Waters 2535/2545/2555 Quaternary Gradient Module

Operator's Guide

Revision B

Waters

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Waters contact information

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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.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Considerations specific to the quaternary gradient module

High voltage hazard

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

Safety advisories

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

Operating this instrument

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

Applicable symbols

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

Audience and purpose

This guide is intended for personnel who install, operate, and maintain quaternary gradient modules.

Intended use of the quaternary gradient module

Waters designed the quaternary gradient module to be used as a research tool in purification systems. It is not for use in diagnostic procedures.

ISM classification

ISM Classification: ISM Group 1 Class B

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

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1 Setting up the Quaternary Gradient Module

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Overview

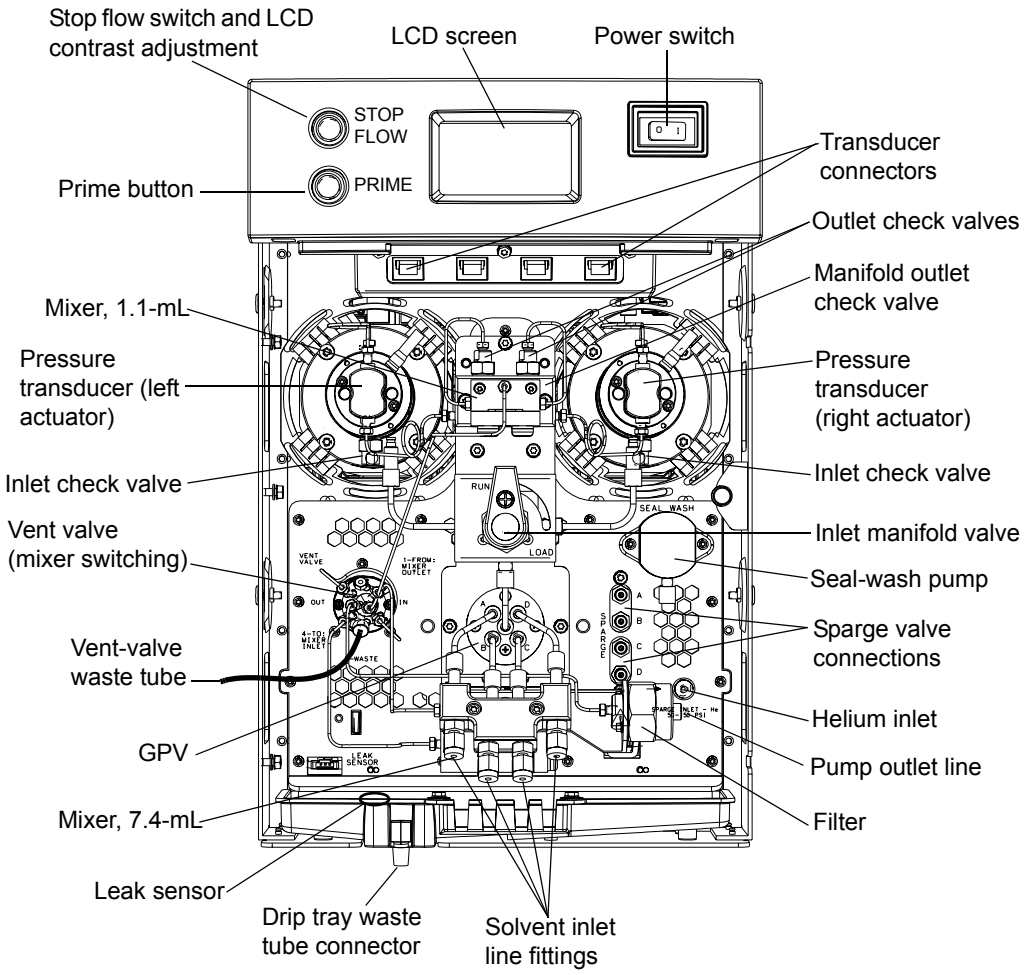
The Waters® Quaternary Gradient Module (QGM) is a pump that delivers solvents from their reservoirs to the HPLC system. Three QGM models (2535, 2545, and 2555) can operate over the flow ranges shown in the table below.

QGM operating ranges

Model	Maximum flow	Pressure
2535 QGM	50 mL/min	41,400 kPa (414 bar, 6000 psi) up to 50 mL/min.
2545 QGM	150 mL/min	41,400 kPa (414 bar, 6000 psi) up to 100 mL/min, linear roll-off to 34,500 kPa (345 bar, 5000 psi), at 150 mL/min.
2555 QGM	300 mL/min	20,700 kPa (207 bar, 3000 psi) up to 200 mL/min, linear roll-off to 17,200 kPa (172 bar, 2500 psi), at 300 mL/min.

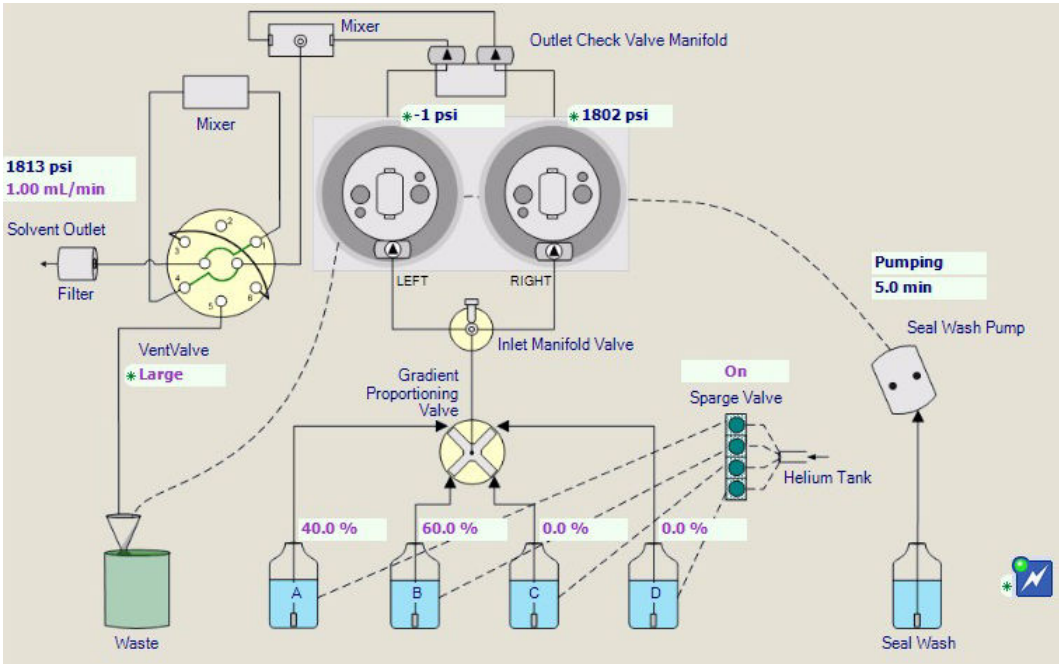
2535 QGM major components

The following diagram shows the 2535 QGM's major components.



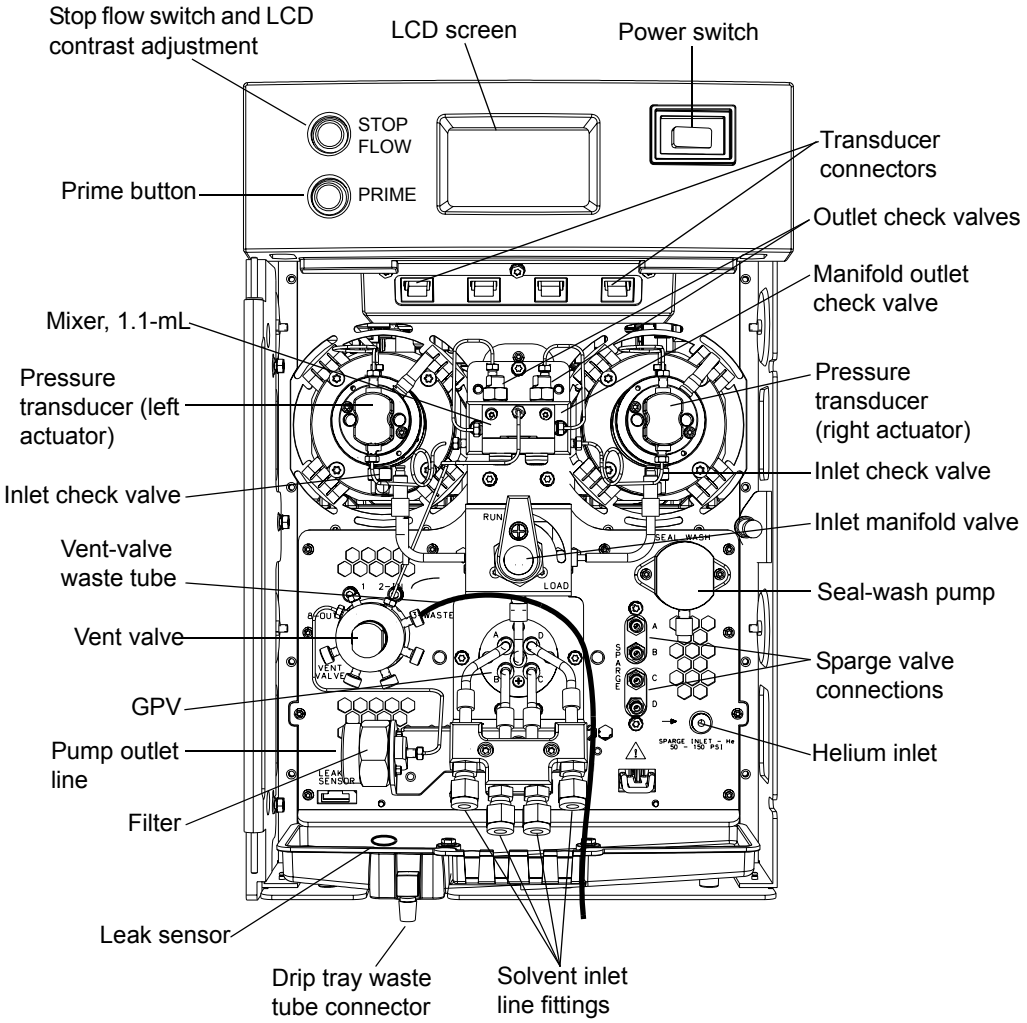
2535 QGM flow

The following screen shows the 2535 QGM's flow.



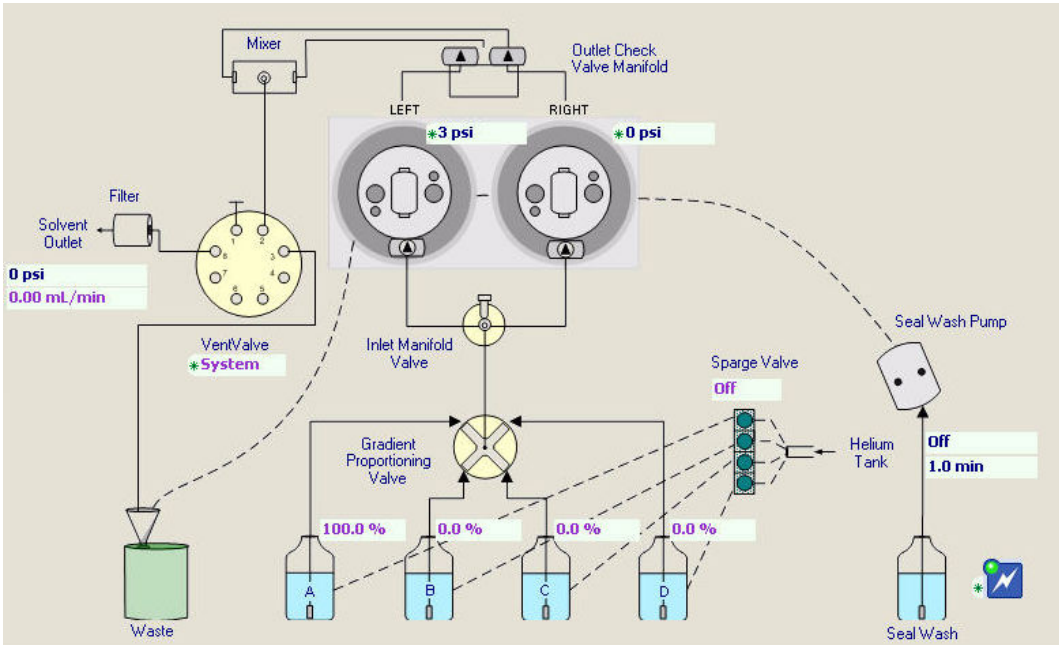
2545 QGM major components

The following diagram shows the 2545 QGM's major components.



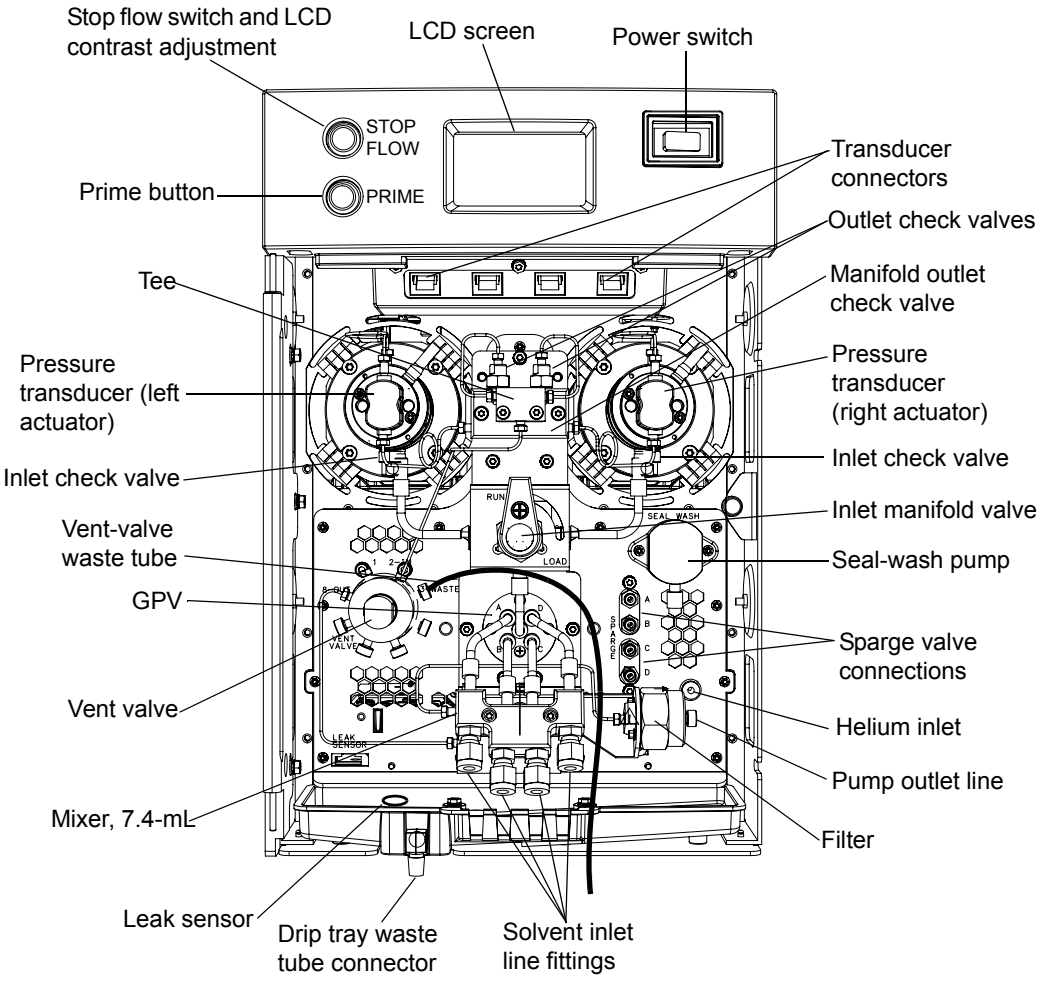
2545 QGM flow

The following screen shows the 2545 QGM's flow.



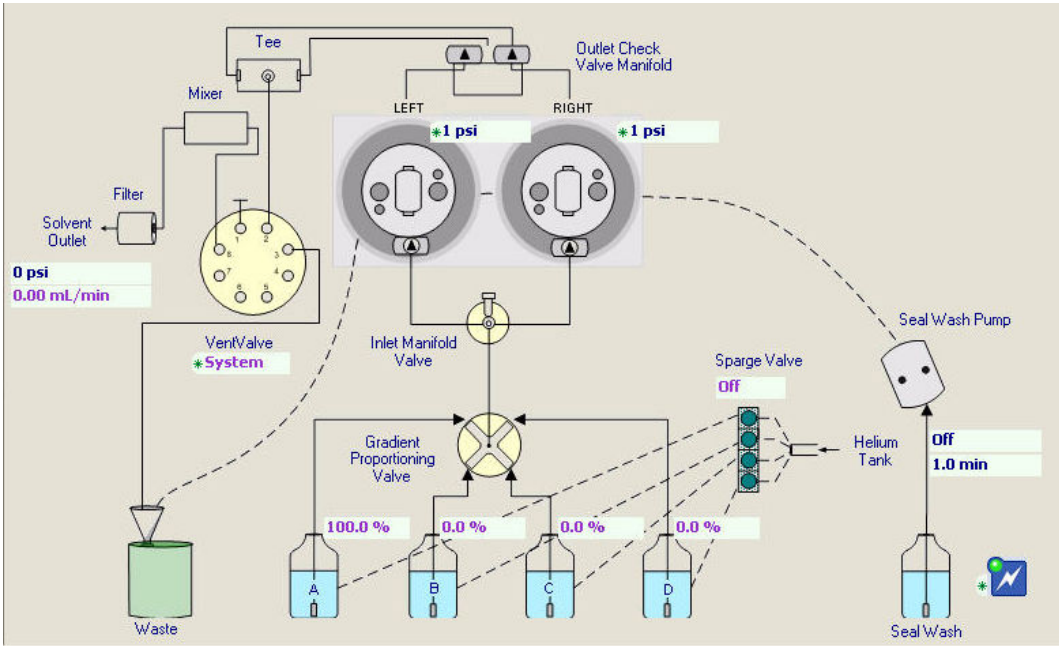
2555 QGM major components

The following diagram shows the 2555 QGM's major components.



2555 QGM flow

The following screen shows the 2555 QGM's flow.



QGM major components

Component	Description	2535	2545	2555
Drip tray waste tube connector	Connects the waste tube to the leak management system.	X	X	X
Filter	Filters the solvent before it goes to the pump outlet.	X	X	X
GPV	A gradient proportioning valve that mixes the solvent to create the gradient.	X	X	X
Helium inlet	Inlet for the helium (sparge gas) used to reduce the total dissolved gas in the eluent reservoirs and maintain that condition during operation. (See page 1-23.)	X	X	X

QGM major components (Continued)

Component	Description	2535	2545	2555
Inlet check valve	The check valve located at the bottom of the pump heads. (See page 3-36.)	X	X	X
Inlet manifold valve	A valve that switches between normal gradient solvent flow (Run mode) and large-sample loading (Load mode).	X	X	X
LCD screen	A liquid crystal display screen that displays current flow rate, pressure, composition, and run time. (See page 2-2.)	X	X	X
Leak sensor	Continuously monitors the QGM for leaks and stops the system flow when its optical sensor detects about 3.0 mL of accumulated, leaked liquid in its surrounding reservoir. (See page 3-4.)	X	X	X
Manifold outlet check valve	Manifold that houses the outlet check valves.	X	X	X
Mixer, gradient swirl, 1.1-mL	A component that mixes the solvents downstream of pumps, improving compositional ripple.	X	X	
Mixer, 7.4-mL	A component that mixes the solvents downstream of pumps, improving compositional ripple. **Note:** For the 2535 QGM, the 7.4 mL mixer is in line only when the QGM is in the large flow scale state.	X		X
Tee	A tee that connects the left pump head and right pump head.			X
Outlet check valves	The check valves located on the outlet check valve blocks. (See page 3-39.)	X	X	X

QGM major components (Continued)

Component	Description	2535	2545	2555
Power switch	A switch that powers-on and powers-off the QGM. (See page 2-2.)	X	X	X
Pressure transducer (left actuator)	A transducer that monitors the head pressure of the right-side pump.	X	X	X
Pressure transducer (right actuator)	A transducer that monitors the head pressure of the left-side pump.	X	X	X
Prime button	A button that primes the QGM and prepares it for use. When pressed, the prime button defaults to the previous Prime dialog box selections.	X	X	X
Pump outlet line	The tube that connects the outlet of the pump to the rest of the chromatographic system.	X	X	X
Seal-wash pump	The pump that circulates solvent to keep the actuator's high pressure seals free of contaminants.	X	X	X
Seal-wash solvent supply tube	The tube that supplies solvent to the plunger wash system.	X	X	X
Solvent inlet line fittings	The fittings that the solvent inlet lines are connected to.	X	X	X
Sparge valve connections	The four connections (A, B, C, and D) for the helium gas lines that go to the solvent reservoirs. (See page 1-25.)	X	X	X
Stop flow switch and LCD contrast adjustment	Immediately stops flow from the QGM. Holding the button in adjusts the contrast of the LCD screen. (See page 2-2.)	X	X	X

QGM major components (Continued)

Component	Description	2535	2545	2555
Transducer connectors	The electrical connections for the pressure transducers located on the front of the actuators.	X	X	X
Vent valve	A valve that automatically switches to waste during priming.		X	X
Vent valve (mixer switching)	A valve that automatically switches between large flow scale, small flow scale, and waste.	X		
Vent-valve waste tube	The tube that connects the pump to a waste container used during pump priming. (See page 1-20.)	X	X	X

Before you begin

Requirement: To install the QGM, you must know how to set up and operate laboratory instruments and computer-controlled devices and how to handle solvents. Refer to the *Quaternary Gradient Module Site Preparation Guide,* or contact Waters Technical Service for more information.

Before installing the QGM, 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.

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.

Customers in the USA and Canada can report damage and discrepancies to Waters Technical Service (800 252-4752). Others can phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA). Alternatively, they can visit www.waters.com.

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

Installing the QGM

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

Warning: Risk of fire. 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 QGM.

When installing the QGM, place it on a level surface. Doing so ensures proper functioning of the drip management system and drain tube, to which you can connect a waste reservoir that diverts solvent leaks from the pump. Align the front of the QGM with that of the workbench.

System configurations must meet Waters' safety and performance standards, which prescribe that no more than two accessory modules be stacked atop the QGM.

Warning: Collapse hazard. Do not configure systems in which any one Waters "base" module, such as the QGM, physically supports more than two vertically oriented "accessory" Waters modules, such as 515 pumps, photodiode array detectors, evaporative light-scattering detectors, or dual-wavelength absorbance detectors.

Managing solvent and waste reservoirs

Warning: Explosion hazard. Ensure all containers, columns, and instruments are properly grounded.

Warning: Electric shock hazard. To avoid electrostatic discharge from the waste container, be sure to connect the stainless-steel tubing included in the startup kit to the vent-valve waste, and route it to the waste container.

Warning: To avoid spills,

- empty the waste container at regular intervals.
- ensure the waste container capacity is adequate for the volume of system solvent flow.

Requirements:

- Solvent inlet tubing should not exceed 2.3 m (7.5 feet).
- For best performance, solvent containers should be placed at the same level of the pump or higher.

Installing the detector drip tray

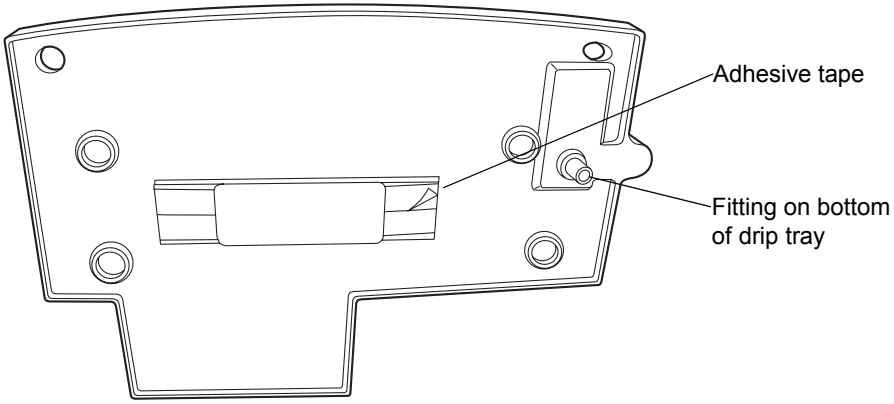
When installing a detector atop the QGM, you must first install the detector's drip tray to route spills to a waste container.

