Waters Alliance Series Column Heater and Column Heater/Cooler

Operator's Guide

715003768/Revision A

Waters

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Trademarks

Customer comments

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

Contact Waters

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

Waters contact information

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

Safety considerations

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

Safety advisories

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

FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the users authority to operate the equipment. This

device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Canada spectrum management emissions notice

This class A digital product apparatus complies with Canadian ICES-003.

Cet appareil numérique de la classe A est conforme à la norme NMB-003.

Electrical power safety notice

Do not position the instrument so that it is difficult to operate the disconnecting device.

Safety hazard symbol notice

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

Equipment misuse notice

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Operate this device

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

Applicable symbols

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

Please refer to "Safety Advisories" on page 85 for a list of safety symbols which occur in emergency situations.

Audience and purpose

This guide is intended for personnel who install, operate, and maintain the Waters Alliance Series Column Heater and Column Heater/Cooler.

Intended use of the Waters Alliance Series Column Heater and Column Heater/Cooler

Waters designed the Alliance Series Column Heater and Column Heater/Cooler to maintain column temperature within a column compartment. The Alliance Series Column Heater and Column Heater/Cooler is for research use only.

Calibrate

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

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

Quality-control

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

ISM classification

ISM Classification: ISM Group 1 Class B

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

EC authorized representative

Waters Corporation (Micromass UK Ltd.)
Floats Road
Wythenshawe
Manchester M23 9LZ
United Kingdom

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

Table of Contents

1 Introduction

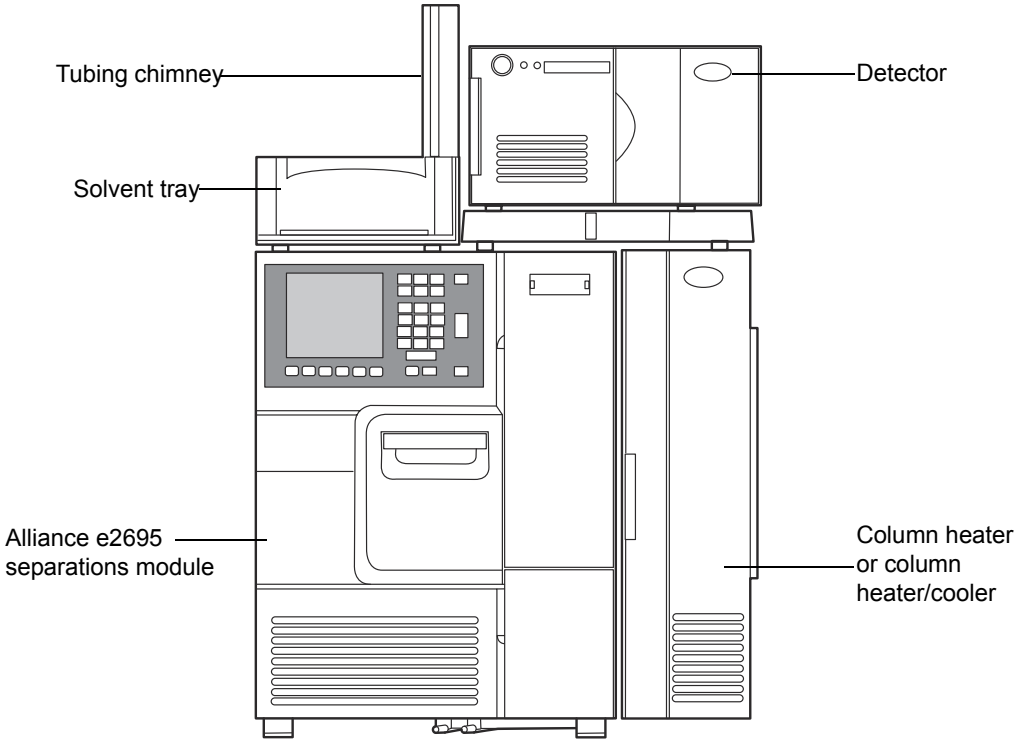
This chapter provides an overview of the Waters® Alliance® series column heater and column heater/cooler instruments for Waters separations modules.

Contents:

Overview

The Alliance series column heater (second generation) and column heater/cooler are designed to manage and maintain column temperature within the column compartment. In addition, three optional valve configurations are available that provide flow path switching and column regeneration under software control from the separations module.

Separations module with column heater or column heater/cooler:



Alliance series column heater

The Alliance series column heater (second generation) is improved from previous Waters column heaters. This design offers these features for increased performance and ease of use:

- Expanded temperature range. Refer to Appendix B for specifications.
- Adjustable column clips that slide for easy column mounting.
- Extended column compartment that allows for the use of two 300-mm columns with column guards, four 150-mm columns without column guards, or six 50-mm columns without column guards.
- The addition of a grommet in the column mounting plate for attaching a calibrated probe (thermistor) for validation.

- Single-piece column compartment door and hinge assembly that allows tubing routing to enter or exit the column compartment from all four sides.
- Improved valve tubing management that allows you to add an optional integral column switching valve without needing a visit from the field service representative, or even removing the instrument's covers.

The column heater is compatible with separations modules beginning with firmware version 2.02. You can upgrade e2690/e2695, e2690D/e2695D, or 2790/2795 separations modules operating with firmware version 2.00.

Column heater differences at a glance

The table below lists differences between the column heaters:

Column heater differences:

Item	Column heater (first generation)	Column heater (second generation)	Comments
Physical dimensions	Height = 21.75 in. (55.25 cm) Width = 6.0 in. (15.24 cm) Depth = 12.0 in. (35.6 cm)	Height = 21.75 in. (55.25 cm) Width = 6.0 in. (15.24 cm) Depth = 12.0 in. (35.6 cm)	—
Weight	17 lbs (3.65 kg)	17 lbs (3.65 kg)	—
Compartment door	Two independent doors, one for each compartment	One integrated door for both compartments	—
Column retainer hardware	Permanently affixed single spring clip	Adjustable vertical positioning, multiple column rail clips	Adjustable column clips are held in place after a column is installed.

Column heater differences: (Continued)

Item	Column heater (first generation)	Column heater (second generation)	Comments
Acceptable column size	• 8-mm ID maximum • 2 each × 300 mm • Maximum of 4 each, depending on size and use column guards	• 8-mm ID maximum • 2 each × 300 mm with column guards • 4 each × 150 mm without column guards • 6 each × 50 mm without column guards	A limit of six 50-mm columns is recommended to match the six-port valve.
Compartment operational temperature range	20 to 60 °C, with a minimum controllable temperature of at least 5 °C above ambient laboratory temperature	5 °C above ambient laboratory temperature to 65 °C, with a set point temperature range of 4 to 65 °C	This allows the heater to turn on at 9 °C if ambient room temperature is 4 °C.
Valve position	Positioned within the valve compartment.	Repositioned forward for easier access to fluidic connections.	—

Alliance series column heater/cooler

The column heater/cooler is optional for e2695, e2695D, and 2795 separations modules. This integrated column heater/cooler expands the heating temperature range over the previous column heater, while offering column cooling capabilities. The column heater/cooler retains the physical characteristics and the latest features as the new (second generation) column heater.

The column heater/cooler is compatible with Waters separations modules beginning with firmware version 2.02. Existing separations modules

operating with firmware version 2.00 can be upgraded by you or your Waters Service Representative.

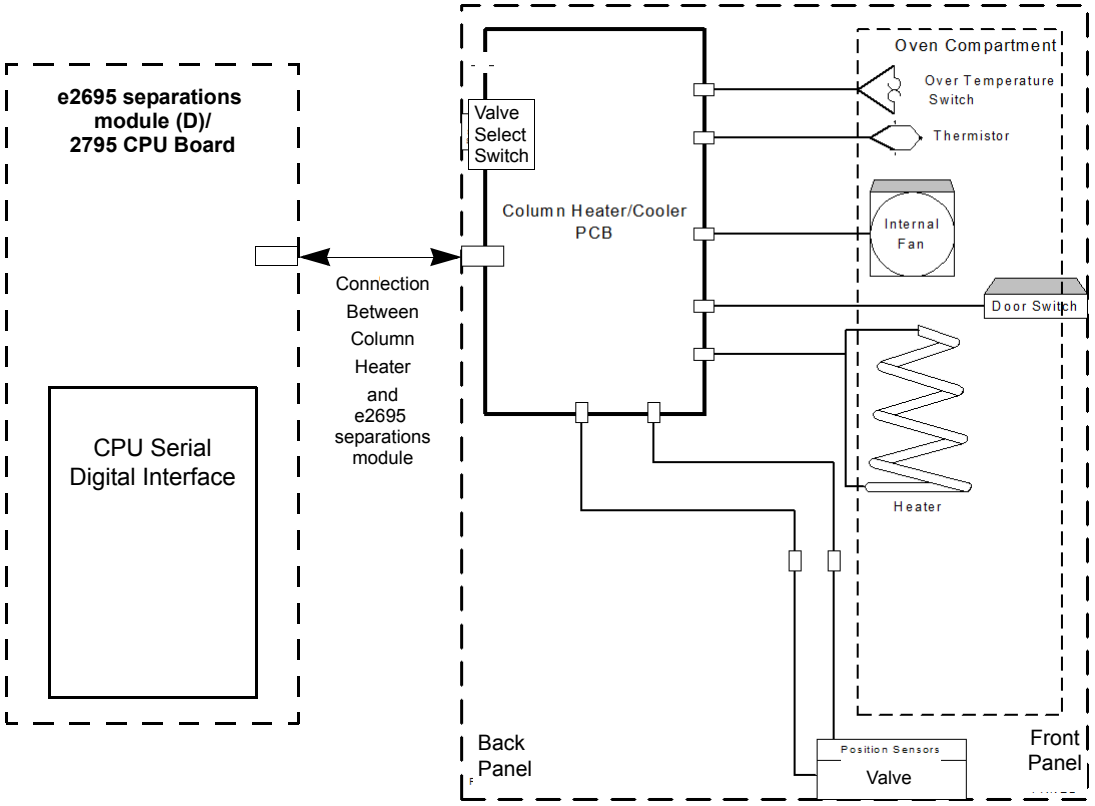
Theory of operation

The column heater and column heater/cooler are optional devices for the separations module. They control the temperature in the column compartment.

Column heater

The column heater is a forced-air convection heater. When the temperature is set at the separations module, a command is sent to a column heater CPU board that controls the temperature by turning the heater element on and off to achieve the desired column temperature.

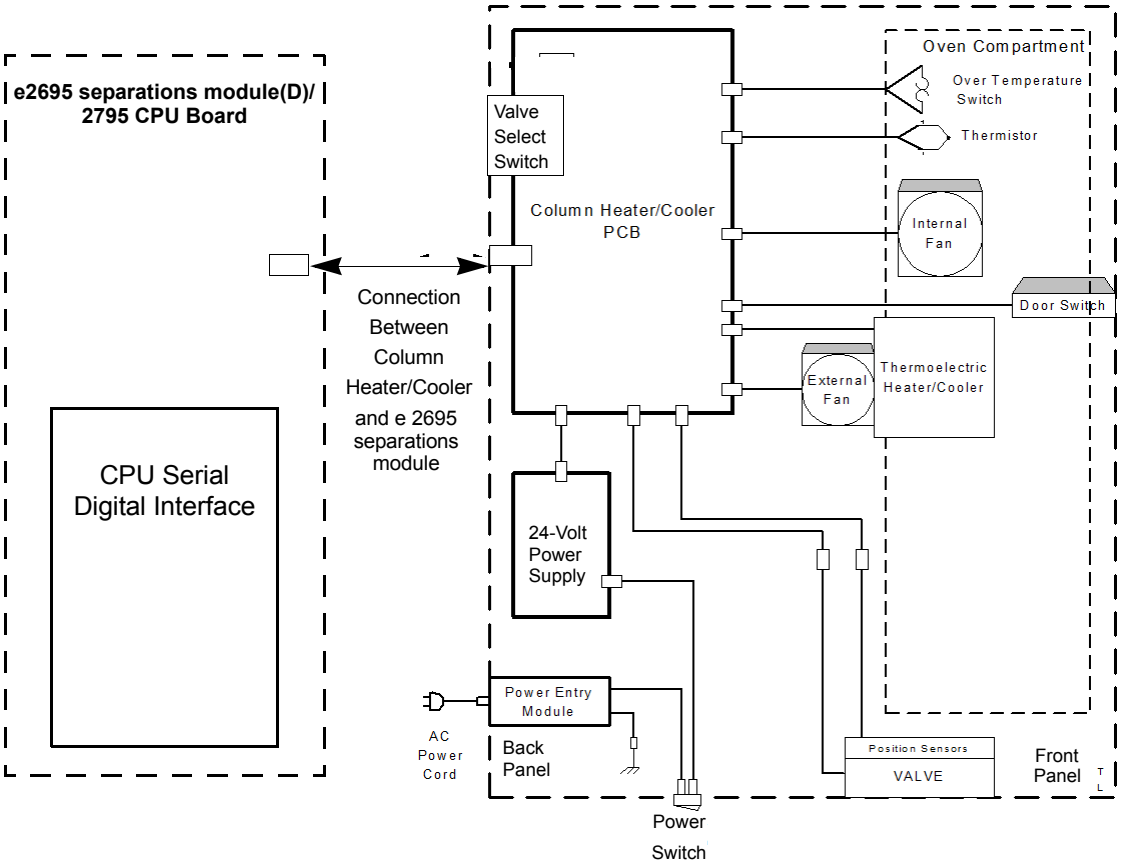
Column heater block diagram:



Column heater/cooler

The column heater/cooler is a combination forced-air convection heater and cooler. When the temperature is set at the separations module, a command is sent to a column heater/cooler CPU board that controls the temperature by applying power to the thermoelectric devices to achieve the desired column temperature. The column heater/cooler contains many of the same components as the column heater with the exception of a thermoelectric engine for heating and cooling, and the additional fan for exhausting power supply heat. In addition, the column heater/cooler has an internal onboard power supply. The power supply is necessary for additional power required to operate the thermoelectric heater/cooler engine circuit.

