

Waters Alliance e2695 Separations Module

Operator's Guide

General Information

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Safety considerations

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

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.

Considerations specific to the Waters Alliance e2695 Separations Module

Power cord replacement hazard



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

Solvent leakage hazard

The source exhaust system is designed to be robust and leak-tight. Waters recommends you perform a hazard analysis, assuming a maximum leak into the laboratory atmosphere of 10% LC eluate.



Warning:

- To confirm the integrity of the source exhaust system, renew the source O-rings at intervals not exceeding one year.
- To avoid chemical degradation of the source O-rings, which can withstand exposure only to certain solvents, determine whether any solvents you use that are not listed are chemically compatible with the composition of the O-rings.

Flammable solvents hazard



Warning: To prevent ignition of flammable solvent vapors in the enclosed space of a mass spectrometer's ion source, ensure that nitrogen flows continuously through the source. The nitrogen supply pressure must not fall below 690 kPa (6.9 bar, 100 psi) during an analysis requiring the use of flammable solvents. Also a gas-fail device must be installed, to interrupt the flow of LC solvent should the nitrogen supply fail.

When using flammable solvents, ensure that a stream of nitrogen continuously flushes the instrument's source, and the nitrogen supply pressure remains above 690 kPa (6.9 bar, 100 psi). You must also install a gas-fail device that interrupts the solvent flowing from the LC system in the event the supply of nitrogen fails.

Hand crush hazard



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

High temperature hazard



Warning: To avoid burn injuries, avoid touching the source enclosure when operating or servicing the instrument.

High voltage hazard



Warning: To avoid electric shock, observe these precautions:

- Do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.
- When the instrument is in Operate mode, avoid touching the areas marked with the high voltage warning symbol. To touch external areas marked with the symbol, first put the instrument in Standby mode.

Bottle placement prohibition



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



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

FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the 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.

Electrical power safety notice

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

Equipment misuse notice

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

Safety advisories

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

Operating this instrument

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

Applicable symbols

Symbol	Definition
	Manufacturer
	Date of manufacture
	Authorized representative of the European Community.
	UK Conformity Assessed marking confirms that a manufactured product is in conformity with the applicable requirements for products sold within Great Britain.
	Confirms that a manufactured product complies with all applicable European Community directives.
 ABN 49 065 444 751	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements.
	Consult instructions for use.
	Alternating current
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with the Waste Electrical and Electronic Equipment Directive (WEEE) 2012/19/EU, contact Waters Corporation for the correct disposal and recycling instructions.

Symbol	Definition
SN	Serial number
REF	Part number catalog number

Audience and purpose

This guide is intended for use by individuals who need to install, operate, maintain, or troubleshoot the Waters Alliance e2695 Separations Module.

This guide sets forth procedures for unpacking, installing, using, maintaining, and troubleshooting the Waters Alliance e2695 Separations Module. It also includes appendixes for specifications, spare parts, and solvent considerations.

Intended use of the Waters Alliance e2695 Separations Module

Waters designed the Alliance e2695 Separations Module, an integrated solvent and sample management platform, to facilitate all critical separations functions. The Waters Alliance e2695 Separations Module is for research use only and is not intended for use in diagnostic applications.

Calibrating

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

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

Quality control

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

EMC considerations

Canada spectrum management emissions notice

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

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

ISM Classification: ISM Group 1 Class A

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

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

Class A products are suitable for use in all establishments other than residential locations and those directly connected to a low voltage power supply network supplying a building for domestic purposes.

There may be potential difficulties in ensuring electromagnetic compatibility in other environments due to conducted as well as radiated disturbances.

EC authorized representative



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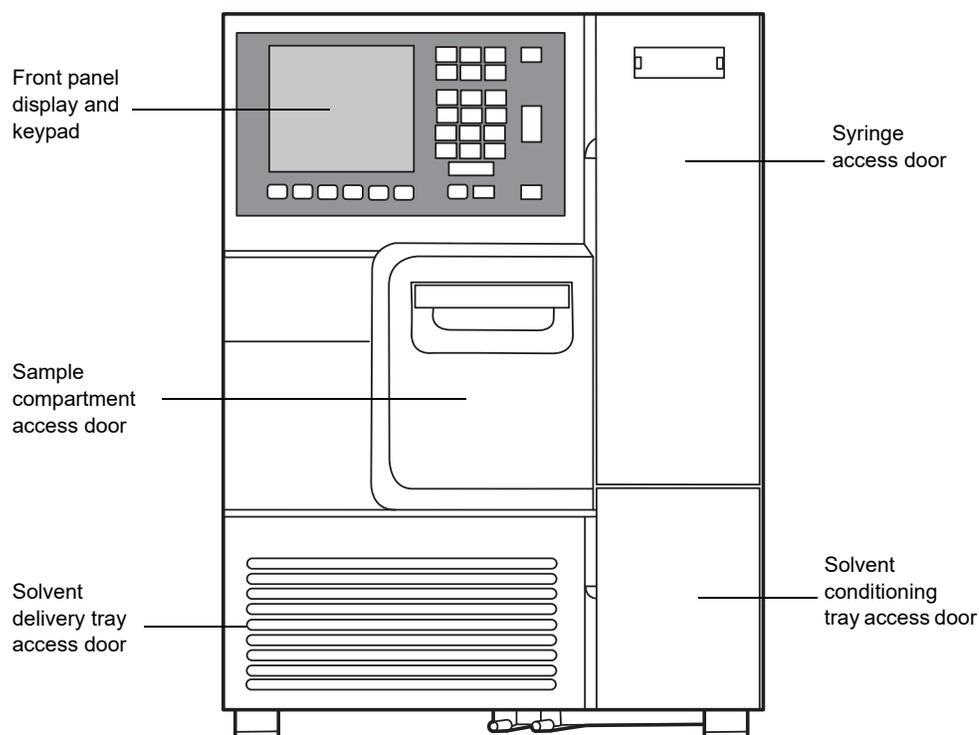
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1 Getting started with the Alliance e2695 Separations Module

1.1 Separations module overview

The Alliance e2695 Separations Module is an integrated solvent and sample management platform. Integrating the two, traditional high performance liquid chromatography (HPLC) components—a solvent management system and a sample management system—facilitates all critical separations functions.

Figure 1–1: Alliance e2695 Separations Module (front view)



1.1.1 HPLC system configurations

The separations module supports RS-232, IEEE-488, Ethernet, and I/O connections for compatibility with a variety of HPLC system configurations. It can function in these ways:

- As the source of Input/Output and timing signals in a simple, standalone HPLC system
 - As the IEEE-488 system controller in an HPLC standalone system that includes these Waters detectors:
 - 2414 or 2410* refractive index (RI), or 410* differential refractive index (dRI)
 - 2414 RI, 2410 RI, or 410 dRI and the 2487* dual wavelength or 486* tunable absorbance
 - 2487 dual wavelength or 486 tunable absorbance
- *A legacy product that is no longer available for purchase from Waters.
- As a component of an HPLC system controlled by Empower or MassLynx software that uses IEEE-488 communications
 - As a component of an HPLC system controlled by Empower software and that uses Ethernet communications
 - As a component of an HPLC system controlled by a non-Waters data system that uses RS-232 communications

1.1.2 Solvent bottle tray kits

There are several solvent bottle tray kits available for use with Alliance HPLC system configurations.

Table 1–1: Solvent bottle tray kit options

System configuration	Kit part number	Where used	Description
A series: Bottle organizer	176003103	Systems with a separations module only	Bottle tray comes with a removable drip tray and is stacked atop the detector. Accommodates up to two 4-liter bottles and nine 1-liter bottles.
B series: Bottle tray and detector base	176003104	Systems with a separations module and a column heater or column heater/cooler installed	Bottle tray comes with a removable drip tray and adjacent detector base. Enables placement of detector atop column heater. Accommodates up to two 4-liter bottles and four 1-liter bottles.
C series: Bottle bucket	176003105	Any Alliance HPLC system	Free-standing bottle tray. Accommodates a combination of 1 to 4-liter bottles.

Figure 1–2: A-series solvent bottle organizer configuration

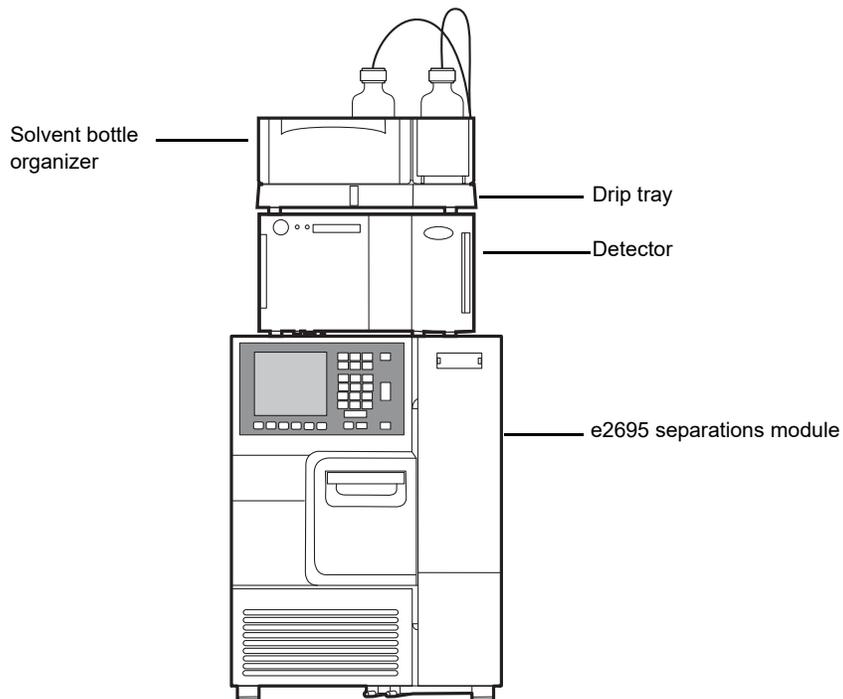


Figure 1–3: B-series solvent bottle tray configuration (only single detector shown)

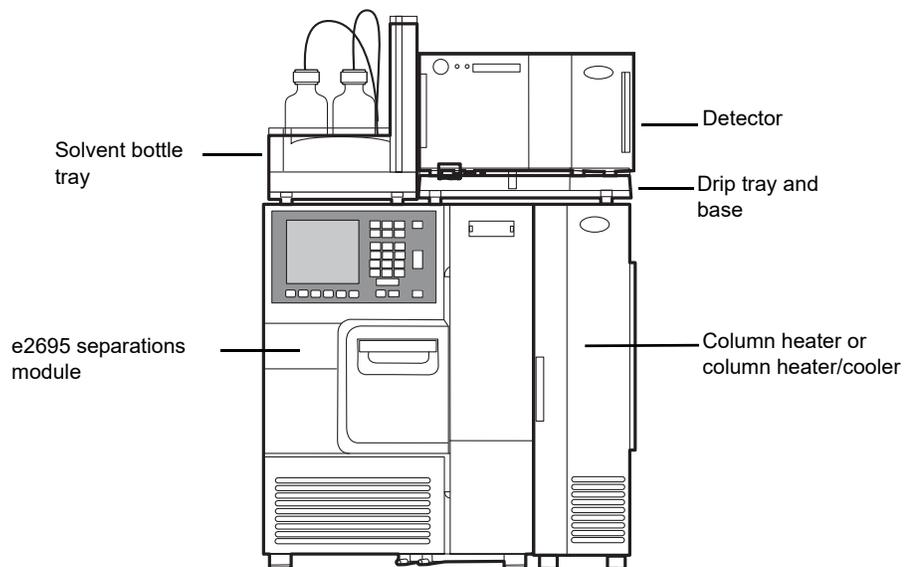
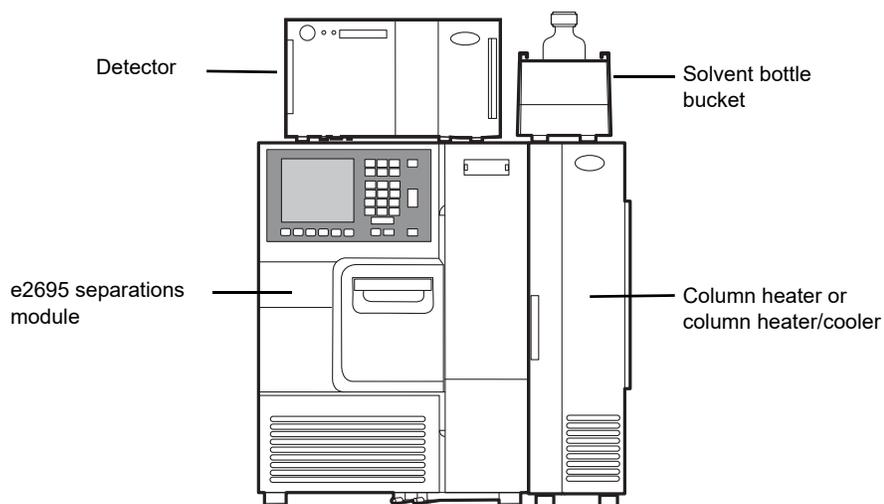


Figure 1–4: C-series solvent bottle bucket configuration:



1.1.3 Control of chromatographic functions

Once you program a method, the separations module controls these functions:

- Solvent composition
- Flow rate
- Plunger seal wash flow
- Needle wash flow
- Sample injection
- In-line vacuum degassing
- External events
- Operation of detectors over the IEEE-488 interface bus
- Column heating/cooling (if installed)
- Sample heating/cooling (if installed)

The separations module controls parameters that influence a chromatographic separation. You can program the values of these parameters to change from method to method without setting up the instrument before each run.

1.1.4 Spill protection

All fluid-handling areas of the separations module can contain spills. Spilled solvent drains to the waste line connectors located below the front panel. A drip tray for the top cover provides leak protection for the separations module when a detector is placed atop it. A solvent bottle tray, which provides storage for solvent reservoirs and wash solvent reservoirs, also provides leak protection for a spill.

1.1.5 Record-keeping functions

The separations module automatically records the following information:

- System errors
- Programmed operating conditions for each run (local methods only)

System errors may be viewed from the front panel by pressing the **Log** soft key. Local methods are viewable on the front panel, and they can be printed if a printer is attached or transferred to an external device using the RS-232 port. Remote methods (sent from Empower) are not recorded or viewable on the front panel.