Required materials

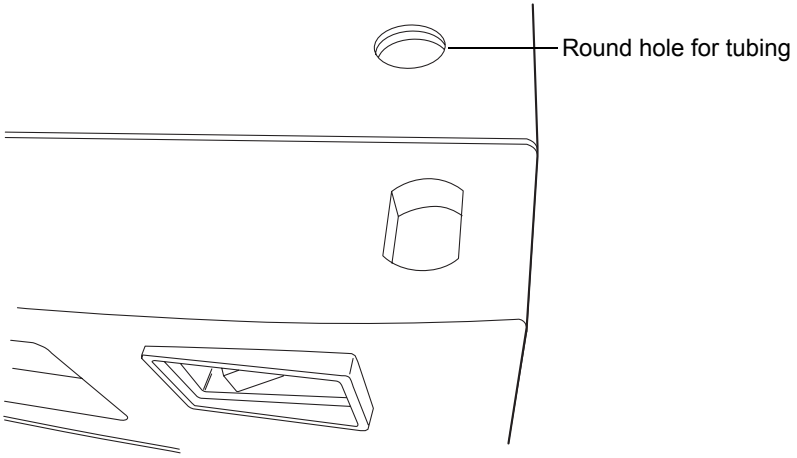
- Detector drip tray (startup kit)
- Tygon® tubing (startup kit)

To install the detector drip tray

1. Insert the Tygon tubing onto the fitting on the bottom of the detector drip tray.

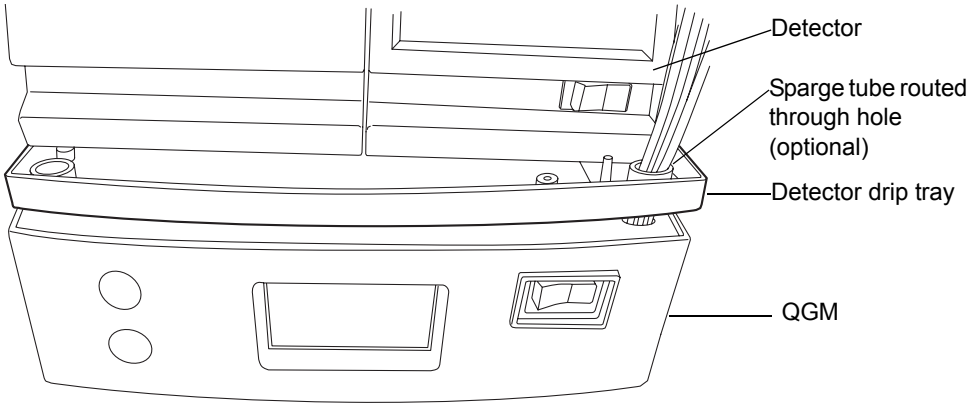


2. Peel the adhesive tape, removing it from the bottom of the detector drip tray.
3. Route the Tygon tubing through the round hole, at the top, right-hand side of the QGM, to the drip tray.



4. Cut the Tygon tubing to an appropriate length.

5. Place the drip tray on top of the QGM, along its front.



Making plumbing connections

Caution: Inspect all fittings and tighten them if necessary. Fittings can loosen during shipment, particularly if the module has undergone temperature extremes.

Installing solvent inlet tubes

Warning: Explosion hazard. Ensure all containers, columns, and instruments are properly grounded.

Warning: To avoid spills,

- empty the waste container at regular intervals.
- ensure the waste container capacity is adequate for the volume of system solvent flow.

Caution:

- To avoid adversely affecting the module's performance, do not allow solvent inlet tubing to exceed 2.3 m (7.5 feet).
- To avoid adversely affecting the module's performance at low flow rates, place solvent containers at the same level of the module or higher.
- Wear clean, chemical-resistant, powder-free gloves when handling the solvent filter. Skin oils can contaminate the solvent filter.

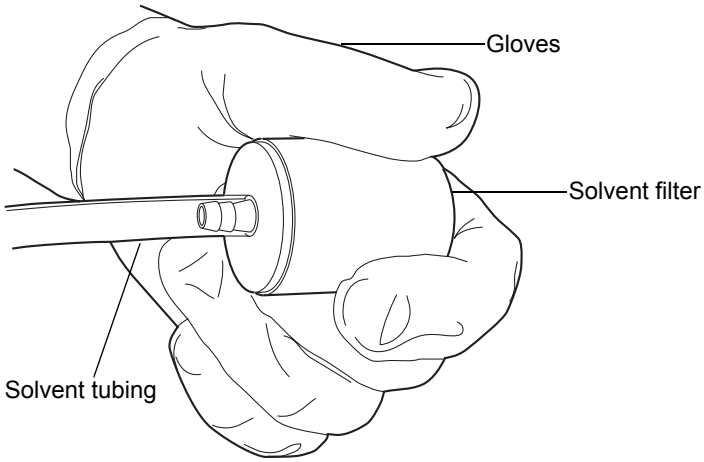
Requirement: You must comply with local regulations for solvent and waste management.

Required materials

- Gloves: clean, powder-free, chemical-resistant
- Solvent inlet tubing assembly
- Wrench, 7/16-inch (for 2535 QGM)
- Wrench, 9/16-inch (for 2545 and 2555 QGM)

To make the initial plumbing connections

1. Install the solvent filters on the ends of the solvent inlet tubing as shown below.



Warning: To avoid harmful vapors, position solvent and waste containers in a safe place, such as in a fume hood or under a counter vented to the outside.

Caution:

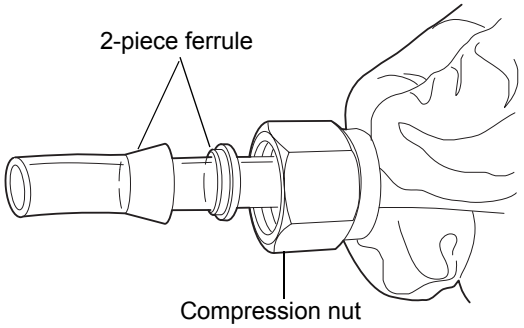
- Do not use pressurized solvent containers with the QGM. Pressures above 34 kPa (0.34 bar, 5 psi) can force solvent through the internal components of the gradient proportioning valve, possibly causing flow when it is unexpected (that is, in zero-flow conditions) and inaccurate gradient composition.
- Do not reuse the solvent inlet tube ferrule more than two times. Doing so can result in leakage. Cut the end of the tube squarely, and use a new ferrule. The nut can be reused.

2. Insert the filter ends of the solvent tubes into the appropriate solvent containers.
3. Open the front panel door of the QGM.
4. Loosen the solvent inlet fitting on the manifold by hand.

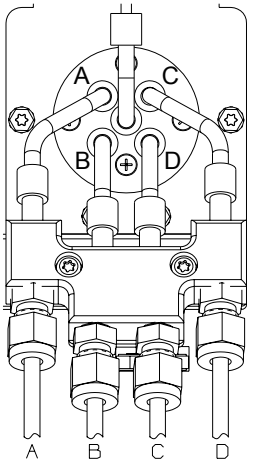
   Recommendation: Shorten the length of the solvent tubing to minimize pressure drop. Ensure the ends of the tubing are squarely cut.
5. Verify that the tubing is placed in the appropriate fittings.
6. Slide the tubing through the compression nut, and tighten the nut using the 9/16-inch wrench (2545 and 2555 QGM) or the 7/16-inch wrench (2535 QGM).
7. Tighten the fitting 3/4-turn beyond finger-tight.

   Requirement: Tighten fittings in the order B, C, A, and D.

Tubing in compression nut



Solvent manifold fittings



8. Repeat step 4 through step 7 for the remaining solvent tubes.

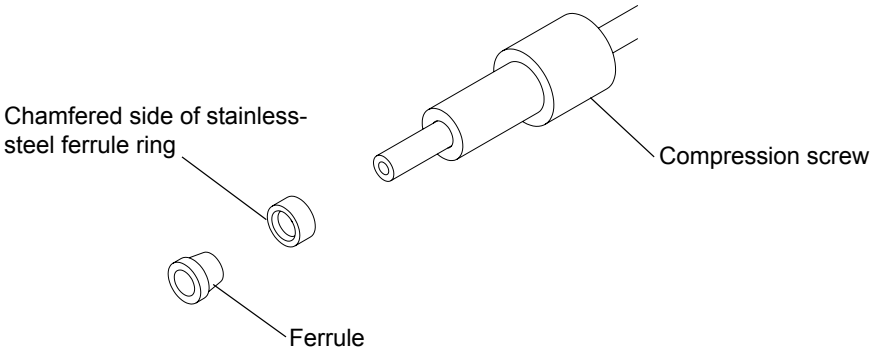
   You can shorten the solvent inlet tubes to accommodate the solvent container locations.

Installing seal-wash solvent inlet tubes

The seal-wash solvent inlet tube uses a PEEK™ compression screw and 2-piece ferrule. Be careful not to lose those parts when working with the tubes.

Note: The chamfered side of the stainless-steel ferrule ring is pressed onto a Tefzel® ferrule.

Compression screw and 2-piece ferrule



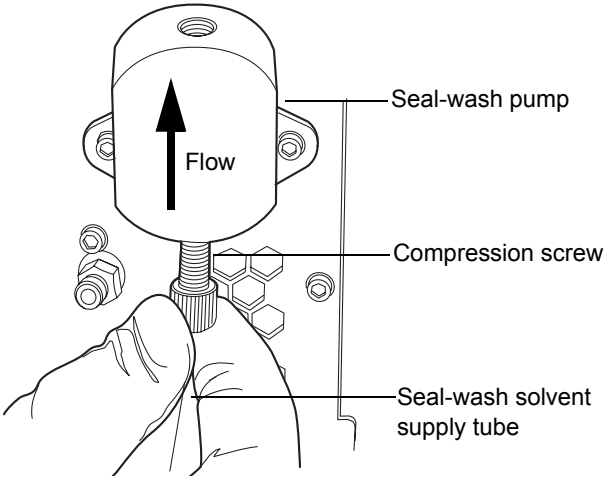
Required material

Seal-wash inlet tube assembly

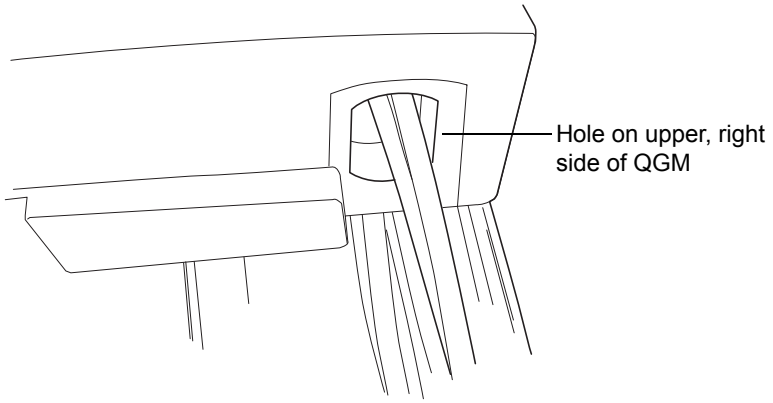
To install the seal-wash solvent supply tubes

1. Insert the seal-wash solvent supply tube into the bottom port of the seal-wash pump.

   Requirement: Press the tube into the pump before tightening the compression screw to set the ferrule.



2. Route the seal-wash supply tube through the hole at the upper, right-hand side of the QGM.



Installing the vent-valve waste tube

Required materials

- Open-end wrench, 1/4-inch
- Waste tube assembly (stainless-steel tube, gold compression screw)

To install the vent-valve waste tube

1. Install the waste tube onto the front panel's vent-valve port, "Waste".
   - Do not use PEEK tubing for the vent-valve waste tube.
   - Use only 0.40-inch ID tubing.

   Warning: To avoid releasing solvent vapors into the room, route the vent-valve waste tube to a waste container located in a fume hood or other suitable exhaust system.

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

2. Route the free end of the tube to the waste container.

Installing the system outlet tube

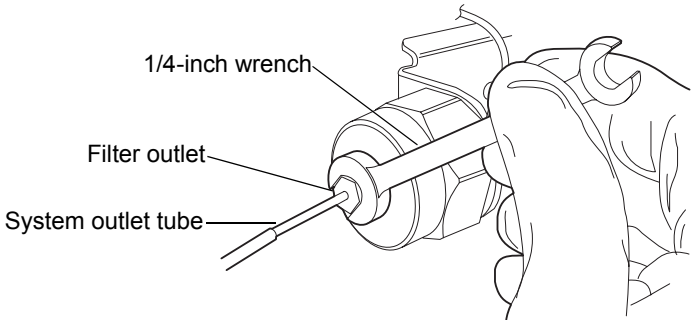
Required materials

- System outlet tube assembly
- Wrench, 1/4-inch

To install the system outlet tube

1. Insert the system outlet tube assembly into the filter outlet, and tighten the tube's compression nut using the 1/4-inch wrench.

QGM outlet tube (left side orientation shown)



2. Connect the system outlet tube to the next in-line device.

Installing the drip tray waste line

Required material

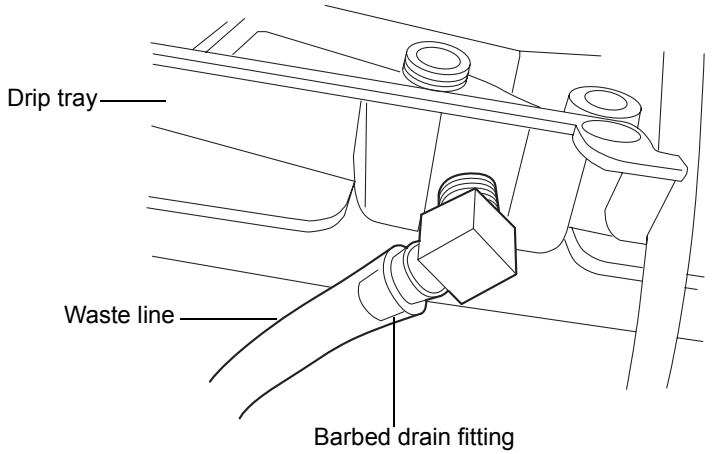
Drip tray waste line

To install the drip tray waste line

Caution:

- To avoid damaging the drip tray, support the barbed drain fitting while you slide the waste line over it.
- To avoid damaging the barbed drain fitting, ensure that the front of the QGM aligns with the front of the workbench.

1. Support the barbed drain fitting with your hand, slide the waste line over the fitting, and then route it to a suitable waste container.



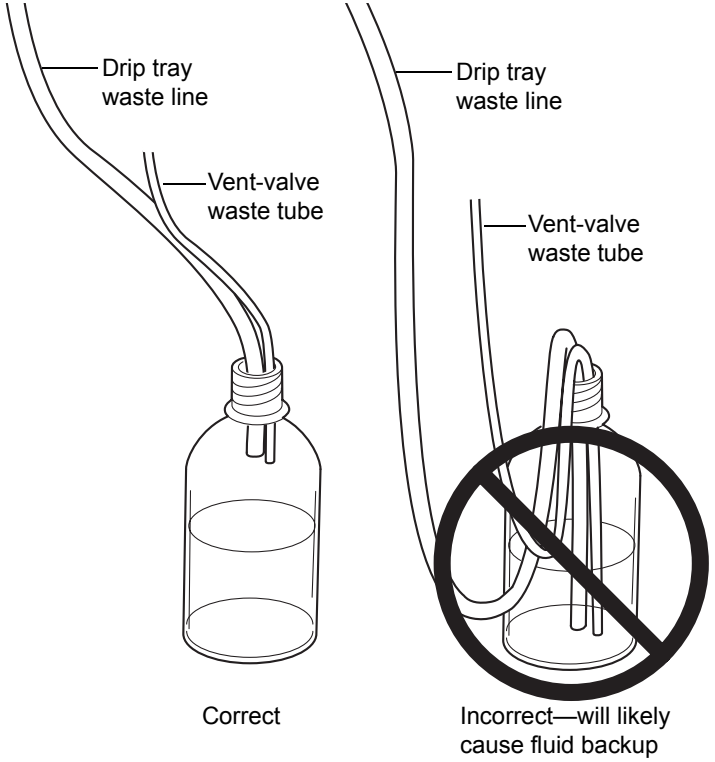
Warning: To avoid spills, empty the waste container at regular intervals.

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

- Place the waste container below the module.
- Ensure that the drip tray waste line and vent-valve waste tube do not crimp or bend, impeding flow to the waste container.
- Ensure the exit of the drip tray waste line is not immersed in waste solvent. If necessary, shorten the line so that no portion of it drops below the top of the waste container (see next figure).

2. Route the drip tray waste line to a suitable waste container.

Correct positioning of drip tray waste line and vent-valve waste tube



Tip: The vent-valve waste tube can be immersed in waste solvent.

Making the sparge system connections

Caution: To avoid damaging the sparge valve assembly

- do not place sparge lines in pressurized solvent containers.
- do not use the sparge assembly to pressurize solvent containers.
- adequately vent solvent containers.

System sparging overview

Helium sparging reduces the total dissolved gas in the eluent reservoirs and maintains that condition during operation. Minimizing dissolved gases decreases the gas that can be released when different mobile phases are mixed in the gradient proportioning valve. Use an ultra-pure, carrier (UPC) grade of helium to prevent eluent contamination.

A diffuser filter disperses the helium sparge gas into small bubbles, which are introduced into the eluent. The bubbles increase the effectiveness of sparging by increasing the surface area of eluent exposed to helium. A reduction in total dissolved gas occurs as the dissolved gases equilibrate with the helium at the gas-liquid interface of the bubbles, the displaced gases bubbling to the surface and being expelled through the bottle vent.

Helium specifications

The minimum specifications for helium sparge gas are listed below. The tank or house supply source must be independently regulated between 350 and 1030 kPa (3.5 and 10.3 bar, 50 and 150 psi) for connection to the sparge inlet.

These are required specifications for ultra-pure carrier-grade helium:

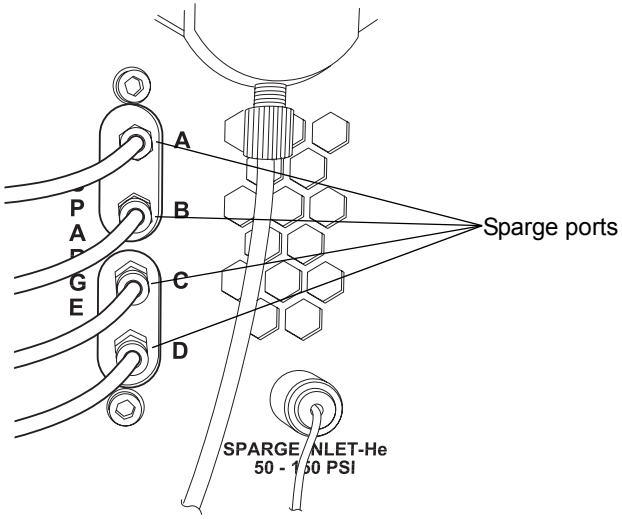
- Nitrogen–less than 5.0 M ppm (molar parts per million).
- Oxygen–less than 5.0 M ppm.
- Total water–less than 1.0 M ppm
- Total hydrocarbon–less than 0.5 M ppm

Recommendation: Before performing a run, sparge the solvent(s), at 100%, for 10 minutes per liter of solvent. Then sparge for an additional 10 minutes per liter of solvent, at 20%. Continue to sparge at 20% during the actual run.

Installing the solvent sparge outlet lines

To install the solvent sparge outlet lines

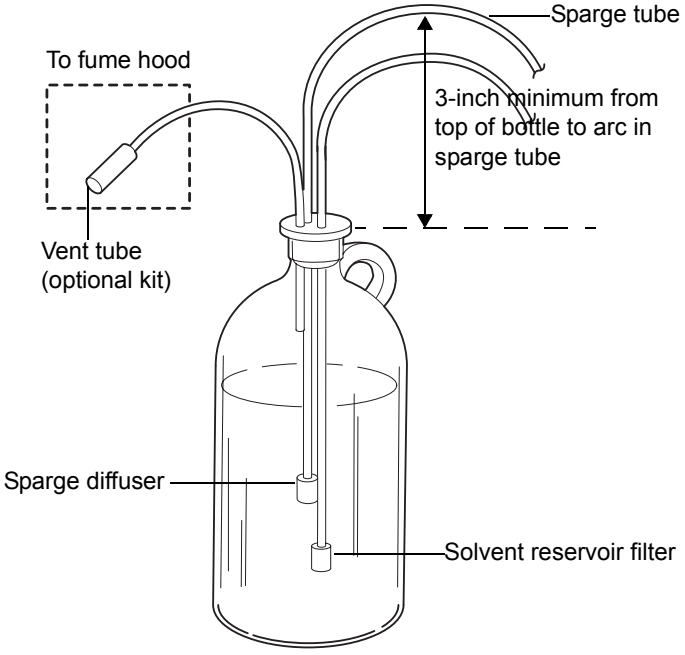
1. Insert the solvent sparge outlet lines in their corresponding sparge ports on the front of the QGM.



2. Insert the corresponding vent tubes, solvent lines, and sparge lines into the solvent reservoir.

Tips:

- A stainless-steel sparge diffuser is attached to the end of each sparge line.
- A solvent reservoir filter is attached to the end of each solvent line.



Requirement: To avoid introducing gas bubbles into the solvent lines, ensure the sparge diffusers are located above or away from the solvent reservoir filter.

3. Route the sparge tube out of the hole on the top, right side of the QGM.

Connecting to the helium sparge supply

The QGM connects to the helium supply via 1/16-inch OD, 0.040 ID stainless-steel tubing (startup kit). This tubing requires compression-fitting connections on each end.

Required material

Open-end wrench, 1/4-inch

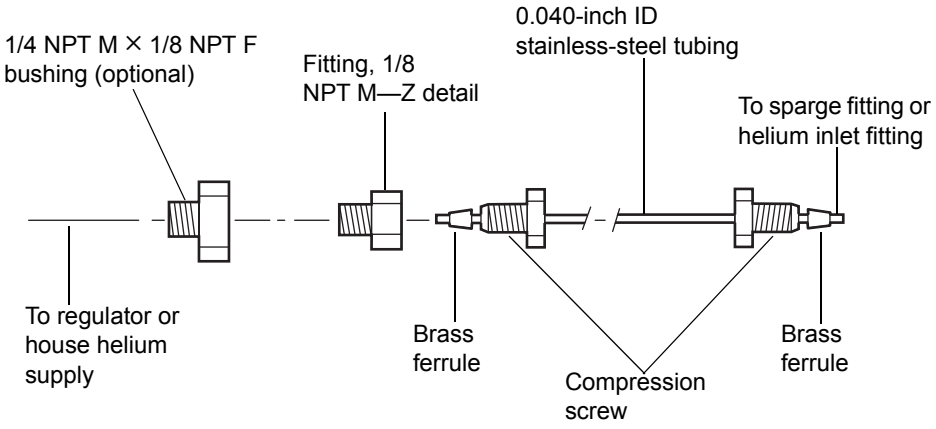
Recommendation: Before performing a run, sparge the solvent(s), at 100%, for 10 minutes per liter of solvent. Then sparge for an additional 10 minutes per liter of solvent, at 20%. Continue to sparge at 20% during an actual run.

To attach compression fittings to the helium line

1. Slide the compression screw onto an end of the 1/16-inch OD, 0.040 ID, stainless-steel tubing.

   Tip: Additional NPT fittings might be required (startup kit).

2. Slide the brass ferrule from the helium connector kit onto the tubing. See the diagram below for orientation.



3. Push the tubing into the fitting body (1/8 NPT, M–Z-detail) until it bottoms there.

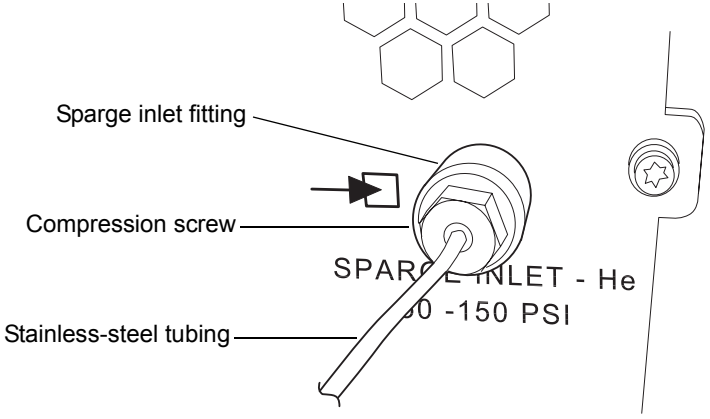
4. While pushing the tubing into the bottom of the female connector on the helium supply tank, tighten the compression screw approximately 3/4-turn beyond finger-tight to seat the ferrule on the tubing.

   Tip: Subsequent tightening of this ferrule does not require the extra 3/4-turn. A 1/8-turn is usually adequate.

To connect the helium sparge supply to the QGM

1. Slide a 1/16-inch, gold, compression screw and brass ferrule onto the 1/16-inch OD, 0.040 ID, stainless-steel tubing from the helium supply, push the tubing into the sparge inlet fitting until it bottoms, and tighten the compression screw using a 1/4-inch open-end wrench.

Helium supply connection



2. Regulate the helium tank or house supply source between 350 and 1030 kPa (3.5 and 10.3 bar, 50 and 150 psi).

Making signal connections

The rear panel of the QGM provides connection terminals and communication ports for connecting with external devices. The signal connections you need to make to the QGM depend on the instruments that constitute your system.

