-7](#), [C-8](#), [C-9](#), [C-10](#), [C-11](#), [C-13](#)
 - compatibility kit [C-5](#)
- tightening recommendations, fittings [2-21](#)
- total flow rate [4-5](#)
- TQ detector
 - overview [1-11](#)
 - verifying [5-9](#)
- troubleshooting
 - baseline noise [7-5–7-10](#)
 - binary solvent manager [7-10–7-14](#)
 - chromatography [7-35–7-41](#)
 - column heater/cooler [7-21–7-23](#)
 - column manager [7-21–7-23](#)

- drift problems 7-5–7-10
- ELS detector 7-32–7-33
- FLR detector 7-33–7-35
- HT column heater 7-21
- PDA detector 7-27–7-31
- sample manager 7-14–7-17
- sample organizer 7-17–7-20
- TUV detector 7-24–7-27
- turn lights off control 4-8
- TUV detector
 - control panel, using 4-9
 - flow cell
 - clearing bubbles 7-24
 - replacing 6-217
 - fuses, replacing 6-224
 - I/O signal connectors 2-63
 - information window 3-38
 - lamp
 - cooling time 6-222
 - installing 6-223
 - LED 3-4, 4-10, 7-24
 - life threshold warning 6-8
 - removing 6-221
 - replacing 6-221
 - turn on/turn off control 4-9
 - maintaining 6-203
 - overview 1-10
 - plumbing 2-22
 - power LED 7-24
 - signal connectors 2-63
 - specifications
 - electrical B-17
 - environmental B-17
 - flow cell B-19
 - operational B-18
 - starting 3-37
 - troubleshooting 7-24–7-27
 - verifying 3-38

U

- UPLC system, guidelines 1-4
- UV cutoff values C-11

V

- vent valve cartridge, replacing 6-118
- verifying
 - FLR detector 5-9
 - PDA detector 5-8
 - SQ detector 5-9
 - system 5-2
 - TQ detector 5-9
 - TUV detector 3-38
- version information, software and firmware 7-4
- viewing
 - column information 6-7
 - instrument information 6-6, 6-7, 7-4
 - serial numbers 6-6, 6-7, 7-4
- viscosity considerations C-10

W

- warning symbols A-2, A-5
- warnings
 - column manager switch valve
 - cycles 6-9
 - column usage 6-9
 - configuring 6-8
 - detector lamp life 6-8
 - injections threshold 6-8
 - pumped volume maintenance 6-8
- wash needle control 4-8
- wash solvents, selecting 3-15
- wash syringes, replacing 6-152
- washing, sample manager needle 3-20
- waste line positioning 2-32
- water, as solvent C-3
- Waters Technical Service, contacting 6-6

wavelength
 cutoffs [C-11](#)
 display [4-9](#)
 mobile phase absorbances [C-12](#)
 selection [C-11](#)
weak needle wash connection [2-27](#)

X

XYZ mechanism, calibrating [2-67](#)