1.1.6 Power supply

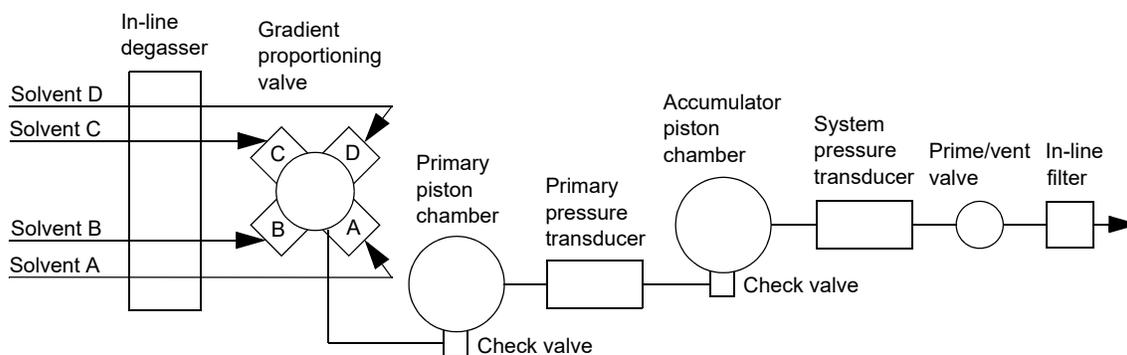
The separations module is factory-equipped with a power supply of sufficient capacity to simultaneously power an optional sample heater/cooler.

The power supply is protected against short circuits. It does not use external replacement fuses, and is instead reset by powering-off the instrument and then powering it back on.

1.2 Solvent management system overview

The solvent management system blends and delivers solvents from the reservoir bottles to the sample management system via a pulse-free, low-dispersion flow path. The system incorporates a serial flow path, two independently driven plungers, and two check valves for optimal flow control. Synchronized Composition Control (SCC) determines the gradient proportioning valve (GPV) actuation rate based on the selected flow rate, composition and piston volume.

Figure 1–5: Flow path through the solvent management system:



1. The solvent is processed through the in-line vacuum degasser.
2. The gradient proportioning valve blends the solvents.
3. The blended solvents flow through the inlet check valve and into the primary piston chamber while the accumulator piston delivers solvent, under pressure, to the system pressure transducer.

4. Just before the accumulator chamber empties, the solvent in the primary piston chamber is precompressed to a pressure slightly less than that indicated by the system pressure transducer.
5. When the accumulator piston chamber is empty, the primary piston delivers solvent, under pressure, through the primary pressure transducer. It refills the accumulator piston chamber and delivers solvent, under pressure, through the system pressure transducer, maintaining a constant flow through the system. The cycle repeats, beginning at [step 3](#).
6. The system pressure transducer measures the operating pressure. The software compares the primary head pressure with the system pressure and regulates the precompression step, balancing the pressures and providing a smooth, ripple-free flow.
7. Solvent flows from the system pressure transducer outlet to the prime/vent valve and into an in-line filter.
8. From the in-line filter, the solvent flows to the sample management system.

1.2.1 Solvent blending

The gradient proportioning valve (GPV) blends as many as four solvents in any combination and proportion. It produces predictable gradient segments regardless of solvent compressibility and system back-pressure. Solvent selection and proportioning take place on the low-pressure (intake) side of the solvent delivery system. Solvents continue to blend in each of the piston chambers.

1.2.2 Prime/vent valve

The prime/vent valve is designed so that you can attach a syringe to it and draw solvent through the solvent management system.

1.2.3 Plunger seal wash system

The seal wash solvent lubricates the plunger and flushes away any solvent or dried salts forced past the plunger seal from the high pressure side of each piston chamber. Washing the seals extends their life. The plunger seal wash system operates in this sequence:

- Plunger seal wash solvent flows from a reservoir to the solenoid wash pump and then to a cavity behind the main plunger seal in the primary head.
- The solvent flows from the head and into the cavity behind the plunger seal, in the accumulator head.
- From the accumulator head, the solvent flows to waste.

When the solvent management system is delivering solvent, the plunger seal wash pump intermittently circulates the wash solvent according to a time that you specify.

1.2.4 Loss of prime protection

When a loss of prime occurs because a solvent reservoir empties, the resultant loss of operating pressure to below 172 kPa (1.7 bar, 25 psi) for 125 cycles of the solvent delivery mechanism causes the separations module to stop operating.

1.2.5 Preferred plunger stroke volume

The separations module's discrete, programmable stroke volumes provide optimal flow delivery and solvent blending. Nevertheless, you can improve performance for specific applications by overriding the default (preprogrammed) stroke volume of 130 μL . To do so, you select a preferred stroke volume of 25 μL , 50 μL , or 100 μL , as shown in the following table. A lower stroke volume can be more effective with solvents that require additional mixing, like trifluoroacetic acid (TFA) gradients. This additional mixing, unlike static or dynamic mixing, occurs without added delay volume.

Table 1–2: Alternate stroke volumes

Flow range (mL/min)	Stroke volume (μL)
0.050 - 0.530	25
0.531 - 1.230	50
1.231 - 3.030	100
3.031 - 5.000	130

1.2.6 In-line vacuum degasser

The design of the PerformancePlus in-line vacuum degasser combines a variable speed, continuously operating vacuum pump with low-internal-volume degasser chambers. The result is shortened instrument priming and equilibration times, and minimum delay when resuming operation from an idle state or following a solvent change.

The degasser vacuum pump is designed specially for membrane degassing of HPLC mobile phases. The continuously running pump provides rapid vacuum pull-down at high speed and consistent vacuum level at low speed.

1.2.6.1 Degasser considerations

Degassing efficiency is based on the solvent gas load and the length of time that the solvent remains in its vacuum chamber. Gas removal efficiency decreases as the solvent flow rate increases because the solvent is in the vacuum chamber for less time. Adopting a normal analytical flow rate range of 0.000 to 5.000 mL/min, the degasser removes most of the dissolved gases. Because you can perform dry and wet priming at flow rates greater than 5.000 mL/min, you must expose the solvents to the degasser vacuum for a short time (after priming) at zero flow rate (see [“Equilibrate solvents in the in-line vacuum degasser” on page 77](#)).

1.2.6.2 Vacuum degasser pressure transducers

Separations modules are equipped with an absolute pressure transducer (APT), which is unaffected by altitude or barometric changes. The following table lists the attributes of APT-equipped separations modules.

Table 1–3: Absolute pressure transducer attributes

Item	Absolute pressure transducer
Units displayed	kPa, bar, psa
Sign of displayed unit	Positive

Table 1–3: Absolute pressure transducer attributes (continued)

Item	Absolute pressure transducer
Theoretical maximum vacuum (based on 1 atm at sea level)	0 psia
Operating range (using the default vacuum pressure threshold)	22.1 to 0 kPa (0.2 to 0 bar, 3.2 to 0 psia) (If pressure is out of range, an asterisk [*] appears in the pressure field.)
“Typical” value	5.0 to 10.0 kPa (0.05 to 0.10 bar, 0.7 to 1.5 psia)
More vacuum	Smaller number is displayed

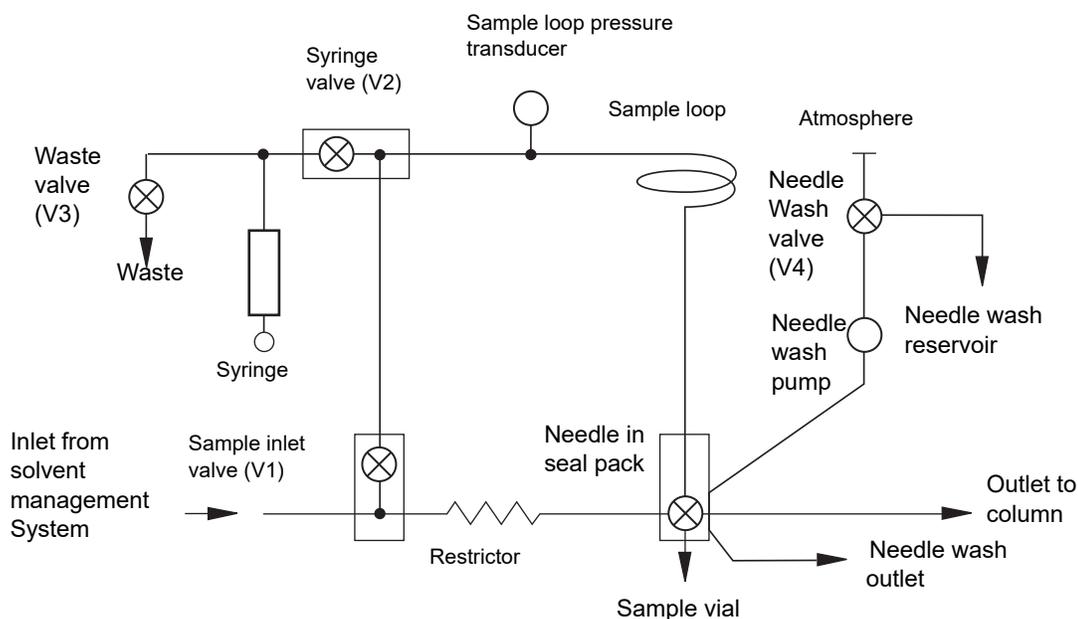
1.3 Sample management system overview

The sample management system holds and positions the sample vials and injects the samples into the solvent flow. The system’s five carousels each hold as many as 24 sample vials for a total of 120 vials. The vials are industry-standard, 2-mL vials with snap-on, crimp-on, or screw-on caps. A carrier rotates the carousels to the injection station in the sample compartment.

1.3.1 Flow path

Four valves in the sample management system control the flow of solvent, sample, and needle wash solvent. These valves, designated V1 through V4, are shown in the following figure. You can monitor the position of each valve in the Status screen. (See [figure “Second page of the Status screen” on page 69.](#))

Figure 1–6: Flow path through the sample management system



The flow path through the sample management system changes according to the function being performed.

1.3.2 Normal flow

During normal flow, V1 is open, allowing solvent to flow along two paths:

- 95 percent of the solvent flows through the body of V2, the sample loop pressure transducer, the sample loop, and needle. The needle is in the Stream position, and solvent flows out the needle port, through the seal pack, and out to the column.
- 5 percent of the solvent flows through the restrictor and the seal pack, and out to the column.

1.3.3 Injection

The injection cycle comprises four sequential steps:

1. Isolating the sample loop from the normal flow – V1, V2, and V3 close, and then V4 opens, the needle wash pump powers-on, and the needle moves to the Seal position. V2 and V3 then open to vent the sample loop pressure and to equilibrate the loop to atmospheric pressure.
2. Sample withdrawal – The needle extends into the sample vial, and then V3 closes and the needle wash pump powers-off. The syringe plunger retracts, drawing the sample from the vial into the sample loop. The sample does not enter the syringe.
3. Preparing the needle for injection – The needle moves to the Seal position.

Note: If the extended wash is selected, V4 opens and the needle wash pump runs while the needle retracts to the Seal position. Once the needle has reached the Seal position, V4 closes and the needle wash pump is powered-off.

4. Injecting the sample – V2 closes, V3 opens, and the needle moves to the stream position to pressurize the sample loop. V1 opens, returning the solvent to normal flow. The solvent pushes the sample out of the sample loop, through the needle, and out to the column. The syringe returns to the home position, expelling drawn solvent to waste, and V3 closes.

1.3.4 Carousel configuration

Each carousel is identified by color and letter and occupies a unique position on the carousel carrier. The separations module displays a warning when you attempt an injection with a carousel in the wrong position (see [“Set configuration parameters” on page 47](#)). You can perform as many as 99 injections per sample vial, and you can offset the depth of the needle in the vial for small sample volumes, small injection volumes, or to compensate for variations in vial bottom thickness. Offsets may also be necessary when you use low-volume inserts in the sample vials.

Spills and condensation in the sample compartment are routed to a waste connection below the front panel.

You can load a carousel into the carrier while an injection is underway. An open-door sensor prevents the carrier from automatically advancing while you load the carousel (see [“Load carousels” on page 62](#)).

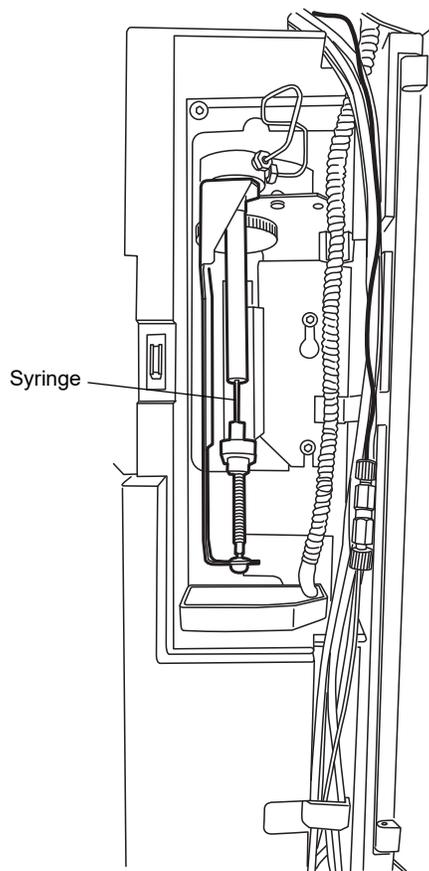
1.3.5 Vial presence

A light-emitting diode assembly searches for the presence of a vial before the needle moves to the Draw position. This searching prevents air from being accidentally injected into the solvent stream when a programmed vial position is accidentally left empty. See the Verify vial presence parameter in [“Set configuration parameters” on page 47](#).

1.3.6 Syringe assembly

The syringe assembly is located behind a front panel door for ease of access (see [figure “Syringe assembly” on page 28](#)). The separations module uses a standard 250- μ L syringe; as an option, you can install a 25- μ L, 100- μ L, or 2500- μ L syringe. See [“Replace the syringe” on page 137](#) for the procedure to change the syringe. Note that the separations module serial number is located on the syringe mounting plate.

Figure 1–7: Syringe assembly



1.4 Operating configurations

The separations module can operate in three general control modes:

- System Controller
- No Interaction
- Remote Control

Both System Controller and No Interaction are standalone modes.

1.4.1 System Controller mode

Note: This mode is not available if it is disabled on the Service Diagnostics page.

In this standalone mode, the separations module functions as the HPLC system controller. You can perform runs manually or automatically in the System Controller mode. For a manual run, you input parameter values and selections through the front panel of the separations module. For an automatic run, you use separation methods, sample sets and/or sample templates to control the separations module. See [Chapter 4](#) for information on using the front panel to control runs. See [Chapter 6](#) for procedures to create and store separations' methods, sample sets, and sample templates.