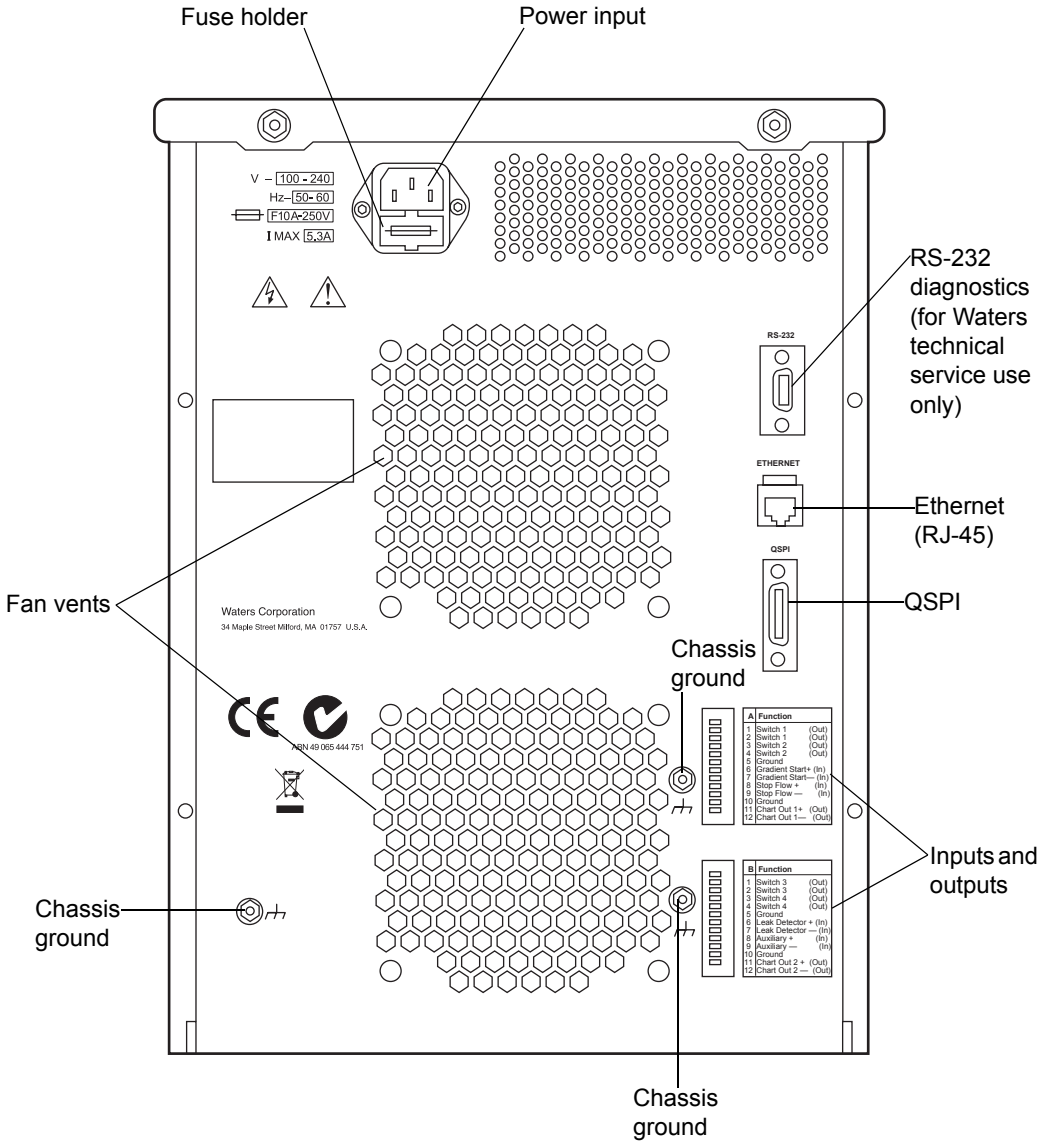
This section describes the event input/output (I/O) and analog output connections that you can make from the terminal strips and communications connectors.

To make signal connections

1. Connect one end of the Ethernet communication cable (RJ-45) to the rear panel and the other end according to the instructions in the *Ethernet Instrument Getting Started Guide*.
2. Connect the event input/output cables from an external device (for example, column-switcher or fraction collector) to the appropriate connector block on the rear panel.
3. To allow the detection of a nitrogen supply failure in mass spectrometer-based systems, connect one end of a signal cable to the stop flow terminals of the connector strip on the mass spectrometer and the other end to terminals 8 and 9 on connector A, on the rear panel.

The following diagram shows the rear panel's major components.

QGM rear panel major components

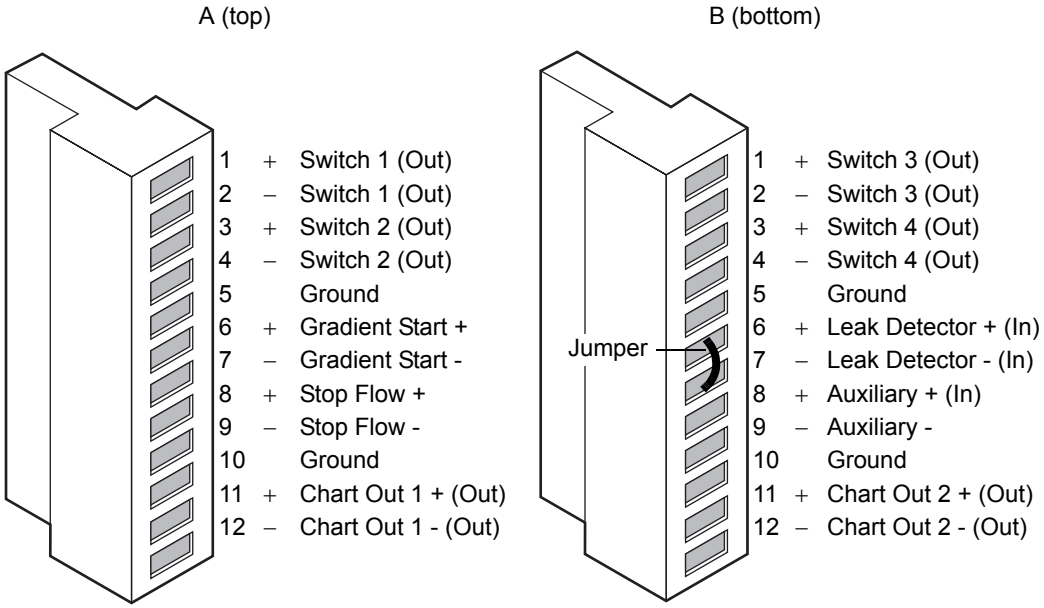


Making I/O signal connections

Warning: To avoid electric shock, power-off and unplug the QGM before making signal connections.

Refer to the signal connection location shown on a label on the rear panel of each instrument. The rear panel includes two removable connectors that hold the pins for the I/O signals (see the following figure). These connectors, A and B, are keyed so that you can insert them only one way.

I/O signal inputs and outputs



The following table describes each signal available on the I/O connectors. See Appendix B for the signal's electrical specifications

I/O signals for the QGM

Signal	Connections	Description
Switch 1 to Switch 4	A1, A2, A3, A4, B1, B2, B3, and B4	Four programmable event output connections (two terminals per closure). See "Event switches" on page 1-35 for information on switch positions and functions.

I/O signals for the QGM (Continued)

Signal	Connections	Description
Ground	A5, A10, B5, and B10	Connected to signal ground and used as reference for outputs.
Gradient Start	A6 and A7	An input that initiates the pumps to begin gradient operation by either contact closure input or TTL (transistor-transistor logic) low. Connect the positive input wire to the start gradient (+) terminal and the negative input wire to the start gradient (–) terminal.
Stop Flow	A8 and A9	An input that enables other LC components to stop the solvent flow from the QGM. Connect the positive input wire to the "Stop Flow+" terminal and the negative input wire to the "Stop Flow–" terminal.
Leak Detector	B6 and B7	The connections for an external leak detection device that is part of a purification system. These are not part of the leak sensor built into the QGM. The QGM is shipped with a jumper installed across terminals B6 and B7. If an external leak detection device is not used, this jumper must remain in place. Flow can start only if the jumper is installed or an external leak detection device is connected. Input voltage range: ±30 Vdc. Logic high = >3.0 Vdc ±10%. Logic low = <1.9 Vdc ±10%. Minimum pulse width = 10 msec. A logic high signal indicates the external leak detection device has detected a leak and initiates stopping the pump. A logic low signal indicates normal operation.
Auxiliary Input	B8 and B9	Reserved for future use.

I/O signals for the QGM (Continued)

Signal	Connections	Description
Chart Out	A11, A12, B11, and B12	Outputs the analog signal. Through software, you can select one of the following signals as the chart out signal: • Flow rate • System pressure • Composition (%A, %B)

2535 QGM chart-out signal conditions

Signal	Parameter Setting at 0 Volts (Minimum)	Parameter Setting at 2.000 Volts (Maximum)
Flow Rate	0.000 mL/min	50 mL/min
System Pressure	0 kPa (0 bar, 0 psi)	41,400 kPa (414 bar, 6000 psi)
Composition	0.0%	100.0%

2545 QGM chart-out signal conditions

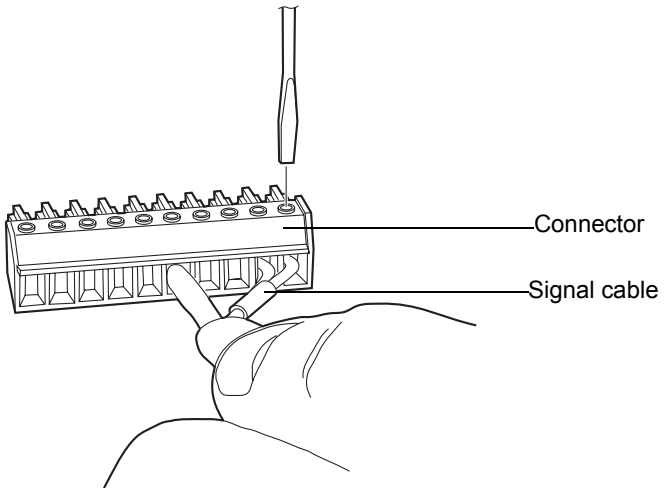
Signal	Parameter Setting at 0 Volts (Minimum)	Parameter Setting at 2.000 Volts (Maximum)
Flow Rate	0.000 mL/min	150 mL/min
System Pressure	0 kPa (0 bar, 0 psi)	41,400 kPa (414 bar, 6000 psi)
Composition	0.0%	100.0%

2555 QGM chart-out signal conditions

Signal	Parameter Setting at 0 Volts (Minimum)	Parameter Setting at 2.000 Volts (Maximum)
Flow Rate	0.000 mL/min	300 mL/min
System Pressure	0 kPa (0 bar, 0 psi)	20,700 kPa (207 bar, 3000 psi)
Composition	0.0%	100.0%

To make signal connections

Attach the positive and negative leads of the signal cable to the connector.



Event switches

You can use switches 1 through 4 on connectors A and B as contact-closure switches to control column-switching valves, fraction collectors, or similar external devices, connecting each pair of switch terminals to an external device. You can operate the switches in these ways:

- Manually via the console, in the QGM Rear Panel dialog box. To access the QGM Rear Panel dialog box, click Troubleshoot > Rear Panel.
- Automatically via the instrument method.

Event switch positions and functions

Position	Function
On	Closes the switch.
Off	Opens the switch.
Toggle	Changes the current state of the switch.
Pulse	Closes/opens the switch for a user-defined period.
No Change	Leaves the switch in its current state.

Connecting to the electricity source

The QGM requires a separate, grounded electricity source. The ground connection in the electrical outlet must be common and connected near the system.

Warning: Avoid electrical shock:

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

To connect to the electricity source

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

1. Move the off/on (○/|) switch to the “off” (○) position.
2. Connect the female end of the power cord to the receptacle on the rear panel of the QGM.
3. Plug the power cord into a suitable AC wall outlet.

2 Preparing for Operation

This chapter explains how to configure the QGM as a chromatographic system pump.

Contents

Powering-on

When you complete all electrical, plumbing, and signal connections, you can power-on the QGM.

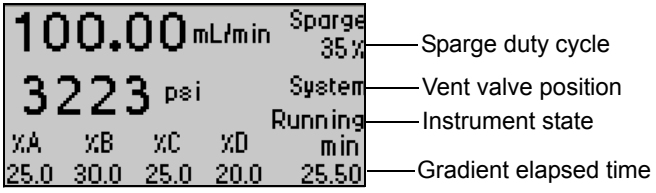
Warning: In the event of a solvent leak, to avoid contacting solvent, ensure the QGM's front door is closed when operating under normal conditions.

Caution: To avoid damaging electrical parts, do not power-on the QGM until you complete all electrical, plumbing, and signal connections.

To power-on the QGM, toggle the power switch (on the top, right-hand side of the front panel) to the **I** ("on") position.

At startup, the QGM performs a series of internal diagnostic tests.

Example: QGM LCD (liquid crystal display)



Adjusting the LCD contrast

To adjust contrast

Verify that the flow is 0 mL/min, and then press the Stop Flow button, and hold it down until the display's contrast suits you.

Configuring in Empower software

Note: W25X5Q is used to denote the 2535, 2545, and 2555 quaternary gradient modules in Empower software.

Perform these tasks to configure in Empower software:

- Install the W25X5Q instrument control software (for installation instructions, see the 2535/2545/2555 Quaternary Gradient Module Release Notes).
- 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: Double-click the Empower desktop shortcut.
2. In the Empower Login dialog box, type your user name and password.
3. Click OK.

Selecting system instruments

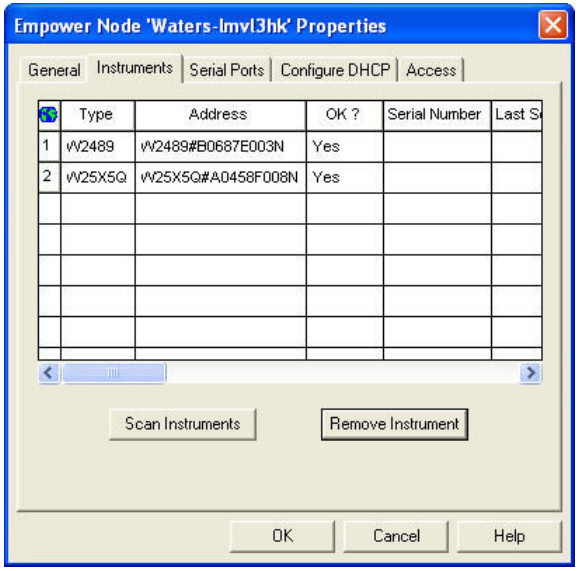
To select system instruments

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

3. In the Empower Node Properties dialog box, click the Instruments tab.

   Tip: The system instruments that are successfully communicating with your system are indicated by a Yes in the "OK?" column.

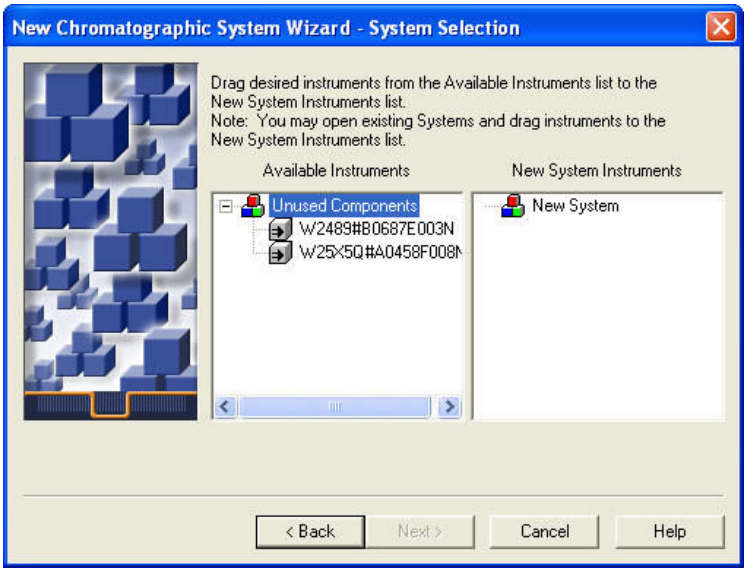
Instruments tab



4. Ensure that a quaternary gradient module appears in the instrument list and is 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



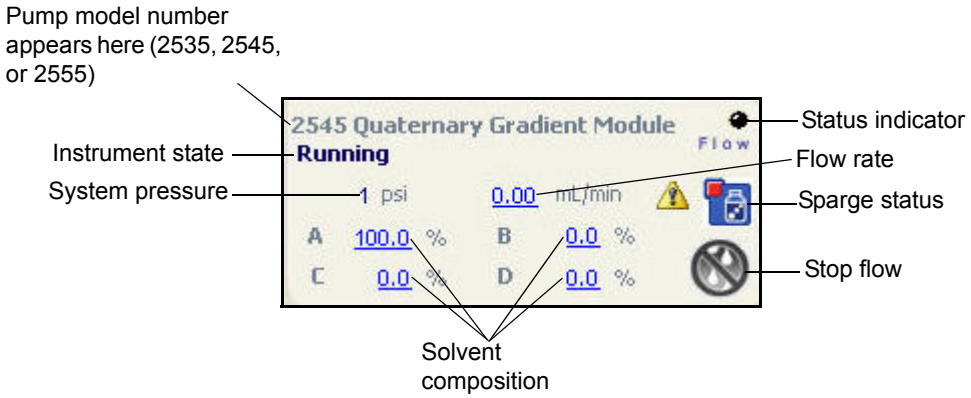
7. In the System Selection dialog box, drag the name of the instrument(s) you want to include in the new system from the Available Instruments pane to the New System Instruments pane, and click Next.
8. When the Access Control dialog box appears, click Next.
9. In the Name Selection dialog box, specify 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. From the Run Samples window, you can monitor the QGM using its control panel.

About the QGM control panel

The QGM's control panel appears at the bottom of the Run Samples window in Empower. When MassLynx software controls the system, the QGM's control panel appears on the Additional Status tab of the Inlet Editor window.

QGM control panel



The QGM control panel indicates the flow rate, flow status, system pressure and sparge status. It also indicates the solvent composition parameters, which you can edit by clicking on the underlined value when the system is idle (but not when samples are running).

The following table lists the items in the QGM control panel.

QGM control panel items

Control panel item	Description
Status indicator	Indicates the flow status of the QGM. LED states are: • Steady green–indicates that the QGM is delivering flow. • Flashing green–indicates the QGM is initializing. • Steady red–indicates communication with the QGM is lost. • Flashing red–indicates that the QGM is in error state.

QGM control panel items (Continued)

Control panel item	Description
Instrument state	Displays the current instrument state (running, error, or priming).
System Pressure	Displays the QGM pressure, in kPa, bar, or psi. Pressure units can be customized through the QGM console by clicking Configure > Pressure units.
Solvent Composition	Displays the percentage of solvents to be drawn via the gradient proportioning valve (A, B, C, and D). Composition values range from 0.0 to 100.0%.
Flow Rate	Displays the flow rate of the QGM, in mL/min.
 (Sparge status)	Displays the sparge status. Conditions are indicated as follows: • Green circle – sparging is on. • Red square – sparging is off.
 (Stop Flow)	Immediately stops flow from the QGM.

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

Additional functions in the QGM control panel

Control panel function	Description
Prime solvents	Displays the Prime Solvents dialog box. (See page 2-16.)
Prime seal wash	Displays the Prime Seal Wash dialog box. (See page 2-15.)
Set sparge	Displays the Set Sparge dialog box. (See page 2-18.)
Set flow scale (2535 QGM only)	Displays the Set Flow Scale dialog box. (See page 2-20.)

Additional functions in the QGM control panel (Continued)

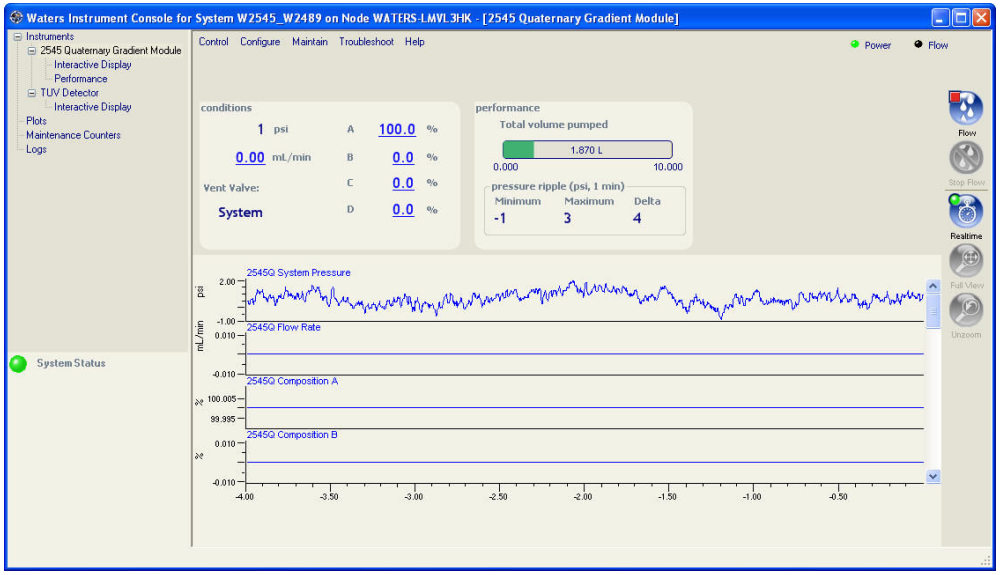
Control panel function	Description
Reset QGM	Resets the QGM to recover from an error condition.
Help	Displays the QGM online Help.
Launch Instrument Console	Launches the instrument console software.

Starting the console from Empower software

To start the console from Empower software

In the Run samples window, right-click in the QGM control panel, and select Launch Instrument Console.

Console window



Configuring in MassLynx software

Note: W25X5Q and 25X5Q are used to denote the 2535, 2545, and 2555 quaternary gradient modules in MassLynx software.

Perform these tasks to configure in MassLynx software:

- Install the W25X5Q instrument control software and the Waters Pump Control software (for installation instructions, see the 2535/2545/2555 Quaternary Gradient Module Release Notes).
- Start the application.
- Select system instruments.

Starting MassLynx software and logging in

To start MassLynx software

1. Select Start > All Programs > MassLynx > MassLynx.

   Alternative: Use the MassLynx desktop shortcut.

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

2. Type your user name and password, and select your domain.
3. Click OK.

   Result: The MassLynx window appears.

Selecting system instruments

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.
3. In the Inlet Configuration window, click Configure, and then click Next.
4. In the Select Pump dialog box, select Waters Pump Control, and then click Next.

5. Select the autosampler (or select None if no autosampler is present), and then click Next.
6. Highlight the detector to select it, click the right arrow (or select None if no detector is present), and then click Next.
7. In the Inlet Configuration Wizard window, click “Scan for Instruments” and then click OK.

   Result: All scanned instruments appear.

   Requirement: If any instruments are missing, ensure all system instruments are connected and powered-on. Repeat the scan. If instruments are still missing, you must troubleshoot and resolve any issues before proceeding.
8. Select the desired instrument from the “ID” drop-down list, click Next, and then click Finish.
9. In the Inlet Configuration window, click Events & Triggering, and then click Next.
10. If you are using a mass spectrometer, select the desired events. If not, click Next.
11. Select “Triggering by contact closure” for both the pump and the detector, and then click Next.
12. Click Finish, and then click Finish again.
13. In the Inlet Method window, click the Inlet icon.
14. In the Modify Instrument window, click Config.
15. In the Waters Pump Control Configuration window, click Scan.

    Result: A list of connected instruments appears, among them the Quaternary Gradient Module.
16. Click Close.
17. Select the Quaternary Gradient Module in the “Associate with an instrument” drop-down list for the pump. Select any additional system instruments, and then click OK.
18. In the Reset Communications dialog box, click OK.

    Tip: This dialog box appears only if the configurations have changed.

19. In the Modify Instrument Method window, click OK.
20. In the Inlet Method window, select LC > Reset Communications.

Starting the console from MassLynx software

To start the console from MassLynx software

1. In the MassLynx window, click Inlet Method.
2. In the Inlet Method window, click the Additional Status tab.
3. Right-click in the QGM control panel, and select "Launch Instrument Console".

   Tip: See page 2-6 for information about the control panel.

Enabling and disabling the leak sensor

Rule: When you power-on the system, the QGM leak sensor defaults to enabled, unless previously disabled.

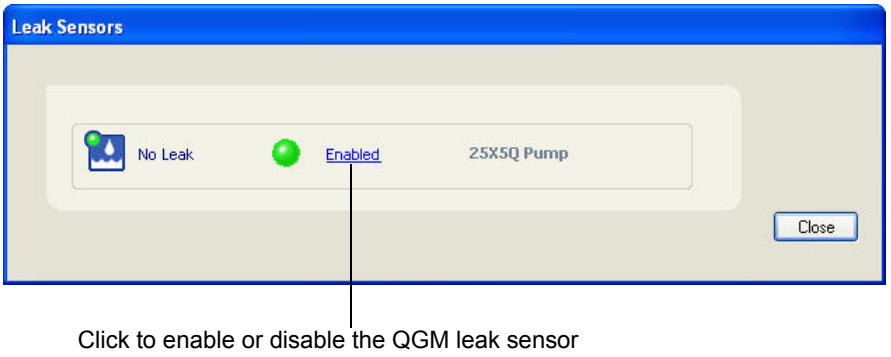
To enable and disable the leak sensor

1. If operating your device under Empower software control, in the console, select Quaternary Gradient Module from the instrument tree.

   If operating your device under MassLynx software control, in the console, select Chromatographic pump from the instrument tree.

2. In the Quaternary Gradient Module window, click Control > Leak sensor.

Leak Sensors dialog box



3. To enable or disable the QGM leak sensor, click the status on the Enabled/Disabled hyperlink.
4. In the Leak Sensors dialog box, select the leak sensor status, and then click (OK).
5. Click Close.