Column heater/cooler block diagram:



Optional column selection valves

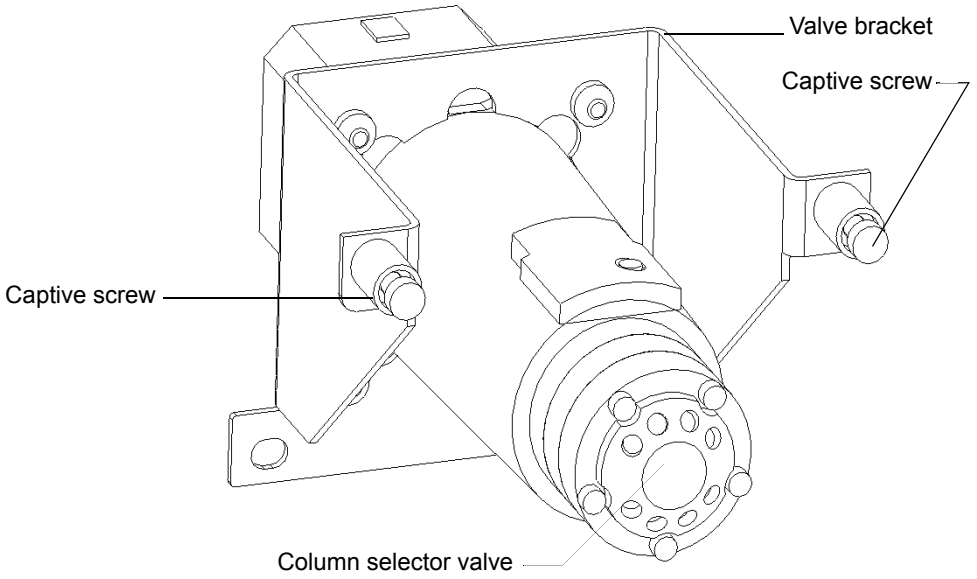
The column selection valve is motorized to enable the system to switch solvent flow to one of several columns. This optional valve is installed within the column heater or column heater/cooler.

Three optional column selection valves are available that allow you to switch the flow path among multiple columns:

- Three-column selection valve
- Six-column selection valve
- Column regeneration valve

Refer to page 29 for information on valve configurations.

Column selection valve assembly:



Tip: The optional outboard two-column switching valve (part number WAT050046) for the Alliance e2695 separations module that allows the selecting between two columns is not considered part of the optional selection valves available for the column heater or column heater/cooler. This valve option is attached externally, self-powered and controlled by event I/O directly from the separations module, and cannot be mounted inside the column heater or column heater/cooler.

2 Install the Column Heater or Column Heater/Cooler

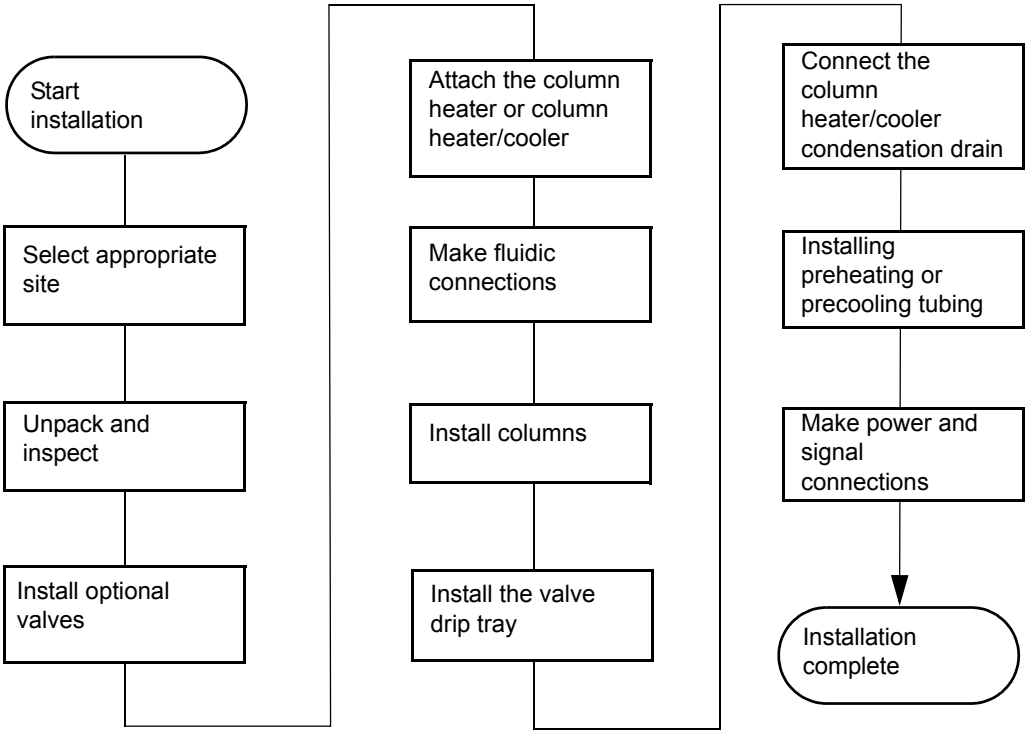
This chapter describes how to unpack and install the column heater or column heater/cooler.

Contents:

Tip: To install the column heater or column heater/cooler, you should know how to set up and operate general laboratory instruments and computer-controlled devices, and how to handle solvents.

The figure below shows the primary steps in installing the column heater or column heater/cooler:

Steps to install the column heater or column heater/cooler:



Select a Site

The column heater and column heater/cooler are optional devices to the Waters separations module that physically attach to the separations module. Typically, the column heater is attached to the right hand side of the Alliance separations module by three mounting screws. Select a site that meets the requirements indicated in the table below:

Installation site requirements:

Factor	Requirement
e2695/2795 Separations Module to attach the column heater or column heater/cooler onto. See page 39.	e2695, e2695D, or 2795 separations module with firmware version 2.02 or later.
Temperature	4 to 40 °C (39 to 104 °F)
Relative humidity	20 to 80%, noncondensing
Bench space	Width: 6 in. (15.24 cm) Depth: 21.25 in. (52.975 cm) Height: 22.5 in. (57.15 cm) Level to within ±2°
Vibration	Negligible
Clearance	At least 2 in. (5 cm) at rear for ventilation
Static electricity	Negligible
Power – column heater	Refer to page 27
Power – column heater/cooler	Refer to page 27
Required electrical receptacles	Refer to page 27
Electromagnetic fields	No nearby source of electromagnetic noise, such as arcing relays or electric motors

Power requirements

The column heater needs no connection to an external power source. The column heater receives all its required power from the separations module.

The column heater/cooler requires:

- Grounded alternating current (AC) power source.
- Minimal power transients and fluctuations.
- Line voltage of 100 to 240 Vac at a frequency range of 50 to 60 Hz. Power consumption is 240 Volt Amps (VA).

Unpack and inspect the module

The column heater or column heater/cooler is shipped in a single carton. Save the carton and all packing materials in case you need to transport or ship the instrument later.

To unpack and inspect the column heater or column heater/cooler:

1. Inspect the outside of the shipping carton for signs of damage. Remove the plastic wrap, if any, and open the shipping carton. Remove the instrument from the carton.
2. Locate the packing list and perform an inventory of the items provided against the packing list.
3. Locate the Certificate of Validation. Check that the instrument serial number (found on the back panel) corresponds to the number on the certificate.
4. Immediately report any shipping damage to both the shipping company and your Waters representative. To report any discrepancies with the instrumentation, see the contact information on page 77. Refer to *Waters Licenses, Warranties, and Support* for complete information on shipping damages and claims.

Required materials

- #2 Phillips screwdriver
- Flat-head screwdriver
- 5/16-inch and 1/4-inch open-end wrenches
- Tubing cutter
- Startup Kit for column heater or column heater/cooler
- Flashlight (optional)

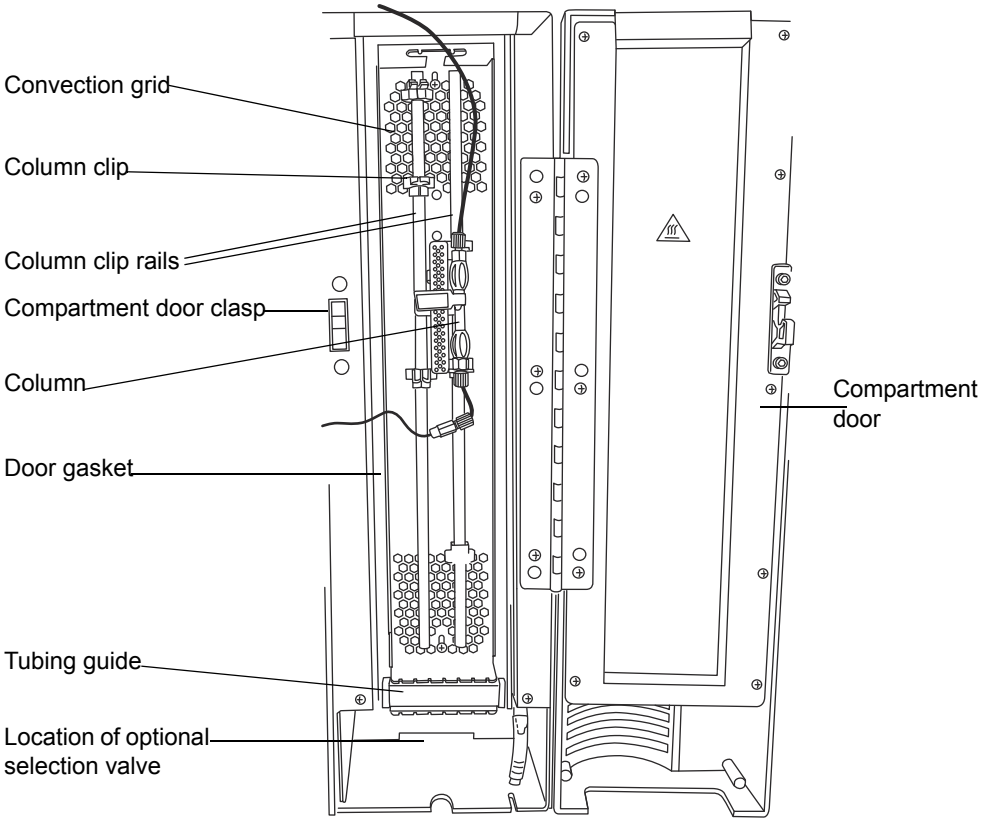
Install column selection valves

Three optional valves are available for the column heater and column heater/cooler that allow you to switch the flow path among multiple columns. The three valve types are:

- Three-column selection valve
- Six-column selection valve
- Column regeneration valve

The figure “Column heater (cooler) with optional column selection valve:” on page 30 shows a typical column heater or column heater/cooler with an optional column selection valve installed.

Column heater (cooler) with optional column selection valve:



Install a column selection valve

When an optional column selection valve is used with the column heater or column heater/cooler, the valve assembly will be installed on site. The mechanical installation of the valve is significantly easier to perform before the column heater or column heater/cooler is attached to the separations module. Use the following procedure to install each column selection valve:

Required materials

- Phillips screwdriver
- Flashlight (optional)

To install a column selection valve:

1. Ensure that the power switch is set to **Off** at the separations module and the column heater/cooler (because it too has an independent power supply).
2. Locate the column selection valve to be installed.
3. Remove the drip tray from the bottom the compartment (if installed). Note the direction the tray and drain fitting are facing. Place the tray in a safe location for reinstallation.

   Tip: The two open electrical signal connectors within the compartment match the electrical connectors on the valve assembly.

   Tip: Tilting the valve assembly 5 to 10° top forward while sliding the valve assembly into place will greatly ease the mechanical installation of the valve assembly.
4. Partially install the valve into the compartment.
5. Attach the corresponding electrical connectors within the compartment to the connectors prewired to the valve assembly. Align the connector before attempting to join the connectors. With the connectors aligned, seat the connectors firmly. Dress each wiring harness to the sides of the valve to avoid pinching the harness in the bracket assembly.
6. Secure the valve assembly bracket into the chassis tabs. Ensure that each corner is firmly secured.
7. Slowly press the upper valve bracket inward while aligning the captive screws to the treated inserts within the compartment. Once aligned, finger-tighten the screws to retain the bracket in place. Use a Phillips screwdriver to firmly secure the assembly.
8. Using the flashlight, inspect your installation.
9. Reinstall the drip tray.