Depending on your system components, you can connect the separations module to other components via these signal connections:

- IEEE-488 (digital) for Waters detectors
- I/O (analog, hard wire cables)
- RS-232 (digital) for non-Waters data systems, printers, or integrators

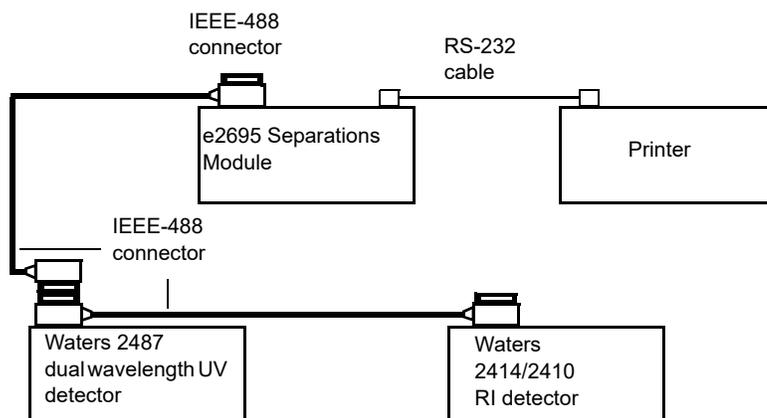
Follow the procedures in [Chapter 4](#) to control the HPLC system in the System Controller mode.

In System Controller mode, the separations module cannot control any other instruments/detectors via Ethernet communications. You can use Ethernet communications only when the separations module is configured for remote control (see [“Remote Control mode” on page 31](#)).

1.4.1.1 Digital signal connections for system controller operation

The following figure illustrates a typical HPLC system configuration where the separations module (in System Controller mode) uses only IEEE-488 and RS-232 digital signal connections to control the HPLC system components.

Figure 1–8: Digital signal control of an HPLC system



In the System Controller mode, the separations module can control as many as three detector channels on the IEEE-488 bus (two UV detector channels and one RI detector channel). These Waters detectors can be used:

- 2414 RI, 2410 RI, or 410 dRI
- 2487 dual wavelength or 486 tunable absorbance (any combination of up to two channels)

In this type of system, you specify detector parameters in the separation method's detector table. During a run, the separations module sends commands such as to change wavelength or to change sensitivity to the detector(s) at specified times. See [“Set detector parameters” on page 107](#) for details on setting up a detector table.

The separations module does not process detector data. Detector results and other system data are sent directly to a data acquisition system, integrator, or chart recorder.

Besides the IEEE-488 controlled detectors, you can use RS-232 and I/O connections to control other components that do not support the IEEE-488 interface bus.

See [“Digital signal connections” on page 211](#) for details on making digital signal connections.

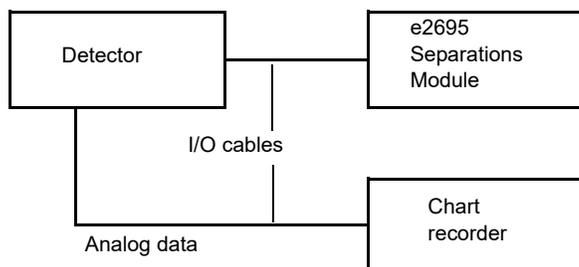
1.4.2 No Interaction mode

In this standalone mode, the separations module controls non-IEEE-488 devices in the HPLC system using the input/output (I/O) connections on its rear panel. This mode disconnects the separations module from the IEEE-488 and Ethernet interface buses. Use it when you want to suspend communications with a connected Empower system and operate system components from their front panels.

1.4.2.1 Input/output (I/O) signal connections for No Interaction mode

The following figure shows an example HPLC system configuration where the separations module controls the HPLC components through I/O signal connections.

Figure 1–9: I/O signal control of an HPLC system



In this type of system, you set up each HPLC component individually using its front panel. You connect the components to the separations module using the I/O connector on its rear panel. During a run, I/O signals from the separations module trigger synchronized or timed events in all the HPLC components. The separations module does not process detector data. Detector results and other system data go directly to a data acquisition system, integrator, or chart recorder. See [“I/O signal connections” on page 208](#) for details on making I/O signal connections.

1.4.3 Remote Control mode

In remote control mode, the separations module and the other HPLC system components are controlled by one of these data systems:

- Empower Chromatography Data Software
- Waters MassLynx Mass Spectrometry Software for mass spectrometers
- RS-232 communications by non-Waters data systems
- An external autosampler (the Waters 2700 Sample Manager, for example) with the separations module in the Operate Gradient by Event In mode

Use the procedures in [Chapter 5](#) to set up the separations module for remote control.

1.4.3.1 Supported IEEE-488 and Ethernet configurations

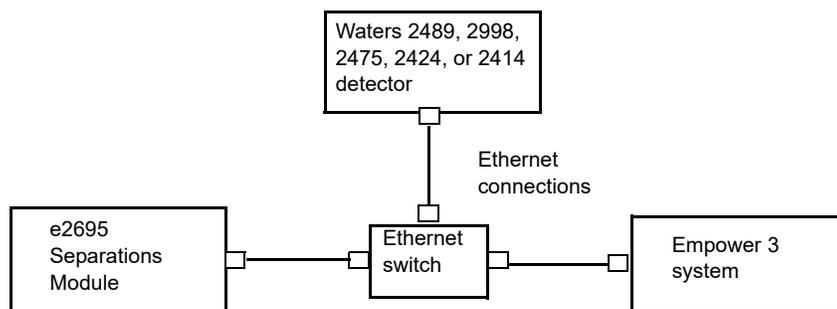
In remote control mode, the separations module can participate in any one of these supported IEEE-488 and Ethernet configurations:

- Where the separations module and detector(s) both communicate with the data system via IEEE-488
- Where the separations module and detector(s) both communicate with the data system via Ethernet
- Where the data system and separations module communicate via IEEE-488, and include one or more detectors that can only communicate with the data system via Ethernet (that is, a Waters 2998, 2489, 2475, 2424, e-SAT/IN module-connected, or Mass Spectrometer (for example, 3100) Detector)

1.4.3.2 Empower control

The following figure illustrates a typical HPLC system using Ethernet communications and Empower chromatography data software.

Figure 1–10: Typical e2695 Separations Module HPLC system under Empower 3 software control



The separations module can communicate with Empower software via Ethernet or via the IEEE-488 interface bus (but not both simultaneously). Use Empower software to create instrument methods and method sets to control the separations module and other HPLC system components. See the Empower software documentation for more information on creating instrument methods and method sets.

1.4.3.3 MassLynx control

When you control the separations module with MassLynx Mass Spectrometry Software, you use the MassLynx inlet editor to define operating parameters for the separations module and detector(s)—but not the mass spectrometer—used in the LC/MS system. See the MassLynx software documentation for more information on controlling the separations module.

1.4.3.4 RS-232 control

To set parameters that remotely control the separations module from a data system that uses RS-232 (ASCII or binary) communications, refer to the documentation that comes with the data system.

1.4.3.5 Operate Gradient by Event In control

In Operate Gradient by Event In mode, the separations module is connected to an external autosampler (a Waters 2700 Sample Manager, for example). In this mode, the separations module provides gradient functionality, and the external autosampler provides the sampling/injection functionality. The external autosampler, which is connected to the I/O signal connector on the separations module's rear panel, signals the solvent management system to begin a gradient.

1.5 Options and accessories

Various options for the separations module are available to suit your applications and site requirements. You can display a list of the hardware options currently installed in your separations module by pressing the Configuration screen's **Options** key. See [“Configure the separations module” on page 47](#) for details on displaying the Configure screen.

1.5.1 Column heater

The column heater maintains the column at temperatures from 5 °C above ambient (minimum of 20 °C) to 60 °C. An alarm warns you when the temperature varies outside the range that you specify. The *Waters Alliance Series Column Heater and Column Heater/Cooler Operator's Guide* presents the procedures for installing, operating, maintaining, and basic troubleshooting of the Alliance column heaters and column heater/coolers.

1.5.2 Column heater/cooler

The column heater/cooler expands the heating range over the column heater, while offering column cooling capabilities. The column heater/cooler maintains the column at temperatures from 4 °C to 65 °C, with a set point temperature range that is ambient minus 15 °C or 4 °C (whichever is greater) to 65 °C, in 1 °C increments. The *Waters Alliance Series Column Heater and Column Heater/Cooler Operator's Guide* presents the procedures for installing, operating, maintaining, and basic troubleshooting of the Alliance column heaters and column heater/coolers.

1.5.3 Column pre-heater

For certain applications, especially in laboratories with less than ideal temperature control, the column pre-heater is useful in stabilizing the temperature of the mobile phase as it enters the chromatographic column. This stabilization, or conditioning, of the mobile phase improves retention-time precision and overall chromatographic performance. The Pre-Heater Plate Assembly (WAT271064) does not come as standard equipment in either the column heater or the column heater/cooler; it is an option that must be ordered separately and installed at the e2695 Alliance instrument site.

1.5.4 Sample heater/cooler

To optimize sample stability and/or solubility, the sample heater/cooler maintains the sample compartment at temperatures from 4 °C to 40 °C, with a set point temperature range that is ambient minus 25 °C or 4 °C (whichever is greater) to 40 °C, in 1 °C increments. This option is installed on the separations module at the factory or is available as an option installed on site by Waters personnel. The heater/cooler mounts through the rear panel of the separations module. It uses four Peltier devices for temperature control.

1.5.5 Syringes

You can install a 25- μ L, 100- μ L, 250- μ L, or 2500- μ L sample syringe in the separations module. The 250- μ L syringe is standard. (See [“Replace the syringe” on page 137](#) for the procedure to replace the syringe.)

1.5.6 Sample loops

You can install an additional 100- μ L sample loop or you can install a larger one to inject sample volumes greater than 100 μ L. (See [“Install an optional sample loop” on page 214](#) for the procedure to install an optional sample loop.)

1.5.7 Column selection valve

The motorized column selection valve enables the system to switch solvent flow to one of several columns. Such valves are only recognized by Alliance if they are installed in the column heater.

The column selection valve is usually mounted in the column heater or column heater/cooler (see [figure “e2695 column heater” on page 35](#)). It is installed on site as an add-on option.

The e2695 has four column selection valve options, as described in the following table.

Table 1–4: Column selection valve options

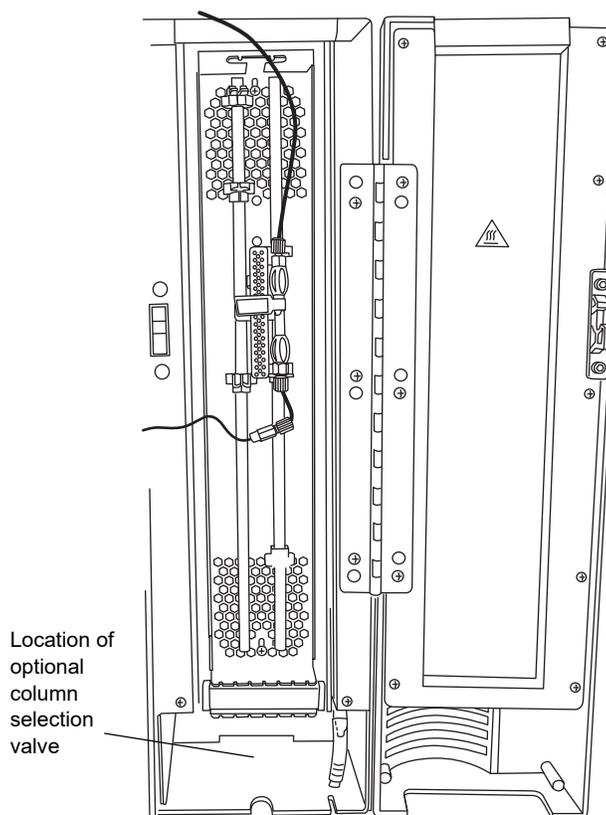
Description	Software detection	Controlled by
2 Column (6 port, 2 position)	No	I/O
3 Column	Yes	e2695 software
6 Column	Yes	e2695 software
2 Column- Regeneration (10 port, 2 position)	Yes	e2695 software

The software automatically detects the presence and type of column selection valve when you power-on the separations module. You can specify column selection:

- Using the front panel (see [“Perform direct functions” on page 72](#))
- Through the separation method (see [“Create and edit separation methods” on page 90](#))
- As part of a function in the sample set method (see [“Create and edit a sample set” on page 113](#))

The Status screen shows the currently selected column or flow path for only those valves detected by the software.

Figure 1–11: e2695 column heater



1.5.7.1 Two-column selection valve

The firmware does not recognize the 2-column selection valve (6 port, 2 position) option. Instead, the option is controlled via one of the four I/O event switches on the separations module's rear panel. You select one column by turning the switch to Off and the second column by turning the switch to On.

1.5.7.2 Three-column selection valve

The 3-column selection valve selects any of three column positions. It is preconfigured before shipment, but you can reconfigure it. For more information, see [“Connect the column selection valve” on page 203](#).

1.5.7.3 Six-column selection valve

The 6-column selection valve selects any one of six column positions. You can specify any position as bypass or waste. For more information, see [“Connect the column selection valve” on page 203](#).

1.5.7.4 Two-column regeneration valve

With the 2-column regeneration valve, you perform LC operations using one column while a second column undergoes regeneration by a second solvent delivery system. For more information, see [“Connect the column selection valve” on page 203](#).

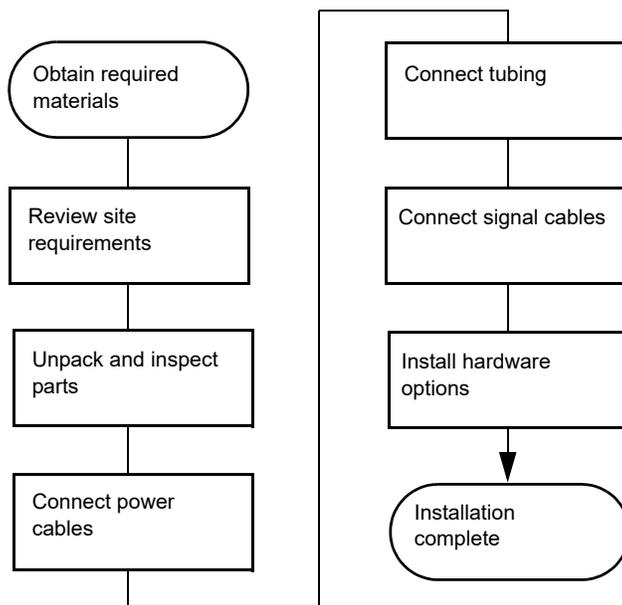
2

Install the e2695 Separations Module

2.1 Installation overview

This chapter explains how to connect electrical cables, signal cables, tubing and fittings, and hardware options to the separations module.