Operating from the standalone console

The standalone software can control the pump and a compatible Waters detector. The software monitors various pressure readings generated by the QGM, but saves them for only 96 hours. The software does not collect data from the detector. To collect and save the pump data permanently or the detector signals, you must use a data collection device.

Launching the standalone console

The CD that ships with the QGM contains the instrument control software. You must install this software to operate the QGM as a standalone device. For installation instructions, see the 2535/2545/2555 Quaternary Gradient Module Release Notes.

Once the instrument control software is installed, you can launch the standalone console and use it to control the pump and a compatible detector.

To launch the standalone console:

1. Locate the icon for the Standalone ACQUITY Console in the C:\Program Files\Waters Instruments\Bin folder.
2. Create a shortcut for the icon, and copy the shortcut to the desktop. Click the icon to launch the standalone console.

Standalone console window

Creating methods

Use the method editor to create and edit methods for the system.

To access the Methods editor window

In the console, select Methods from the instrument tree. The Methods editor window appears.

Tip: For information about creating methods, consult the console's online Help.

Preparing sample lists

Use the Samples window to create a sample list.

To access the Samples window

In the console, select Samples from the instrument tree. The Samples window appears.

Tip: For information about preparing sample lists, consult the console's online Help.

Preparing the QGM

For optimal performance of the system, you must prime the seal-wash pump as well as the QGM.

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

Requirement: To maintain the efficiency of the QGM and to obtain accurate, reproducible chromatograms, use only HPLC-grade (or better) solvents, water, and additives. For details, see Appendix C.

Operational considerations with normal phase solvents

For best chromatography results:

- Run normal or reverse-phase solvents with a minimum backpressure of 4140 kPa (41.4 bar, 600 psi).
- Do not leave normal phase solvent in the QGM for extended periods. Flush the QGM with isopropyl alcohol.
- See page C-7 for mobile phase solvent degassing information.

Priming the seal-wash pump

Caution:

- Ensure the seal wash solvent is fully miscible with the mobile phase.
- To avoid reducing the life of the seal-wash pump and plunger seals, do not use a nonvolatile buffer as the seal-wash solvent.
- To prevent contamination, do not recycle seal-wash solvent.

The seal-wash solvent prevents excessive seal wear caused by the buildup of salt crystals and lubricates the plunger and its wash seal. Prime the seal-wash pump in the QGM to fill the tubing paths and plunger wash cavity with solvent.

Prime the seal-wash pump when these conditions apply:

- When the QGM has been inactive for 24 hours or longer.
- When the plunger and seal-wash pump is dry.

Requirements:

- Seal-wash solvent must contain at least 10% organic solvent to prevent microbial growth. See "Solvent miscibility" on page C-3 for more information.
- Before priming the seal-wash pump, ensure the volume of wash solvent is adequate for priming.
- Continue priming until wash solvent drains from the solvent wash waste line.

Required materials

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

To prime the seal-wash pump

1. Ensure the seal-wash inlet tube is in the solvent reservoir.
2. Push the syringe plunger fully into the syringe barrel.

3. Connect the tubing adapter to the syringe, and then connect the syringe assembly to the outlet tubing from the seal-wash system (the tube from the left side of the left actuator).
4. In the console, select Quaternary Gradient Module from the instrument tree.
5. In the console's Quaternary Gradient Module window, click Control > Prime seal wash, and then click Yes to begin the priming process.
6. Slowly withdraw the syringe plunger to draw seal-wash solvent through the system.
7. When the seal-wash solvent begins to flow into the syringe unaccompanied by major air bubbles, disconnect the tubing, and reinstall it on the drip tray fitting.
8. Press the Stop Flow button at the top, left-hand side of the front panel.

   Alternative: In the console's Quaternary Gradient Module window, click  (Stop flow) to stop the priming process.

   Tip: The priming process automatically stops after 10 minutes of operation.

Priming the QGM

Prime the QGM when preparing it for use, when changing mobile phase, or when restoring flow after the device has been idle for more than four hours. You select which among of the four solvent lines to prime and the duration of each priming operation. During priming, the vent valve moves to its vent position to direct flow to waste.

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-4.

Recommendations:

- Ensure that the solvent reservoirs contain enough solvent for adequate priming and that the waste container can accommodate the volume of used solvent. The default prime flow rate for the 2545 QGM and 2555 QGM is 75 mL/min. The default prime flow rate for the 2535 QGM is 50 mL/min.
- Empty the waste container whenever you fill the solvent reservoirs.
- To avoid overflowing waste, empty the container frequently.

To prime the QGM

1. Open the module's front door.
2. Ensure the solvent waste line is routed to the waste container or drip tray.
3. In the console, select Quaternary Gradient Module from the instrument tree.
4. In the Quaternary Gradient Module window, click Control > Prime Solvents.

   Alternative: In the Run Samples window, right-click in the control panel, and then click Prime.
5. In the Prime Solvents dialog box, select solvents for priming: A, B, C, and/or D.

   Recommendation: Prime all solvent lines, even if they will not be used. Place unused solvent lines in available solvent reservoirs.

   Tip: The system defaults to all solvents selected for the first time after power-up. After that, the last selections are retained.
6. In the Time box, specify the number of minutes, from 0.1 through 60.0.

   Note: Each solvent will prime for the time specified in the Time box.

   Default: 1.0 minute.

   Recommendation: Continue priming until a steady flow exits the solvent waste line.
7. Click Start.

   When solvent flows out of the waste line without bubbles, the path is primed.

To manually prime the QGM using a syringe (for a dry system only)

1. Open the module's front door.
2. Connect the short priming tube (startup kit) to the open port on the left side of the inlet manifold valve. (See page 1-3 through page 1-7.)
3. Fill the 30-mL syringe with solvent, and connect it to the priming tube.
4. Place the inlet manifold valve in the Load position, and inject solvent into the pump stream.

   Rationale: Doing so wets the check valve and aids in priming.
5. Turn the flow on and place the vent valve in the Vent position.
6. Return the inlet manifold valve back to the Run position.

Setting the sparge parameters

Solvents are helium-sparged in their reservoirs and proportioned at the gradient portioning valve.

Requirements:

- Do not blanket solvent reservoirs when sparging.
- Do not pressurize solvent containers.

Recommendation: Prior to running, sparge the solvent(s) at 100% for 10 minutes per liter of solvent. Follow with an additional 10 minutes per liter of solvent at a rate of 20%. Continue to sparge at 20% during an actual run.

To set the sparge parameters in the console or control panel

1. In the console, select Quaternary Gradient Module from the instrument tree.
2. In the Quaternary Gradient Module window, click Control > Set sparge.

   Alternative: In the Run Samples window, right-click in the control panel, and then click Set sparge.
3. In the Set sparge dialog box, select which solvent lines to sparge: A, B, C, and/or D.
4. In the Initial Rate box, specify the sparge duty cycle and duration.

5. In the Normal Rate box, specify the normal duty cycle, and click Set.

   Tip: The normal duty cycle begins once the system has sparged at initial duty cycle for the duration specified in the Initial Rate pane.

To set the sparge parameters in the Instrument Method Editor

1. Click the Sparge tab.
2. Select which reservoirs to sparge: A, B, C, and/or D, and then specify the rate.
3. To modify sparge rates at a certain time after the start of a run, go to the Events tab. Select Sparge from the Event list and edit the values.

Determining the sparge status from the control panel

The icon of a green bottle displayed on the control panel indicates that sparging is operative and a gray bottle indicates that sparging is inoperative.

Determining the sparge status on the LCD

A sparge duty cycle of “0%” displayed on the LCD indicates that sparging is inoperative. Any sparge duty cycle greater than 0% indicates that sparging is operative. For example, a sparge duty cycle of “50%” indicates that sparge is on and the current rate is 50.

Monitoring sparge pressure

When sparging is enabled, the QGM monitors the gas pressure continuously. When the pressure falls below 48 kPa (0.48 bar, 7 psi), the QGM logs a message in the console “Logs” and the Empower Message Center (if you are running Empower software). A low gas pressure condition does not affect a running sample queue. Rather, the LCD will display “Error” instead of “Sparge %”.

When you replace the gas tank, the QGM automatically recognizes that the sparge gas pressure is back to normal and logs another message in the console “Logs” and Empower Message Center (if you are running Empower software).

Setting the 2535 QGM flow scale

The flow scale option is available for the 2535 QGM only. When you select small flow scale, flow is routed through the small-scale mixer tee directly to the 2535 QGM outlet. When you select large flow scale, flow is routed first through the small-scale mixer tee, then through an additional large-scale mixer, and finally to the 2535 QGM outlet.

Recommendations:

- For flow rates ≤5 mL/min, set the flow scale to Small.
- For flow rates >5 mL/min, set the flow scale to Large.

To set the 2535 QGM flow scale in the console

1. In the console, select 2535 Quaternary Gradient Module from the instrument tree.
2. In the 2535 Quaternary Gradient Module window, click the Vent Valve hyperlink.
3. In the Set Flow Scale dialog box, select the flow scale: Large or Small.

   Default: Large
4. Click Set.

To set the 2535 QGM flow scale in the control panel for instruments controlled by Empower software

1. In the Run Samples window, right-click in the control panel, and then select Set flow scale.
2. In the Set Flow Scale dialog box, select the flow scale: Large or Small.

   Default: Large
3. Click Set.

To set the 2535 QGM flow scale in the control panel for instruments controlled by MassLynx software

1. In the Inlet Method Editor, right-click in the control panel, and then select Set flow scale.
2. In the Set Flow Scale dialog box, select the flow scale: Large or Small.

   Default: Large
3. Click Set.

To set the 2535 QGM flow scale in the Interactive Display for instruments controlled by Empower software

1. In the console, select 2535 Quaternary Gradient Module > Interactive Display from the system tree.
2. Click Control  .

   Result: The modifiable settings are underlined.

   Rationale: The Control icon invokes interactive control.

   Tip: A green circle appearing in the upper left corner of the icon means control mode is enabled. A red square in that place means control mode is disabled.
3. Click the Vent Valve hyperlink.
4. In the Set Flow Scale dialog box, select the flow scale: Large or Small.

   Default: Large
5. Click Set.

To set the 2535 QGM flow scale in the Interactive Display for instruments controlled by MassLynx software

1. In the console, select Chromatographic Pump > Interactive Display from the system tree.
2. Click Control  .

   Result: The modifiable settings are underlined.

   Rationale: The Control icon invokes interactive control.

Tip: A green circle appearing in the upper left corner of the icon means control mode is enabled. A red square in that place means control mode is disabled.

3. Click the Vent Valve hyperlink.
4. In the Set Flow Scale dialog box, select the flow scale: Large or Small.

   Default: Large
5. Click Set.

To set the 2535 QGM flow scale in the Instrument Method Editor

1. Click the General tab.
2. Select the flow scale from the drop-down list: Large or Small.

   Default: Large
3. Click Set.

Making large sample injections

The QGM supports two methods of making large sample injections:

- Inlet manifold valve loading, which minimizes the amount of sample required for a single, large sample injection. (The startup kit provides the required tubing assembly.)
- Solvent line (unused) loading, which facilitates performing multiple, large-scale injections.

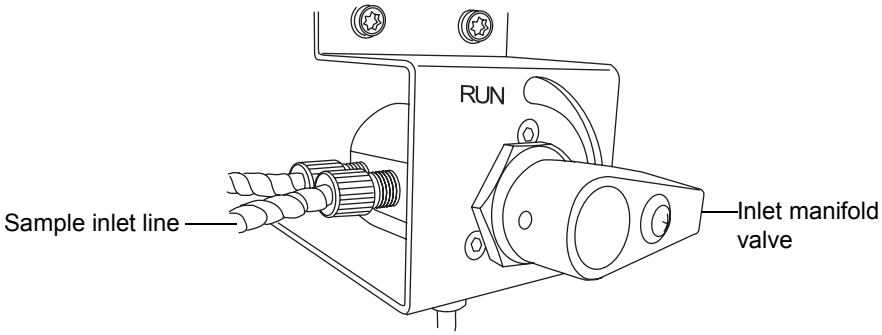
Sample matrices considerations

Before making large-sample injections, note these particulars:

- Filter all samples to remove nonsoluble material.
- Ensure the sample remains dissolved—that is, it must not precipitate—when it mixes with the mobile phase at initial conditions or any other time during the separation.
- A sample that would cause the pump to malfunction would likely damage the column. Nevertheless, most samples course through the fluid-handling unit and load onto the column without fouling the seals or check valves.

Using the manual sample inlet line for large-sample injections

The sample inlet line (startup kit) must be attached to the front of the pump as shown below.



To prime the inlet line

1. Route the length of sample inlet line tubing from the inlet manifold valve to a reservoir containing the initial solvent.
2. While the pump is operating, turn the inlet manifold valve to the Load position.

   Rationale: Doing so does these things:

   - Draws solvent through the manual sample inlet line.
   - Purges air from the inlet line and inlet manifold valve.
   - Removes any previous sample or solvent.
3. Turn the inlet manifold valve to the Run position, disconnecting the manual sample inlet line.

   Tip: The inlet tubing remains filled with solvent.
4. Remove the inlet tubing from the solvent reservoir, and immerse it in the sample reservoir.

To make a large sample injection

1. Before injecting the sample, select the sample inlet line by turning the inlet manifold valve to the Load position.
2. Leave the inlet manifold valve in the Load position for as long as required to load the sample onto the column at the current flow rate.

Requirement: You must ensure mobile phase passes through the pump before sample enters the system, taking into account that, for the first injection, the sample inlet line contains mobile phase.

3. Turn the inlet manifold valve to the Run position, selecting the mobile phase.
4. From the console, trigger the inject start and, on injecting, transfer the sample inlet line to a reservoir containing the initial gradient solvent, to ensure complete sample injection and line flushing.

Using the solvent inlet line for large-sample injections

To prepare the solvent inlet line for the sample

1. Immerse the solvent inlet line for loading the large sample in a reservoir containing the initial gradient solvent.
2. Purge the line of air.
3. Place an appropriate volume of sample into a clean reservoir, and put the solvent line used as the sample inlet in the sample reservoir.

   Tip: When making injections using a solvent inlet line, create a gradient table that does these things:

   - Pumps sample onto the column at the begging of a run.
   - Executes a gradient delay.
   - Performs the normal separation gradient.

To make a sample injection

1. From the console, trigger the inject-start function.

   Result: The gradient table activates and pumps sample onto the column.

   Tip: For the first injection only, the solvent reservoir line to the sample reservoir is filled with solvent (the result of purging the line), so the amount of sample loaded during the first injection is reduced by the volume of the solvent line. Solely the product of time and flow rate, subsequent injection volumes equal each other.
2. From the console, trigger the inject start and, on the final injection, transfer the sample inlet line to a reservoir containing the initial gradient solvent to ensure complete sample injection and line flushing.

Creating gradients

You create a gradient to change the composition ratio of solvents during a run.

To access the gradient table

In the Instrument Method Editor, click 25X5Q > General tab > Gradient table.

Tip: For information about creating gradients, see the Empower or MassLynx online Help.

3 Maintaining the Quaternary Gradient Module

Contents

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. The Waters' Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Visit www.waters.com.

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

- Completed normal operation checklist for the method you are using
- Nature of the symptom
- Instrument serial number
- Flow rate
- Operating pressure
- Mobile phase(s)
- Detector settings
- Type and serial number of column(s)
- Sample type
- Control mode (Empower, MassLynx, FractionLynx™, No interaction, or other)
- Software version and serial number

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

Maintenance considerations

Perform the procedures in this chapter when you discover a problem with a QGM component or during preventive maintenance. For information about isolating problems in the QGM, consult the console's online Help.

Safety and handling

Observe these warning and caution advisories when you perform maintenance operations on the QGM.

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

Warning: Avoid electric shock:

- Do not open the QGM cover. The components within are not user-serviceable.
- Power-off and unplug the QGM before performing any maintenance operation on the instrument.

Warning: Using incompatible solvents can cause injury and severe damage to the instrument. See Appendix C for more information.

Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

Spare parts

Replace only parts mentioned in this document. For spare parts details, see the Waters Quality Parts Locator on the Waters Web site's Services & Support page.

Resolving leak sensor errors

All fluid-handling areas of the QGM are designed to route spilled solvent to the drip tray (fitted with a leak sensor). The solvent then flows to the waste tube below the front panel.

After approximately 3.0 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 QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

- Cotton swabs
- Gloves: clean, powder-free, chemical-resistant
- Nonabrasive, lint-free wipes

To resolve a leak sensor error

1. View the Leak Sensors dialog box to confirm that the QGM leak sensor detected a leak.

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

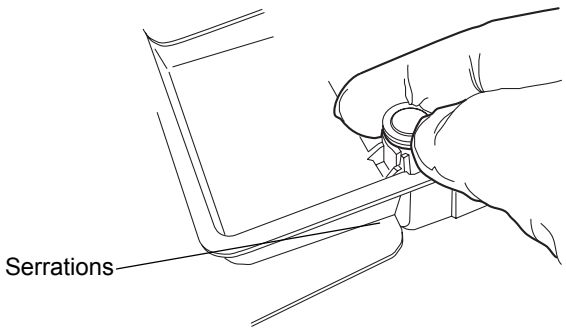
   Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

2. Power-off the QGM.
3. Open the module's door, gently pulling its right-hand edge toward you.
4. Locate the source of the leak, and make the repairs necessary to stop it.

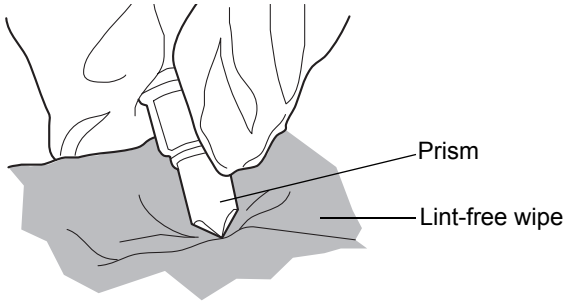
   Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

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

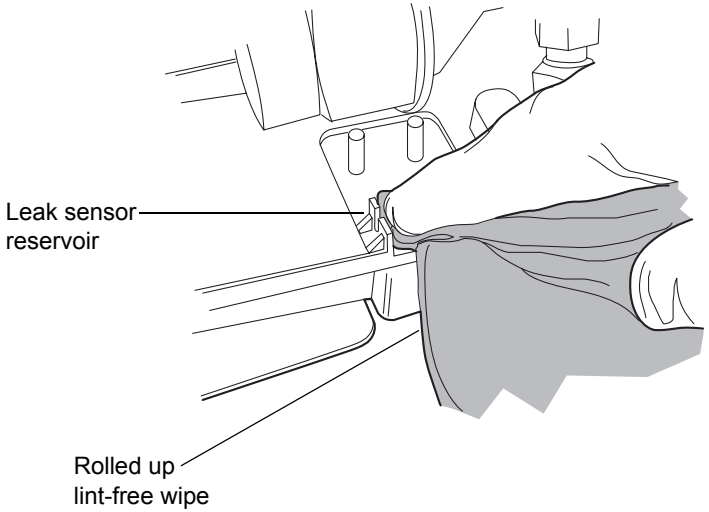
   Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see page 3-8).



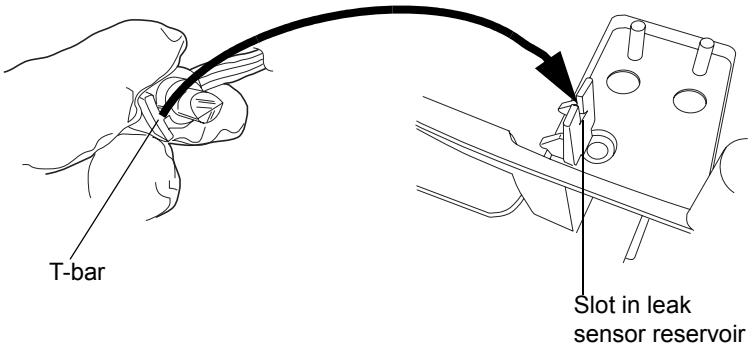
6. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.

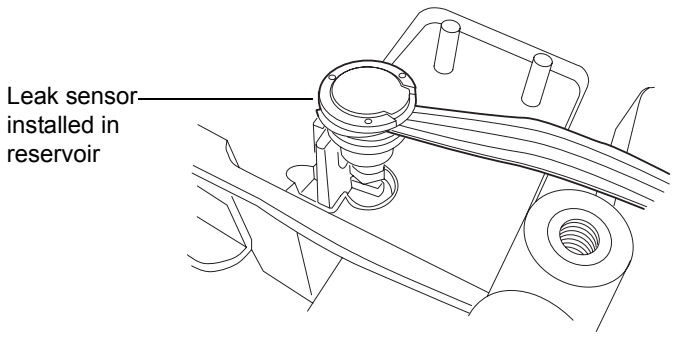


7. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



8. 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. Power-on the QGM.
11. In the console, select Quaternary Gradient Module from the instrument tree.
12. In the Quaternary Gradient Module window, click Control > Reset 25X5Q, to reset the QGM.

Replacing the QGM's leak sensor

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

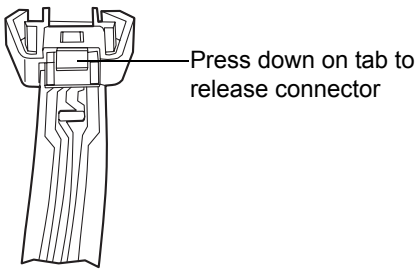
Required materials

- Gloves: clean, powder-free, chemical-resistant
- Leak sensor

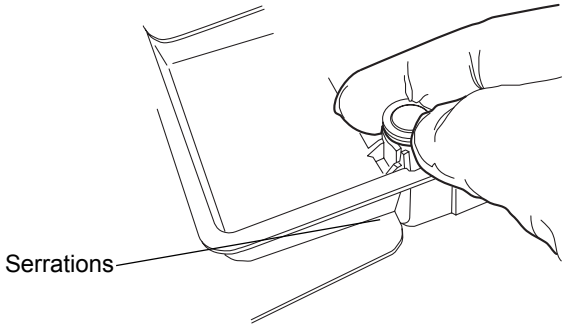
To replace the QGM's leak sensor

Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

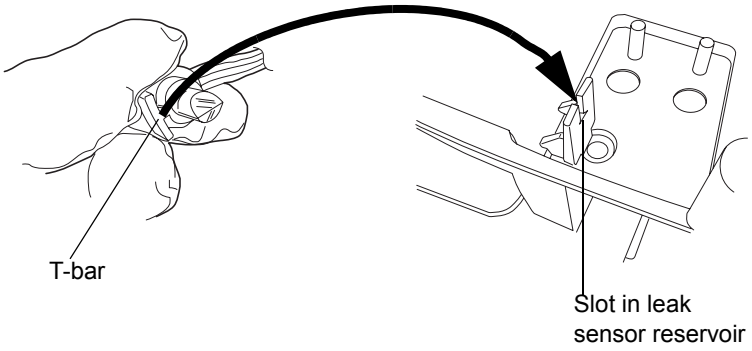
1. Power-off the QGM.
2. Open the module's door, gently pulling its right-hand edge toward you.
3. Press down on the tab to detach the leak sensor connector from the front of the module.

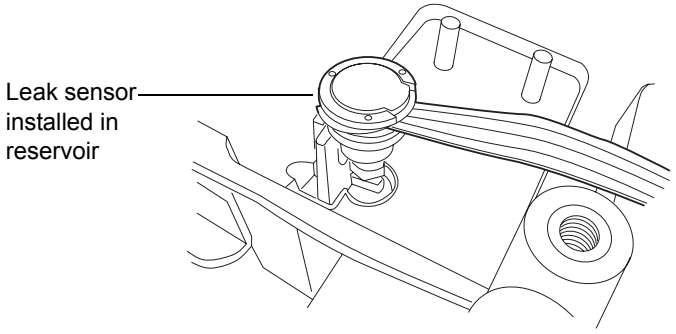


4. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



5. Unpack the new leak sensor.
6. 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.





7. Connect the leak sensor connector to the front of the instrument.
8. Power-on the QGM.

9. In the console, select Quaternary Gradient Module from the instrument tree.
10. In the Quaternary Gradient Module window, click Control > Reset 25X5Q, to reset the module.

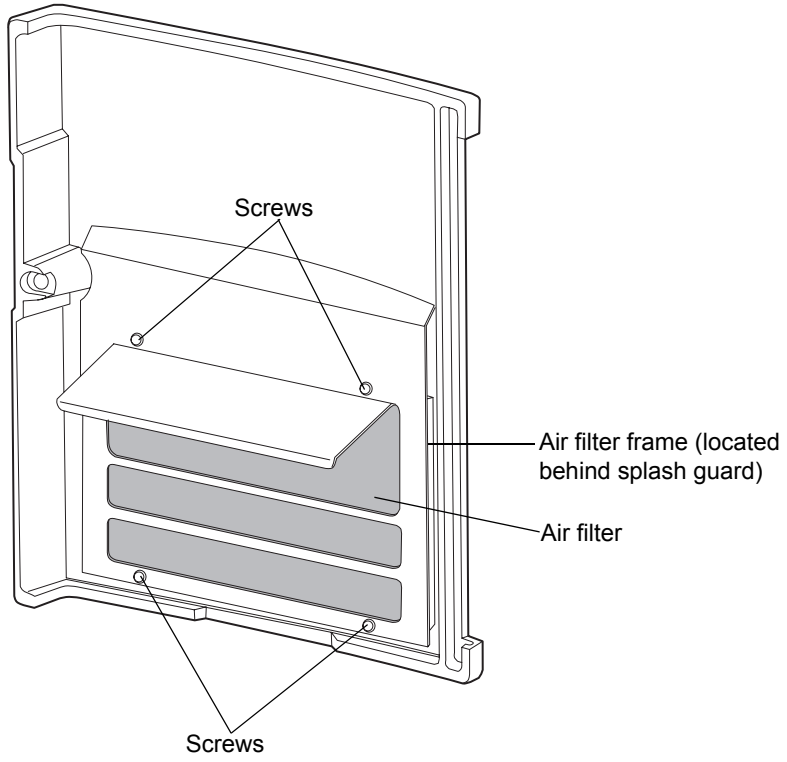
Cleaning the door's air filter

Required material

T10 TORX® driver

To clean the air filter

1. Using the T10 TORX driver, remove the 4 screws that secure the air filter frame and air filter to the inside of the module's door.