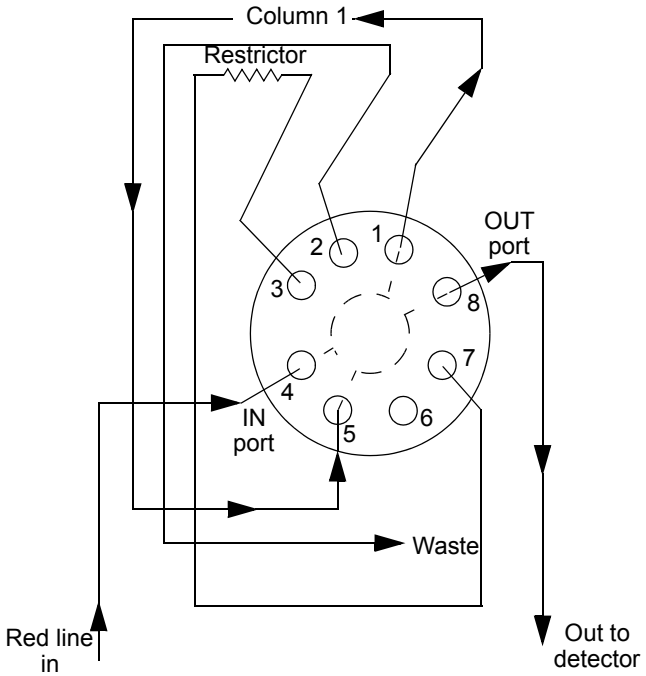
With the optional selection valve installed within the column heater or column heater/cooler, connect the installed valve as described on page 31, page 33, or page 35.

Connect the three-column selection valve

The optional three-column selection valve is an eight-port, three-position valve. The figure below shows typical fluidic connections.

- Ports 1 and 5 – Route to and from the column
- Port 2 – Route to waste
- Ports 3 and 7 – Route through a restrictor bypass to the detector
- Port 4 – Input port from the separations module
- Port 6 – Not used
- Port 8 – Output port to the detector

Typical three-column selection valve connections:



Required materials

- 5/16-inch and 1/4-inch open-end wrenches (if using stainless steel tubing)
- Tubing and fitting supplied with the optional selection valve

To connect the three-column selection valve to the sample management system fluidic path:

1. Use a wrench to connect the stainless-steel red line (from the separations module inject valve) to the IN port on the column selection valve.
2. Use the tubing and fittings (supplied with the three-column selection valve) to connect the OUT port of the column selection valve to the detector inlet.
3. Route the waste line (installed) from port 2 of the column selection valve through the door of the column heater (or column heater/cooler) to a suitable waste reservoir. Refer to page 44. You can reconfigure the three-column selection valve as appropriate for your application.

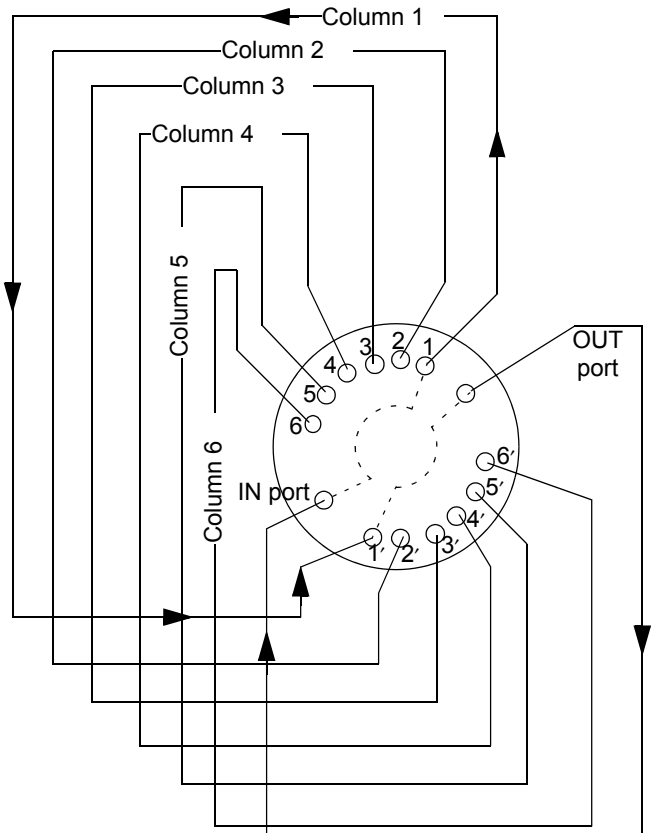
Connect the six-column selection valve

The optional six-column selection valve is a fourteen-port, six-position valve. The figure “Typical six-column selection valve connections:” on page 34 shows a typical fluidic connection diagram.

- Ports 1 and 8 – Route to and from column 1
- Ports 2 and 9 – Route to and from column 2
- Ports 3 and 10 – Route to and from column 3
- Ports 4 and 11 – Route to and from column 4
- Ports 5 and 12 – Route to and from column 5
- Ports 6 and 13 – Route to and from column 6
- Port 7 – Input port
- Port 14 – Output port to the detector

Tip: Any of the six column positions can be used for a restrictor or for waste as in the three-column selection valve (see the example in the figure “Typical three-column selection valve connections:” on page 32).

Typical six-column selection valve connections:



Required materials

- 5/16-inch and 1/4-inch open-end wrenches (if using stainless steel tubing)
- Tubing and fitting supplied with the optional selection valve

To connect the six-column selection valve to the sample management system fluidic path:

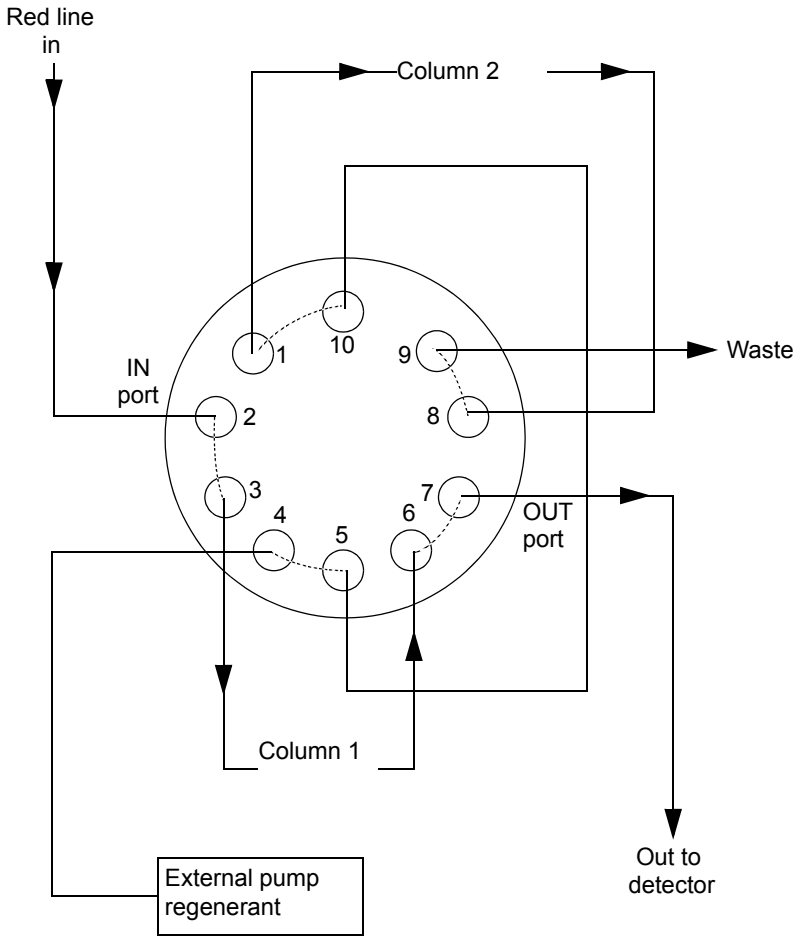
1. Use a wrench to connect the stainless-steel red line (from the separations module inject valve) to the IN port on the column selection valve. Ensure the line is routed and dressed through the door properly.

2. Use the tubing and fittings (supplied with the six-column selection valve) to connect the OUT port of the column selection valve to the detector inlet.
3. Route the detector output line port 14 of the column selection valve through the door of the column heater (or column heater/cooler). You can reconfigure the six-column selection valve as appropriate for your application.

Connect the column regeneration valve

The optional column regeneration valve is a ten-port, two-position valve that is connected to an external regeneration pump. The figure “Typical column regeneration valve connections:” on page 36 shows a typical fluidic connection diagram.

Typical column regeneration valve connections:



Required materials

- 5/16-inch and 1/4-inch open-end wrenches (if using stainless steel tubing)
- Tubing and fitting supplied with the optional selection valve

To connect the column regeneration valve to the sample management system fluidic path:

1. Use a wrench to connect the stainless-steel red line (from the separations module inject valve) to the IN port on the column

regeneration valve. Ensure the line is routed and dressed through the door properly.

2. Use the tubing and fittings (supplied with the column regeneration valve) to connect the OUT port of the column selection valve to the detector inlet.
3. Route the line through the door of the column heater (or column heater/cooler). Ensure the line is routed and dressed through the door properly.
4. Use the tubing and fittings (supplied with the column regeneration valve) to connect the drain or waste port of the column selection valve to an appropriate waste container.

Address the column selection valve

Column selection valves must be addressed after they have been installed within the column heater or column heater/cooler. The column selection switch, located on the back panel of both instruments, sets the internal electronics to allow communication between the valve controls and the user interface software. The following table lists the required switch setting for the particular valve that is installed.

Column selection valve switch settings:

Switch position	Valve type
0	No valve installed
1	Three-column selection valve (8-port, 3-position)
F	Six-column selection valve (14-port, 6-position)
7	Column regeneration valve (10-port, 2-position)

Required material

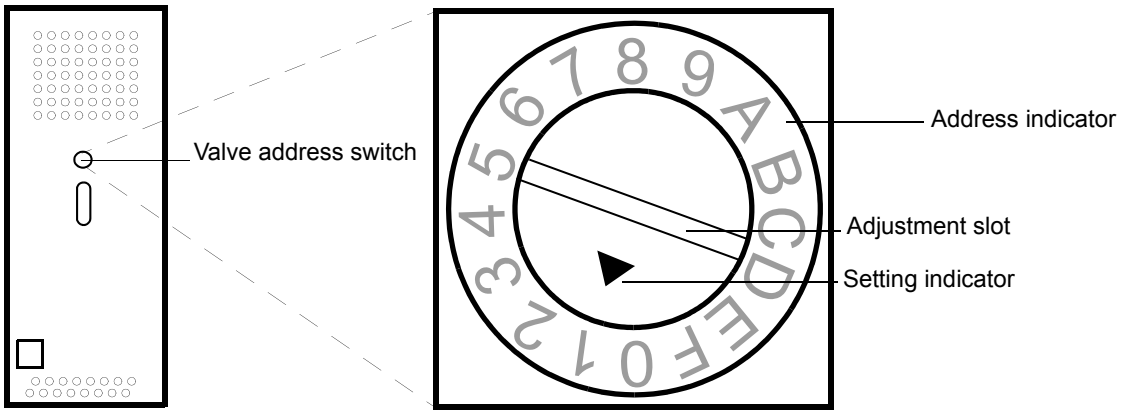
Small flat-head screwdriver

To set the column selection switch:

1. Power off (**0**) the separations module and the column heater/cooler.
2. Locate the column selection switch at the back panel of the column heater or column heater/cooler.

3. Using a small flat-head screwdriver, turn the switch dial to the required address setting for the corresponding installed valve. The arrow in the switch dial indicates the setting.
4. Power on (**1**) the separations module (and the column heater/cooler) to enable the new address.

Column selection valve address switch:



Attach the column heater or column heater/cooler

The column heater or column heater/cooler is attached directly to the right side panel of a separations module with three screws. A standoff (spacer) is required along with each mounting screw to attain the proper spacing for the installation. Ensure that there is sufficient bench space for the instrument before attempting to attach it to the separations module.

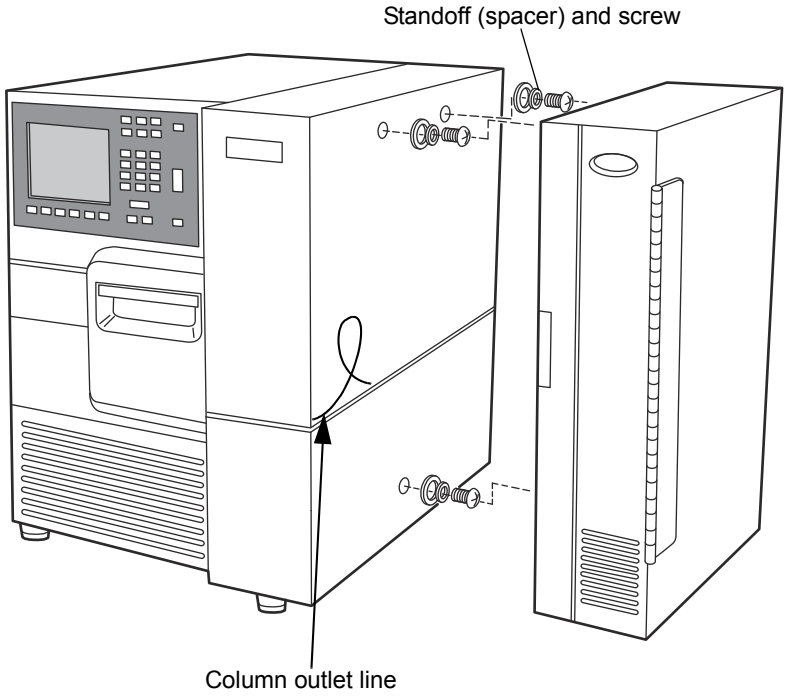
Caution: Set the separations module power switch to Off (**0**) before attaching the column heater or column heater/cooler.

To attach the column heater or column heater/cooler:

1. Power off (**0**) the separations module.
2. Remove the three screws from the right side of the separations module (if installed).