Figure 2–1: Major steps to installing the separations module



Required materials

- Screwdriver, cross-head, #2
- Screwdriver, flat-blade
- Open-end wrench, 5/16-inch
- Tubing cutter
- e2695 Separations Module Start-up Kit

Once you install the separations module, proceed to [Chapter 3](#).

2.2 Site requirements

The installation site must meet the specifications in the following table.

Table 2–1: Installation site requirements

Factor	Requirement
Temperature	4 to 40 °C (39 to 104 °F)
Relative humidity	20 to 80%, noncondensing
Bench space	Width: 45.7 cm (18 inches). With optional column heater or column heater/cooler, 58.4 cm (23 inches). Depth: 57.1 cm (22.5 inches). With optional sample heater/cooler, 64.8 cm (25.5 inches) Height: 57.1 cm (22.5 inches) Level to within $\pm 2^\circ$
Vibration	Negligible
Clearance	At least 16 cm (6 inches) at rear for ventilation
Static electricity	Negligible
Power	Grounded AC, 100-240 Vac, 50-60 Hz
Electromagnetic fields	No nearby source of electromagnetic noise such as arcing relays or electric motors

! **Notice:** To avoid overheating the separations module, provide at least 16 cm (6 inches) of clearance at the rear of the separations module.

Typical system dimensions for the e2695 Separations Module:

Note: To avoid exceeding the sizing limits of the tray, use a combination of four 1-L bottles or two 4-L bottles plus two 1-L bottles.

The top panel of the separations module can support the weight of two detectors, the detector drip tray, and the solvent bottle tray with four filled solvent bottles. Be sure to provide leak protection below any fluid-carrying units you place on top of the separations module.

2.3 Firmware and software requirements

! **Notice:** In Alliance e2695 Separations Module firmware update version 3.04, the degasser system test runs for up to 30 minutes after system start-up. If a sample set is started during this time, the system continues running the degasser test while it runs the sample set; if the degasser test fails while the sample set is running, the system goes into an error state, possibly causing loss of sample.

The Waters detectors that connect to the separations module must meet minimum firmware requirements to successfully communicate with the software installed in the separations module. Similarly, the external remote management system that controls the separations module must also meet minimum software requirements. See the *Alliance e2695 Separations Module Release Notes* for details on minimum firmware and software requirements.

2.4 Unpack the instrument

The separations module is shipped on a wooden pallet in a single carton. Save the carton and pallet in case you decide to transport the unit in the future. External accessories and options such as columns, column heater, and detectors are shipped in separate cartons.

To unpack the separations module:

1. Remove the bands securing the carton to the pallet.
2. Remove the carton, the carousel box, the solvent bottle tray, the start-up kit, and packing material.
3. Check the contents of the carton against the packing slip to confirm that all items are included.



Warning: To avoid skeletal or muscle injury associated with lifting heavy objects, enlist at least two people to lift the separations module from the pallet to the laboratory bench.

4. Lift the separations module from the pallet, and place it at the installation site.
5. Inspect all items for damage, and immediately report any shipping damage to both the shipping company and your Waters representative.

Requirement: If shipping damage occurred, contact Waters Customer Service. Refer to *Waters Licenses, Warranties, and Support Services* for complete information on shipping damages and claims.

2.5 External connections

See [Appendix B \(“External Connections” on page 191\)](#) for instructions on making all external connections to the Alliance e2695 Separations Module.

3

Prepare the e2695 Separations Module for Operation

3.1 Power-on the separations module

Power-on the separations module by moving the power switch (located at the top of the left-hand side panel) to I. The start-up diagnostic tests begins.

3.1.1 Start-up diagnostic tests

On start-up, the separations module performs the following functions and tests:

- CPU board
- Memory (RAM and ROM)
- Keypad
- Display
- External communication
- Digital signal processor (DSP)

Once the electronic part of the diagnostics test is complete, the display shows the test results.

3.1.2 The Main screen

When the initial part of the test routine is successful, the Main screen appears in the display. The start-up diagnostics routine continues, initializing these systems:

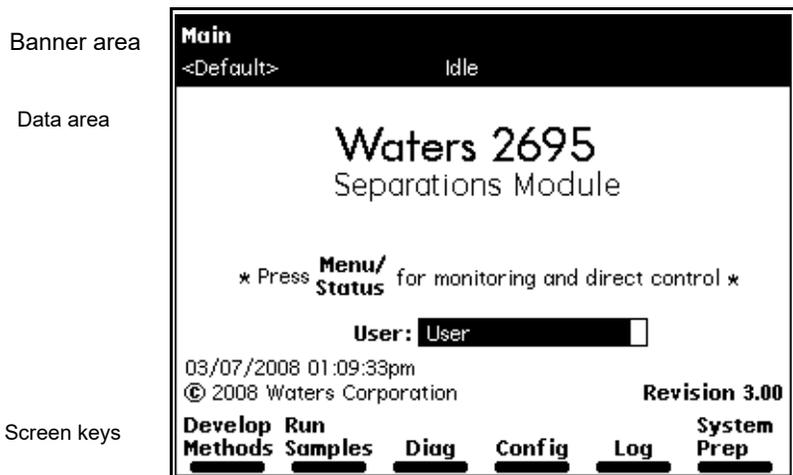
- Needle, syringe, and valves
- Carousel carrier
- Solvent management system

The statuses of these mechanical diagnostics tests appear in the banner area of the Main screen while the tests are running. If the test routine is not successful, see [“Troubleshoot” on page 169](#). When the start-up diagnostics run to completion, the separations module becomes idle.

Figure 3–1: Main screen

Notes:

- Develop Methods and Run Samples options are dimmed when locked on the Service Diagnostics page.
- The option to disable Run Samples from the front panel ensures that injections can only be controlled through the software. Analysts cannot use the front panel.



3.2 Operating overview

Whether you operate the separations module as a standalone device or via remote control, you prepare it for operation by interacting with the Main screen.

3.2.1 Screen display overview

The Main screen (and all others) contain these three areas:

- Banner
- Data
- Screen keys

3.2.1.1 Banner area

The always displayed banner area of the screen indicates the current status of the separations module and reports this information:

- Name of the current screen
- Current function status – Describes the current state of solvent flow; idle, purging, sample, or equilibration
- Work status – Displays the number of preprogrammed events that remain for a current sample set; for example, Vial 6/Inj 5/6.

- Remote control – Indicates Empower software or another remote control mode
- Current separation method, sample set, or template

3.2.1.2 Data area

The Data area of the screen’s two modes of display show operating information:

- Menu – Shows all control features with which you perform tasks; configuring the separations module, and creating or editing separation methods, sample sets, or sample templates
- Status/Control – Shows detailed status and interactive control information; flow rate, degassing rate, and solvent composition

Press the **Menu/Status** key to switch between the two display modes.

3.2.1.3 Screen key area

The screen key area of the screen shows the current function of the six keys located directly below the display. The screen keys are described in more detail in [“Use the keypad” on page 44](#). The following table describes the functions of the screen keys for the Main screen only. Each screen key displays a different screen.

Table 3–1: Screen keys in the Main screen

Screen keys	Function	Reference
Develop Methods	Displays the Methods screen so that you can create, edit, view, copy, delete, and run stored methods.	Chapter 6, “Create Methods, Sample Sets, and Sample Templates”
Run Samples	Displays the Methods screen so that you can run stored separation methods, sample sets, and sample templates.	Chapter 4, “Front Panel Control”
Diag	Displays the Diagnostics screen so that you can run a variety of diagnostics. You can also view current transducer readings.	Chapter 8, “Diagnostic Testing and Troubleshooting”
Config	Displays the Configuration screen so that you can enter values for a number of parameters.	“Configure the separations module” on page 47
Log	Displays the error log.	“Use the error log” on page 150
System Prep	Displays the System Preparation (System Prep) screen so that you can run System Prep, initiating a series of automated steps to effect system readiness for running samples.	“Run System Prep” on page 75

3.2.1.4 More screen key

The More screen key appears on various screens. Pressing it displays an additional set of screen keys.

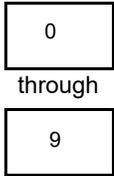
3.2.2 Use the keypad

The following table describes the functions of the keypad keys.

Table 3–2: Keypad functions

Key	Function
	Moves the cursor one field to the left.
	Moves the cursor one field to the right.
	Moves the cursor to the field above a current field.
	Moves the cursor to the field below a current field.
	Moves the cursor to the left most field in a current row. Press twice to move the cursor to the first field in the screen.
	Moves the cursor to the right most field in a current row. Press twice to move the cursor to the last field in the screen.
	Increases the display contrast.
	Decreases the display contrast.
	Displays a context-sensitive Help screen. Press twice to display a general Help screen. To close the Help screen, press Exit or Clear .
	Closes an open menu or dialog box and reestablishes the previous screen.
	Clears the contents of a current field, or enters <code>INIT</code> or <code>OFF</code> in selected fields.
	Stores the contents of a current field and advances the cursor to the next field in the screen.
	Toggles the display between the menu mode and the status/control mode.

Table 3–2: Keypad functions (continued)

Key	Function
	Immediately stops the flow of solvent and terminates a current function (sample processing, priming, purging, and so on). Pressing this key also activates the Run Stopped output switch.
	Enters the corresponding numeral into a current field, selects the corresponding gradient curve (0 = curve 10), positions the cursor to the corresponding entry in a list (0 = tenth item).
	Enters a decimal point, selects gradient curve 11, or serves as a separator for vial ranges. For example, to enter 2 through 5 in a vial field, press 2.5. Also positions the cursor to the last entry in a list.

3.2.3 Enter values in parameter fields

To enter parameter values:

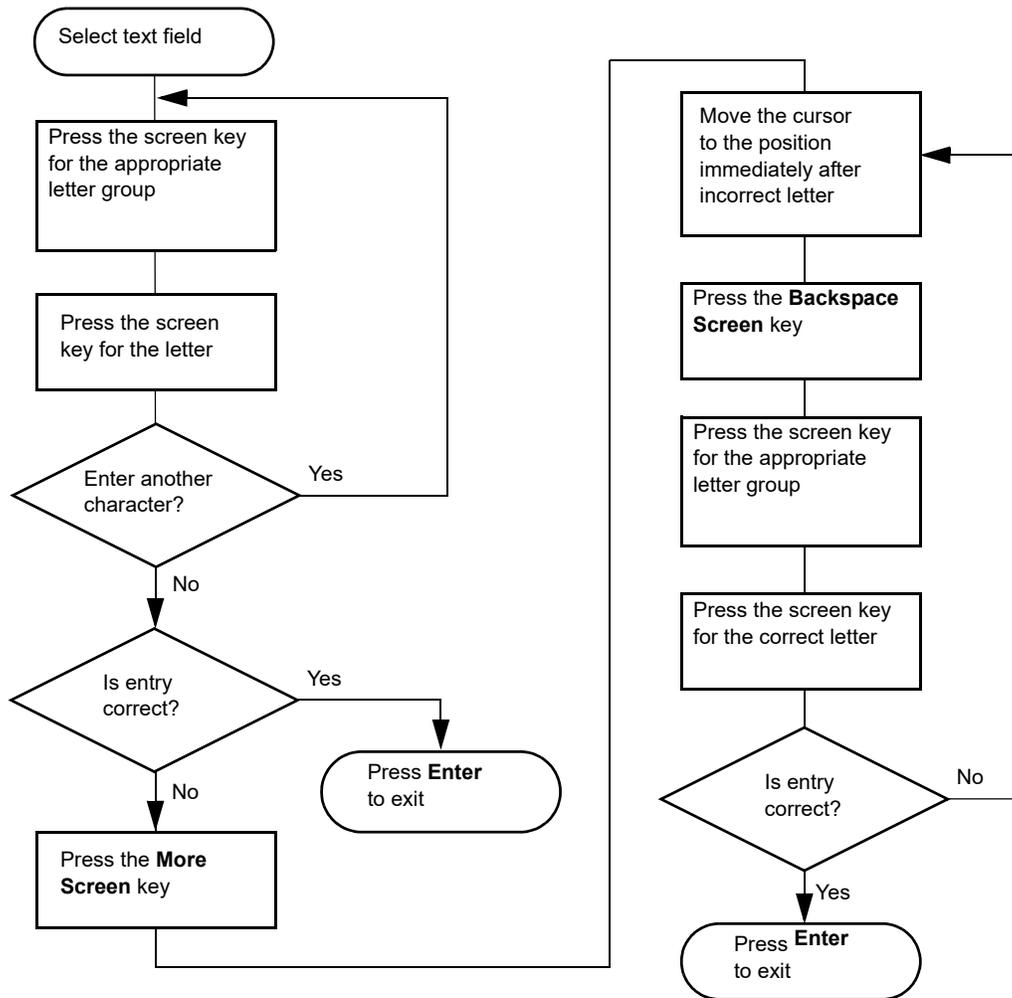
1. Use the screen keys or the Menu/Status key to display the appropriate screen.
2. Use the arrow keys to select the parameter field you want to change.
3. When a diamond appears after the field name, do as follows:
 - a. Press **Enter** to display a drop-down list.
 - b. Use the arrow keys to select the value you want, then press **Enter**.
4. When you need to enter data into a field, do as follows:
 - Use the numeric keys to enter a numeric value, then press **Enter**.
 - Use the screen keys to enter alphabetic data, then press **Enter**. See [“Enter an alphanumeric string” on page 45](#) for more information.
5. To select a check box, do as follows:
 - a. Use the arrow keys to select the check box.
 - b. Press a numeric that corresponds to an option to activate the option.
6. To clear a check box, press any numeric key to clear the box and deactivate the option.

3.2.3.1 Enter an alphanumeric string

When you select a field that requires an alphabetic entry, such as the user name or method name, each screen key appears with a group of letters, as shown in the figure, below. Press the **More** key for additional selections, such as uppercase and lowercase letters, and for selected non-alphabetic characters. Press the appropriate numeric keys to enter numbers into the field.