2. Remove the air filter from the air filter frame, and clean it.

3. Align the air filter with the air filter frame.
4. Using the T10 TORX driver and the 4 screws, attach the air filter and frame to the inside of the door.

Replacing the door's air filter

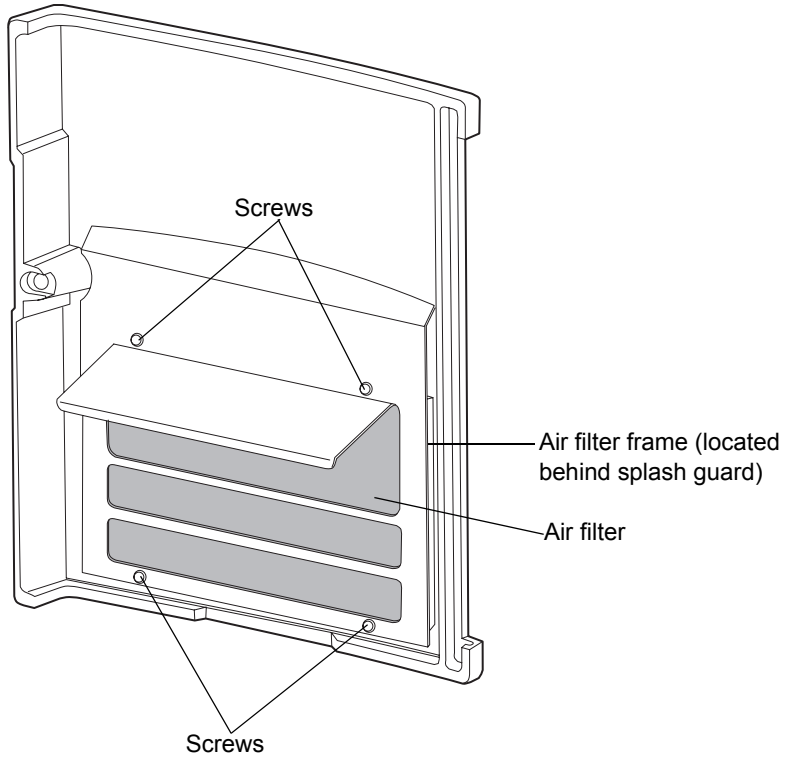
If you cannot clean the air filter, replace it.

Required materials

- T10 TORX driver
- QGM air filter

To replace the air filter

1. Using the T10 TORX driver, remove the 4 screws that secure the air filter frame and air filter to the inside of the door.



2. Remove the old air filter from the air filter frame and discard it.
3. Align the new air filter with the air filter frame.
4. Using the T10 TORX driver and the 4 screws, attach the air filter and frame to the inside of the door.

Replacing the transducer

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

Note: For illustration purposes, the 2545 QGM is shown in the drawings in this procedure.

Required materials

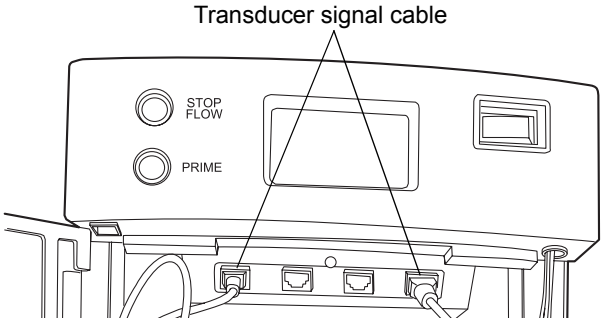
- Open-end wrench, 5/16-inch
- T10 TORX driver
- Transducer assembly

To replace the transducer

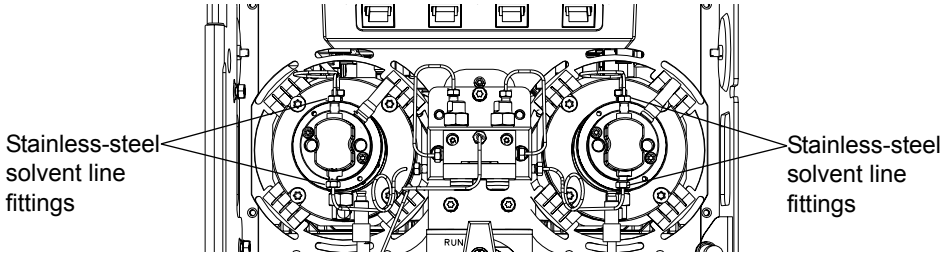
1. Flush the QGM with methanol.

   Requirement: If the solvent you are using is not miscible with methanol, use an intermediate solvent.

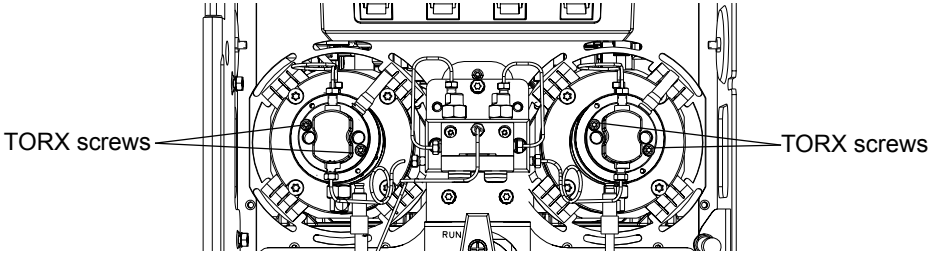
2. Disconnect the transducer signal cable from the front panel.



3. Using the 5/16-inch open-end wrench, disconnect the two stainless-steel solvent lines on the top and bottom of the transducer.



4. Using the T10 TORX driver, loosen the two TORX screws that hold the transducers to the pump manifolds.



5. Place the new transducer onto the pump manifold, with the signal cable in the 7 o'clock position.

6. Using the T10 TORX driver, tighten the two TORX screws to hold the transducer in place.
7. Reconnect the transducer signal cable to its connector on the front panel, ensuring the connector clicks securely into place.
8. Using the 5/16-inch open-end wrench, reconnect the two stainless-steel solvent lines to the top and bottom of the transducer.

Replacing the pump head seals and O-ring

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

This procedure involves replacing these parts:

- O-ring
- Plunger seal
- Plunger wash seal

Required materials

- Open-end wrench, 1/4-inch
- T27 TORX driver
- Gloves: clean, powder-free, chemical-resistant
- Methanol in a wash bottle
- Pliers

- Replacement seal kit
- Seal insertion/extraction tool
- Tweezers, plastic

Caution: To avoid damaging the sealing surfaces, use the seal insertion/extraction tool. Do not use a sharp or metallic tool to remove or install seals.

To remove the pump head and seal-wash assembly

1. Flush the QGM with non-hazardous solvent.
2. In the console, select Quaternary Gradient Module from the instrument tree.
3. In the Quaternary Gradient Module window, click Maintain > Heads.
4. In the Head Maintenance dialog box, select the pump head (left or right) that you plan to perform maintenance on.
5. Click Move Plunger Back, and then wait for the plunger to stop.

   Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

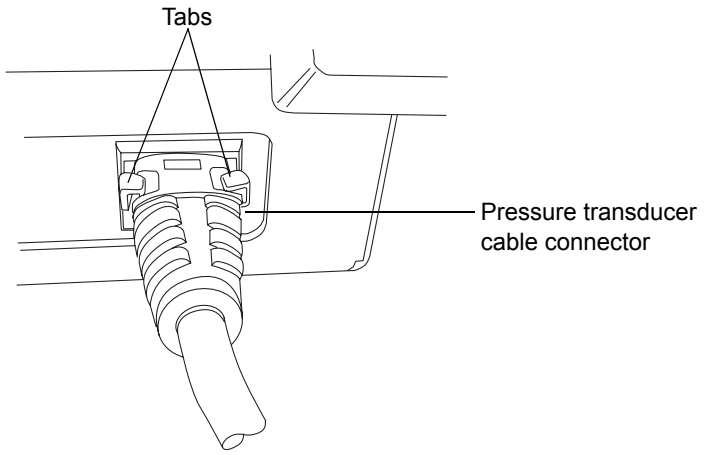
6. Power-off the QGM.

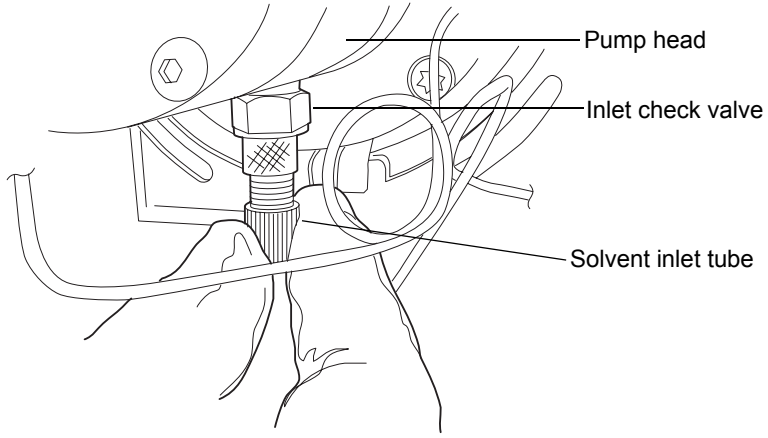
   Warning: To avoid solvent siphoning, relocate the solvent bottles below the QGM.

7. Relocate the solvent bottles below the QGM.

8. Squeeze the tabs of the pressure transducer cable, and pull gently, disconnecting the cable from the bulkhead.

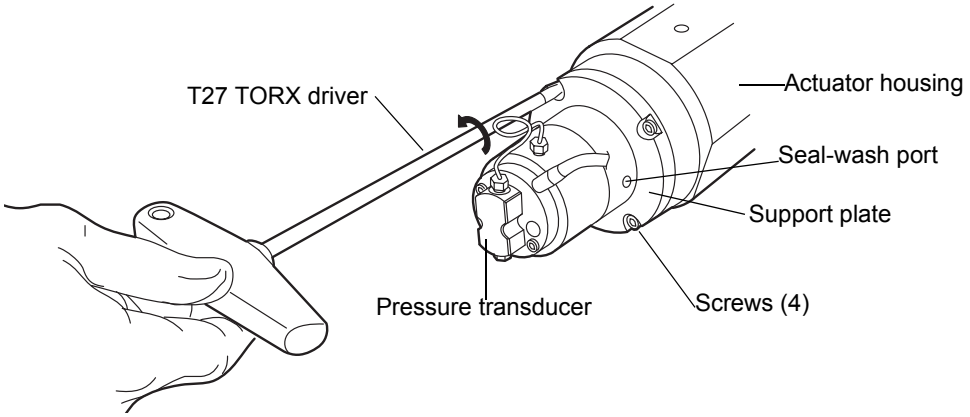


9. Disconnect the solvent inlet tube from the inlet check valve at the bottom of the pump head.



10. Using a 1/4-inch open-end wrench, disconnect the transducer outlet tube connection at the outlet of the transducer.
11. Disconnect the two seal-wash tubes from the sides of the pump head.

12. Supporting the pump head's weight, use the T27 TORX driver to remove the 4 screws from the support plate.



Caution: To avoid damaging the plunger, support the head from below as you remove it.

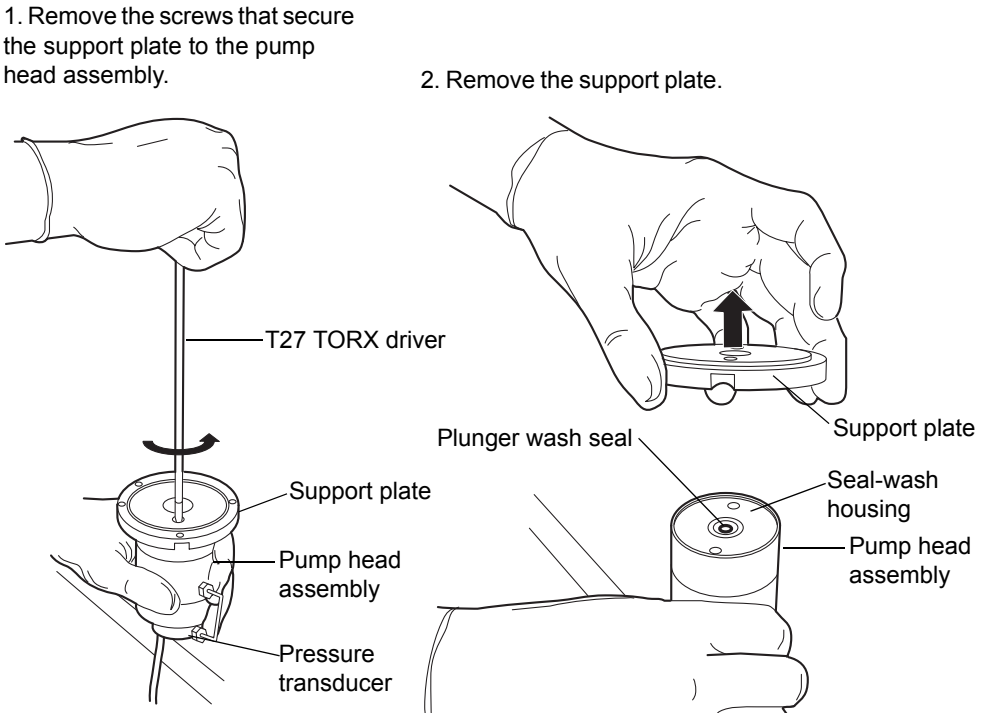
13. Gently pull the pump head assembly away from the plunger.

Caution: When you are placing the head assembly on a flat surface, take care not to damage the cable that protrudes from the transducer face.

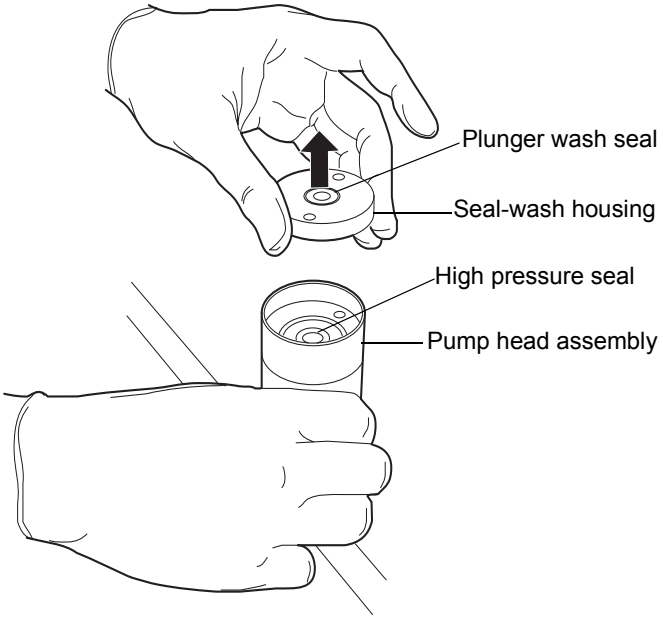
14. Place the transducer face of the head assembly on a flat work surface.

To replace the plunger seal

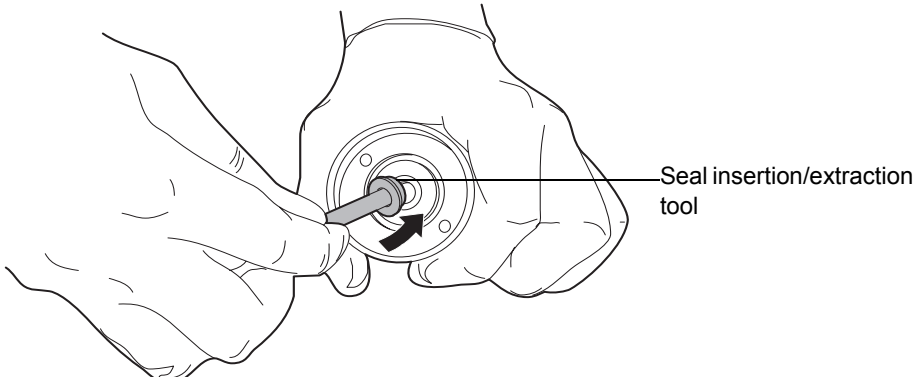
1. Use the T27 TORX driver to remove the 2 screws securing the support plate to the pump head assembly, and then remove the support plate.



2. Remove the seal-wash housing.

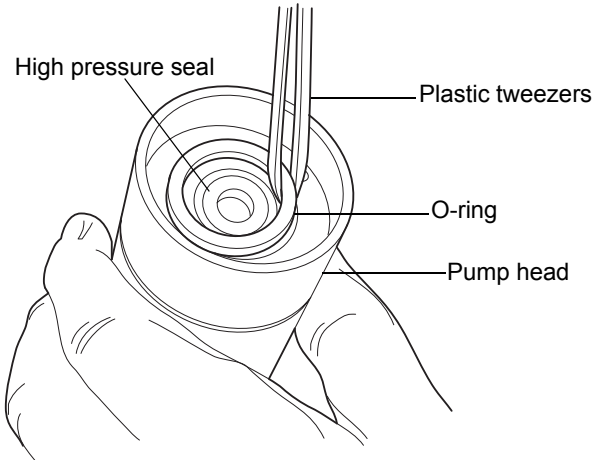


3. While holding the pump head, insert the seal insertion/extraction tool into the head, and with a rocking motion, lift the seal from the head.

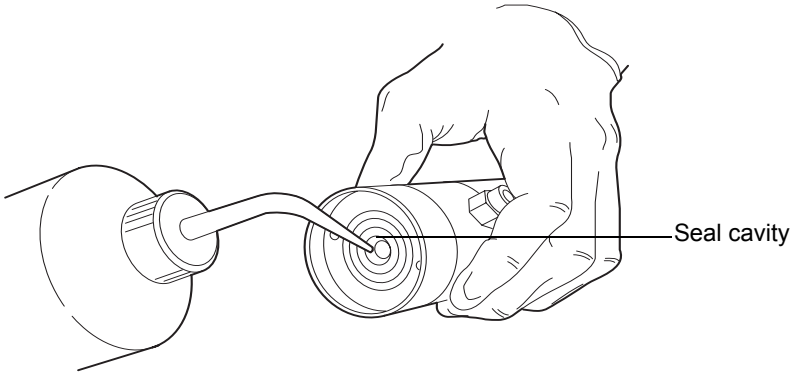


Caution: To avoid scratching surfaces, use plastic tweezers when removing the O-ring.

4. Using the plastic tweezers, remove the O-ring.

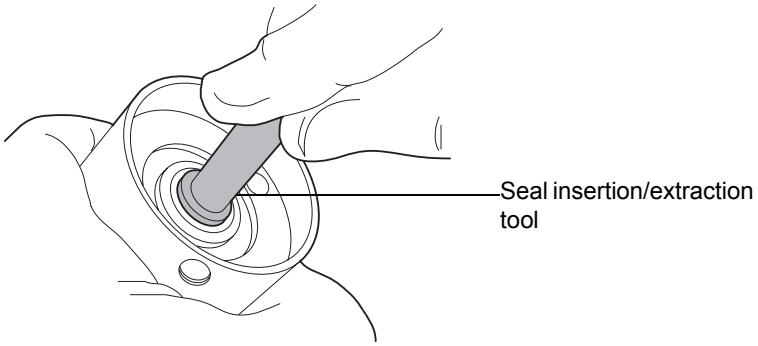


5. Wet the seal cavity and new plunger seal with methanol.

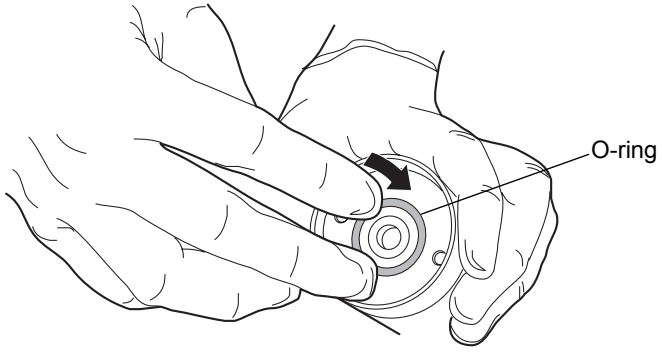


6. Using the seal insertion/extraction tool, insert the new plunger seal into the pump head.

   Requirement: Ensure the flange side of the plunger seal faces outward.

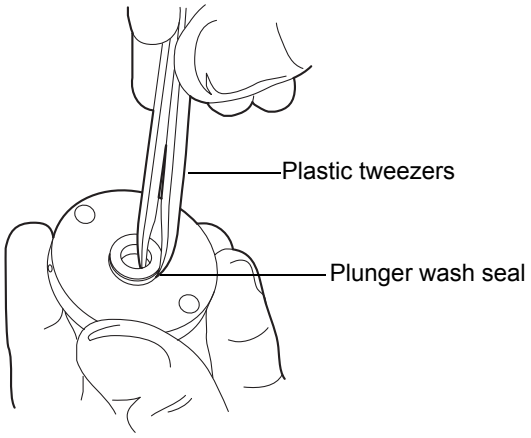


7. Insert the new O-ring into the O-ring groove in the pump head.

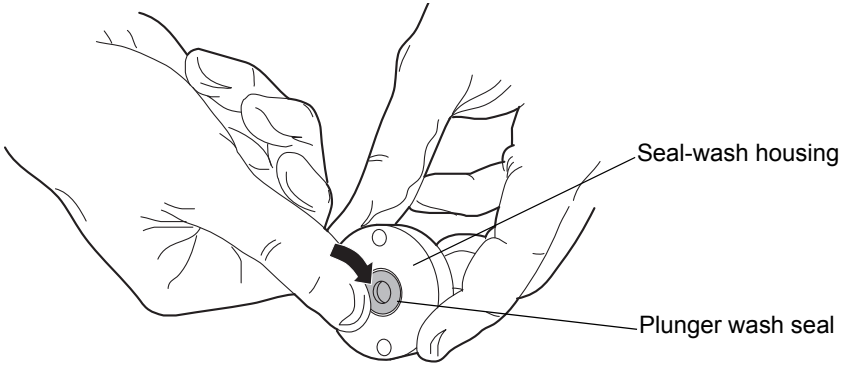


Caution: Use plastic tweezers to remove the plunger wash seal.

8. Using the plastic tweezers, remove the existing plunger wash seal.



9. Wet the new plunger wash seal with methanol, and insert it into the seal-wash housing.

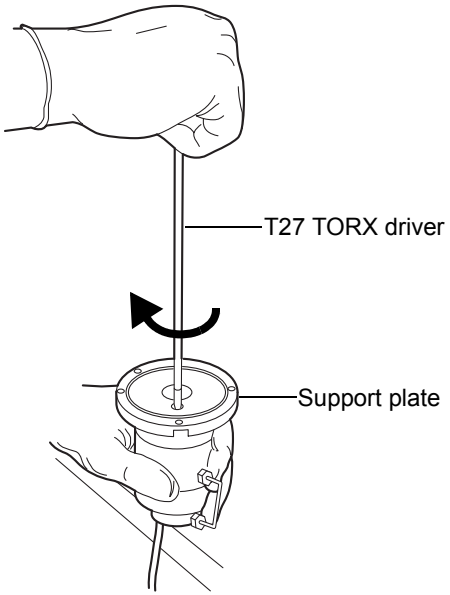


10. Insert the seal-wash assembly into the pump assembly, and align the screw hole in both assemblies.

    Requirement: The plunger wash seal must be exposed.

11. Reassemble the pump head, seal-wash assembly, and support plates, and secure them with screws tightened evenly using the T27 TORX driver.

    Requirement: Ensure that the drain fitting and check valve housing align.

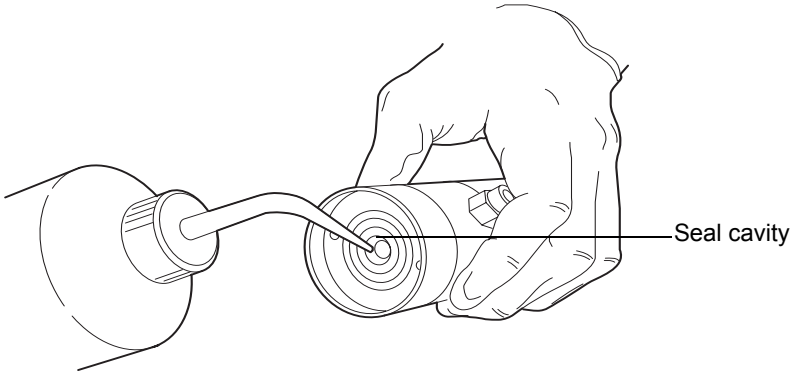


12. If you are replacing the plunger assembly, proceed to "Replacing the plunger" on page 3-27.

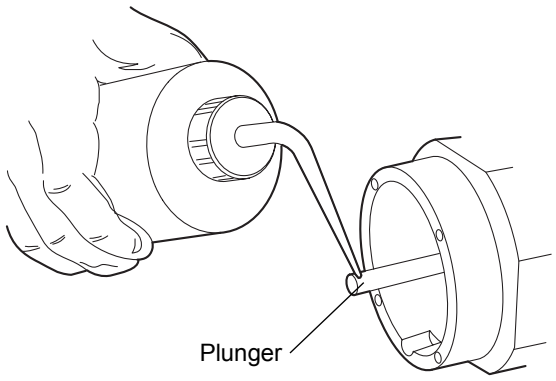
    Recommendation: Replace the plunger when you replace the head seals.