3. Install the three screws and standoffs (from the Startup Kit) into the threaded holes in the right side panel of the separations module. Tighten (without force) each screw firmly up against the standoffs.
4. Route the red outlet tubing through the slot located halfway up the front chassis of the separations module. This will be routed to a column (or column selection valve) during the fluidic connection.
5. Carefully mount the column heater or column heater/cooler on the three standoffs on the right side panel. Lock the instrument in place by positioning the three standoffs into the slotted holes of the side panel.

Attach the column heater or column heater/cooler to the separations module:



Make tubing connections

There are two types of fittings used in HPLC systems: PEEK (polymer-based) fittings and tubing, and stainless steel fittings and tubing. Stainless steel is used in HPLC applications that require high pressure, and PEEK is used

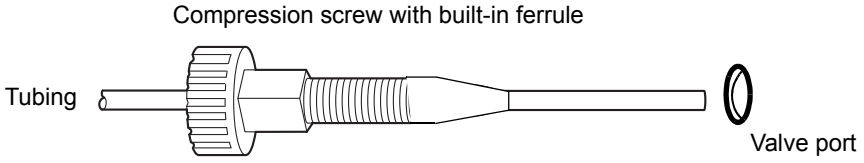
when chemistry is extremely caustic. Use the following procedures when making fluidic connections at the optional column selection valves.

PEEK fittings

To use PEEK fittings to make fluidic connections at the optimal column selection valves:

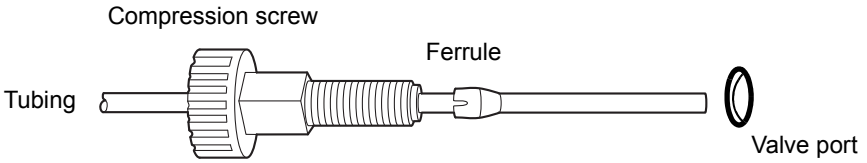
1. Measure the length of tubing required for the connection.
2. Open the cutter and insert the tubing into appropriate guide hole. Close the cutter to allow the blade to rest on the tubing.
3. Gently press down on the cutter while rotating the tubing back and forth. Ensure the cut end is straight and free of burrs.
4. Attach a compression screw over one end of the tubing.
   - If you use a finger-tight fitting with a molded ferrule end, install the one-piece fitting on the tubing.

     One-piece compression screw with molded ferrule:

   - If you use individual fittings, slide the compression screw over one end of the tubing, followed by a ferrule with its tapered end toward the valve port.

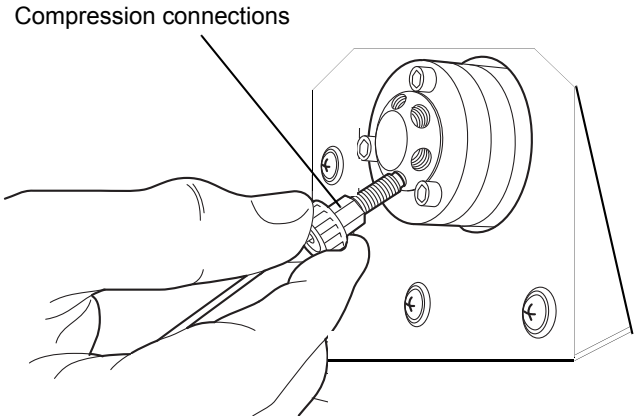
     Ferrule and compression screw assembly:

5. Firmly seat the tubing end into the inlet on the valve, then finger-tighten the compression screw.

Caution: To avoid damaging the ferrule, do not overtighten the compression screw.

PEEK fluidic connections:

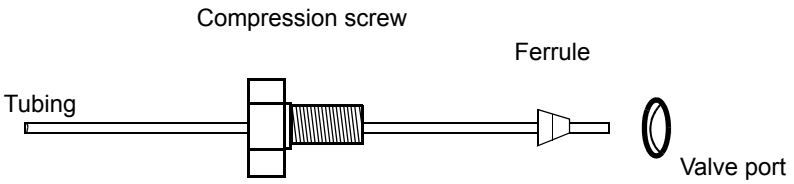


Stainless steel fittings

To use stainless steel fittings to make fluidic connections at the optimal column selection valves:

1. Measure the length of tubing required for the connection.
2. Insert the tubing into the tubing cutter, making sure that the tubing that extends from the metal side of the cutter is the correct length.
3. Rotate the tubing cutter several times to score the stainless tubing. Snap the tubing at the score. Inspect the cut end to ensure it is straight and free of burrs.
4. Slide a compression screw over one end of the tubing, followed by a ferrule with its tapered end toward the valve port.

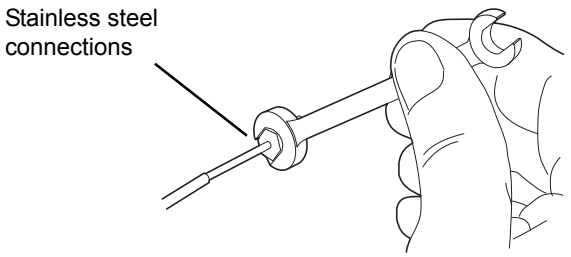
Stainless steel compression screw and ferrule assembly:



5. Firmly seat the tubing end into the inlet on the valve, then finger-tighten the compression screw. Tighten stainless steel fittings only a one-quarter to one-half turn past finger-tight using an open-end wrench.

Caution: To avoid damaging the ferrule, do not overtighten the compression screw.

Tighten connector using an open-end wrench:



Install columns

Multiple chromatographic columns can be installed within the compartment of the Waters column heater or column heater/cooler. The table below shows the possible column configurations. Columns are not provided with the Waters column heater, column heater/cooler, or optional column selection valves.

Column configurations:

Quantity	Description
1	300-mm length column, up to 7.8-mm ID maximum, with a column guard up to 20 mm maximum

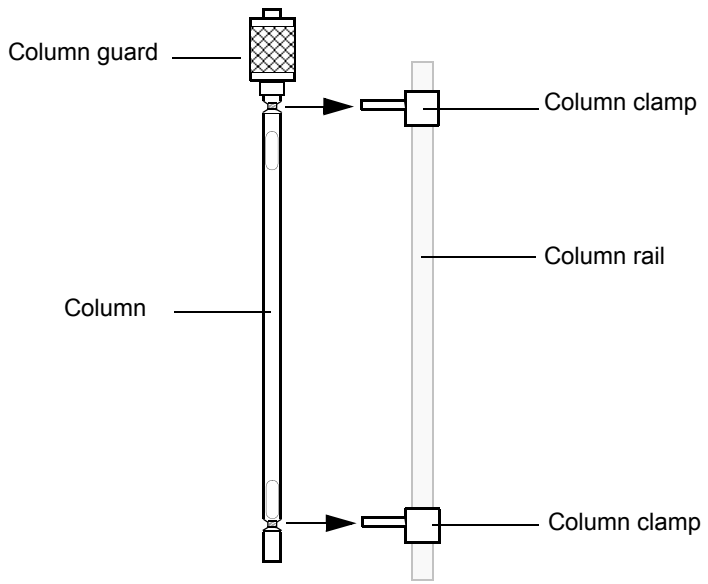
Column configurations: (Continued)

Quantity	Description
2	300-mm length column, up to 7.8-mm ID maximum, with column guards up to 20 mm maximum
4	150-mm length column, up to 7.8-mm ID maximum, without column guards
6	50-mm length column, up to 7.8-mm ID maximum, without column guards

Attach columns in the clamps

Columns are held in place within the heater/cooler compartment using adjustable column clamps attached to the column rail. You can add multiple clamps to the rail. Clamps are held in place on the rail when the column is inserted. The column clamps are designed to hold onto the compression screw threads, to allow use of columns from multiple vendors, and will not damage the threads.

Column retainer clamps (side view):

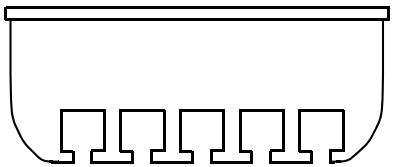


Route tubing in the column compartment

Tubing retainers

Tubing retainers are provided along the left side, the bottom, and the top of the column compartment to hold the fluidic tubing in place. The use of these retainers is highly recommended when a column switching valve is installed.

Tubing retainer (top view):



Tubing routing

The heater or heater/cooler column compartment is only functional when the door can be properly closed. The door gasket and hinge assembly are designed to allow the passage of tubing without damaging the tubing.

The door gasket, however, is not designed to have fluidic tubing routed out the door bottom. Routing tubing out the door bottom can cause the compartment door gasket not to seal completely, or cause a closed door to spring open inadvertently.

Install the valve drip tray

A valve drip tray is provided for both the column heater and column heater/cooler.

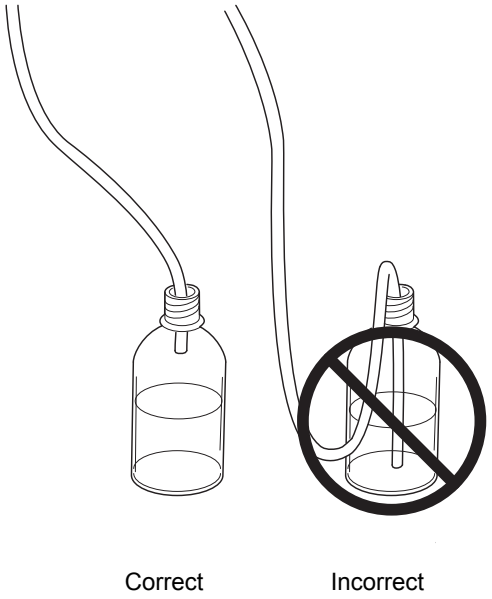
To install the valve drip tray:

1. Ensure that the column heater or column heater/cooler is secured to the separations module.
2. Install the fitting into the drip tray.
3. Install the drip tray in the compartment base just below the valve.
4. Install the drip tray so proper gravity drainage can be achieved from the bottom of the instrument.

5. Ensure that there is clearance between the drip tray and the bench top.
6. Push the tubing over the barb on the fitting and cut it to the appropriate length.
7. Ensure that liquid can drain freely down the drain tube and into an appropriate waste container.

Caution: To properly drain the waste fluid, ensure that the waste tube does not get crimped or bent. A crimp or bend in the tube may prevent adequate flow to the waste container.

Drain tube configuration:



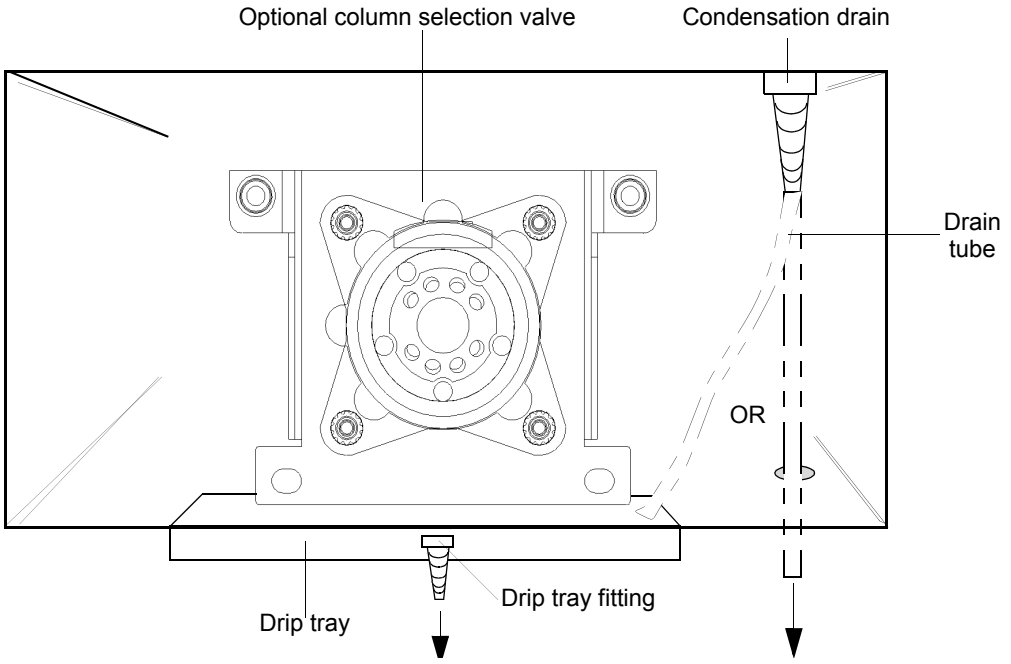
Connect the column heater/cooler condensation drain

The column heater/cooler has a condensation drain for the cooler assembly. The condensation drain collects condensation that forms in or around the internal cooler engine.

The condensation drain fitting is located just above and to the right of the valve compartment. Connect the fitting to a drain tube that may either be

routed to the valve drip tray, or directly out to a waste vessel using the exit hole in the bottom of the compartment.

Condensation drain:



Install preheater and precooler tubing

The column heater or column heater/cooler does not contain preheater or precooler tubing, since not all applications require their use. Preheater and precooler tubing can be fabricated using stainless steel tubing and fittings, and installed by you when needed.