You can enter up to 30 characters in an alphanumeric field. To end and save an alphanumeric entry, press **Enter**.

Figure 3–2: Steps for entering alphabetic data



Example: To enter the name “Dave” in the User Name field:

1. Press the **ABCDEF** screen key.
2. Press the **D** screen key.
3. Press the **More** screen key, then press the Lowercase screen key.
4. Press the **abcdef** screen key.
5. Press the **a** screen key.
6. Press the **stuvwx** screen key.
7. Press the **v** screen key.
8. Press the **abcdef** screen key.
9. Press the **e** screen key.
10. Press **Enter** to store the entry.

3.3 Configure the separations module

Before you can operate the separations module, you need to configure the separations module for standalone or remote control and also for various operating conditions. Configure the separations module using the Configuration screen whenever you want to perform one of these tasks:

- Set up the separations module for the first time
- Change the configuration of the separations module
- Add, change, or delete a user
- Reset the clock

Press the Configure screen key in the Main screen to display the Configuration screen.

3.3.1 Set configuration parameters

Select the following parameters, as necessary, for your configuration:

- Syringe size, Loop size – Specify the size of the syringe and sample loop. These values are preset at the factory. Change them only when you install a different syringe and/or sample loop.
- Verify vial presence – When checked (the default setting), the software notifies you when a vial is not present at a specified carousel position.
- Verify carousel placement – When checked (the default setting), the software notifies you when a carousel is mounted in the wrong position at the time when an injection is attempted.
- System – Selects the operating mode and network address, when required. See [“Configure the operating mode” on page 52](#).
- Printer – Selects the type of printer and the baud (data transfer) rate.
Tip: The baud rate is automatically set to 1200 when you set the Waters 746 Integrator as the printer type.
- Date format – Selects between “Mmm DD YY” (for example, Aug 28 17) and “DD Mmm YY” (28 Aug 17)
- Pressure units – Selects between psi, kilopascal (kPa), and bar units
- Alarm sound – Selects the duration of alarms (continuous or a single beep)

Press the screen keys in the Configuration screen to access other parameters whose values you can specify.

Table 3–3: Screen keys in the Configuration screen

Screen key	Description
User Names	Displays a list of stored user names and adds, changes, and removes them.
Detectors	Scans the IEEE-488 bus and displays a list of Waters detectors connected to it, their addresses, and their firmware revision numbers. Active only when the separations module is configured as the system controller and the detectors are powered-on.

Table 3–3: Screen keys in the Configuration screen (continued)

Screen key	Description
Options	Displays a list of the hardware options installed on the separations module.
Set Clock	Displays the Set Clock dialog box, in which you establish the current time and date.
Events In	Specifies external signal conditions for Stop Flow and Inject Hold (see “Configure the operating mode” on page 52).
More	Toggles between the two sets of screen keys for the Configuration screen.
Reports	Specifies the type of information that is included in reports, and whether the reports go to a printer or integrator.
Comp Check	Specifies the criteria for the compression check.
Auto Shutdown	Specifies parameters for automatic shutdown of the separations module (see “Auto shutdown” on page 48).

3.3.1.1 Auto shutdown

You can set up the separations module to shut down automatically after a specified period of inactivity, which is defined as follows:

- No keyboard use
- No injections performed
- No changes sent to the separations module from a remotely connected Empower system, MassLynx computer, or external autosampler
- An error condition that suspends the operation of the separations module

You can leave the separations module in the shutdown state indefinitely. The separation method you specify in the Auto Shutdown dialog box defines the initial conditions that are applied after the specified period of inactivity. Use the auto shutdown function:

- When there is a long delay between injections
- To minimize solvent flow after an unattended or long run
- To disable the in-line vacuum degasser
- To disable temperature controls
- To turn off detector lamps

Tip: For instrument storage lasting one week or more, use methanol or methanol mixed with HPLC-quality water to flush the solvent delivery system before shutdown (see [“Power-off the separations module” on page 64](#)).

To enable the auto shutdown function:

1. Press the Config screen key in the Main screen, and then press the More screen key once.
2. Press the Auto Shutdown screen key.
3. Select a separation method to use while the separations module is shut down.
Tip: Only the initial conditions in the method you select are used.
4. Enter a time period (in minutes) after which you want the separations module to shut down.
Alternative: Press Clear to disable the Auto Shutdown function.
5. Press OK.
Result: The separations module shuts down in the absence of activity for the specified period.
6. Press Exit.

If you want to use Auto Shutdown to turn off the lamp of a 2487 or 486 Detector, program a Lamp Off event and specify a time of INIT in the I/O Events Timed Table screen (see “Set I/O parameter values” on page 104).

3.3.1.2 Events in

To define signal conditions that enable Stop Flow and Inject Hold:

1. Press the Events In screen key.
2. In the Events In dialog box, press Enter to display the conditions under which a signal on the stop flow terminals (on the I/O connector) immediately stops the flow of solvent:
 - Ignore – Disables the stop flow terminals
 - On High – Stops flow when the signal is high
 - On Low – Stops flow when the signal is low
3. Select the appropriate condition, and then press Enter.
Result: When a condition is met, the current function is suspended. A dialog box appears, allowing you to abort or resume the function.
4. Select the Hold 1 field, and then press Enter to display the conditions under which the Hold 1 input stops the next injection.
5. Select the appropriate condition, and then press Enter.
6. Select the Logic field, and then press Enter to display the combination of Hold 1 and Hold 2 signals that prevents sample being drawn into the sample loop.
7. Select the appropriate logic, and then press Enter.
Tip: Select And or Or:
 - And – The injection is delayed while the conditions specified on both Hold 1 and Hold 2 are in effect
 - Or – The injection is delayed while the conditions specified on either Hold 1 or Hold 2 are in effect
8. Repeat [step 4](#) through [step 7](#) for the Hold 2 field.
9. Press the OK screen key.

3.3.1.3 Set report options

Use the Report Options dialog box to define information sent to the printer or integrator. (Before you print a report, select the printer in the Printer section of the Configuration screen. You select the destination of the report in the print dialog box that appears when you press the Print screen key.)

To define the information sent to a printer or integrator:

1. Press the Reports screen key to display the Report Options dialog box.
2. In the list that appears, select the report's destination.
Rule: If the RS-232 port is configured for remote control, or you previously set the printer selection to None, you can select only Disable Reporting.
3. Select any option, press any numeric key (to enable the option), and then press Enter.
Tip: These are the options and their functions:
 - Gradient table – Generates the gradient table described in the table on [page 98](#).
 - I/O event table – Generates the I/O table described in the table on [page 106](#).
 - Detector table – Generates the detector table described in the table on [page 113](#).
 - Event overview – Generates an overview of all merged tables
 - Misc. parameters – Generates a list of all parameters not included in tables
 - System config – Generates a list of the instrument configuration parameters
 - Per-inject data – Generates a list of the minimum, maximum, and average values for temperature and pressure, the time and date of each injection, the bar code number, and so on
 - Alarms – Generates a list of the error conditions that occurred during each injection
4. Press the OK screen key to exit the Report Options dialog box.
5. Press Exit to return to the Main screen.

The following figure is an example of a report made with all options selected.

Figure 3-3: Sample report

```

REPORT: Alliance e2695 Separations Module
SERIAL NUMBER: F0ISM7885M
PRINTED: 03/17/08 08:26:40am
USER: User
CONFIGURATION: Syringe (250 uL), Loop (100 uL)
SEPARATION METHOD: Default

(1) MOBILE PHASE
    Low pressure alarm: Disable
    High pressure alarm: Disable
    Bubble detect alarm: Alert user
    Flow Ramp: 2.00 min (5.0 mL/min/min)
    Degasser Mode: On
    Degasser fail alarm: Alert user
    Pump sealwash period: 1.00
    Preferred stroke volume: 130 uL
Gradient: (min) (mL/min) %A %B %C %D Curve
          1. INIT 0.000 100.0 0.0 0.0 0.0
(2) SAMPLE
    Sample temperature: 4C
    Sample temp alarm:Log quietly +/- 5C
    Syringe draw (rate): Normal (2.50 uL/sec)
    (depth): 3 mm from bottom of vial

(3) AUTOSAMPLER
    Pre-column volume: 0.0 uL
    Post-run delay: 0.00 min
    Compression check alarm: Disable
(4) COLUMN
    Column temperature: 35C
    Column temp alarm:Log quietly +/- 5C
(5) I/O
Chart output: System pressure

Table: (min)Event TypeAction Param
1. 0.00Switch 1On
(6) DETECTORS
    2487-1:Enabled
    2487-2:Disabled
    2410:Enabled
    2410 Sensitivity:1
    2410 Cell temperature:30.0C
    2410 External temp 1: 25.0C
    2410 External temp 2: 25.0C
    2410 Filter: 3.0 sec
    2410 Autozero on inject: Yes
  
```

```

(2410 Polarity: [+])
Table: (min) Detector Action Param
1. INIT 2487 (1) Set wavelength 254
2. INIT 2487 (1) Set filter
3. INIT 486 (1) Set AU full scale 1.234
4. INIT 486 (1) Set chart polarity [+]
5. INIT 486 (1) Auto zero
OVERVIEW (min) Action
1. INIT 2487(1) 254 nm
2. INIT 2487(1) filter 1.0
3. INIT 2487(1) 1.234 AUFS
4. INIT 2487(1) chart polarity pos
5. INIT 2487(1) auto zero
6. INIT 1.000 mL/min 100.0%A 0.0%B 0.0%C 0.0%D c6
7. INIT Set Switch 1
->Sample injected at 03/17/08 02:26:42pm( 10.0 uL from vial 1 )
  
```

3.3.1.4 Compression check parameters

The separations module provides a compression check to test for leaks in the sample loop, seal pack, and valves (V1, V2, and V3).

You enter the parameter values that determine whether the separations module passes or fails a compression check in the Compression Check dialog box.

To specify the compression check parameters:

1. Press the More screen key in the Configuration screen, and then press the Comp Check screen key.
2. Enter an appropriate value in each field, and then press OK.

Tip: The following table lists the ranges and typical values for each parameter. Waters recommends you use the typical values for each parameter to best characterize the system.

Table 3–4: Compression check parameters

Parameter	Description	Range	Typical value
Compression pressure	Sets the target pressure for the compression check.	206.8 to 2068.4 kPa (2 to 20.7 bar, 30 to 300 psi)	689.5 kPa (6.9 bar, 100 psi)
Compressible volume: <ul style="list-style-type: none">• 25-μL syringe• 100-μL syringe• 250-μL syringe• 2500-μL syringe	Sets the maximum displacement volume to produce the compression pressure.	1 to 15 μ L 2 to 20 μ L 5 to 30 μ L 5 to 100 μ L	15 μ L 18 μ L 20 μ L 50 μ L
Decay time	Sets the amount of time that the system is kept at the compression pressure.	0.0 to 999.99 min	1.0 min
Maximum delta decay	Sets the maximum difference in pressure between the beginning and the end of the decay time.	0 to 5000 psi	< 345 kPa (< 3.45 bar, < 50 psi)

3.3.2 Configure the operating mode

Use the System field in the Configuration screen to select one of nine operating modes:

- No Interaction – For standalone operation.
- System Controller – For standalone operation when controlling Waters detectors over the IEEE-488 interface.
- Controlled by Empower – For Empower software operation when the software controls the HPLC system via Ethernet or the IEEE-488 interface.

- Controlled by MassLynx – For MassLynx software control via the IEEE-488 cable interface.
- Operate Gradient by Event In – Use when an autosampler (external to the separations module) initiates a chromatographic run via contact closure.
- Controlled via RS232 (ASCII) – Use when a non-Waters data system controls the separations module via RS-232 communications and using ASCII control, as specified by the data system.
- Controlled via RS232 (binary) – Use when a non-Waters data system controls the separations module by RS-232 communications and using binary control, as specified by the data system.

3.3.2.1 No Interaction mode

In this standalone mode, the separations module is not connected to the IEEE-488 or Ethernet interface bus. The separations module can trigger other non-IEEE devices in the system via I/O connections on the rear panel. See [“I/O signal connections” on page 208](#) for the procedure to make I/O connections.

Use the No Interaction mode when you want to suspend communications with a connected remote data system, such as an Empower system, and operate the separations module and the other system components from their front panels.

To set the separations module to the No Interaction mode:

1. Select the System field in the Configuration screen, and then press Enter.
2. From the list of operating modes, select No Interaction, press Enter, and then press Exit.

3.3.2.2 System Controller mode

In this standalone mode, the separations module controls up to three detector channels on the IEEE-488 bus. These can include two UV detector channels (Waters 2487 dual wavelength and/or 486 tunable absorbance) and one RI detector channel (Waters 2414 RI, 2410 RI, or 410 dRI). See [“Digital signal connections” on page 211](#) for information on IEEE-488 connections. You can also trigger other system modules via I/O connections. See [“I/O signal connections” on page 208](#) for the information on I/O connections.

To set the separations module to the System Controller mode:

1. Select the System field in the Configuration screen, and then press Enter.
2. From the list of operating modes, select System Controller, and then press Enter.
3. Press the Detectors screen key.

Result: A list of active devices and their IEEE-488 interface bus addresses appears:

- a. Press the **Scan** screen key to update the list.
- b. Press the **OK** screen key to return to the Configuration screen, and then press Exit.

Result: The separations module is ready to control Waters detectors using separation methods and sample sets.

3.3.2.3 Controlled by Empower mode

In this remote control mode, Empower software controls the operation of your HPLC system using the Ethernet interface or the IEEE-488 interface bus (but not both simultaneously). Connect the Ethernet or IEEE-488 cable as described in [“Digital signal connections” on page 211](#).

To set the separations module to the Controlled by Empower mode:

1. Select the System field in the Configuration screen, and then press Enter to display the list of operating modes.
2. From the list of operating modes, select Controlled by Empower, and then press Enter.
Result: The network address field is highlighted.
3. Press Enter to display the network address drop-down list.
4. Depending on which interface you are using, make one of these selections:
 - For Ethernet, select the Auto/Ethernet setting.
 - For IEEE-488, select an IEEE-488 address that is unused by other chromatographic components connected to the Empower system.
5. Press Enter, and then press Exit.

Result: The separations module is ready to be controlled from the Empower system.

3.3.2.4 Controlled by MassLynx mode

In this remote control mode, MassLynx software (version 3.5 or later) controls the separations module (in 2690-emulation mode). MassLynx software is used with mass spectrometers, using the IEEE-488 interface between the MassLynx computer and the separations module.