13. Wet both the seal cavity and the plunger with methanol before reinstalling the pump head.

Wetting the seal cavity



Wetting the plunger

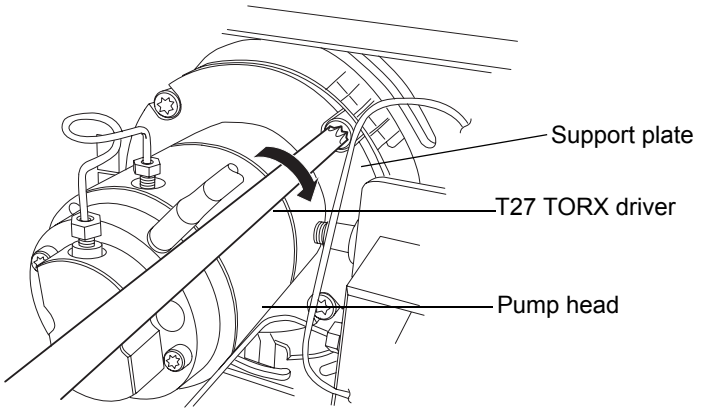


Caution: To avoid damaging the plunger, ensure it is retracted before you push the pump head onto it. See step 2 through step 5 under "To remove the pump head and seal-wash assembly" on page 3-15.

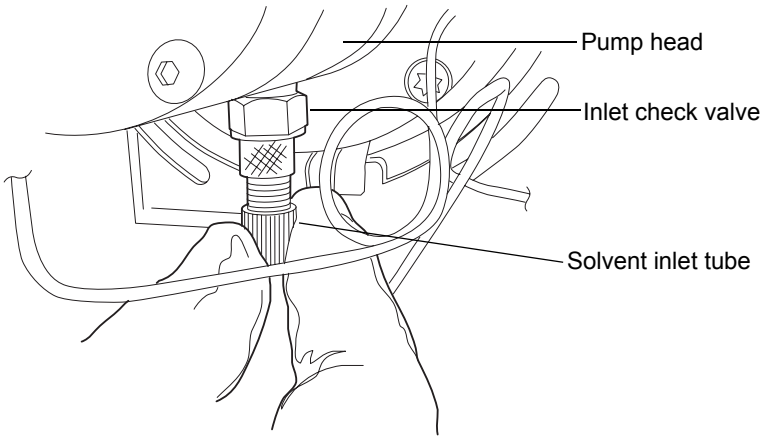
14. Carefully align the pump head with the plunger, and gently push the pump head onto the plunger until the four screw holes align with the holes in the support plate.

Caution: To avoid damaging the plunger, support the head from below as you install it.

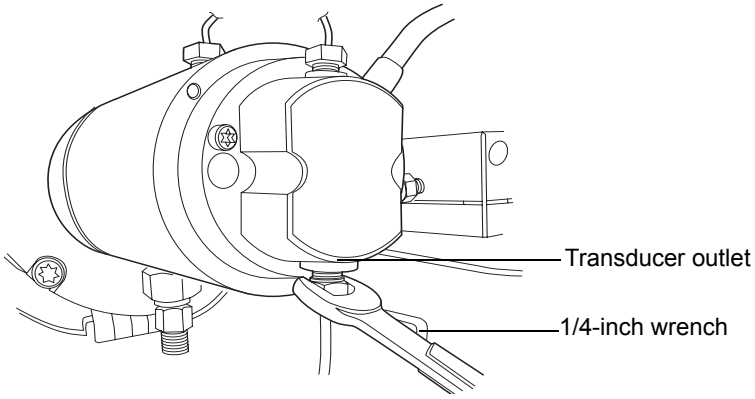
15. Supporting the pump head's weight, attach the 4 T27 TORX screws to the support plate, and tighten them 1/8-turn beyond finger-tight. Tighten the screws evenly.



16. Reattach all fittings and seal-wash tubes. Use pliers to attach the seal-wash tubes.
17. Connect the solvent inlet tube to the inlet check valve at the bottom of the pump head.



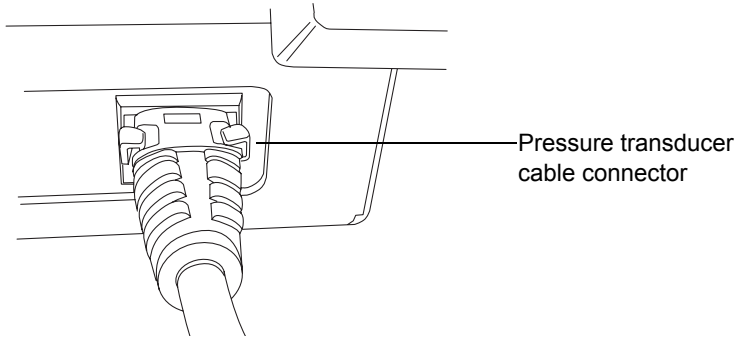
18. Reconnect the transducer outlet tube to the transducer outlet, using a 1/4-inch open-end wrench.



19. Connect the pressure transducer cable to the bulkhead.

    Requirement: The left-hand pressure cable connects to the leftmost port. The right-hand pressure cable connects to the rightmost port.

    Tip: The two center ports are empty.



20. Perform a wet prime to draw solvent into the plunger cavity.

Replacing the plunger

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

Recommendation: Replace the head seals when you replace the plunger.

Required materials

- Open-end wrenches, 1/4-inch and 13/16-inch
- T27 TORX driver
- Gloves: clean, powder-free, chemical-resistant
- Grease pack
- Methanol (or other suitably miscible solvent) in a wash bottle
- Plunger assembly

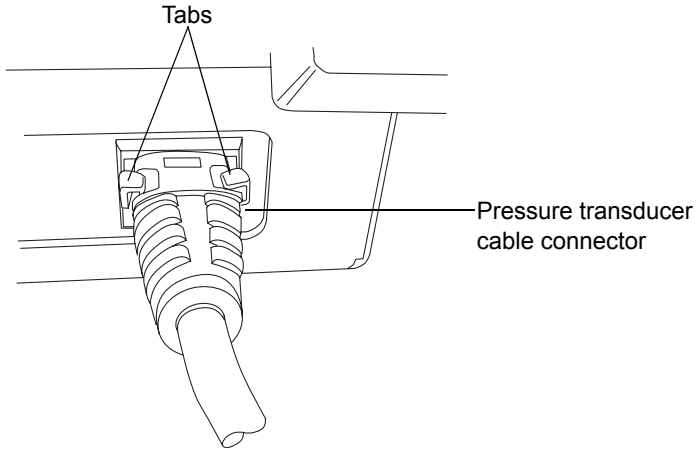
To remove the pump head

1. Flush the QGM with non-hazardous solvent.
2. In the console, select Quaternary Gradient Module from the instrument tree.
3. In the Quaternary Gradient Module window, click Maintain > Heads.
4. In the Head Maintenance dialog box, select the pump head (left or right) that you plan to perform maintenance on.
5. Click Move Plunger Forward, and then wait for the plunger to stop.

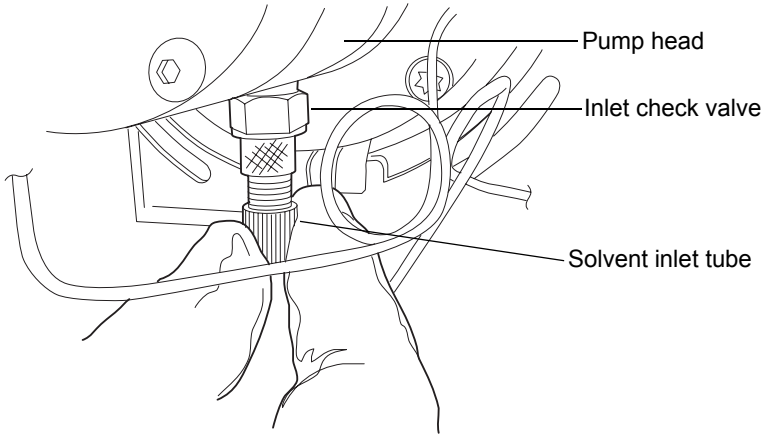
Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

6. Power-off the QGM.
7. Relocate the solvent bottles below the QGM.

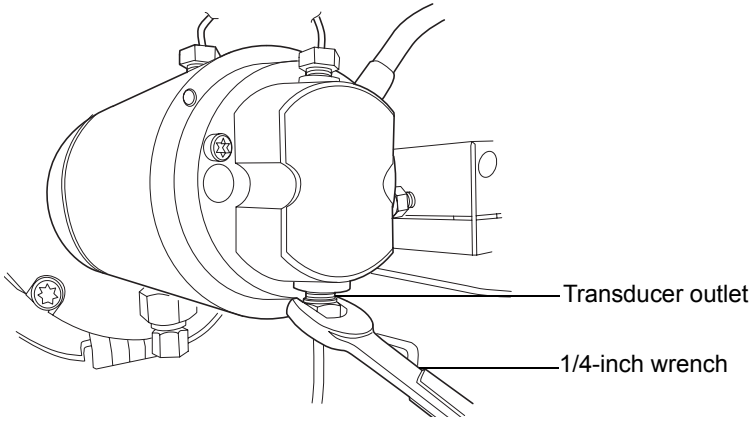
   Rationale: Relocating the solvent bottles below the QGM avoids solvent siphoning.
8. Squeeze the tabs of the pressure transducer cable, and pull gently, disconnecting the cable from the bulkhead.



9. Disconnect the solvent inlet tube from the inlet check valve at the bottom of the pump head.

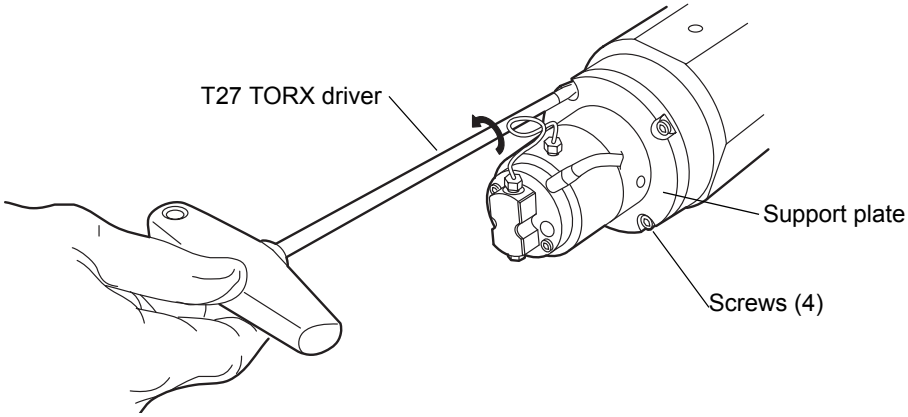


10. Using the 1/4-inch open-end wrench, disconnect the tube connection at the outlet of the transducer.



11. Disconnect the two seal-wash tubes from the sides of the pump head.

12. Supporting the pump head's weight, use the T27 TORX driver to remove the 4 screws from the support plate.



Caution: To avoid damaging the plunger, support the head from below as you remove it.

13. Gently separate the pump head assembly from the housing.

Caution: When you are placing the head assembly on a flat surface, take care not to damage the cable that protrudes from the transducer face.

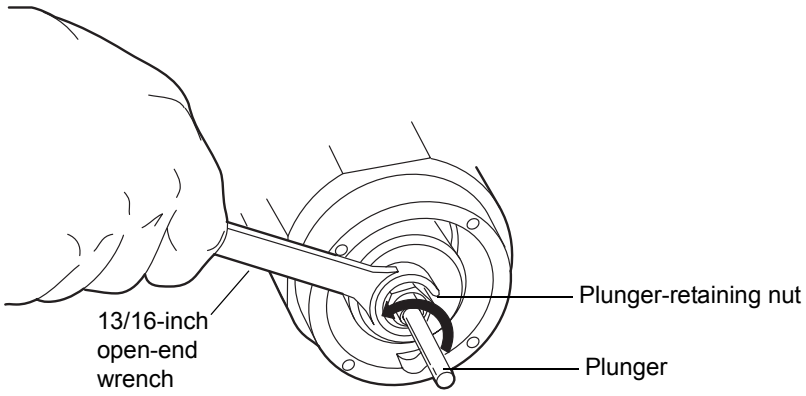
14. Place the transducer face of the head assembly on a flat work surface.

To replace the plunger

1. Stand the head assembly upright on a clean surface, and set it aside.

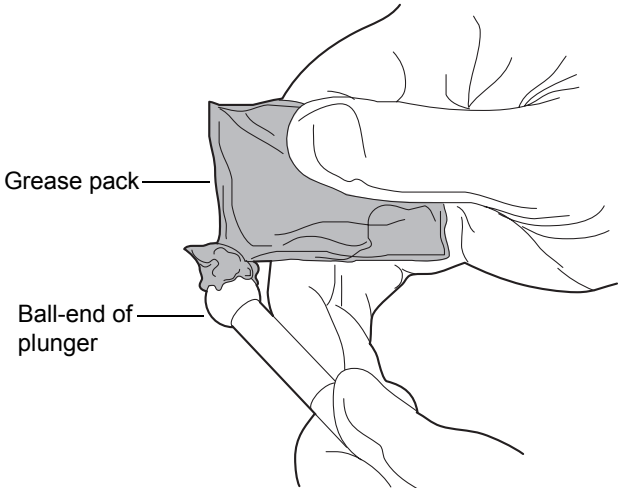
   Recommendation: Replace the head seals when you replace the plunger.

2. With the plunger in its forward position, carefully slide the 13/16-inch open-end wrench over the plunger, loosen its retaining nut, and remove the plunger.



3. Using the grease pack supplied with the new plunger, apply grease to the ball-end of the plunger.

   Tip: Be careful not to get grease on the plunger or your gloves.



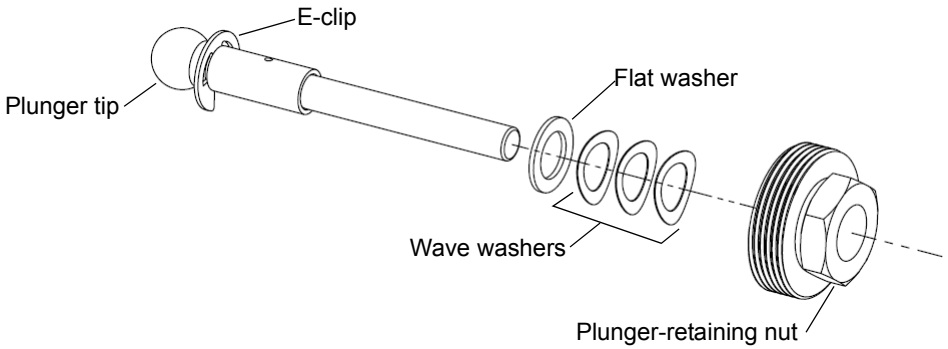
4. For the 2535 and 2545 QGM, install the flat washer and then the 3 wave washers, in order stated.

   Requirement: Ensure the wave washers are bowed toward the plunger tip.

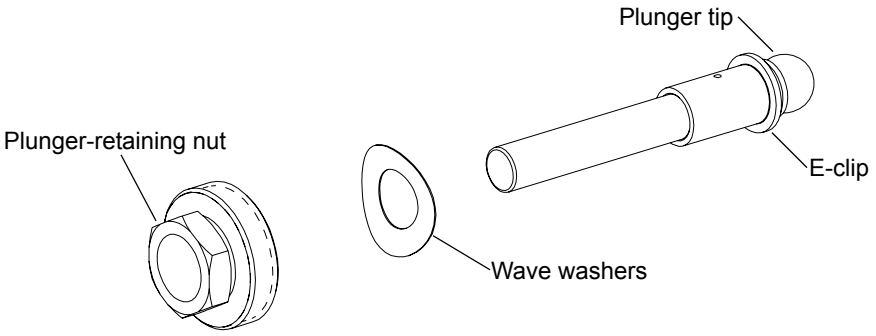
   For the 2555 QGM, install the flat washer and 1 wave washer.

   Requirement: Ensure the wave washer is bowed toward the plunger tip.

2535 and 2545 QGM plunger



2555 QGM plunger



Caution: Avoid damaging the plunger when sliding the wrench over it.

5. Insert the new plunger into the actuator piston, and tighten the plunger-retaining nut with a 13/16-inch open-end wrench.
6. Align the new plunger so that it points straight out from the piston.

   Tip: Aligning the plunger facilitates installing the head without damaging the plunger.

Reinstalling the pump head

To reinstall the pump head

1. In the console, select Quaternary Gradient Module from the instrument tree.
2. In the Quaternary Gradient Module window, click Maintain > Heads.
3. In the Head Maintenance dialog box, select the pump head (left or right) to work on.
4. Click Move Plunger Back, and then wait for the plunger to stop.
5. Lubricate the plunger seals (inside the pump head) with methanol immediately before reinstalling the pump head.

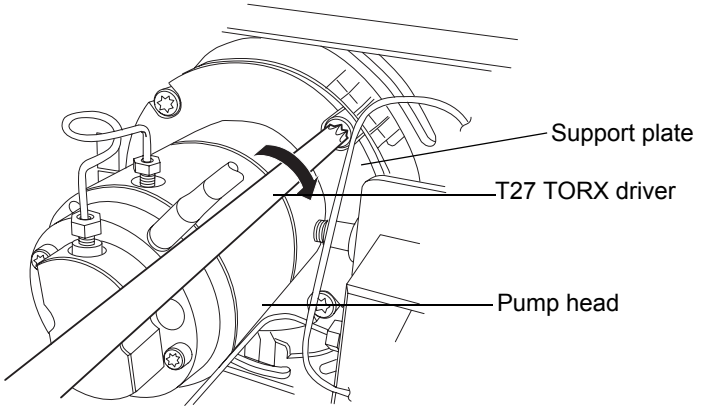
   Caution: To avoid damaging the plunger, ensure it is retracted and aligned with the pump head before installing it. See step 2 through step 5 under "To remove the pump head and seal-wash assembly" on page 3-15.

6. Orient the pump head so that the inlet check valve is at the bottom.

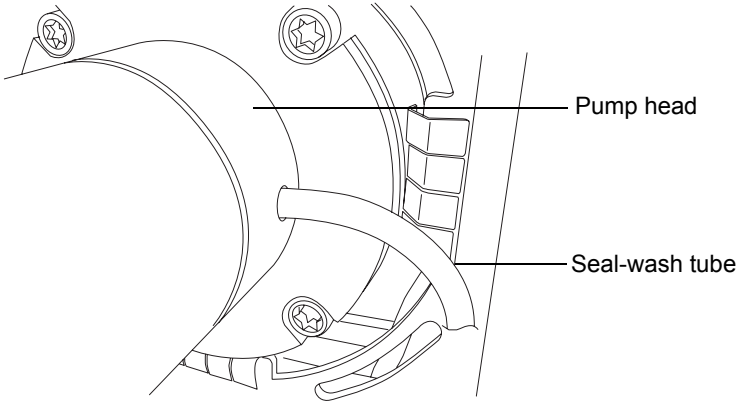
7. Carefully align the pump head with the plunger, and gently push the pump head onto the plunger until the 4 screw holes align with the holes in the support plate.

Caution: To avoid damaging the plunger, support the head from below as you install it.

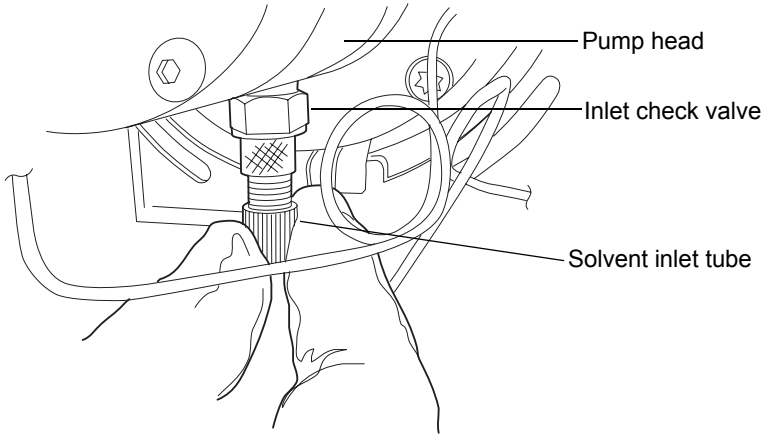
8. Supporting the weight of the pump head, use the T27 TORX driver to tighten the 4 screws in the support plate 1/8 of a turn beyond finger-tight. Tighten the screws evenly.



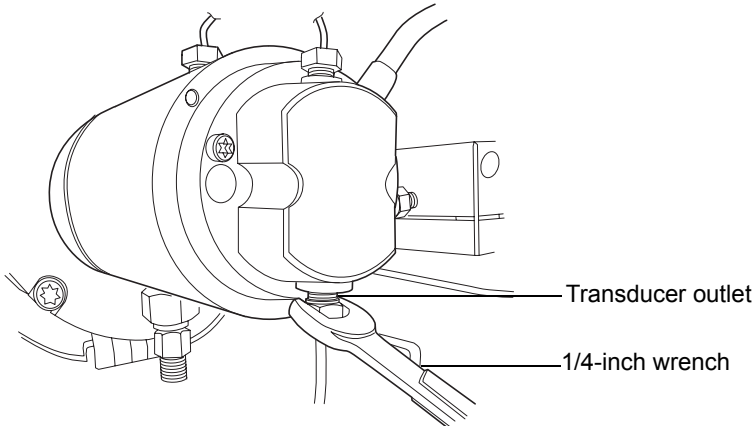
9. Reattach the 2 seal-wash tubes to their fittings at the sides of the pump head.



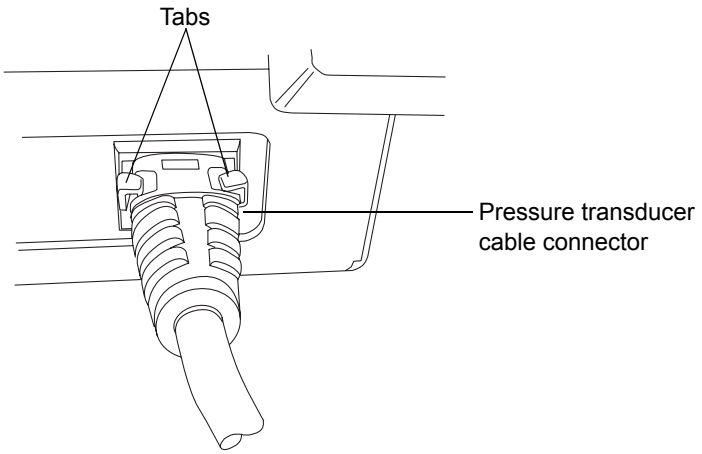
10. Connect the solvent inlet tube to the inlet check valve at the bottom of the pump head.



11. Reconnect the transducer outlet tube to the transducer outlet, using a 1/4-inch open-end wrench.



12. Reconnect the transducer cable to the appropriate connector on the front panel, making sure it clicks into place.



13. Prime the QGM (see page 2-16).

Replacing an inlet check valve cartridge

The QGM has one inlet check valve per pump head. The check valves, made of synthetic ruby and sapphire, are contained in easy-to-replace cartridges.

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

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

Required materials

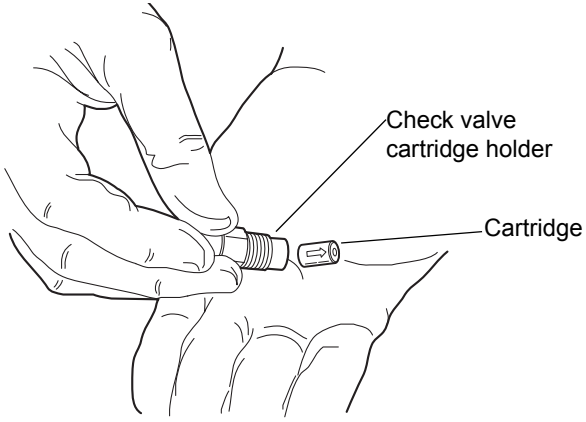
- Open-end wrench, 1/2-inch
- Gloves: clean, powder-free, chemical-resistant
- Methanol (or other suitably miscible solvent) in a wash bottle
- Replacement check valve cartridge
- Tweezers, plastic

To replace an inlet check valve cartridge

1. Remove the tube fitting at the inlet check valve cartridge holder.
2. Use the 1/2-inch open-end wrench to remove the check valve cartridge holder from the head.