The column outlet line from the separations module, if placed in its entirety inside the column heater or column heater/cooler, will provide sufficient preheating at 1 mL/min with a temperature differential of 65 °C. The length of

the red line is approximately 60 cm. Preheater and precooling tubing specifications are based on these assumptions:

- Mobile phase flow rate – Variable between 50 and 1000 µL/min
- Preheat tube inlet temperature differential (ΔT) – Variable between 10 and 60 °C
- Preheat outlet temperature differential – ≤3 °C
- Preheat tubing material – Stainless steel
- Preheat tubing ID – 0.005 to 0.012 inches
- Preheat tubing OD – 0.063 inches

Equivalent linear flow rates (approximate) for different ID columns (relative to 1 mL/min flow rate):

ID	Equivalent flow rate	Generally used flow rate
3.9 mm	1000 mL/min	-
2.1 mm	290 µL/min	200 µL/min
1.0 mm	66 µL/min	50 µL/min

Tubing lengths for flow rates and differential temperatures:

Tubing length (cm)	Various flow rates		
	3.9 mm (Column Diameter)	**2.1 mm** (Column Diameter)	**1.0 mm (Column Diameter)**
ΔT	**1000 µL/min (Flow Rate)**	**200 µL/min** (Flow Rate)	**50 µL/min** (Flow Rate)
1. 10	2. 21	3. 4	4. 1
5. 20	6. 33	7. 7	8. 2
9. 30	10.40	11.8	12.2
13.40	14.45	15.9	16.2
17.50	18.49	19.10	20.2
21.60	22.52	23.10	24.3

References

The information on page 46 can be found in the following references:

- R.G. Wolcott, *et al., Journal of Chromatography*. A. 869: (2000), pp. 211–230
- B. Yan, *et al., Analytical Chemistry*. 72(6): (2000), pp. 1253–1262

Make power and signal connections

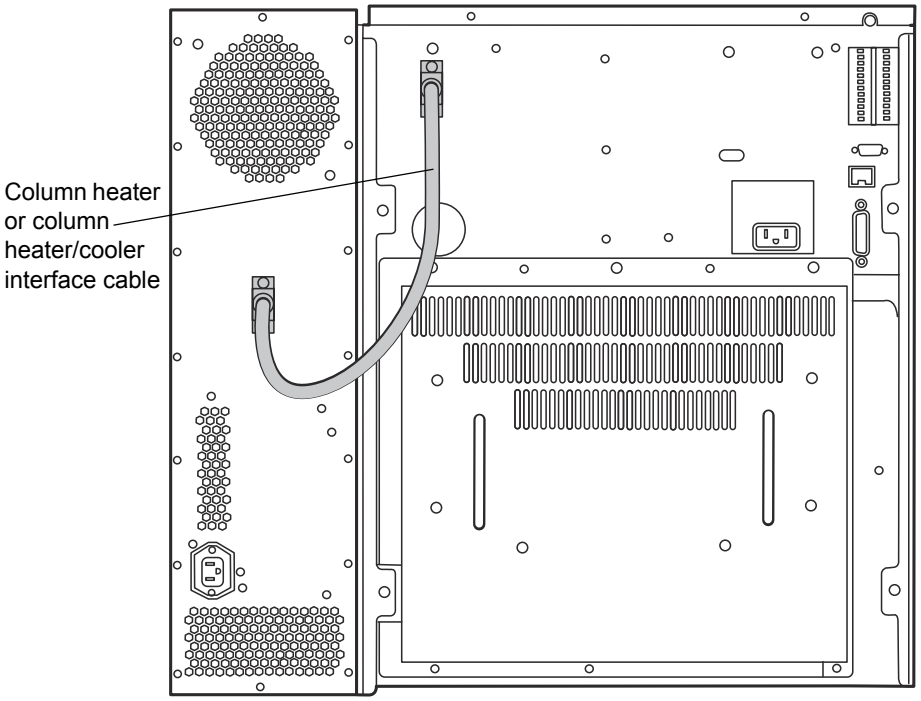
The column heater receives power only from the separations module, and the column heater/cooler receives power from two sources: a grounded AC receptacle and the separations module (DC power). Communications and control signals for the column heater and column heater/cooler are provided through a cable, from the 26-pin male, D-series subconnector at the separations module.

Connect the column heater cable

Caution: To avoid possible damage to components, power-off the separations module before you connect or disconnect the column heater cable.

Install one end of the interface cable to the connector at the column heater back panel. Install the other end of the interface cable to the connector at the separations module back panel.

Rear panels of the separations module and column heater (cooler):



Make column heater/cooler connections

The column heater/cooler contains its own internal power supply that is connected directly to a grounded AC receptacle. An appropriate country line cord is provided to make the connection to the AC source. Use the interface cable provided in the Startup Kit to make the connection to the separations module.

Making the signal connection

With the separations module (and column heater/cooler) power switched off, install one end of the interface cable to the connector of the Column

Heater/Cooler back panel. Install the other end of the interface cable to the connector at the separations module back panel.

Make the power connection

For proper operation, the column heater/cooler requires a grounded AC power supply with no abrupt voltage fluctuations.

Caution: Power off the 2695 and column heater/cooler when disconnecting or connecting cables.

To connect the column heater/cooler to the grounded AC power source:

1. Ensure that the power switch on the side panel of the instrument is in the Off (**0**) position.
2. Connect the power cord to the power entry module on the rear of the instrument.
3. Insert the other end of the power cord to the grounded power receptacle.

3 Configure the Separations Module

This chapter explains how to configure the Waters separations module for the installed column heater or column/heater cooler. Before you can perform a run using the instrument, you must configure related parameters using the separations module and in Empower software when interfaced to a chromatography system.

Contents:

Configure the separations module for a column heater

Configure column heater-related parameters at the separations module. These parameters are found in:

- Status screens
- Instrument screens

Tip: The firmware of the separations module must be v**2.02** or later to properly control the second generation column heater or column heater/cooler module.

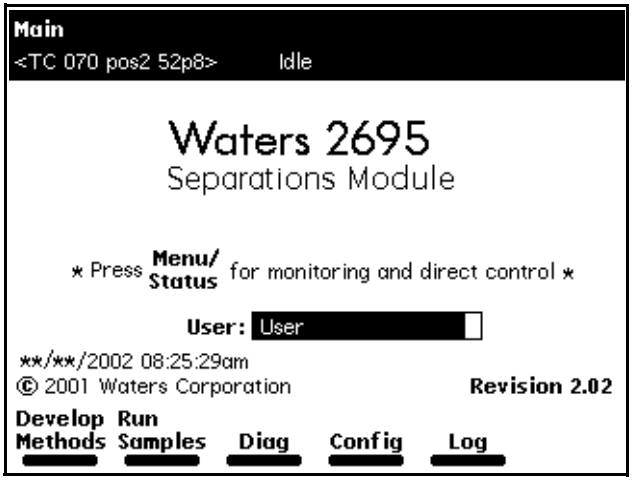
1. Power-on the separations module. Refer to the specific operator's guide for the separations module you are running.

   The separations module begins its startup diagnostics routine.

   When the initial part of the startup diagnostics routine is successful, the Main screen appears in the front panel display.

2. If the startup diagnostics routine is unsuccessful, see Chapter 5.

2695 Separations Module main screen:



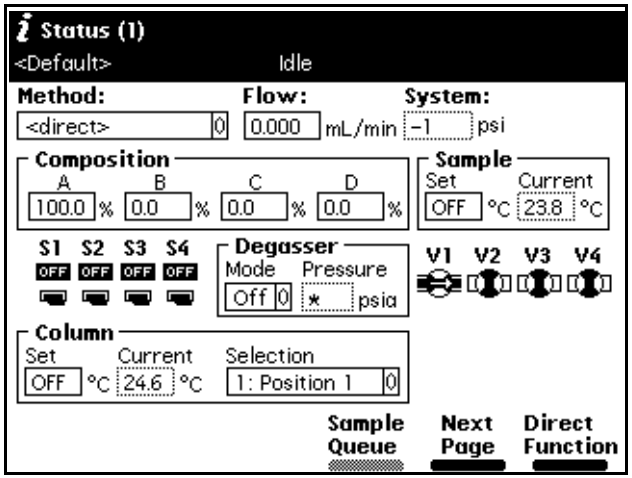
You can access column heater and column heater/cooler-related screens from screen keys on the Main screen, or monitor and set certain column heater and column heater/cooler parameters by pressing the Menu/Status screen key.

Configure the separations module for a column heater using the Status screens

Press Menu/Status on the front panel keypad to access the first page of the Status screen. The content and layout of the Status screen vary with the options installed in the separations module and with the mode of operation.

Using the Status screen, you can monitor the current status of the column heater and program the compartment temperature. You can make changes only in the fields that have a solid border. Fields that have gray segmented borders are disabled parameter functions or reporting functions.

First page of the Status screen:



The following table describes the functions of the parameters in the Status screen fields related to the column heater.

Status screen parameters:

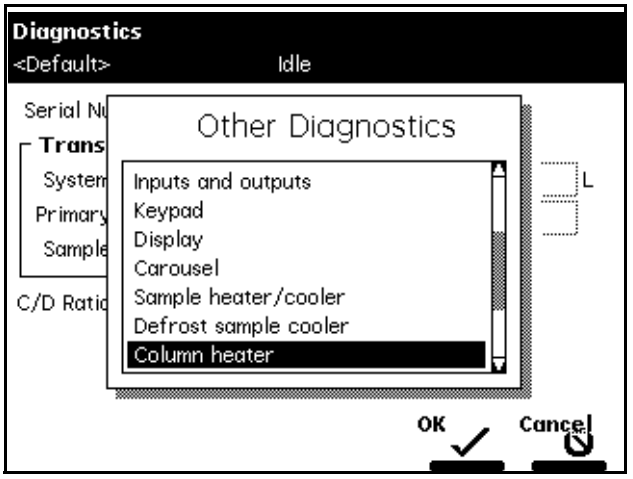
Parameter	Description
Set (programmable field)	Indicates the desired column compartment temperature. Use the numerical keys to enter a temperature or Clear to set the field to Off.
Current (reporting field)	Indicates the current real-time temperature of the column compartment.
Selection	Indicates the selected active column with a descriptive label provided (created) in the column Configuration screen.

Configure the separations module for a column heater using the Main screen

The Main screen contains screen keys that access column heater/cooler-related screens. Use these screens to add, delete, or change hardware in or attached to the separations module such as the column heater/cooler, column selection valves, or when you need to change parameters of the separations module.

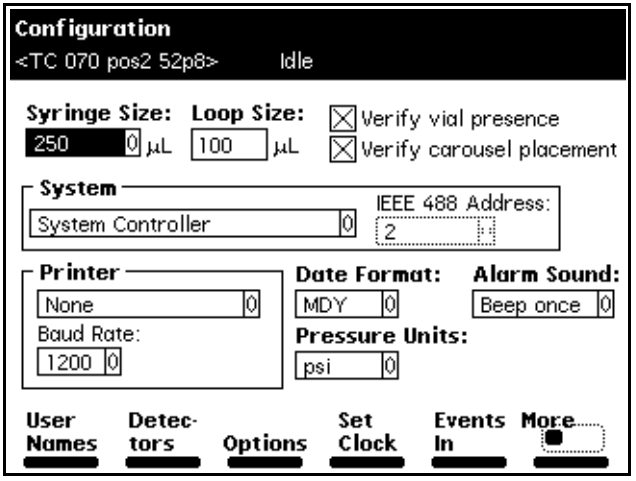
Press **Diag > Other Tests** to access column heater/cooler-related information.

Diagnostics screen:



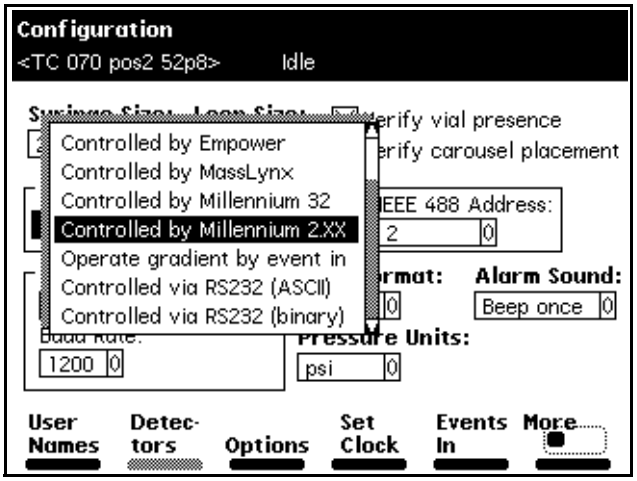
Press **Config** in the Main screen to display the Configuration screen.

Configuration screen:



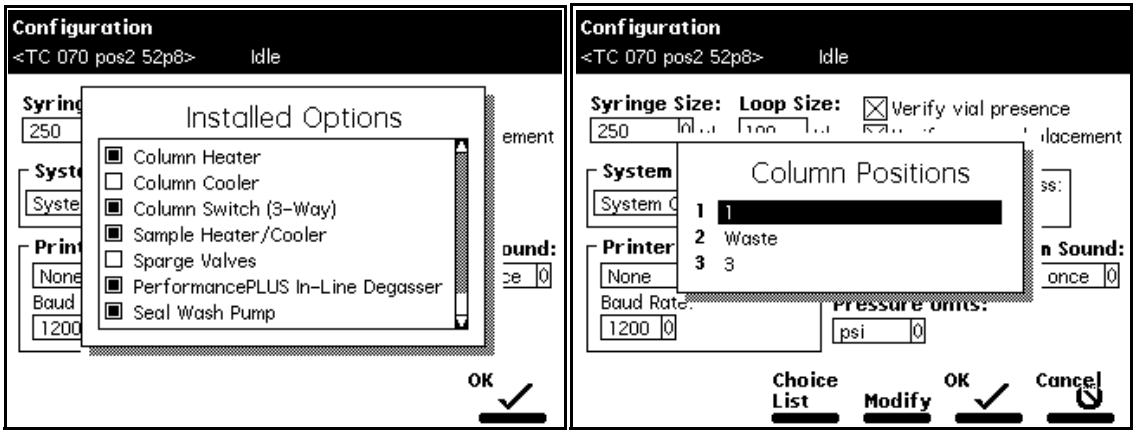
Press **Config > System** to configure the separations module for the designated controller system. Press **More** to extend the configuration selections.