To set the separations module to the Controlled by MassLynx mode:

1. Select the System field in the Configuration screen, and then press Enter to display a list of operating modes.
2. From the list of operating modes, select Controlled by MassLynx, then press Enter.
Result: The Network Address field is highlighted.
3. Press Enter to display a list of addresses.
4. Select an address that is unused by other chromatographic components connected to the MassLynx computer, and then press Enter and then Exit.

See [“Perform automatic runs under MassLynx control” on page 87](#) for details on performing runs under MassLynx software control.

3.3.2.5 Controlled by Operate Gradient by Event In mode

In this mode, an external autosampler (a Waters 2700 Sample Manager, for example) initiates the start of a chromatographic run and performs the inject function (instead of the separations module). The separations module has no control of IEEE-488 devices, but can control non-IEEE devices using the I/O connections on its rear panel. See [“I/O signal connections” on page 208](#) for information on I/O connections.

To set the separations module to the Operate Gradient by Event In mode:

1. Select the System field in the Configuration screen, and then press Enter to display a list of operating modes.
2. From the list of operating modes, select Operate Gradient by Event In, and then press Enter.
3. Press the Events In screen key to display the Events In dialog box.
Tip: For information on defining Events In conditions, see [“Events in” on page 49](#).
4. Set the Stop Flow field to the appropriate condition (Ignore, High, or Low), and then press Enter.
 - Choose Ignore if the Stop Flow I/O terminals of the separations module are not used.
 - Choose High if the output connection from the external autosampler to the Stop Flow I/O terminals of the Separations Module changes to a high (more positive) TTL-level.
 - Choose Low if the output connection from the external autosampler to the Stop Flow I/O terminals of the separations module changes to a low (more negative) TTL-level.
5. Set the Hold 1 field to the appropriate condition (High or Low), and then press Enter.
 - Choose High if the output connection from the external autosampler to the Hold 1 Inject terminals of the separations module changes to a high (more positive) TTL-level to initiate a chromatographic run (and prevent an injection by the separations module).
 - Choose Low if the output connection from the external autosampler to the Hold 1 Inject terminals of the separations module changes to a low (more negative) TTL-level to initiate a chromatographic run (and prevent an injection by the separations module).**Requirement:** If you are configuring the separations module with a Waters 2700 Sample Manager, select Low.
6. Set the Logic field to Or, and then press Enter.
7. Set the Hold 2 field to Ignore, and then press Enter.
8. Press the OK screen key to save your selections.

3.3.2.6 Controlled via RS-232 (ASCII) or (binary) mode

In these remote control modes, a non-Waters data system (a mass spectrometry system, for example) controls the separations module using RS-232 ASCII or binary communications. The separations module disconnects from the Ethernet or IEEE-488 interface bus when either of these modes is selected. The separations module can control other non-IEEE devices in the HPLC system using the I/O connections on the rear panel. See [“I/O signal connections” on page 208](#) for information on I/O connections.

To set the separations module to the Controlled via RS-232 (ASCII) or (Binary) mode:

1. Select the System field in the Configuration screen, and then press Enter to display a list of operating modes.
2. Select Controlled via RS232 (ASCII) or Controlled via RS232 (Binary), and then press Enter and then Exit.

Tip: If you experience repeated problems when using a third-party data system, reconfigure the separations module to No Interaction mode. If the problem persists, refer to the appropriate section of this guide. If the problem occurs when using a third-party data system, but not when the instrument is configured in No Interaction mode, contact the data system's vendor.

3.4 Prepare the solvent management system



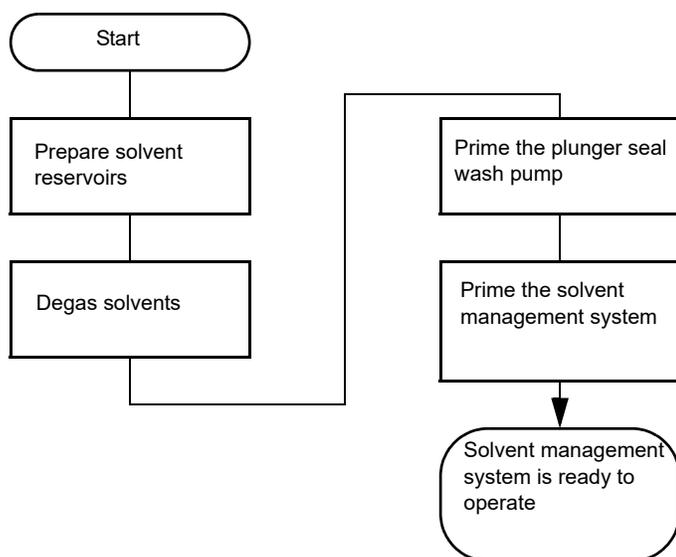
Warning: Observe safe laboratory practices when you handle solvents. Refer to the Safety Data Sheets for the solvents you use.

Tip: To maintain the efficiency of the solvent management system, and to obtain accurate, reproducible chromatograms, use only chromatography-grade solvents.

The separations module is designed to provide a pulse-free, accurate, and precise flow of solvent. For optimal performance, perform these tasks:

- Properly prepare the solvent reservoirs
- Use degassed solvents
- Prime the solvent management system
- Prime the plunger seal wash pump

Figure 3–4: Prepare the solvent management system for operation



3.4.1 Prepare solvent reservoirs

General recommendations:

- When using the instrument for general chromatography (i.e., reverse and/or normal phase and gel permeation [GPC]), Waters recommends the use of high-quality lab glassware made of borosilicate glass for all reservoirs (solvent, seal wash & needle wash).
- When using techniques such as ion chromatography, where glass containers can contribute ionic contamination (sodium and/or chloride ions), it is acceptable to use laboratory-grade polypropylene or polyethylene containers as reservoirs.
- When using the instrument in combination with a mass spectrometric detector, please refer to recommendations within the most recent version of “Controlling Contamination in UPLC/MS and HPLC/MS Systems” at the Waters’ website (www.waters.com) in the Support Library under Waters Division/Services & Support.

Choose solvent reservoirs that provide a snug fit for the reservoir caps supplied in the start-up kit. Waters recommends 1-L reservoirs. Place the reservoirs in the solvent bottle tray, and set the tray above the solvent management system components.

The solvent reservoir caps help control solvent evaporation. Three feather-edged holes in each cap make a gas tight seal around the solvent and vent tubes.

Install the solvent and vent lines as described in “[Connect tubing and attachments](#)” on page 193.

3.4.2 Degassing solvents

In-line vacuum degassing reduces the total dissolved gas in the mobile phase. For more information on vacuum degassing, see “[Degasser considerations](#)” on page 25.

Tip: For proper operation of the in-line vacuum degasser, you must fill each vacuum chamber with solvent. Use the dry prime direct function to prime all vacuum chamber solvent lines.

To control the in-line vacuum degasser from the Status screen:

1. Press Menu/Status.
2. On the status screen, press the Next Page screen key (if necessary) to display the degasser fields.
3. Select the Degasser mode field, and then press Enter.
4. From the list of degasser modes, select one:
 - On – The degasser is always on
 - Off – The degasser is always off
5. Select the desired operating mode, and then press Enter.

Results:

- The Vac pump field displays the current status of the vacuum degasser pump.
- The Pressure field displays the current vacuum level in psia, bar, or kPa.

3.4.3 Prime the plunger seal wash pump

The seal wash solvent lubricates each plunger and flushes away any solvent or dried salts forced past the plunger seal from the high-pressure side of the piston chamber. This wash cycle extends the life of the seals. Position the plunger seal wash reservoir in a visible location above the solvent management system, and refill the reservoir as necessary.

! **Notice:** Use separate solutions and containers for the plunger-seal wash and needle wash. Because the functions of these solutions differ, using one solution for both functions can compromise the effectiveness of either needle washing or plunger-seal washing.

For reversed-phase HPLC applications, use an aqueous plunger seal wash solution with enough organic content to inhibit bacterial growth. For example, use an 80% water/20% methanol solution or an 80% water/20% acetonitrile solution, depending on your application. For GPC seal wash solvent recommendations, see [“GPC solvents and seal choices” on page 224](#).

To prime the plunger seal wash pump for first-time use or when the entire line is dry:

Tip: If you are adding the same plunger seal wash-solution or changing to a solution that is totally miscible with the existing solution, perform only [step 1](#) and [step 5](#). If the syringe adapter is not used, steps 2 and 4 can be skipped.

1. Press the **Diag** screen key in the Main screen.
Result: The Diagnostics screen appears. See [“Perform main diagnostic tests” on page 150](#) for more information on the Diagnostics screen.
2. Attach the syringe adapter to the syringe (both are in the start-up kit).
3. Press the **Prime Seal-Wash** screen key, and then press the **Start** screen key.
4. Attach the syringe with adapter to the seal wash waste line and pull.
5. When seal wash solvent flows out the seal wash waste tube, press the **Halt** screen key.
6. Press **Close** to return to the Diagnostics screen.

3.4.4 Prime the solvent management system

Prime the solvent management system using one of these priming methods:

- Dry prime, when the solvent lines are dry
- Wet prime, when you want to change between miscible solvents

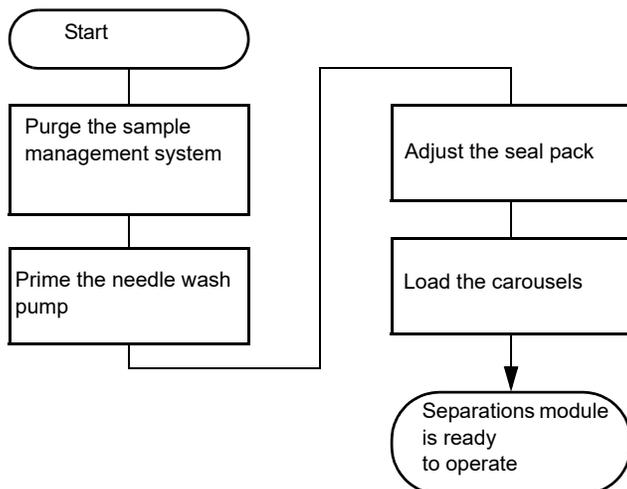
Follow the procedure in [“Dry prime the system” on page 74](#) or [“Wet prime the system” on page 76](#), as appropriate.

Once you prepared the solvent management system for operation, you must prepare the sample management system for operation.

3.5 Prepare the sample management system for operation

Prepare the sample management system for operation after you prepared the solvent management system (see “Prepare the solvent management system” on page 56). The following figure shows the steps involved in preparing the sample management system for operation.

Figure 3–5: Preparing the sample management system



3.5.1 Purge the system

! **Notice:** To avoid precipitating salts in the separations module, use an intermediate solvent such as water when you change from buffers to high-organic-content solvents.

Purge the sample management system on these occasions:

- You primed the solvent management system.
- You changed solvents.
- You see bubbles in the syringe.
- You start using the separations module at the beginning of each day.

To purge the sample management system:

1. In the Main screen, press Menu/Status.
2. In the Status screen’s Composition fields, specify the solvent composition.
3. Press Direct Functions to display the Direct Functions menu.
4. From the Direct functions menu, select Purge Injector, and then press Enter. The Purge Injector dialog box appears.
5. In the Purge Injector dialog box, adopt the default value of 6.0 volumes, and then press Enter.

Result: The solvent management system gradually attains a predetermined flow rate, flushing the sample loop with the selected volume.

6. To prevent a compression test from running, leave the Compression Check check box blank the first time you use the system.

Note: Calibrate the seal pack before running a compression test or it will likely fail.

7. Press OK.

3.5.2 Prime the needle wash pump

The needle wash pump flushes the needle in the sample management system, preventing carryover of sample between injections. The needle wash also extends the life of the injector seals by flushing buffered mobile phase and sample from the needle.

3.5.2.1 Select a needle wash solvent

Use a needle wash solvent based on the sample and mobile phase chemistries, making sure all solutions and buffers are miscible and soluble.

A gradient separation typically involves the initial use of a weak solvent combination, whereas final conditions usually combine stronger solvents. In general, it is best to choose a needle wash solvent similar to final gradient conditions. For example, in a separation with an initial gradient of 80% water/20% methanol, and a final gradient of 10% water/90% methanol, an effective needle wash solvent would be 20% water/80% methanol.

The choice of a needle wash solvent similar, but not equivalent, to final gradient composition ensures solubility of early eluting peaks. When sample solubility in the mobile phase is pH-dependent, choose a needle wash solution that is similarly pH-adjusted, unless using buffer solutions that are pH-adjusted containing nonvolatile salts.

If acids are used, avoid long-term exposure of the needle wash system to halide-containing acids.

Using samples containing strongly hydrophobic components can require experimentation to determine the best needle wash solvent. In these cases, the use of a needle wash solvent such as 20% water/80% methanol with tetrahydrofuran (THF) or dimethylsulfoxide (DMSO) [1-5 mL THF or DMSO/99-95 mL needle wash solvent] may be superior.

The following table lists some needle wash solvents recommended for use with certain mobile phase conditions. High sample concentrations can require other needle wash solvents.

! **Notice:** Use separate solutions and containers for needle wash and plunger seal wash. The solutions' functions differ, so using one solution for both functions can compromise the effectiveness of needle or plunger-seal washing.

Table 3–5: Suggested needle wash solvent

Chromatographic condition	Needle wash solvent
Buffered aqueous, reversed-phase	80% organic/20% aqueous ¹
Nonaqueous, reversed-phase	100% methanol
Normal phase	Mobile phase
GPC	Mobile phase
Ion exchange	Water

1. If the aqueous portion of the analytical solution is pH-adjusted to ensure sample solubility, match the pH of the aqueous portion of the needle wash solution to it. If acids are used, avoid long term exposure of the needle wash system to halide-containing acids.

To prime the needle wash pump:

1. Be sure the needle wash tubing is properly installed (see [“Install needle wash and plunger seal wash reservoirs” on page 201](#)).
2. Press the **Diag screen** key in the Main screen.
3. In the Diagnostics screen, press the **Prime NdlWash** screen key.

Tip: The 30-second needle wash procedure begins. If solvent does not flow out the waste tube within 30 seconds, press the **Start Again** screen key.

3.5.3 Adjust the seal pack

Adjust the seal pack on these occasions:

- You start up the separations module for the first time. (The separations module does not perform injections or compression checks until the seal pack has been adjusted.)
Note: Calibrate the seal pack before running a compression test or it will likely fail.
- A “Compression Check Failed” error message appears.
- A “Missing Restrictor” alarm appears during a diagnostic procedure.
- You change the seal pack or needle.
- You rebuild the seal pack.