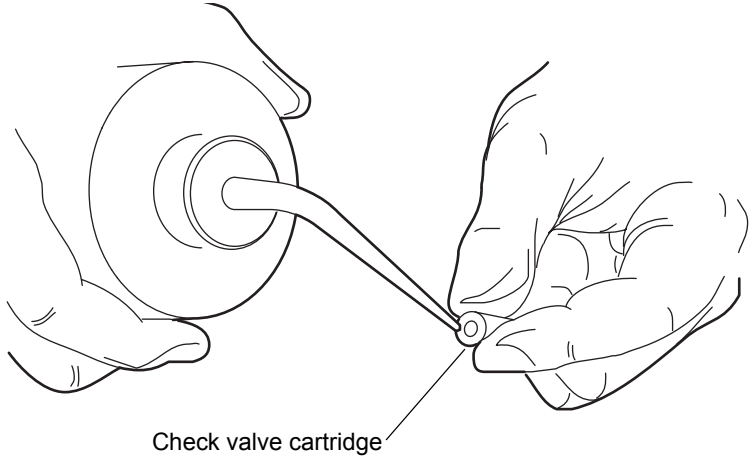
   Caution: Avoid scratching any surfaces when removing the old cartridge. Use the plastic tweezers to free stuck cartridges.

3. Invert the check valve cartridge holder to remove the old cartridge.

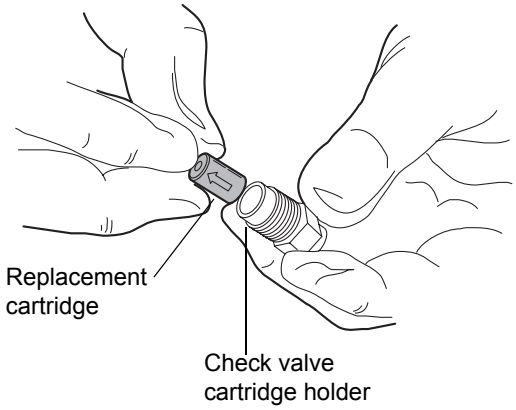


4. Inspect the check valve cartridge holder, and clean it if necessary. Rinse the components with methanol or an appropriate solvent.

5. Wet the new check valve cartridge with an appropriately miscible solvent.

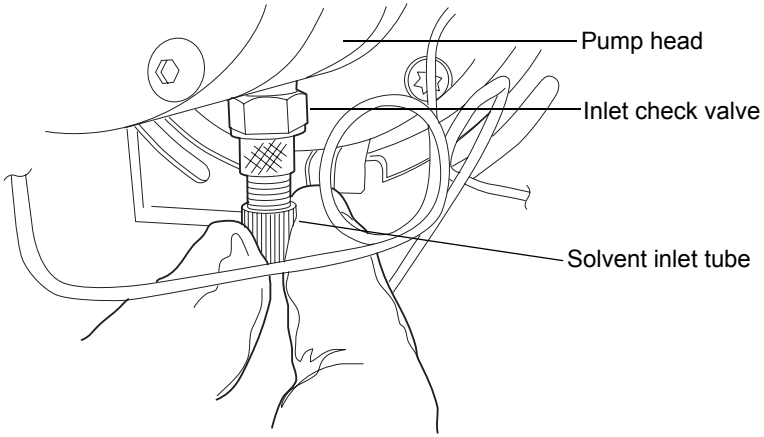


6. Insert the replacement cartridge into the check valve cartridge holder with the arrow pointed away from the hex nut.



7. Insert the check valve cartridge holder in the head, and finger tighten the fitting.
8. Use the 1/2-inch open-end wrench to tighten the check valve cartridge holder 1/8-turn beyond finger-tight.

9. Reattach the fitting from the solvent inlet tube to the check valve cartridge holder.



10. Prime the QGM (see page 2-16).

Replacing an outlet check valve cartridge

Within the module's flow path are two outlet check valves per pump manifold. The check valves, made of synthetic ruby and sapphire, are contained in easy-to-replace cartridges.

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

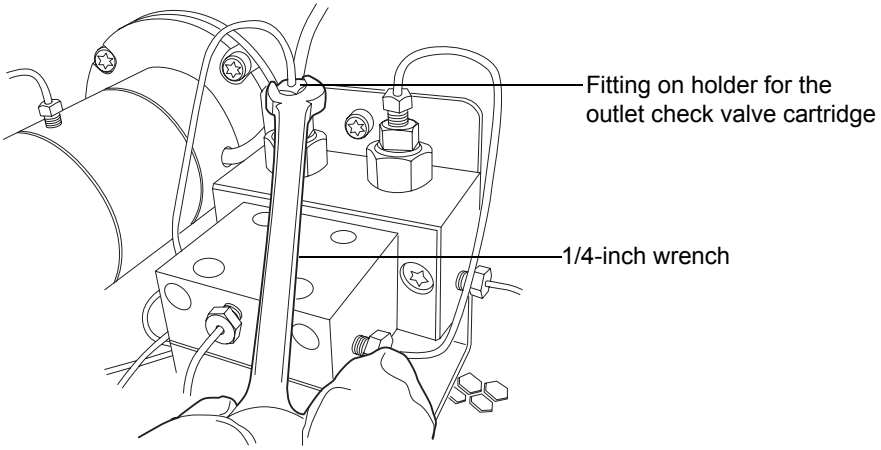
Warning: The outlet check valve cartridge can be contaminated with biohazardous and/or toxic materials. Always wear clean, powder-free, chemical-resistant gloves when performing this procedure.

Required materials

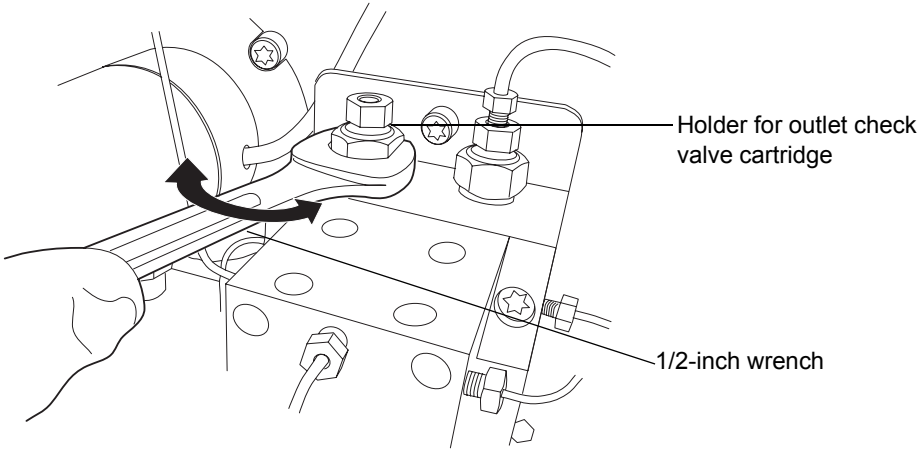
- Open-end wrenches, 1/2-inch, 1/4-inch
- Gloves: clean, powder-free, chemical-resistant
- Methanol (or other suitably miscible solvent) in a wash bottle
- Replacement check valve cartridge
- Tweezers, plastic

To replace an outlet check valve cartridge

1. Using the 1/4-inch open-end wrench, remove the fitting on the holder for the outlet check valve cartridge.

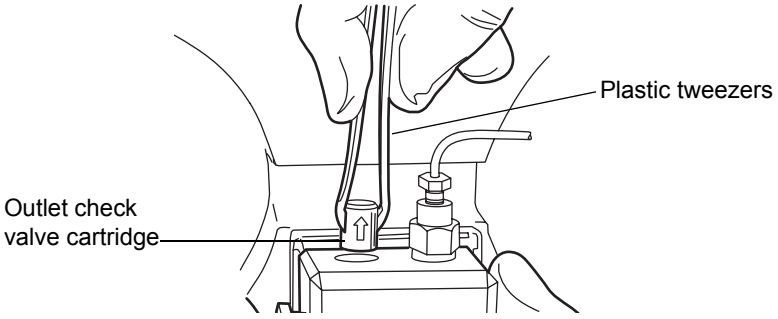


2. Use the 1/2-inch, open-end wrench to remove the holder for the outlet check valve cartridge from the manifold.



Caution: To avoid damaging the manifold, always use plastic tweezers to remove the outlet check valve cartridge.

3. Use the plastic tweezers to remove the existing outlet check valve cartridge from inside the manifold.



4. Inspect the holder for the check valve cartridge, and clean it, if necessary, rinsing the components with methanol or an appropriate solvent.

5. Wet the replacement check valve cartridge with an appropriately miscible solvent.

6. Insert the replacement cartridge into the holder, with the arrow pointed upward.
7. Insert the cartridge holder in the manifold, and finger tighten the fitting.
8. Use the 1/2-inch wrench to tighten the cartridge holder 1/8-turn beyond finger-tight.
9. Using the 1/4-inch open-end wrench, reinstall the fitting on the cartridge holder. Tighten the fitting 1/4-turn beyond finger-tight.
10. If the solvent tubes are empty, manually prime them to push solvent into the pump head (see page 2-18).
11. Prime the QGM (see page 2-16).

Replacing the 2535 QGM's selector-valve rotor-seal

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

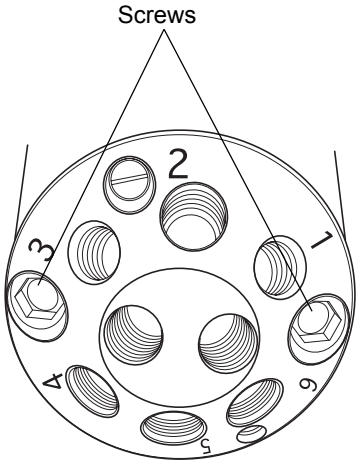
Warning: The selector-valve rotor-seal can be contaminated with biohazardous and/or toxic materials. Always wear clean, powder-free, chemical-resistant gloves when performing this procedure.

Required materials

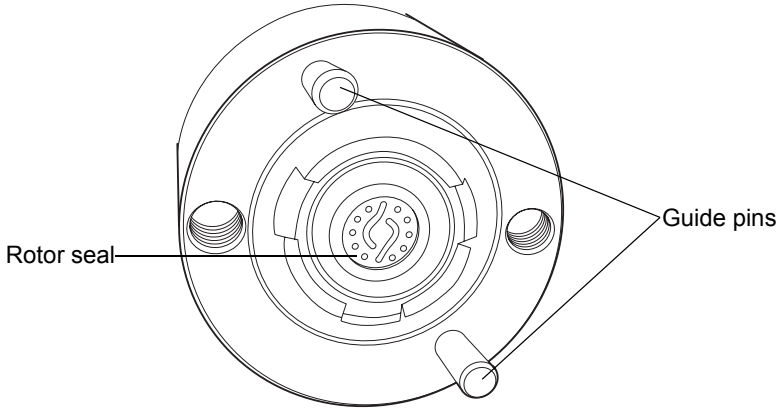
- Allen wrench, 9/64-inch
- Gloves: clean, powder-free, chemical-resistant
- Tweezers, plastic
- Selector-valve rotor-seal

To replace the selector-valve rotor-seal on the 2535 QGM

1. Using the 9/64-inch Allen wrench, remove the two screws on the front of the selector-valve, and then remove the stator.



2. Using the plastic tweezers, remove the rotor-seal from inside the valve.



3. Place the new rotor-seal into the valve, aligning the key with the slot in the actuator.

   Tip: The rotor-seal only fits into the valve one way.

4. Refit the stator onto the guide pins on the front of the valve.

   Tip: The stator only fits on the front of the valve one way.

5. Tighten the two 9/64-inch Allen screws on the front of the selector-valve until you encounter some resistance, then alternate tightening each screw 1/8-turn until it bottoms out.
6. Prime the QGM.

Replacing the 2545 and 2555 QGM's vent-valve rotor-seal

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

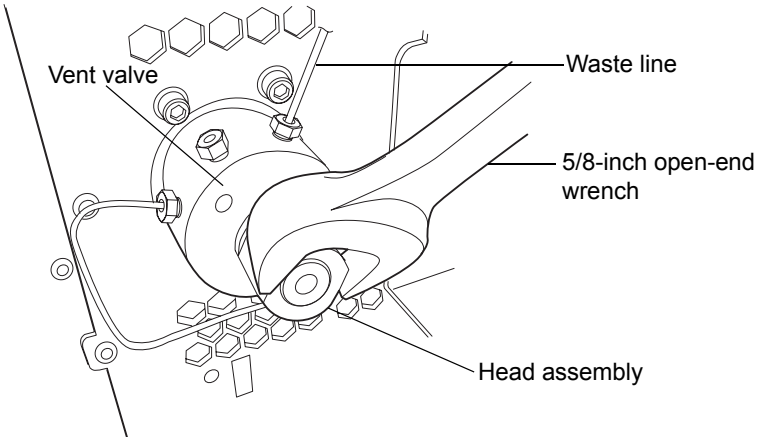
Warning: The vent-valve rotor-seal can be contaminated with biohazardous and/or toxic materials. Always wear clean, powder-free, chemical-resistant gloves when performing this procedure.

Required materials

- Open-end wrench, 5/8-inch
- Gloves: clean, powder-free, chemical-resistant
- Tweezers, plastic
- Vent-valve rotor-seal

To replace the vent-valve rotor-seal on the 2545 QGM and 2555 QGM

1. Using the 5/8-inch open-end wrench, loosen the head assembly at the front of the vent valve, and then remove it.



2. Using the plastic tweezers, remove the rotor-seal assembly from inside the valve.

   Tip: If the rotor sticks inside the valve, change the valve state between "system" and "vent" several times.

3. Place the new rotor-seal into the valve, aligning the key with the slot in the actuator.

   Tip: The rotor orientation does not matter as long as the key aligns with the slot.

4. Refit the preload assembly onto the front of the valve, and finger tighten it until it stops turning. When the preload assembly stops turning, the rotor-seal pressure or preload is correct.

5. Prime the QGM.

Replacing solvent filters

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

Caution:

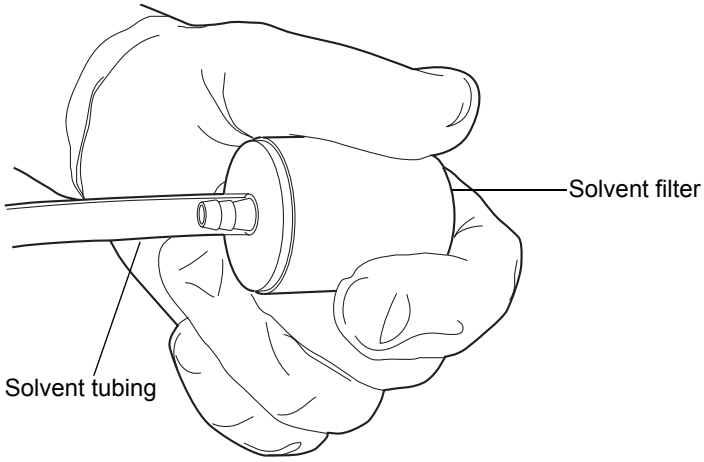
- Wear clean, powder-free, chemical-resistant gloves when handling the solvent filter. Oil from your hands can contaminate the solvent filter.
- If you must reduce flow restriction when operating at high flow rates or when using viscous solvents, remove the solvent reservoir filters. Note, however, that doing so can introduce particulates into the fluid path, reducing the life of components such as check valves and seals.

Required materials

- Gloves: clean, powder-free, chemical-resistant
- 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 solvent tubing.
3. Insert the new solvent filter into 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 QGM (see page 2-16).

Performing the static leak test

If you replaced the seals, run the QGM at close to the highest system pressure for 30 minutes before performing the static leak test.

Tip: Do not substitute the leak test for a qualification test. The purpose of the leak test is simply to identify failed components when a QGM malfunctions.

To perform the static leak test

1. Make sure all fittings on the QGM are tight, and then prime all the solvents for a least 3 minutes.
2. In the console, select Quaternary Gradient Module from the instrument tree.

3. In the Quaternary Gradient Module window, click Maintain > Static Leak Test.
4. In the Test Parameters area, specify your typical operation pressure and select the solvent.

   Range:

   - 3,450 to 34,500 kPa (34.5 to 345 bar, 500 to 5,000 psi), for the 2535 and 2545 QGM
   - 3,450 to 17,240 kPa (34.5 to 172.4 bar, 500 to 2500 psi), for the 2555 QGM

   Default:

   - 34,500 kPa (345 bar, 5,000 psi), for the 2535 and 2545 QGM
   - 17,240 kPa (172.4 bar, 2500 psi), for the 2555 QGM
5. Click Start.

   Result: The test time appears in the Run Time bar graph. When the test ends, the Results pane appears.
6. Evaluate the results for each pump head as follows:
   - The main test criterion is the leak rate. A rate of 300 psi/min or less for each pump head indicates a successful or passed test.
   - Other criteria for the static leak test include percent of stroke and highest pressure.

   Tip: The test is performed first for the left head and then for the right head.
7. Click Close.

Resolving leak problems

To resolve leaks

1. Inspect all fittings, tubing, and the vent-valve rotor.
2. If leaks persist, inspect the check valves and pump seals.

3. If the right and left pump heads fail, evidencing the same decay rate, a leak occurring in the areas common to both pump heads, is likely:
   - Tubing between the outlet check valve and vent valve
   - Fittings between the outlet check valve and vent valve
   - Vent-valve rotor

Replacing the fuses

Warning: To avoid electric shock, power-off and unplug the QGM before examining the fuses. For continued protection against fire, replace fuses only with those of the same type and rating indicated on the module.

Suspect an open or otherwise defective fuse when these three failures occur:

- The module fails to power-on
- The module's status LEDs are unlit
- The power-supply's fan does not operate

Required materials

- 10 A, 5 × 20 mm, slow-blow, IEC type fuse (2)
- Small screwdriver

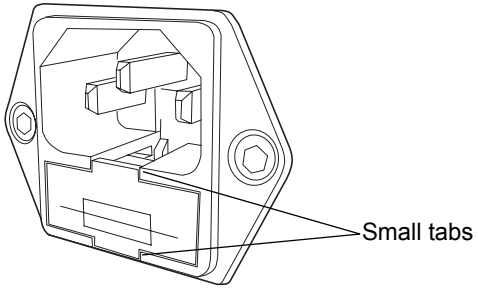
To replace the fuses

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 the QGM. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds thereafter before you disconnect an assembly.

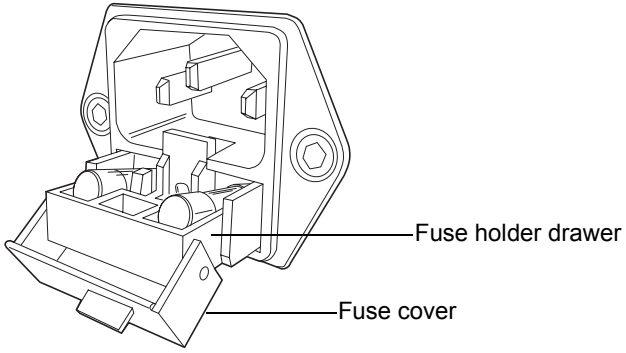
1. Power-off the QGM.
2. Disconnect the power cord from the power entry module.

3. Grasp the two small tabs on the fuse holder drawer and pull the drawer out.

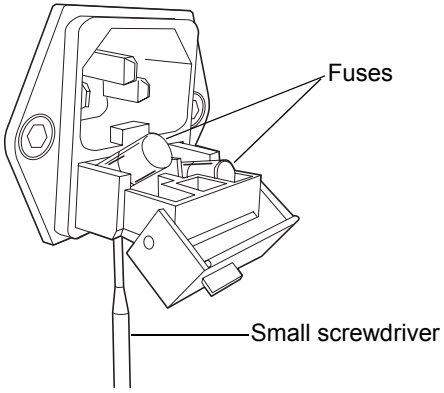


Note: The fuse holder drawer can only be partially withdrawn.

4. Open the fuse cover.



5. Insert the tip of a small screwdriver through the hole at the bottom of the fuse holder drawer under each fuse, and push each fuse up.



6. Discard the fuses.

   Warning: For continued protection against fire, replace fuses with those of the appropriate type and rating.

7. Insert the new fuses into the holders and the holders into the fuse holder drawer.
8. Close the fuse holder drawer cover and fuse holder drawer.
9. 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 QGM.

4 Troubleshooting

Contents

Proper operating procedures

So that your system always runs optimally, follow the operating procedures and guidelines in Chapters 2 and 3. Contact Waters Technical Service to report malfunctions or other problems. (See page 3-2).

System troubleshooting

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

Follow these basic steps to perform system troubleshooting:

- Examine the system, and consider the likely causes of a problem. For example, unresponsive instruments can mean that power or signal cables are disconnected or connected improperly.
- Compare the system's performance with its performance before the problem arose. When a system is installed, document parameters and operating conditions, establishing a performance baseline. Likewise, in a daily log, document system performance. You must know whether the parameters specified when tuning a system approximate those specified for a previous test sample. If they do not, and the settings needed to achieve optimum sensitivity and a good result differ significantly from those previously specified, some part of the system requires attention.
- Using known samples, preferably the ones used for instrument acceptance, measure the performance of individual instruments. To identify atypical parameters, methodically investigate and eliminate possible causes of a problem:
  - System pressure (high, low, erratic)
  - Baseline (flow path or electronics problem)
  - Changes in peak retention time (incorrect or changing over time)
  - Loss of peak resolution

  - Abnormal peak shape (smaller than expected, broad, tailing, and so on)
  - Incorrect qualitative or quantitative results
- Consult the troubleshooting information in this chapter's tables to identify potential causes of a problem and find corrective actions. If you isolate a problem to a system instrument or device other than the QGM, consult the operator's guide for that instrument.

Troubleshooting mechanical, electrical, and electronic components

This table contains suggestions for resolving hardware problems in the QGM. If the suggested solutions do not solve the problem, contact Waters Technical Service.

Requirement: Consult the column's care and use instructions before performing any of the corrective actions listed in this table.

Troubleshooting the hardware

Symptom	Possible Cause	Corrective Action
Communication problems	Configuration problem	Verify Ethernet configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.
Display is blank	Unit not powered on	Power-on unit.
	Startup diagnostics failure	Power off and on again. If failure persists, contact Waters Technical Service (see page 3-2).
	Contrast set too dark	Press contrast button (see page 2-2).

Troubleshooting the hardware (Continued)

Symptom	Possible Cause	Corrective Action
Erratic flow rate or pressures	Air bubble in pump head	1. Ensure solvent reservoir contains solvent. 2. Ensure that solvent filters are submerged in solvent. 3. Inspect for external leaks. 4. Prime the QGM (see page 2-16).
	Faulty inlet check valve cartridge	1. Prime the QGM (see page 2-16). 2. Perform the leak test to identify faulty check valve (see console online Help). 3. Replace faulty inlet check valve cartridge (see page 3-36).
	Prime/vent valve open or leaking	Close or rebuild prime/vent valve.
	Plugged solvent reservoir filter	Clean or replace filter (see page 3-46).
	Plunger seal leaking	Replace plunger seal assembly (see page 3-14).
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
	Backpressure too low.	Apply a small amount of backpressure on the flow cell using a restrictor tube to generate a minimum backpressure of 4140 kPa (41.4 bar, 600 psi).

Troubleshooting the hardware (Continued)

Symptom	Possible Cause	Corrective Action
Fans do not run	Open (spent) or defective fuse	Contact Waters Technical Service (see page 3-2).
	Power supply fan wiring or motor problem	1. Power-off and power-on again. 2. If the problem persists, contact Waters Technical Service (see page 3-2).
	Power supply not working	1. Power-off and power-on again. 2. If the problem persists, contact Waters Technical Service (see page 3-2).
Front panel display fails to illuminate	Broken electrical connection	Inspect electrical connections.
	Open (spent) or defective fuse	Contact Waters Technical Service (see page 3-2).
	Bad LCD or control board	Contact Waters Technical Service (see page 3-2).
	Faulty power supply	Contact Waters Technical Service (see page 3-2).
Front panel displays odd characters	Faulty EPROMs or bad LCD control board	Contact Waters Technical Service (see page 3-2).
Leak detected in external drip tray	Leak in QGM.	Follow the procedure to resolve a quaternary gradient module leak sensor error (see page 3-4).

Troubleshooting the hardware (Continued)

Symptom	Possible Cause	Corrective Action
Leak detected in internal drip tray	Leak in QGM.	1. Power-off the QGM. 2. Place the solvent supply bottles on the floor to reduce pump head pressure. 3. Contact Waters Technical Service (see page 3-2)).
Priming problems; will not start flow	Solvent connect tubes may be leaking	1. Ensure solvent reservoir contains solvent. 2. Ensure that solvent filters are submerged in solvent. 3. Inspect for external leaks. 4. Inspect solvent connect tubes. 5. Perform the leak test (see console online Help).
	Pump heads not primed	Prime pump heads.
	Solvent tube may be defective	Switch to another solvent tube (if you are using tube A, switch to tube B).
	Defective check valve	Replace the check valve cartridge.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).

Troubleshooting the hardware (Continued)

Symptom	Possible Cause	Corrective Action
Unit does not power-on	Power cord not connected	Check power cord.
	No power at outlet	Test line voltage.
	Power supply fuse blown or missing	Replace power supply fuses.

Chromatography troubleshooting

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the QGM. Consult the Material Safety Data sheets for the physical and chemical properties of solvents you use.

The table below lists chromatographic symptoms, possible causes, and suggested corrective actions for the QGM.

Before you consult the table, read the General Troubleshooting section, and follow the basic troubleshooting steps to isolate the cause of the chromatographic symptom.

Requirement: Consult the column's care and use instructions before performing any of the corrective actions listed in this table.