Configuration screen:



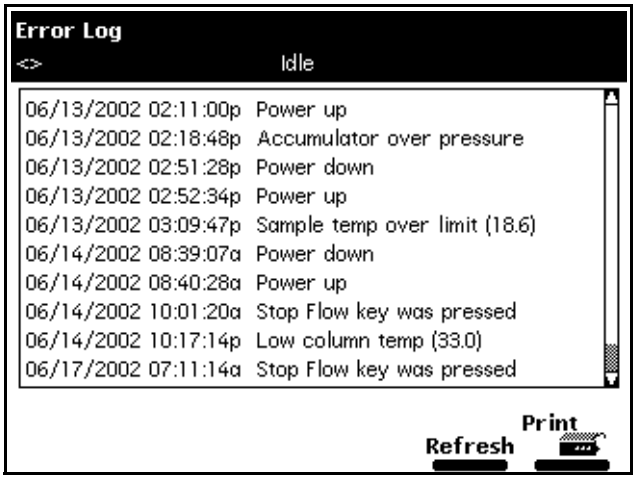
Press **Config > Options** to configure the separations module for installed options, such as the column type and column switching valves. Press **More** to extend the option selections.

Configuration Installed Options screens:



Press **Log** in the Main screen to display the system Error Log.

Error Log screen:



Configure the separations module for a column heater using the Methods screen

You can configure the column heater using the Methods screen. Parameter adjustments are similar to the operation of the Status screens. The adjustable parameters are:

- Column Temperature Target
- On error (alarm notification, disable, or enable)

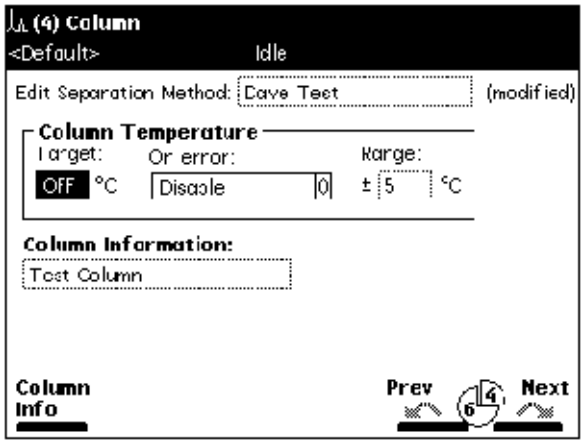
The Temperature Range is a default of ±5 °C, adjustable in 0.10 °C increments.

Set column parameter values

To enter parameters in the Column screen:

1. Press the **Next** or **Prev** screen key (as appropriate) to display the Column screen.

Column screen:

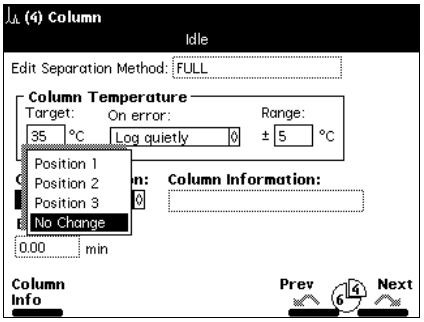


2. Enter values in the Column screen, as appropriate. The table below describes the parameters in the Column screen.

Column screen parameters:

Parameter	Description	Value range
Column Temperature Target	Sets the temperature of the column heater using the numerical keys of the front panel. To turn off the column heater, press the **Clear** key.	Column heater – 4 to 65 °C (5 °C above ambient to 65 °C) Column heater/cooler – 4 to 65 °C (the greater of 15 °C below ambient or 4 to 65 °C)
On error	Response that occurs when the column temperature is out of the specified range.	See the table titled "Column heater-related error messages:" on page 78.

Column screen parameters: (Continued)

Parameter	Description	Value range
Column Temperature Range	Sets the maximum allowable variation in column temperature. If the temperature variation exceeds the range, the alarm condition selected in the adjacent box is triggered. Default = ±5 °C	1 to 20 °C (in 1 °C increments)
Column Selection 3-Column Valve	Selects a column position if a column selection valve is installed. The example drop-down list shows the selections for a three-column valve.  Select **No Change** when you link methods in a sample set and do not want to change the column from the previous method.	Position 1 to Position 3, or No Change
Column Information	Displays the column information you enter using the **Column Info** screen key.	N/A
Column Info (screen key)	Allows you to enter or modify the Column Information field.	30 characters

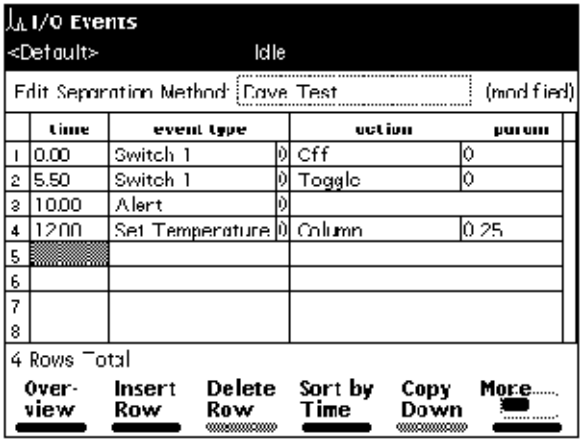
Edit the I/O Events table

The I/O Events table allows you to set times for events to occur during a run. The column heater temperature can be programmed to a set temperature for a particular timed event.

To enter the confugration event in the I/O Events table:

1. From the Column screen, press the **Next** or **Prev** screen key to display the I/O screen.
2. Press the **Table** screen key. The I/O Events table appears.

I/O Events table



3. Enter values in the I/O Events table as appropriate. The table titled “I/O Events table parameters:” on page 60 describes the parameters in the I/O Events table. The table titled “Action field parameters:” on page 60 lists the parameters you can use in the action field in the I/O Events table.
4. Press **Exit** to return to the I/O screen.

I/O Events table parameters:

Parameter	Description	Value range
Time	Determines the time after the start of a run at which the change is to occur. Press the **Clear** key to select INIT. Conditions in the INIT line apply when the system is initialized to a method, while events at time 0.00 occur immediately upon an injection.	INIT, 0.00 to 999.99 in 0.01-minute increments
Event type	Sets the type of event to occur.	Switches 1 to 4 Set Sparge Set Temp Alert
Action	Selects the action to perform with the specified event.	Refer to the table titled "Action field parameters:" on page 60.
Parameter	Selects the value for the action.	Refer to the table titled "Action field parameters:" on page 60.

Action field parameters:

Event type	Action	Value range (Param column)
Switches 1 to 4	On Off Toggle[1] Pulse[2] No Change	 0.01 to 10.00 in 0.01-minute increments (Pulse only)

Action field parameters: (Continued)

Event type	Action	Value range (Param column)
Set Sparge (if installed)	A B C D All	0 to 100% in 1% increments
Set Temperature (if column heater or column heater/cooler is installed)	Column	Column heater – 4 to 65 °C in 1 °C increments Column heater/cooler – 4 to 65 °C in 1 °C increments (the greater of 15 °C below ambient or 4 °C)
Alert	No action	N/A

1. Changes the state of the switch (open to closed, or closed to open).
2. A single pulse with a width defined in the param column of the I/O Events table.

Configure the instrument in Empower

The separations module with a column heater or column heater/cooler can be controlled as part of a chromatography system, using Empower software.

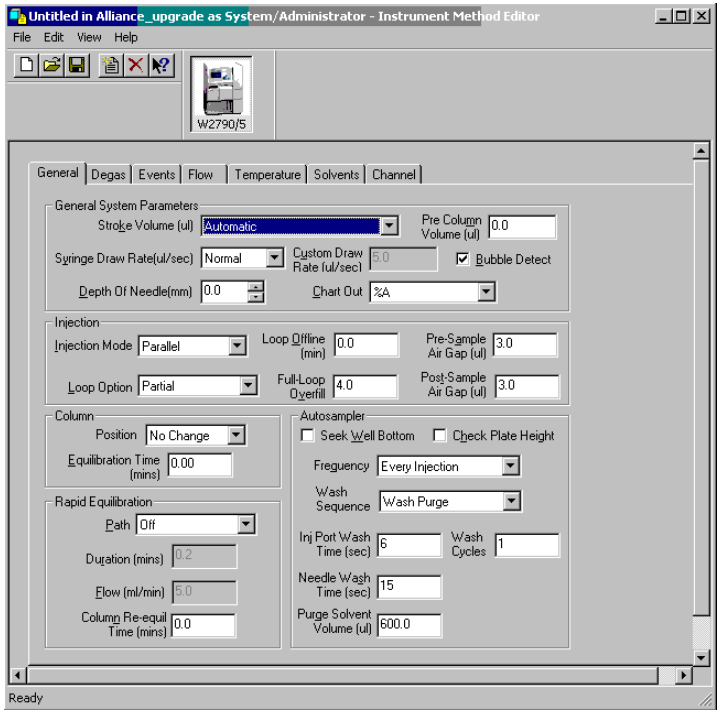
The Empower Instrument Method Editor is used to operate the column heater and column heater/cooler. On the General page, you will find fields that control column parameters such as column position. The Temperature page contains the column temperature range controls.

The table below shows the column heater and column heater/cooler devices that can be controlled for different separations modules.

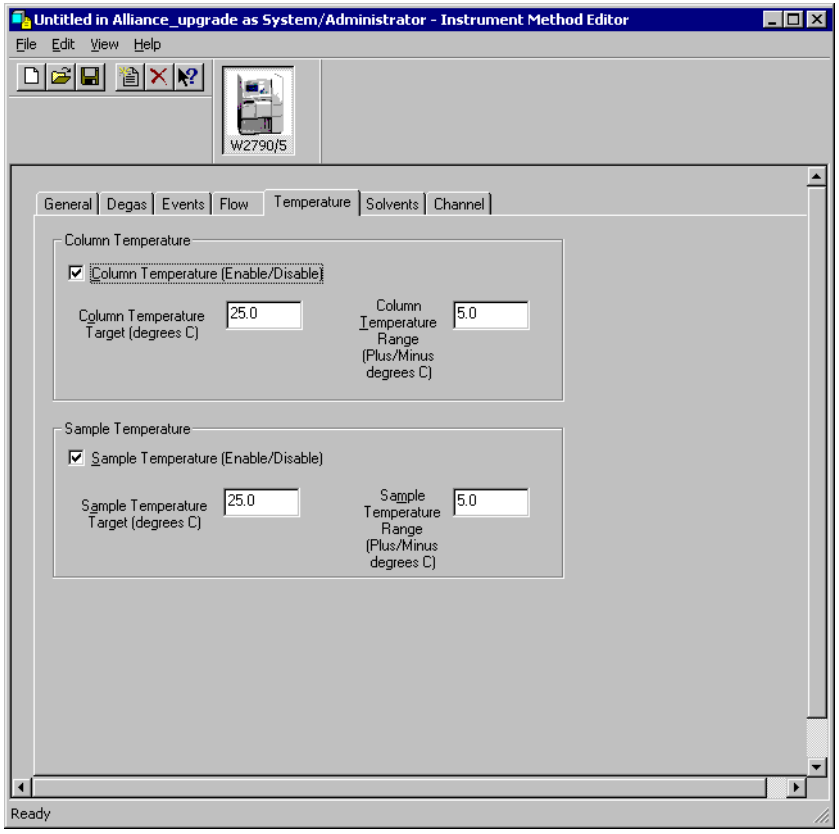
Empower matrix:

Function	Empower
Column Positioning	2695, 2695D, 2795
Column Temperature	2695, 2695D, 2795

Empower Instrument Method Editor, General page (2790/5 example):



Empower Instrument Method Editor, Temperature page:



Configure the separations module for a column heater/cooler

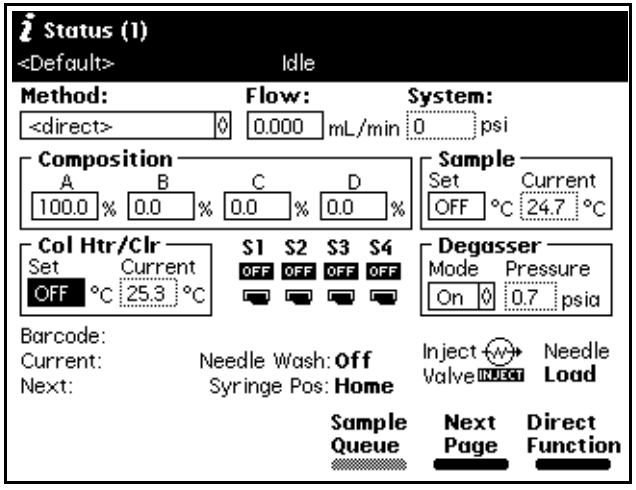
When a column heater/cooler is installed and integrated with the separations module, the firmware recognizes that the device has the cooler assembly and generates the appropriate cooler-related screens.

Screen keys are similar to those used for the column heater except that certain fields and controls are labeled Column Heater/Cooler or Column Cooler.