Before you adjust the seal pack, purge the sample management system to ensure the syringe is empty of air. Air in the system can cause the Alarm Seal Geometry or Alarm Missing Restrictor dialog boxes to (erroneously) appear.

To adjust the seal pack:

1. In the Main screen, press the Diag screen key.
2. In the Diagnostics screen, press the Adjust Seals screen key.

Result: The adjustment procedure begins. When the procedure completes, the results are shown (see [“Adjust the seals” on page 154](#)).

3.5.4 Load carousels

The following table lists the carousels and their color codes.

Table 3–6: Carousel codes and colors

Code	Color	Vial numbers
A	Blue	1 to 24
B	Yellow	25 to 48
C	Red	49 to 72
D	Green	73 to 96
E	White	97 to 120

To load the carousels:

1. Open the carousel door.
Tip: The message the “Door is Open” appears.
2. Press the Next screen key (or select the desired carousel screen key) to position the carousel turntable for loading the appropriate carousel.
3. Load the carousel into the sample compartment.
4. Repeat [step 2](#) and [step 3](#) until all carousels are loaded.
5. Close the carousel door.

If you enable the “Verify carousel placement” check box (the default setting) in the Configuration screen, an error message appears if you attempt to make an injection from a carousel that is in the wrong position.

After the carousel is engaged, all subsequent carousel movements are monitored using a sensor. If the sensor detects that a carousel movement has failed, the movement is retried. If it fails a second time, a “Carousel malfunction” error is generated. This ensures that the carousel is always positioned correctly.

3.6 Prepare the separations module for operation

This section explains how to prepare the separations module. The instructions provide the sequence of steps to prime, equilibrate, and purge a separations module that is in one of the following states:

- A new or dry separations module.
- A separations module that is powered-off or left idle with no flow.
- A separations module that requires a solvent change (from a buffered solvent to an organic solvent).

For step-by-step instructions on how to use direct functions to prime, equilibrate, and purge your separations module, and to fully ready the separations module for running samples, see [“Perform direct functions” on page 72](#).

3.6.1 Prepare a new or dry separations module for operation

To change from a buffered solvent to a totally organic solvent:

1. Prime the plunger seal wash pump (see [“Prime the plunger seal wash pump” on page 58](#)).
2. Prime the needle wash pump (see [“Prime the needle wash pump” on page 60](#)).
3. Fill all four solvent reservoirs with fresh solvent filtered and degassed by vacuum filtration (see [“Prepare solvent reservoirs” on page 57](#)).
Tip: To maximize degasser efficiency (and fill all four degasser chambers with solvent), fill all four solvent reservoirs (even if you use fewer than four solvents). If you choose water as one of the “unused” solvents, change the water weekly to prevent bacterial contamination.
4. Set the degasser to On (see [“Equilibrate solvents in the in-line vacuum degasser” on page 77](#)).
5. Perform a dry prime to fill each solvent line and degasser chamber (see [“Dry prime the system” on page 74](#)).
6. Run System Prep (see [“Run System Prep” on page 75](#)).

3.6.2 Prepare an idle or powered-off separations module for operation

- !** **Notice:** If you just performed the steps from the procedure, below, for changing from a buffered solvent to an organic solvent, ensure that the Plunger Seal-Wash and Needle-Wash solvents are compatible with the new solvents.

To prime, equilibrate, and purge the separations module that is powered-off or idle with no solvent flow:

1. Check plunger seal wash fluid levels. Refill and prime, if necessary (see [“Prime the plunger seal wash pump” on page 58](#)).
2. Check needle wash fluid levels. Refill and prime, if necessary (see [“Prime the needle wash pump” on page 60](#)).
3. Set the degasser to On (see [“Equilibrate solvents in the in-line vacuum degasser” on page 77](#)).
4. Run System Prep (see [“Run System Prep” on page 75](#)).

3.6.3 Change from a buffered solvent to an organic solvent

! **Notice:** To avoid salts from precipitating in the separations module, use an intermediate solvent such as water when you change from buffers to high-organic-content solvents (see [“Solvent miscibility” on page 228](#)).

To change from a buffered solvent to a totally organic solvent in the separations module:

1. Remove the solvent line from the reservoir containing the buffered solvent.
2. Specifying a flow rate of 2.000 mL/min, perform a wet prime until air begins to enter the line (see [“Wet prime the system” on page 76](#)).
3. Place the previously removed solvent line into filtered HPLC-grade water, and perform a wet prime for 0.5 min at 5 mL/min (see [“Wet prime the system” on page 76](#)).
4. Equilibrate the solvent in the degasser chamber by setting a flow rate of 0.000 mL/min for 5 min (see [“Equilibrate solvents in the in-line vacuum degasser” on page 77](#)).
5. Purge the injector for a minimum of 6 loop volumes (see [“Purge the injector” on page 77](#)).
6. Remove the solvent line from the HPLC-grade water.
7. Fill a clean solvent reservoir with the organic solvent and place the solvent line in the reservoir.
8. Perform the procedure, above, for preparing an idle or powered-off separations module for operation.

3.7 Power-off the separations module

Before you power-off the separations module, remove any buffered mobile phase present in the flow path (see [“Flushing the flow path”](#) below).

! **Notice:** To avoid damaging your column, remove the column before you perform the following procedure.

3.7.1 Flush the flow path

To flush buffered mobile phase from the flow path:

1. Replace the buffered mobile phase with HPLC-quality water, and wet prime the system for 10 minutes at 3 mL/min (see [“Wet prime the system” on page 76](#)).
2. Perform three injector purge cycles (see [“Purge the injector” on page 77](#)).
Requirement: The sample loop must be clean.
3. Replace the plunger seal wash solvent with a fresh solution of 80% water/20% methanol or a miscible solvent, and prime the plunger seal wash pump (see [“Prime the plunger seal wash pump” on page 58](#)).
4. Replace the water mobile phase with a solution of 90% methanol/10% water, and flush the system for 10 minutes at 3 mL/min.

5. Replace the needle wash solvent with a solution of 90% methanol/10% water or a miscible solvent, and perform a needle wash prime (see [“Prime the needle wash pump” on page 60](#)).

3.7.2 Power-off the separations module

To power-off the separations module:

1. Ensure that the system is flushed properly (see [“Flush the flow path” on page 64](#)).
2. Press the **Power** switch to the (Off) 0 position.

Tip: The time and date are entered in the log file.

4 Front Panel Control

This chapter explains how to manually control the components of your HPLC system through the front panel of the separations module.

When the separations module is configured in the No Interaction or Operate Gradient by Event In mode, you control each HPLC system component individually via its front panel. When the separations module is configured in the System Controller mode, you control all components in your HPLC system through the front panel of the separations module.

4.1 Routine start-up

If the separations module is not already powered-on, follow the power-on procedure in [Chapter 3](#). When the start-up diagnostics tests run to completion, the separations module enters the idle mode.

To automatically identify the reports and methods you will generate, press Enter, then select your name in the User list. If your name is not on the list, you can enter it by pressing the Config screen key (to display the Configuration screen), pressing the User Names screen key (to display the Users dialog box), then entering your name using the keypad. If necessary, refer to [“Enter an alphanumeric string” on page 45](#). A user name is not required to operate the separations module.

The time and date are entered into the log file.

4.1.1 Reinitialize the system

It is good practice to reinitialize the system instruments at least once a week. When the separations module and detectors are power-cycled (powered-off for a minimum of one minute, and then powered-on), each undergoes a complete system initialization, including internal diagnostics and verifications. This procedure helps ensure that the mechanical and electrical components, as well as internal firmware, synchronize for proper operation.

4.2 Load sample vials

Loading the sample vials involves these tasks:

- Removing the carousel from the sample compartment
- Loading the vials into the carousel
- Loading the carousel into the sample compartment

4.2.1 Remove the carousel

To remove a carousel from the sample compartment:

1. Open the carousel door.
2. Press the Next screen key, or select the desired carousel screen key (A to E), to move the desired carousel to the front loading position.
3. Remove the carousel from the sample chamber.

4.2.2 Load vials

Use only 12 × 32-mm (2-mL) sample vials.

Insert the prepared vials into the appropriate positions in the carousel(s). Be sure to place vials in the numbered slots that correspond to the sample set (if any) you are using. For an overview of the vial positions specified in the sample set, display the vial numbers in the Loading View of the Sample Set table (see [“View sample sets” on page 83](#)). If you are not using a sample set, enter the vial positions containing the samples in the Inject Samples dialog box (see [“Inject samples” on page 79](#)).

4.2.3 Load the carousel

To load the carousel:

1. Open the carousel door if it is closed.
2. Press the Next screen key (or select the desired carousel screen key) to position the carousel turntable for loading the appropriate carousel.
3. Load the carousel into the sample compartment, and close the carousel door.

Tip: If you inadvertently load a carousel in the wrong location when the “Verify Carousel Placement” parameter in the Configuration screen is selected, an error message appears when you make an injection from that carousel.

4.2.4 Add new sample vials during a run

If you need to add new sample vials while the instrument is processing a sample set, use these guidelines to minimize interruptions during the run:

- Have the new sample vial(s) ready so that you minimize the amount of time that the sample compartment door is open.
- Wait until the run time displayed on the front panel is greater than 30 seconds before adding the sample(s).
- Do not add any sample(s) during the last 30 seconds of the run.
- Ensure that the sample compartment door is closed at the start of each injection when injection commands (selecting vial, washing the needle, drawing sample, and so forth) are executing.

4.3 Monitor your HPLC system

You can use the Status screen to monitor the current status of your HPLC system during a run. Press Menu/Status to display 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.

Figure 4–1: First page of the Status screen

Status (1)
<Default> Idle

Method: <direct> **Flow:** 0.000 mL/min **System:** -1 psi

Composition
A: 100.0% B: 0.0% C: 0.0% D: 0.0%

Sample
Set: OFF Current: 23.8 °C

S1 S2 S3 S4
OFF OFF OFF OFF

Degasser
Mode: Off Pressure: * psi

V1 V2 V3 V4
[Icons]

Column
Set: OFF Current: 24.6 °C Selection: 1: Position 1

Sample Queue **Next Page** **Direct Function**

To display the second page of the Status screen, press **Next Page**.

Figure 4–2: Second page of the Status screen

Status (2)
<Default> Idle

Method: <direct> **Flow:** 0.000 mL/min **System:** 0 psi **Sample:** 0 psi

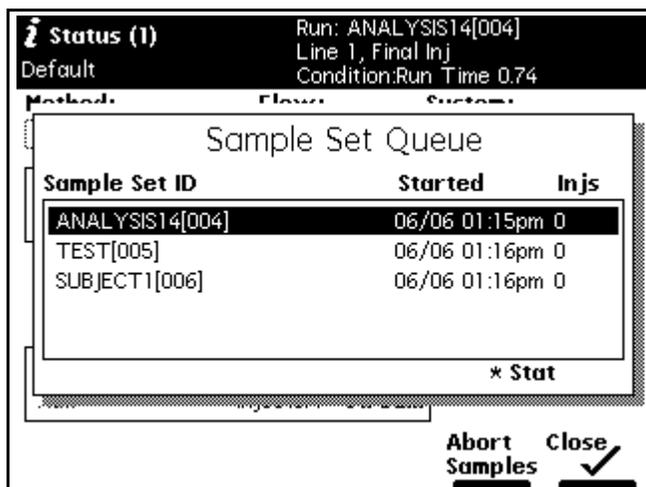
Syr: Home Ndl Wash: Off
Vial: Injector: Stream

Pressure Ripple (past minute)
Max: 0 psi Min: -1 psi Delta: 1 psi

Sample Queue **Next Page** **Direct Function**

To display a list of current sample sets, press **Sample Queue**. To delete selected sample sets, press **Abort Samples**. An asterisk appears in the Injs column to indicate if the “Stat” function was used to initiate a sample set.

Figure 4–3: Sample set queue



The following table describes the functions of the parameters in the Status screen fields. You can make changes only in the fields outlined with a solid border.

Table 4–1: Status screen parameters

Parameter	Description
Method	Indicates the current separation method. When you change any parameter in the Status screen, this field changes to <direct>.
Flow	Indicates the current flow rate of the solvent management system.
System	Indicates the current system pressure in psia, bar, or kPa.
Sample: Set	Indicates the user-specified sample compartment temperature.
Sample: Current	Indicates the current actual sample compartment temperature.
Composition	Indicates the current solvent composition.
S1 - S4	Indicates the status of the event switches on the I/O connector.
Degasser: <ul style="list-style-type: none"> • Mode • Vac pump • Pressure 	Indicates the status of the in-line vacuum degasser (if installed). Selects the mode of operation: On, or Off. Indicates whether the vacuum pump motor is active or idle. Current vacuum level in psia, bar, or kPa.
Valves V1 - V4	Graphically indicate the position of each of the four valves in the sample management system. Open Closed  

Table 4–1: Status screen parameters (continued)

Parameter	Description
Pressure Ripple: <ul style="list-style-type: none"> • Max psi • Min psi • Delta psi 	Indicates pressure readings in the solvent management system for the last minute. Maximum pressure. Minimum pressure. Peak-to-peak pressure difference.
Vial	Indicates the number of the vial currently under the injector.
Syr	Indicates the current position of the syringe. Valid positions are: <ul style="list-style-type: none"> • Empty – Highest position (to purge the syringe) • Home – Default position • Full – Lowest position (to draw in sample)
Ndl Wash	Indicates if the needle-wash pump is on.
Sample	Indicates the current pressure in the sample loop.
Column Temp	Indicates the set temperature and the current temperature of the column (if the column heater or column heater/cooler is so installed).
Injector	Indicates the current position of the needle port. Valid positions are: <ul style="list-style-type: none"> • Stream – In the high-pressure stream • Seal – In the lower seal position • Wet – In the needle wash position • Vial – In the sample vial
486 (1) and (2): <ul style="list-style-type: none"> • λ • Lamp • AUFS 	Indicates the current status of the following parameters for the 486 detector(s) (if so installed): <ul style="list-style-type: none"> • Wavelength • Lamp On or Off • Absorbance Units Full-Scale
2487 (1) and (2): <ul style="list-style-type: none"> • λ • Lamp • AUFS 	Indicates the current status of the following parameters for the 2487 detector(s) (if so installed): <ul style="list-style-type: none"> • Wavelength • Lamp On or Off • Absorbance Units Full-Scale

Table 4–1: Status screen parameters (continued)

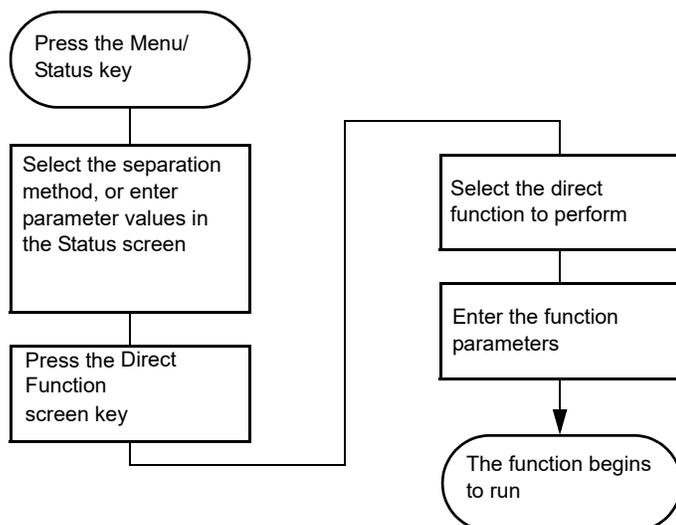
Parameter	Description
2410 and 410: <ul style="list-style-type: none"> • Cell • Col 1 • Col 2 	Indicates the current status of the following parameters for the 2414, 2410, or 410 detector (if so installed): <ul style="list-style-type: none"> • Cell Temperature (detector oven temperature) • External Temp 1¹ • External Temp 2¹

1. Enabled if the optional column heater or column heater/cooler module is configured with the Waters 2414, 2410, or 410 detector.