Chromatography troubleshooting

Symptom	Possible cause	Corrective action
Baseline drift, rapid	Column not equilibrated	Equilibrate column.
	Detector not allowed to warm up	Allow detector to warm up until baseline is stable. Time varies with wavelength and sensitivity.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Baseline drift, rapid (continued)	Solvent contaminated	Use fresh solvent.
	Flow fluctuations (rapid or slow drift)	Prime pump, replace pump seals, check valves (see "Erratic retention times" symptom later in this table).
	Incorrect wavelength for solvent	Ensure that solvent does not have absorbance at the wavelength used.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Baseline drift, slow	Solvent contaminated	Use fresh solvent.
	Decreased UV lamp energy	Test lamp energy using detector diagnostics.
	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration.
	UV detector flow cell leaking (internal, cross-port)	Inspect flow cell, tighten connections.
	Dirty flow cell	Clean flow cell.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Baseline noise cycling, short term (30 to 60 seconds)	Flow fluctuating	Stabilize flow by inspecting for leaks (see "Erratic retention times" symptom later in this table).

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Baseline noise cycling, short term (30 to 60 seconds) (continued)	Insufficient gradient mixing.	Add gradient mixing (see page 2-20).
	Radio frequency noise (short- or long-term cycling)	Eliminate interference.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Baseline noise cycling, long-term (approximately 10 minutes to 1 hour)	Ambient temperature fluctuations	Stabilize ambient temperature.
	Insufficient gradient mixing.	Add gradient mixing (see page 2-20).
	Faulty integrator or recorder	Inspect integrator or recorder for excessive baseline noise.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Baseline noise, random	Air in detector	Flush detector at a higher flow rate to remove air. Apply a small amount of backpressure on the flow cell using a restrictor tube.
	Air in system	Reprime the solvent management system.
	Solvents contaminated	Use fresh solvent.
	Column contaminated	Clean or replace column.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Baseline noise, random (continued)	Dirty flow cell	Clean flow cell.
	Analog output cable not properly connected between QGM and data system, recorder, or integrator	Properly connect cable.
	System improperly grounded	Plug into outlet on different electrical circuit.
		Use power conditioner.
	Recorder voltage incorrect	Set recorder to correct voltage.
	Unit not cooling properly	Operate unit with covers in place. Ensure there is adequate air space behind it.
	Radio frequency noise	Eliminate interference.
	Defective detector	Troubleshoot detector.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
	Insufficient gradient mixing.	Add gradient mixing (see page 2-20).
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.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Decreased retention times (continued)	Column contaminated.	Clean or replace column.
	Incorrect column.	Use correct column.
	Sample diluent is stronger than the initial mobile phase.	• Dilute sample in a weaker solution. • Inject less.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
	Insufficient gradient mixing.	Add gradient mixing (see page 2-20).
Erratic retention times	Air bubble in pump head.	Prime QGM.
	Malfunctioning check valves.	Clean or replace inlet check valve cartridges.
	Leaking plunger seals.	Replace seals (see page 3-14).
	Clogged solvent filters.	Replace filters.
	Equilibration time following a gradient run is insufficient.	Specify a longer equilibration time.
	Temperature fluctuations.	Control column temperature.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Flat baseline (no peaks)	No solvent flow	Verify flow rate.
	Detector lamp not ignited	Use detector diagnostics to test reference/sample energy. Zero energy indicates lamp not ignited.
		Power-on lamp. If this does not resolve the problem, replace the lamp.
	Detector not zeroed	Zero the detector baseline.
	Improper connection between detector and recorder	Inspect cabling between the detector and the recorder.
	Incorrect detector wavelength	Verify wavelength setting.
	Leak in solvent path	Inspect fittings.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Flat-topped peaks	Detector not zeroed	Zero the detector baseline.
	Incorrect recorder input voltage	Adjust recorder input voltage, or adjust detector output cable to correct position.
	Detector sensitivity too high	Select a less sensitive detection range.
	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).	• Inspect fittings for leaks. • Perform static decay test (see console online Help).
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
	Sparging of mixed solvents.	Use external vacuum degassing for pre-mixed solvents.
Increase in system pressure	Column in-line filter is plugged.	Replace the filter in the in-line column filter unit.
	Column is plugged.	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 your column care and use instructions. 3. If symptom persists, replace the column.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Increase in system pressure (continued)	Tubing is plugged. Defective gradient proportioning valve.	Systematically inspect tubing by making and breaking connections. Contact Waters Technical Service (see page 3-2).
Loss of column efficiency	System not stabilized or chemically equilibrated.	Equilibrate column using a minimum of 5× column volumes and 3× system volumes of the mobile phase to be used. When running an automated gradient method, ensure you specify sufficient and reproducible equilibration times between injections.
	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes.
	Temperature fluctuations.	Control column temperature.
	Column is dirty, defective, or contaminated.	1. Clean the column using the cleaning and regeneration procedures described in your column care and use instructions. 2. If symptom persists, replace the column.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Reproducibility errors	Incorrect chemistry/integration.	Review chemistry and integration.
	Sample management system problem.	Troubleshoot sample management system.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Reproducibility errors (continued)	Incorrect wash solvents or volumes.	Change wash solvents and/or volumes.
	Incorrect volume range for injection method or type.	Change volume range.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).
Sample energy decreased, reference energy not decreased	Contaminated mobile phase	Use fresh mobile phase.
	Dirty flow cell	Clean outside of flow cell windows.
		Flush system with water. If necessary, remove the column, then flush the system with stronger solvent to remove particulate or filmy matter from flow cell windows.
Sensitivity loss	Leak in QGM.	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.
	Peaks wider than expected	Troubleshoot sample management system. Inspect fittings for leaks and proper ferrule seating. Inspect tube ID.
	Incorrect filter constant	Set correct filter constant on detector.

Chromatography troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Sensitivity loss (continued)	Change in mobile phase composition	Correct mobile phase pH or ionic composition.
	Leak in detector flow cell	Repair leak. If problem persists, replace detector flow cell.
	Incorrect flow rate	Set correct flow rate.
	Defective gradient proportioning valve.	Contact Waters Technical Service (see page 3-2).

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

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.)

Warning: (Risk of eye injury.)

Specific warnings

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

Biohazard warning

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

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

Chemical hazard warning

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

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

Caution symbol

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

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

Warnings that apply to all Waters instruments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

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

警告：当有压力的情况下使用管线时，小心注意以下几点：

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

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

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

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

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

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

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

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

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

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

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

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

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

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

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

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 boite 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。

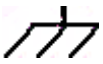
경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

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

Physical specifications

Attribute	Specification
Height	43.2 cm (17 inches)
Depth	66.0 cm (26 inches), without drip tray
Width	28.0 cm (11 inches)
Weight	39.5 kg (87 pounds), without solvent or sparge tubing

Environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	20 to 80%, noncondensing
Shipping and storage temperature	-40 to 60 °C (-40 to 140 °F)
Shipping and storage humidity	10 to 90%, noncondensing
Acoustic noise	<60 dBA

Electrical specifications

Attribute	Specification
Protection class[a]	Class I
Overvoltage category[b]	II
Pollution degree[c]	2
Moisture protection[d]	Normal (IPXO)

Electrical specifications (Continued)

Attribute	Specification
⚠ Line voltages, nominal	Grounded AC
Power requirements	6.5 A
Line voltage	100 to 240 VAC ±10%
Frequency	50/60 Hz
Fuse	10 A, 5 × 20 mm, slow-blow, IEC type
Power consumption	650 W

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 may produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally non-conductive 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.

Operation specifications

Attribute	Specification
Four event outputs	Maximum current: 0.5A per contact Maximum voltage: 30 Vdc Maximum contact resistance: 0.2 ohms
Four event inputs	Input voltage: ±30 Vdc Logic high: >3.0 Vdc, ±10% Logic low: <1.9 Vdc, ±10% Minimum pulse width: 100 milliseconds
Two analog outputs	Output voltage range: 0 to 2.0 Vdc Minimum resolution: 12 bits

2535 QGM performance specifications

Attribute	Specification
Number of eluents	One to four
Modes of operation	Gradient, isocratic, and flow programming
Operating flow range	Programmable from 0 to 50 mL/min, in 0.01 increments
Flow accuracy	±1.0% of required flow. Flow rate 1.0 to 50.0 mL/min using water or methanol, backpressure approximately 4140 kPa (41.4 bar, 600 psi).
Composition range	0 to 100% programmable in 0.1% increments for each of four reservoirs: A, B, C, and D. Total composition must sum to 100%.
Composition accuracy	±3.0% absolute (full scale) from 1 to 50 mL/min using water/water or methanol/methanol, backpressure approximately 4140 kPa (41.4 bar, 600 psi).

2535 QGM performance specifications (Continued)

Attribute	Specification
Composition precision	Retention time variation 0.15 min SD, based on 6 repeat injections. Test conditions: Mobile phases: 70:30 water/methanol dial-a-mix (possibly use methanol and no modifiers). Flow rates (mL/min): 1, 5, 20, 50 Sample: Uracil/caffeine Injection volumes (µL): 1, 5, 20, 100 Columns (XTerra, Prep, MS, C18): 7.8×50, 19×50, 30×50 No column heating
Composition curves	Eleven gradient curves including linear, step [2], concave [4], and convex [4].
Automatic eluent sparging (no blanketing or pressurized containers)	Helium, input gas pressure range of 350 to 1040 kPa (3.5 to 10.4 bar, 50 to 150 psi). Helium flow rate range of 0 to 100%, programmable in 1% increments.
Maximum operating pressure	41,400 kPa (414 bar, 6000 psi) up to 50 mL/min.
Programmable pressure limits	Lower: 0 to 41,400 kPa (414 bar, 6000 psi) Higher: 0 to 41,400 kPa (414 bar, 6000 psi)

2535 QGM performance specifications (Continued)

Attribute	Specification
Delay volume (dwell volume)	Small-scale: <6.0 mL, measured to the outlet of the pump at 1 mL/min, 50% point of the step gradient. Large-scale: <13.0 mL, measured to the outlet of the pump at 10 mL/min, 50% point of the step gradient.
Pump seal wash	Pump and seals required to wash the rear of the high pressure seal (pulsed type flow, about 20 µL per pulse).
Solvent and seal-wash containers	User selectable. Compatible with pressurized containers up to 34 kPa (0.34 bar, 5 psi) maximum.
Priming	Ability to self prime from a solvent container on the floor, without operator intervention, when the pump has wet pump heads and dry tubes.
Audible noise	<60 dB(A), measured as average dB(A) noise over 1 minute at 25 mL/min, 50/50 composition.
User programmable flow path selection feature	Fourth position on vent valve for a large-scale mixer into the flow path. User programmable from the pump method editor. The vent valve positions are as follows: • Closed–Flow is blocked, typically used for pressure test diagnostics. • Vent–Flow is routed to waste, typically used for priming. • Small–Flow is routed from the 1.1-ml mixer tee directly to the pump outlet. • Large–Flow is routed from the 1.1-ml mixer tee through an additional 7-ml mixer and then to the pump outlet.

2545 QGM performance specifications

Attribute	Specification
Number of eluents	One to four
Modes of operation	Gradient, isocratic, and flow programming
Operating flow range	Programmable from 0 to 150 mL/min, in 0.01 increments
Flow accuracy	±1.0% of required flow. Flow rate 1.0 to 150.0 mL/min using water or methanol, backpressure approximately 4140 kPa (41.4 bar, 600 psi).
Composition range	0 to 100% programmable in 0.1% increments for each of four reservoirs: A, B, C, and D. Total composition must sum to 100%.
Composition accuracy	±3.0% absolute (full scale) from 1 to 150 mL/min using water/water or methanol/methanol, backpressure approximately 4140 kPa (41.4 bar, 600 psi).

2545 QGM performance specifications (Continued)

Attribute	Specification
Composition precision	Retention time variation 0.15 min SD, based on 6 repeat injections. Test conditions: Mobile phases: 70:30 water/methanol dial-a-mix (possibly use methanol and no modifiers). Flow rates (mL/min): 1, 5, 20, 50, 150 Sample: Uracil/caffeine Injection volumes (µL): 1, 5, 20, 100, 500 Columns (XTerra, Prep, MS, C18): 3.9×100, 7.8×50, 19×50, 30×50, 50×50 No column heating
Composition curves	Eleven gradient curves including linear, step [2], concave [4], and convex [4].
Automatic eluent sparging (no blanketing or pressurized containers)	Helium, input gas pressure range of 350 to 1040 kPa (3.5 to 10.4 bar, 50 to 150 psi). Helium flow rate range of 0 to 100%, programmable in 1% increments.
Maximum operating pressure	41,400 kPa (414 bar, 6000 psi) to 100 mL/min (linear roll-off) to 34,500 kPa (345 bar, 5000 psi), at 150 mL/min.
Programmable pressure limits	Lower: 0 to 41,400 kPa (414 bar, 6000 psi) Higher: 0 to 41,400 kPa (414 bar, 6000 psi)

2545 QGM performance specifications (Continued)

Attribute	Specification
Delay volume (dwell volume)	<6.5 mL, measured to the outlet of the pump at 1 mL/min, 50% point of the step gradient.
Pump seal wash	Pump and seals required to wash the rear of the high pressure seal (pulsed type flow, about 20 µL per pulse).
Solvent and seal-wash containers	User selectable. Compatible with pressurized containers up to 34 kPa (0.34 bar, 5 psi) maximum.
Priming	Ability to self prime from a solvent container on the floor, without operator intervention, when the pump has wet pump heads and dry tubes.
Audible noise	<60 dB(A), measured as average dB(A) noise over 1 minute at 75 mL/min, 50/50 composition.

2555 QGM performance specifications

Attribute	Specification
Number of eluents	One to four
Modes of operation	Gradient, isocratic, and flow programming
Operating flow range	Programmable from 0 to 300 mL/min, in 0.01 increments
Flow accuracy	±1.0% of required flow. Flow rate 4.0 to 300.0 mL/min using water or methanol, backpressure approximately 4140 kPa (41.4 bar, 600 psi).

2555 QGM performance specifications (Continued)

Attribute	Specification
Composition range	0 to 100% programmable in 0.1% increments for each of four reservoirs: A, B, C, and D. Total composition must sum to 100%.
Composition accuracy	±3.0% absolute (full scale) from 4 to 260 mL/min using water/water or methanol/methanol spiked with dye or marker, backpressure approximately 4140 kPa (41.4 bar, 600 psi).
Composition precision	Retention time variation 0.15 min SD, based on 6 repeat injections. Test conditions: Mobile phases: 70:30 water/methanol dial-a-mix (possibly use methanol and no modifiers). Flow rates (mL/min): 5, 20, 50, 150, 300 Sample: Uracil/caffeine Injection volumes (µL): 5, 20, 100, 500 Columns (XTerra, Prep, MS, C18): 7.8×50, 19×50, 30×50, 50×50 respectively No column heating
Composition curves	Eleven gradient curves including linear, step [2], concave [4], and convex [4].
Automatic eluent sparging (no blanketing or pressurized containers)	Helium, input gas pressure range of 350 to 1040 kPa (3.5 to 10.4 bar, 50 to 150 psi). Helium flow rate range of 0 to 100%, programmable in 1% increments.

2555 QGM performance specifications (Continued)

Attribute	Specification
Maximum operating pressure	20,700 kPa (207 bar, 3000 psi) up to 200 mL/min (linear roll-off) to 17,200 kPa (172 bar, 2500 psi), at 300 mL/min.
Programmable pressure limits	Lower: 20,700 kPa (207 bar, 3000 psi) Higher: 20,700 kPa (207 bar, 3000 psi)
Delay volume (dwell volume)	<13.0 mL, measured to the outlet of the pump at 10 mL/min, 50% point of the step gradient.
Pump seal wash	Pump and seals required to wash the rear of the high pressure seal (pulsed type flow, about 20 µL per pulse).
Solvent and seal-wash containers	User selectable. Compatible with pressurized containers up to 34 kPa (0.34 bar, 5 psi) maximum.
Priming	Ability to self prime from a solvent container on the floor, without operator intervention, when the pump has wet pump heads and dry tubes.
Audible noise	<60 dB(A), measured as average dB(A) noise over 1 minute at 150 mL/min, 50/50 composition.

C Solvent Considerations

Warning: To avoid chemical hazards, always observe Good Laboratory Practices when operating your system.

The information in this appendix applies to the 2535, 2545, and 2555 quaternary gradient modules.

Contents

Introduction

Preventing contamination

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

Clean solvents

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

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

Solvent quality

Use HPLC-grade (or better) 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.

Recommendations:

- Use brown-tinted glassware to inhibit microbial growth.
- Filter buffered solvents with a 0.45-µm filter.
- Degas solvents.
- Keep solvents in a place free from drafts and shock.

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 0.45-µm membrane filter.

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

Using buffers

When you use buffers, dissolve salts first, adjust the pH, and then filter to remove insoluble material.

Tetrahydrofuran (THF)

When using unstabilized THF, ensure that your solvent is fresh. Previously opened bottles of THF contain peroxide contaminants, which cause baseline drift.

Warning: THF contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.

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)
–0.3	N-decane	0.92	174.1	29	—
–0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	—
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	27	—
1.8	Triethylamine	0.38	89.5	26	—
2.2	Isopropyl ether	0.33	68.3	—	220
2.3	Toluene	0.59	100.6	23	285
2.4	*P*-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3	—	—
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	—
3.9	Butanol	3.01	117.2	15	—
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—
4.8	Dioxane	1.54	101.3	17	220
5.2	Ethanol	1.20	78.3	14	210

Solvent miscibility (Continued)

Polarity index	Solvent	Viscosity cP, 20 °C (@1 atm)	Boiling point °C (@1 atm)	Miscibility number (M)	λ Cutoff (nm)
5.3	Pyridine	0.94	115.3	16	305
5.3	Nitroethane	0.68	114.0	—	—
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
7.3	Formamide	3.76	210.5	3	
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.

Buffered solvents

When using a buffer, choose good quality reagents, filtering them through a 0.45-µm membrane filter.

Always use a seal-wash solvent in which the buffer is highly soluble.

Do not leave the buffer in the system after use. Flush all fluid pathways with HPLC-quality water before shutting down the system and leave distilled water in the system (flush with 90% HPLC-quality water:10% methanol for shutdowns scheduled to be more than one day).

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), or visit www.waters.com.

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.

Mobile phase solvent degassing

Mobile phase difficulties account for many of the problems in liquid chromatography. Degassing solvents used in the mobile phase is one of the best measures to eliminate these problems. The benefits are:

- Stability in the baseline and enhanced sensitivity in some types of chromatographic detectors
- Reproducible retention times for eluting peaks
- Reproducible injection volumes
- Stable pump operation

Only a finite amount of gas is dissolved in a given volume of liquid under specific conditions. This amount depends on the temperature of the liquid, pressure on the liquid, and the chemical affinity of the gas for the liquid.

Generally, a gas is most soluble in a solvent where the intermolecular attractive forces between molecules of the solvent are similar to those of the gas (like dissolves like). If the main attractive forces are Van der Waals forces, the gas is more soluble in this solvent than in one with dipole forces or hydrogen bonding. Thus, a larger amount of helium, nitrogen, oxygen, or hydrogen dissolves in alkanes and benzene than in water.

Temperature affects the solubility of gases in two ways. First, the higher the boiling point of a gas, the more soluble the gas is in a given solvent. Second, increasing the temperature of the gas/liquid solution affects the percentage of gas in solution. If the heat of solution results in an exothermic reaction, the percentage of gas in solution reduces. If the reaction is endothermic, the percentage increases. For example, solubility of helium in water decreases with an increase in temperature, but the solubility of helium in benzene increases in direct proportion to the temperature.

The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If the gas pressure decreases, the amount of that gas in solution also decreases.

Dissolved oxygen affects UV-VIS detector performance in several ways[1]. Oxygen dissolved in solvents can form a UV absorbing complex, the amount of which is considerably different in different solvents. The effect is particularly strong with wavelengths below 260 nm. Therefore, any change in dissolved oxygen content can affect a UV baseline considerably. This phenomenon is particularly evident in the solvent tetrahydrofuran (THF). Dissolved oxygen does not seem to affect the absolute sensitivity of a UV system, but primarily causes baseline drift. This effect is especially noticeable during gradient operation where the dissolved oxygen content varies between the different solvents and, as the composition changes, causes erratic baselines or even peak-shaped artifacts on the baseline.

Dissolved oxygen in a fluorescence detector has quite a different effect. It causes a tremendous loss of sensitivity. Bowen and Williams[2] have discussed the quenching of aromatic hydrocarbons by dissolved oxygen in fluorescence detectors. Parker and Barnes[3] have reported a 95% reduction in sensitivity of the fluorescence of borate-benzoin complex in air equilibrated ethanol. The oxygen quenching varies with different types of compounds, and aromatic hydrocarbons, aliphatic aldehydes, and ketones are especially susceptible.

If for any reason the characteristics of the solvent change, the precision and accuracy of the solvent delivery system can be adversely affected. This can cause variations in both peak retention and, to some extent, peak height or area.

Solvent degassing methods

Sparging, or bubbling a gas through solvent, reduces the partial pressure of the unwanted gas by removing it from the surface of a solvent and saturating the solvent with the sparge gas. Sparging with helium removes background absorbance on a UV detector and avoids the quenching phenomenon of dissolved oxygen as it affects the performance of fluorescence detectors.

1. S. R. Bakalyar, M. B. T. Bradley, R. Hoganen, Journal of Chromatography, 158 (1978) 277.
2. E. J. Bowen and A. H. Williams, Trans. Faraday Soc., 35 (1939) 65.
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Helium sparging combines the convenience of short initial degassing time, ease of maintaining the solvent condition during operation, and complete control within the framework of the Waters preparative systems.

The degassing operation must be efficient. To remove the gas as quickly as possible, you must consider these factors:

- Helium sparging gives stable baselines and better sensitivity in a fluorescence detector, and prevents the reabsorption of atmospheric gases. Solvents sparged with helium generally are well-degassed by the time they reach the pump because helium diffuses from the solvent and into the atmosphere through the PTFE tubing connecting the reservoirs to the pump. Thus, the solvent arrives at the pump with less than 1 atmosphere partial pressure of helium.

  

  Warning: To avoid lacerations and chemical injuries, do not apply vacuum to the brown, one-gallon bottles in which solvent is shipped. THey stand a high risk of implosion.

- Vacuum degassing alone is too slow a process to be an acceptable means of degassing solvent.

For in the techniques mentioned above, except for helium sparging, the solvent reequilibrates to air saturation in 12 to 24 hours (depending on the solvent).

Degassing by vacuum or sonication, though often performed for improved pump performance in high-pressure applications, might not yield the required baseline stability for high-sensitivity absorbance detection. Therefore, the most practical solution for most applications is helium sparging which, because of helium's low solubility, does not impair pump performance for most solvents.

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)	Solvent	UV Cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Iso-octane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	*n*-Pentane	190
Cyclopentane	200	*n*-Propanol	210
Diethyl amine	275	*n*-Propyl chloride	225
Dioxane	215	Nitromethane	380
Ethanol	210	Petroleum ether	210
Ethyl acetate	256	Pyridine	330
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

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)-dimethy-lammonio]-1-propanesulfonate) (CHAPS) 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190
(Ethylenedinitrilo) tetraacetic acid disodium salt (disodium EDTA), 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
4-(2-hydroxyethyl)-1-pipera-zineethanesulfonic 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 dibasic, 10 mM	 190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

Refractive indices of common solvents

The following table lists the refractive indices for some common chromatographic solvents. Use this table to verify that the solvent you intend to use for your analysis has a refractive index (RI) significantly different from the RIs of the sample components.

Refractive indices for common chromatographic solvents

Solvent	RI	Solvent	RI
Fluoroalkanes	1.25	Tetrahydrofuran (THF)	1.408
Hexafluoroisopropanol (HFIP)	1.2752	Amyl alcohol	1.410
Methanol	1.329	Diisobutylene	1.411
Water	1.33	*n*-Decane	1.412
Acetonitrile	1.344	Amyl chloride	1.413
Ethyl ether	1.353	Dioxane	1.422
n-Pentane	1.358	Ethyl bromide	1.424
Acetone	1.359	Methylene chloride	1.424
Ethanol	1.361	Cyclohexane	1.427
Methyl acetate	1.362	Ethylene glycol	1.427
Isopropyl ether	1.368	*N,N*-Dimethyl Formamide (DMF)	1.428
Ethyl acetate	1.370	*N,N*-Dimethyl Acetamide (DMAC)	1.438
1-Pentene	1.371	Ethyl sulfide	1.442
Acetic acid	1.372	Chloroform	1.443
Isopropyl chloride	1.378	Ethylene dichloride	1.445
Isopropanol	1.38	Carbon tetrachloride	1.466
n-Propanol	1.38	Dimethyl sulfoxide (DMSO)	1.477
Methylethylketone	1.381	Toluene	1.496
Diethyl amine	1.387	Xylene	~1.50
n-Propyl chloride	1.389	Benzene	1.501
Methylisobutylketone	1.394	Pyridine	1.510

Refractive indices for common chromatographic solvents (Continued)

Solvent	RI	Solvent	RI
Nitromethane	1.394	Chlorobenzene	1.525
1-Nitropropane	1.400	*o*-Chlorophenol	1.547
Isooctane	1.404	Aniline	1.586
Cyclopentane	1.406	Carbon disulfide	1.626

Index

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