Press **Menu/Status** on the front panel keypad to access the first page of the Status screen. The content and layout of the Status screen vary with the options installed in the separations module and with the mode of operation.

Using the Status screen, you can monitor the current status of the column heater/cooler and program the compartment temperature. You can make changes only in the fields that have a solid border. Fields that have gray segmented borders are disabled parameter functions or reporting functions.

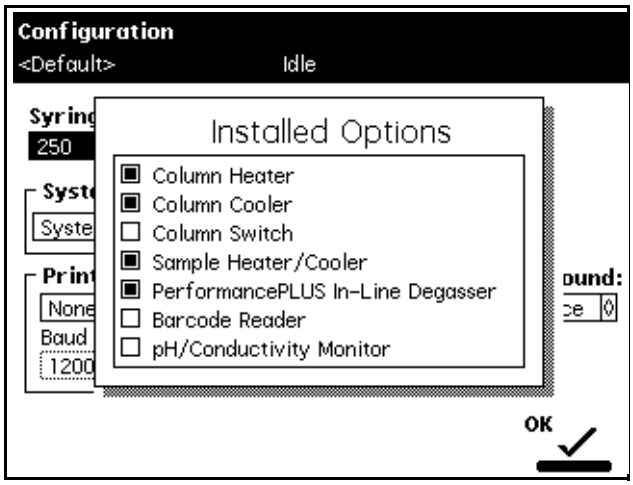
Status screen for the column heater/cooler:



Press **Config** in the Main screen to display the Configuration screen.

Press **Config > Options** to configure the separations module for installed options, such as the column type and column switching valves. Press **More** to extend the option selections.

Configuration Installed Options screen:



4 Perform Maintenance

This chapter presents routine maintenance procedures you can perform to ensure that the column heater or column heater/cooler consistently provides accurate results.

Contents:

Maintenance considerations

Safety and handling

When you perform maintenance procedures on your column heater or column heater/cooler, observe the following:

Caution: To avoid possible damage to components, power-off the separations module before you connect or disconnect the column heater or column heater/cooler interface cable.

Warning: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the column heater and column heater/cooler. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.

Warning: To avoid possible electric shock, do not open the column heater or column heater/cooler. There are no user-serviceable parts inside this device.

Spare parts

Refer to Appendix C, for spare parts information. You should not use replacement parts if they are not listed in Appendix C.

Clean the column heater or column heater/cooler

Warning: Always switch the separations module or column heater/cooler power to the Off position (**0**) before cleaning the device.

Clean surfaces of the column heater or column heater/cooler using only a soft lint-free paper or cloth dampened with mild soap and water.

To clean the surfaces of the device, you should:

- Always ensure the power to the device is set to Off (**0**).
- Always use eye and hand protection during the cleaning process.
- Apply the cleanser to a clean cloth only, then wipe the device down.
- Never spray or apply the cleanser directly to any surface of the device.

Maintain the column heater or column heater/cooler

Perform the procedures in this section when you discover a problem with a specific component in the solvent delivery device. For information about isolating problems in the solvent delivery device, see Chapter 5.

Maintaining the column heater or column heater/cooler involves these tasks:

- Replacing the column selection valve rotors and stator faces
- Replacing fuses for the column heater/cooler
- Defrosting the column cooler coils when frost begins to accumulate

Replace valve rotors and stator faces

Tip: For information about isolating problems in the column heater or column heater/cooler, see Chapter 5.

Required materials

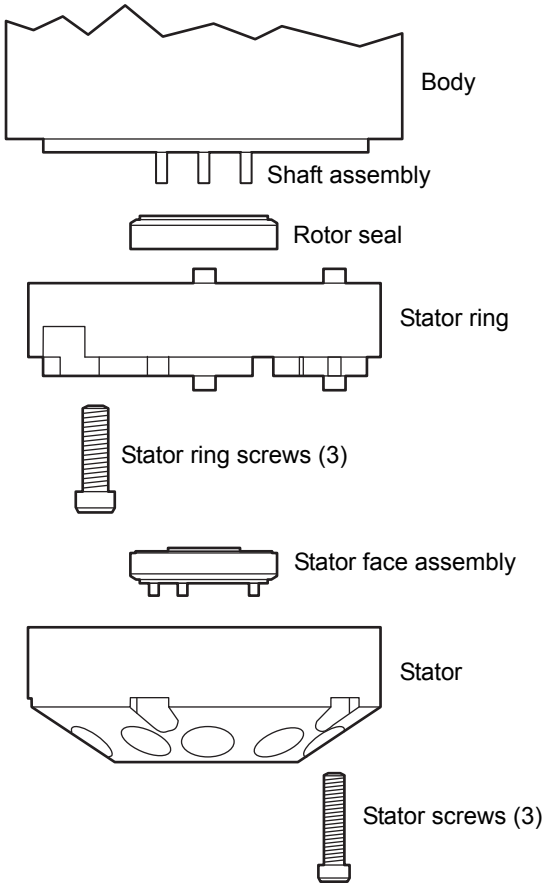
You need the appropriate Performance Maintenance Kit for the valve requiring maintenance. Refer to Appendix C.

Tip: Performance Maintenance Kits contain the necessary tools and instructions for each specific valve.

To replace the valve rotors and stator faces:

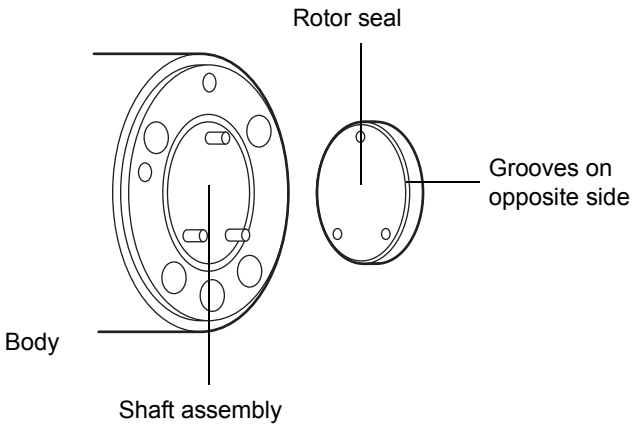
1. Remove the three stator screws with the hex key. See figure below for part locations.

Motorized valve (exploded view):



2. Remove the stator and stator face assembly from the stator ring (the stator face assembly can remain on the stator).
3. Remove the stator ring screws (if necessary).
4. Remove the stator ring.
5. Pull the rotor seal off the pins.
6. Mount the new rotor seal with the grooves facing the stator. The three pins on the shaft assembly fit into the mating holes in the rotor seal only one way.

Correct rotor seal orientation:



7. Replace the stator ring so that the two short pins on the ring enter the mating holes in the body.

   Tip: Do not apply adhesives, shellacs, or other forms of retainers to the screw threads.

8. Replace the stator ring screws (if necessary).
9. Mount the new stator face assembly onto the stator. The pins on the assembly fit into the mating holes in the stator only one way.
10. Replace the stator and stator face assembly on the valve so that the pin in the stator ring enters the mating hole in the stator.
11. Replace the three stator screws in the stator. Tighten each screw finger-tight, then further tighten each screw 1/2 turn.

Install or replace the column heater/cooler fuse

Caution: Replace fuses with those of the same type and rating.

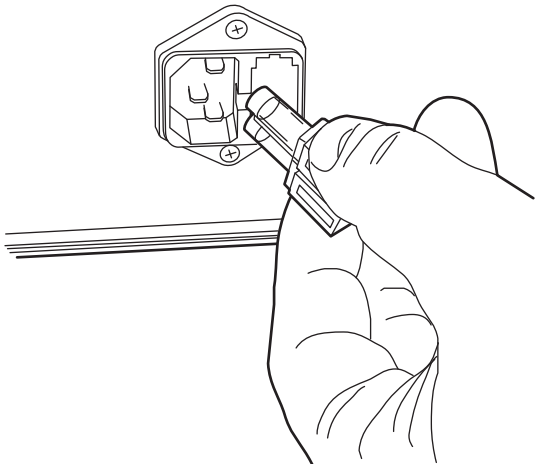
Column heater/cooler fuse ratings require:

- Two fuses
- 3.15 A, 250 V Fast-Blo, 5 × 20 mm (IEC)

To install or replace the power supply fuse in the column heater/cooler:

1. Ensure that the power switch is in the Off (**0**) position, then remove the power cord from the rear panel of the instrument.
2. Locate the power entry module on the back panel of the column heater/cooler.
3. Insert your fingers into the fuse holder slots on the rear panel of the column heater/cooler. Exerting a minimum pressure, pull on the spring-loaded fuse holder and remove it from the rear panel.

Remove and replace the tear panel fuses and fuse holder:



4. Remove and discard the old fuse.
5. Make sure that the replacement fuse is properly rated for the instrument.
6. Insert the new fuse into the fuse holder.
7. Insert the fuse holder into the receptacle and gently push until it locks into position. A click sound will occur when the fuse holder is locked into position.
8. Connect the power cord to the rear panel power entry module.

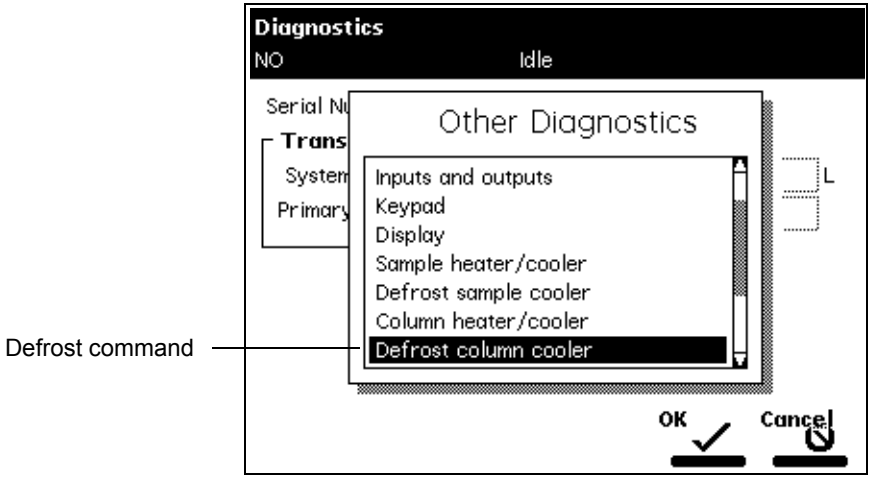
Defrost the column cooler assembly

It is necessary to defrost the column cooler assembly when frost begins to accumulate on the coils. A buildup of frost will reduce the efficiency of the cooler, or cause premature failure of the cooler assembly.

To defrost the column cooler assembly:

1. Ensure the separations module is not scheduled for a methods run during this procedure. Place the device in an offline operational mode.
2. Open the column door, and ensure that the drip tray and cooler condensation fitting are installed properly with the drain lines connected to waste containers.
3. Access the Diagnostic screen from the Main screen by pressing **Diag** > **Other Tests**, then select **Defrost column cooler**.

Diagnostics defrost column cooler command:



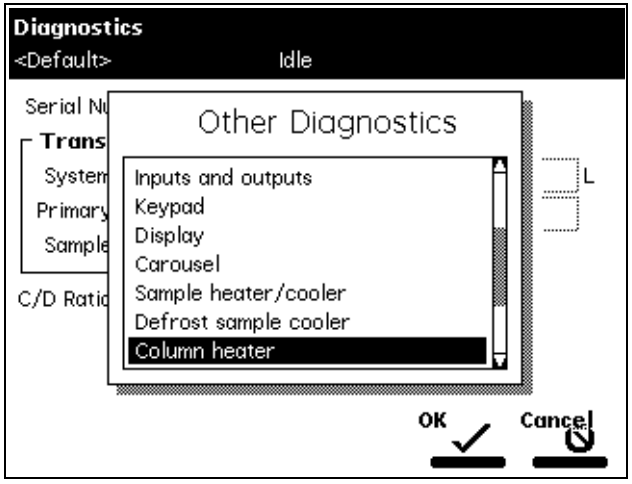
When the defrost process is complete, the separations module displays a message.

Access diagnostics functions

The separations module firmware provides a number of diagnostics functions including the ability to identify the column heater or a column heater/cooler, as shown in the "Column Heater Diagnostics screen:".

Press **Diag** > **Other Tests** to access column heater and column heater/cooler-related information. Refer to the *Alliance e2695 Separations Module Operator's Guide* or the *Waters 2795 Separations Module Operator's Guide* on waters.com

Column Heater Diagnostics screen:



5 Troubleshoot

This chapter describes how to troubleshoot the Waters column heater or column heater/cooler.

Contents:

Proper operating procedures

To keep your column heater or column heater/cooler operating properly, follow the operating procedures and guidelines described in Chapter 4.

Spare parts

Refer to Appendix C, for spare parts information. You should not replace parts excluded from Appendix C.

Safety and handling

When you troubleshoot the column heater or column heater/cooler, observe the following safety consideration:

Warning: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the column heater or column heater/cooler. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.

System troubleshooting

Follow these basic steps to perform system troubleshooting:

1. Examine the system, checking the simple things first. Is something obvious causing the problem? For example, is an instrument unplugged or improperly connected?
2. Compare the current system operation with the way the system operated before the problem started. To help you identify normal operating conditions:
   a. Create a map of your LC system (tubing and electrical connections).
   b. Keep a daily log.
   c. Run test chromatograms regularly.

   Step b points out the importance of keeping track of system parameters and the results of your chromatography during normal operation. Troubleshooting is easier if you know the typical conditions when the system is operating correctly.

   For example, if your system usually runs at *n* psi with a certain method, is the system pressure currently in the same range, or drastically higher (possibly caused by a plug) or lower (possibly caused by a leak)? Are pressure fluctuations in the same pressure range as during normal operation?

   When your system is installed, and each time you develop a new method, record the system conditions during normal operation.
3. Identify in the following order the symptom that varies from normal system operation:
   a. System pressure (high, low, or erratic)
   b. Baseline (fluidic path-related or detector electronics-related)

c. Changes in peak retention time (incorrect or changing over time)

d. Loss of peak resolution

e. Abnormal peak shape (smaller than expected, broad, tailing, and so on)

f. Incorrect qualitative and/or quantitative results

4. For each isolated symptom, identify a list of suspected causes. For suggested troubleshooting procedures, see page 81.

5. Run the performance tests for each chromatographic instrument to quickly determine if a problem exists with a particular instrument.

6. Refer to the troubleshooting information in the table titled "Troubleshooting hardware:" on page 81. This table is organized according to the parameters in step 3 and allows you to narrow the possible causes of a symptom and find suggested corrective actions.

If you determine that there is a problem related to another system component, refer to the appropriate operator's guide.

When to call Waters technical service

Many problems with a column heater and column heater/cooler can be easily corrected. However, if you cannot correct a condition, you can contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

When you call Waters Technical Service, have the following information available:

- Completed normal operation checklist for the method you are using
- Nature of the symptom
- Waters column heater or column heater/cooler serial number
- Flow rate(s)
- Operating pressure(s)
- Mobile phase(s)
- Detector settings
- Type and serial number of column(s)

- Sample type
- Control mode (stand-alone, Empower, or MassLynx)
- Firmware version and serial number

Error messages

The following table lists error messages related to the column heater or column heater/cooler that can appear on the separations module display screen:

Column heater-related error messages:

Error message	Explanation
Column heater door open [AlarmColumnHeaterDoor]	The column heater (cooler) door was opened. This door must remain closed to ensure proper temperature control.
Column heater not ambient [AlarmColumnHeatNotAmbient]	The column heater diagnostic needs to begin with the column heater in the range 18 to 28 °C. Open the column heater door. If this does not bring the column heater temperature within range, the problem could be: • Room temperature is not within the specified operation range • There is not enough clearance around instrument to permit adequate air flow • Faulty column heater module

Column heater-related error messages: (Continued)

Error message	Explanation
Column heater running [AlarmColumnHeaterRunning]	For the column heater, the column heater and sample heater/cooler time-share the same power supply. Although appropriate for normal operation, power time-sharing during the sample heater/cooler diagnostic can adversely affect the test results. The column heater should be shut off.
Column temp h/w fault [AlarmColumnTemperatureHw] 1 = communications (undefined device ID) 2 = watchdog (communications) 3 = h/w over temp (column heater and column heater/cooler) 4 = not used for 2nd generation CH/C 5 = not used for 2nd generation CH/C 6 = thermistor 7 = no 24 Vdc power	A hardware fault was detected by the column heater electronics. !HWERROR#! This may have been caused by: • Internal temperature limit exceeded. • Communications failure with the column heater. Check the interface cable to the separations module.
Column Heater/Cooler power off	A hardware fault was detected by the separations module. !HWERROR - 7!

Column heater-related error messages: (Continued)

Error message	Explanation
Column temp over limit (fault temp) [AlarmColumnTemperatureHigh]	The column temperature during a separation exceeded the upper limit that is defined in the current separation method. This may have been caused by any of the following: Insufficient equilibration time Ambient operating temperature limits exceeded Column heater failure
Error changing column [positionAlarmColumnSwitch]	The column switch indicates an error in moving to the requested position.
Low column temp (fault temp) [AlarmColumnTemperatureLow]	The column temperature during a separation has dropped below the lower limit that is defined in the current separation method. This may have been caused by any of the following: • Insufficient equilibration time. • Column door opened too long or too frequently. • Set point too close to ambient. The desired temperature must be at least 5 °C above ambient for proper operation. • Ambient operating temperature limits exceeded. • Column heater failure.

Column heater-related error messages: (Continued)

Error message	Explanation
Sample heater/cooler running [AlarmHeaterCoolerRunning]	For the column heater, the column heater and sample heater/cooler timeshare the same power supply. Although appropriate for normal operation, power timesharing during the column heater diagnostic can adversely affect the test results. The sample heater/cooler should be turned off.

Hardware troubleshooting

The table below provides suggestions for resolving hardware problems in the column heater or column heater/cooler. If the suggested solutions do not solve the problem, see page 77.

Troubleshooting hardware:

Symptom	Possible cause	Corrective action
Unit does not power on.	Column heater or column heater/cooler interface cable not connected.	Switch separations module to Off (**0**). Check and reseat interface cable at both devices.
	Column heater/cooler power cable not connected.	Check power cable.
	Column heater/cooler power supply fuse open or missing.	Replace power supply fuse.
	No power at AC voltage outlet.	Contact your facilities maintenance department to repair voltage source.

Troubleshooting hardware: (Continued)

Symptom	Possible cause	Corrective action
Startup diagnostics failure.	Internal problem with controller board, solvent management system, or sample management system.	Check rear panel cable connections. Power-cycle devices Off (**0**) and On (**1**). If failure does not clear, contact Waters Technical Service.
Fans not running.	Unit not powered on.	Power-on unit.
	Fan wiring or fan motor problem.	Call Waters Technical Service.
	Power supply not working.	Call Waters Technical Service.
Cannot reach temperature setpoint.	Hardware fault.	Note error message and call Waters Technical Service.
Column cooler cannot attain 15 °C below ambient.	Frost buildup.	Defrost unit.
	Internal fan failure.	Call Waters Technical Service.
Unstable temperature.	Internal fan failure.	Call Waters Technical Service.
	Compartment door not closing completely.	Clear obstruction.
	Damaged door seal.	Replace door seal. Call Waters Technical Service.
	Setpoint beneath 15 °C below ambient (hot/cold).	Check setpoint parameters.
	Setpoint not 5 °C above ambient (hot/cold).	Check setpoint parameters.

Troubleshooting hardware: (Continued)

Symptom	Possible cause	Corrective action
Column switching valve does not move.	Missing power or signal connector.	Check the power and signal connections. Check the fuse and replace, if necessary.
	Defective power supply.	Call Waters Technical Service.
	Defective valve.	Replace valve.
	Worn rotor causing high friction.	Rebuild valve.
Column switching valve valve appears blocked.	Rotor seal clogged.	Rebuild valve.
Column switching valve leaks.	Stator screws are loose.	Tighten stator screws.
	Defective rotor seal.	Rebuild valve.
Column switching valve has high pressure which caused the separations module to shut down.	Clogged rotor.	Rebuild valve.
Connection tubing is leaking.	Worn fitting.	Replace fitting or tubing.
Connection tubing is blocked.	System not thoroughly flushed out.	Replace tubing.

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 sharp-point puncture injury.)


Warning: (Risk of hand crush injury.)


Warning: (Risk of exposure to ultraviolet radiation.)


Warning: (Risk of contacting corrosive substances.)


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

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

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

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Specific warnings

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

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.

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

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

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.

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

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

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.

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

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

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

Biohazard warning

This warning applies to Waters instruments that can be used to process material that can 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 Practice, 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 Practice (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 advisory

Caution advisories appear where an instrument or device can be subject to use or misuse capable of damaging it or compromising a sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.

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

Warnings that apply to all Waters instruments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

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

警告：当有压力的情况下使用管线时，小心注意以下几点：

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

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

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

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

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

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

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

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

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

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

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

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

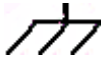
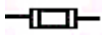
경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

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

Electrical and handling symbols

Electrical symbols

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

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse

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

This appendix includes specifications for the following:

- Physical
- Environmental
- Electrical
- Instrument control and communication

Physical specifications:

Item	Specification
Height	22.5 inches (57.15 cm)
Depth (without drip tray fitting)	21.25 inches (52.975 cm)
Weight: Column heater Column heater/cooler	 22.0 pounds (9 kg) without columns or bottles 32.0 pounds (14 kg) without columns or bottles
Access to fluidics	Inside the heater/cooler compartment
Number of columns	Two 300-mm columns with guards, four 150-mm columns without guards, or six-50-mm columns without guards
Column sizes	ID up to 7.8-mm maximum Column guards up to 20-mm maximum
Valve management	One valve. 6-, 8-, or 10-port valve. Rheodyne only.
Width	6.0 inches (15.24 cm)

Performance specifications:

Item	Specification
Ambient operating temperature	4 to 40 °C (39.2 to 104 °F)
Ambient operating humidity	20 to 80%, noncondensing
Ambient storage temperature	–40 °C to 70 °C
Ambient storage humidity range	0 to 90%, noncondensing
Set point temperature range	4 to 65 °C
Compartment operational temperature range	Column heater/cooler: the greater, 15 °C below ambient or 4 to 65 °C Column heater: 5 °C above ambient to 65 °C
Compartment temperature accuracy	±0.8 °C between 20 and 50 °C. ±1.0 °C for remaining range Temperature measured by a NIST traceable probe located next to the measurement sensor will be within this specification.
Over temperature	Thermal switch shuts off heater at temperatures >80 °C ±10% (temperature measured at thermal switch).
Leak control	Drip trays in compartment and under valve. One exit drain fitting.
Door open to heater cutoff delay	1-minute maximum

Column heater/cooler electrical specifications:

Item	Specification
AC input voltage range	100 to 240 Vac (grounded alternating current voltage source)
AC input frequency range	50 to 60 Hz

Instrument control and communication specifications:

Item	Specification
26-pin male D-series subconnector	Control and communication from the separations module **Tip:** This connection also serves as a power interface for the column heater and column heater/cooler.

C Spare Parts

The following table includes information on recommended spare parts.

Tip: Parts not included in the table below must be replaced by Waters® Technical Service.

The column heater and column heater/cooler contain no user-serviceable parts except for replacement fuses for the column heater/cooler. The column selection valves can be maintained using the recommended spare parts.

Spare parts:

Item	Part number
Column heater/cooler – 3.15 A, 250 V Fast-Blo Fuses, 5 × 20 mm (IEC), Pack of 2	700001800
Column clip, Pack of 4	700002143
Interface cable (separations module to column heater or column heater/cooler)	700002146
Drip tray	700002140
Drip tray drain fitting	700002141
Column switching valve – Performance Maintenance Kit, 2-column regeneration valve	700000424
Column switching valve – Performance Maintenance Kit, 3-column selection valve	700000431
Column switching valve – Performance Maintenance Kit, 6-column selection valve	700000434

D Solvent Considerations

Warning: To avoid chemical hazards, always observe safe laboratory practices when operating your system.

.

Contents:

Introduction

Clean solvents

Clean solvents provide:

- Reproducible results
- Operation with minimal instrument maintenance

A dirty solvent can cause:

- Baseline noise and drift
- Blockage of the solvent filters with particulate matter

Solvent quality

Use HPLC-grade solvents to ensure the best possible results. Filter solvents through 0.45-µm filters before use. Solvents distilled in glass maintain their purity from lot to lot; use them to ensure the best possible results.

Preparation checklist

The following solvent preparation guidelines help to ensure stable baselines and good resolution:

- 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 provide filtered water, filter it through a 0.45-µm membrane filter before use.

Buffers

When you use buffers, dissolve salts first, adjust the pH, 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 the miscibility of the solvents. When you change solvents, be aware that:

- Changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as *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 may precipitate when mixed with organic solvents.

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

Solvent miscibility:

Polarity index	Solvent	Viscosity CP, 20 °C	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	26	—
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

Solvent miscibility: (Continued)

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 atm)	Miscibility Number (M)	λ Cutoff (nm)
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
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	Dimethyl-formamide	0.90	153.0	12	—
6.5	Dimethyl-sulfoxide	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	—	—

How to use 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, use a good quality reagent and filter it through a 0.45-µm filter.

Do not leave the buffer stored in the system after use. Flush all fluidic 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).

Solvent stabilizers

Solvents containing stabilizers, for example, THF with BHT, should never be left to dry out in the system flow path. A dried system flow path, including the detector flow cell, will become contaminated with residual stabilizer requiring substantial cleaning to restore cleanliness to the initial condition.

Solvent viscosity

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However when you are running a gradient, the viscosity changes that occur as the solvents are mixed in different proportions can result in pressure changes during the run. For example, a 1:1 mixture of water and methanol produces approximately twice the pressure of water.

If the extent to which the pressure changes affect the analysis is not known, monitor the pressure during the run.

Mobile phase solvent degassing

Mobile phase solvent degassing and/or sparging is not required with the Waters Column/Fluidics Organizer.

Wavelength selection

The tables in this section provide UV cutoff values for:

- Common solvents
- Common mixed mobile phases
- Chromophores

UV cutoffs for common solvents

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

UV cutoff wavelengths for common chromatographic solvents:

Solvent	UV cutoff (nm)	Solvent	UV cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Isooctane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225

UV cutoff wavelengths for common chromatographic solvents: (Continued)

Solvent	UV cutoff (nm)	Solvent	UV cutoff (nm)
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 table below 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, since 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

Wavelength cutoffs for different mobile phases: (Continued)

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190
EDTA, disodium, 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
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
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	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

Refractive indices for common chromatographic solvents: (Continued)

Solvent	RI	Solvent	RI
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
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