4.4 Perform direct functions

The following figure outlines the procedure for using the front panel to set up the separations module to perform a direct function.

Figure 4–4: Steps to setting up a direct control run



To access the direct control functions:

1. Press Menu/Status to display the Status screen (see [figure “First page of the Status screen” on page 69](#) and [figure “Second page of the Status screen” on page 69](#)).
2. Press the Direct Function screen key. A list of direct functions appears.
3. From the list of direct functions, select the function you want to specify, and then press Enter.

4. Follow the instructions for each function, as described in the following sections.

Table 4–2: Direct functions

Direct function	Description	Reference
Dry Prime	Opens the flow path (from the selected solvent reservoir to the prime/vent valve) to replace air with solvent, and then performs a prime.	“Dry prime the system” on page 74
System Prep	System Prep is a simple-to-use alternative to running the individual functions for Wet Prime, Purge Injector, Equilibrate, and Condition Column (described later in this chapter). It performs a series of automated steps to fully ready the separations module for running samples.	“Run System Prep” on page 75
Wet Prime	Replaces solvent in the path from the reservoirs to V1 and V2 and then to waste (V3). Use to change the solvent(s) in the system.	“Wet prime the system” on page 76
Purge Injector	Removes mobile phase from the sample loop and syringe, replacing it with fresh mobile phase. As an option, performs a compression test.	“Purge the injector” on page 77
Purge 2410/410 Reference (if applicable)	Purges the Waters 2414, 2410, or 410 detector reference cell.	“Purge the 2410 and 410 reference cell” on page 78
Equilibrate	Delivers mobile phase at the current conditions for the specified period of time. Equilibrates the temperature in the sample compartment and column heater (or column heater/cooler) at preset values.	“Equilibrate the system” on page 78
Condition Column	Delivers mobile phase using the gradient table specified in the current separation method without injecting a sample or running the Events table.	“Condition the column” on page 78
Inject Samples	<p>Injects a sample one or more times from the specified vial(s) using the selected method.</p> <p>Note: This function is not available under the following conditions:</p> <ul style="list-style-type: none"> • When the system is under Empower control • When the system is in remote control • When Run Samples is disabled on the Service Diagnostics page 	“Inject samples” on page 79

Tip: To run timed events without injecting a sample, select the Inject Samples option from the Direct Functions menu, and enter an injection volume of 0 (see [“Inject samples” on page 79](#)).

4.4.1 Dry prime the system

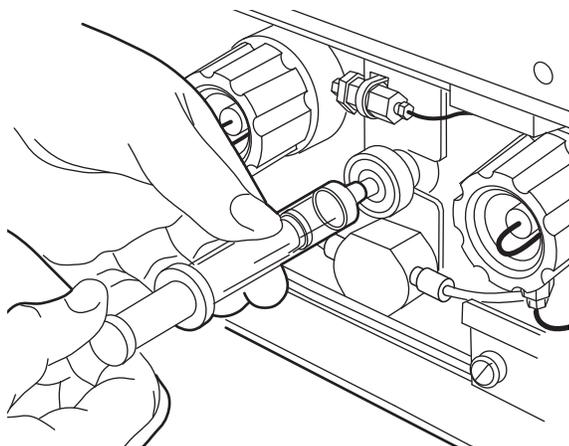
Use the dry prime option to prime the system when the flow path in the solvent management system is dry. See [“Prepare the separations module for operation”](#) on page 62.

To dry prime the system:

1. Set up the reservoirs as described in [“Install needle wash and plunger seal wash reservoirs”](#) on page 201 and [“Prepare solvent reservoirs”](#) on page 57.
2. Insert the solvent tubing into the appropriate reservoir(s).
Requirement: The detector waste line and the sample loop waste line must drain into an appropriate container.
3. Gently shake the filters in the reservoirs to remove any bubbles that may be trapped.
4. Attach an empty syringe to the prime/vent valve, as shown in the following figure.
5. Open the valve by turning it counterclockwise 1/2 turn.

Tip: The syringe does not lock onto the prime/vent valve; hold it in place while you pull on the plunger.

Figure 4–5: Prime/vent valve with syringe



6. Press the Direct Function screen key on the Status screen.
7. Select Dry Prime, and then press Enter.
8. In the Dry Prime dialog box, press the screen key corresponding to the solvent line you want to prime.
9. Withdraw the syringe plunger, pulling solvent through the tubing.

Tips:

- You need to exert force to pull the air and solvent through the system. Continue until you pull all the air through the solvent line and into the syringe.
 - You need not push the syringe plunger. The draw-off valve is located after the primary piston. Once it is filled with solvent, the primary piston delivers solvent to the accumulator piston, and onward to the sample management system.
10. Repeat [step 8](#) and [step 9](#) for each solvent line you want to prime, and then close the prime/vent valve.

11. Press a screen key corresponding to the solvent line with which you want to prime the system.

Tip: To facilitate purging air from the lines, start the wet prime using the solvent with the lowest viscosity.

12. Specify a duration of 5 minutes and press Continue.

Result: The solvent management system begins to operate. At the end of the priming period, the solvent management system turns off and the separations module enters the idle mode.

13. Perform a wet prime.

4.4.2 Run System Prep

The System Prep function performs a series of automated steps, putting the separations module in a condition of complete system readiness for running samples. System Prep is useful when you are changing solvents or after extended periods of system inactivity.

System Prep initiates the following sequence of functions:

- The user may select the duration of priming operations. Default is 0.5 minutes.
- The specified flow rate, composition, sample temperature, and column temperature settings are applied.
- The duty cycle of the seal-wash pump is set to 1 second.
- Simultaneously, the seal-wash pump and needle-wash pump operate for 65 seconds.
- Sequentially, each selected solvent line is wet primed at 7.0 mL/min for 0.5 minutes.
- The specified flow rate, composition, sample temperature, and column temperature settings are applied.
- The system pauses for 30 seconds to condition the solvent management system.
- The Purge Injector function runs with its default parameters (see [“Purge the injector” on page 77](#)).
- The specified flow rate, composition, sample temperature, and column temperature settings are applied.

The System Prep function is a simple to use alternative to running the individual functions of Wet Prime, Purge Injector, Equilibrate, and Condition Column, all of which are described later in this chapter. Additionally, using System Prep, you can specify the separation method parameters stored for when you run System Prep again.

Tip: You can access the System Prep function from either the Main screen or the Direct Functions list. You can also run it from a sample set that is stored in the separations module. When you do so, System Prep adopts the separation method's parameter values.

**Notice:**

- To avoid damaging the plunger seals, run System Prep only when solvent is in the solvent management system's flow path. If necessary, fill all solvent reservoirs and dry-prime the system before you run System Prep.
- To avoid precipitating salts in the separations module, use an intermediate solvent such as water when you change from buffers to solvents of high organic content. See ["Solvent miscibility" on page 228](#) for information on solvent miscibility.

To run System Prep:

1. From either the Main screen or the Direct Functions list, select System Prep and press Enter.
2. In the System Prep dialog box, specify initial settings for the flow rate, composition, sample temperature, and column temperature, and then press the Next screen key.
3. Select Solvents, and then press the Next screen key.
4. Verify that the solvent reservoirs are full and solvent lines wet, and then press the Start screen key.

Result: The System Prep run performs the steps required by your separation method, putting the separations module in a state of complete readiness for running samples. The run ends with these results:

- The solvent management system is primed, equilibrated, and the column conditioned.
- If you ran System Prep from the Direct Function list or Main screen, the separations module enters the idle mode.
- If you ran System Prep in a sample set, the next sample set function determines the next phase of operation for the separations module.

4.4.3 Wet prime the system

Wet prime the system when you want to change reservoirs or solvents or if the separations module has been idle for some time. Wet priming replaces solvent in the flow path from the reservoirs to V3 (waste). If the solvent lines are dry, dry prime before continuing (see ["Dry prime the system" on page 74](#)).

**Notice:**

- To avoid damaging the plunger seals, perform a wet prime only when solvent is in the solvent management system flow path. If necessary, fill all solvent reservoirs and dry prime the system before you wet prime.
- To avoid precipitating salts in the separations module, use an intermediate solvent such as water when you change from buffers to solvents of high organic content. See ["Solvent miscibility" on page 228](#) for information on solvent miscibility.

To wet prime the system:

1. In the Status screen Composition field, select any combination of the four solvents that equals 100% of what you want to use.

Tip: To facilitate purging air from the lines, start the wet prime using the solvent with the lowest viscosity.

2. On the Status screen, press **Direct Function**.
3. Select **Wet Prime**, and then press **Enter**.
4. Gently shake the filters in the reservoirs to remove any bubbles.
5. In the Wet Prime dialog box, specify the flow rate and duration of the priming (using the composition entered in the Status screen), and then press the OK screen key.
Result: The solvent management system begins to operate at the end of the specified time period, the solvent management system returns to the previous conditions, and the separations module enters the idle mode.
6. Repeat [step 1](#) through [step 5](#) for each solvent, as appropriate.

4.4.3.1 Equilibrate solvents in the in-line vacuum degasser

To equilibrate solvents in the vacuum degasser after you wet primed the system:

1. In the Status screen, specify the initial solvent composition for the run.
2. Set the degasser mode parameter to ON.
3. Press the Direct Function screen key.
4. Select Wet Prime, and then press Enter.
5. Enter a flow rate of 0.000 mL/min and a time of 5 min.

After the solvents in the vacuum degasser are equilibrated and the separations module is prepared to run, perform any of the following functions as necessary:

- Purge the injector
- Purge the refractometer reference cell
- Condition the column
- Equilibrate the system

4.4.3.2 Purge the injector

Purge the injector for these reasons:

- To remove any traces of solvent from a previous run
- To clear air bubbles from the flow path or the syringe
- To switch to a new (miscible) solvent after you wet prime

The purge process can include a compression check to test the needle seals.

! **Notice:** To avoid precipitating salts in the separations module, use an intermediate solvent such as water when you change from buffers to solvents of high organic content. See [“Solvent miscibility” on page 228](#) for information on solvent miscibility.

To purge the injector:

1. Press the Direct Function screen key in the Status screen.
2. Select the Purge Injector option, and then press Enter.
3. In the Purge Injector dialog box, specify 6 sample loop volumes.

Tip: To perform a compression test after the purge, move the cursor to the Compression check box and press any numeric key.

4. Press OK.

4.4.4 Purge the 2410 and 410 reference cell

You can purge the reference and sample sides of the Waters 2414 RI, 2410 RI, and 410 dRI detectors using the Purge 410 Reference direct function. Purge the flow path whenever you change solvents or you experience an unexpected loss in sensitivity due to excess noise or drift.

To purge the 2414, 2410, or 410 reference cell:

1. In the Status screen, enter the flow rate and composition at which you want to purge the 2414, 2410, or 410 reference cell.
2. Press the Direct Function screen key.
3. Select Purge 410 Reference, and then press Enter.
4. In the Purge 410 Reference dialog box, specify the amount of time that you want to pump fresh solvent through the reference cell, and then press OK.
5. Press the OK screen key.

4.4.5 Equilibrate the system

To equilibrate the HPLC system, the separations module pumps solvent at the initial conditions specified in the Status screen or in the separation method (see [Chapter 6](#)), flushing the flow path of any sample and solvent run at other compositions and preparing the system for the next run.

To equilibrate the system:

1. Press Menu/Status to display the Status screen.
2. In the Status screen, specify initial conditions, or, select the separation method that contains the initial conditions at which you want to equilibrate.
3. Press the Direct Function screen key.
4. Select Equilibrate, and then press Enter.
5. In the Equilibration dialog box, specify a length of time for equilibrating the system, and press the OK screen key.

Result: The separations module begins the equilibration procedure. When the system is equilibrated, the separations module enters the idle state. The flow rate remains at the value specified in the Status screen.

4.4.6 Condition the column

You condition the column by running a solvent gradient through it without injecting samples or running the events table. You select a separation method (see [Chapter 6](#)) that contains the gradient parameters, and the separations module runs the gradient method.

Tip: To run timed events without injecting a sample, select the Inject Samples option from the Direct Functions menu and enter an injection volume of 0 (see [“Inject samples” on page 79](#)).

To condition the column:

1. Press Menu/Status to display the Status screen.
2. In the Status screen, select the Method field, and then select the separation method with which you want to condition the column.
3. Press the Direct Function screen key.
4. Select Condition Column, and then press Enter.
5. In the Condition Column dialog box, specify a length of time for conditioning the column that equals or exceeds the duration of the gradient, as defined in the specified separation method (including reequilibration time).
6. Press the OK screen key.

4.4.7 Inject samples

Using the keypad, you can inject one or more samples from one or more contiguous vials. The separations module operates according to the instrument conditions displayed in the Status screen.

If you make any changes to the conditions displayed in the Status screen, the Method field indicates “<direct>,” the separation method name in the Banner area appears within brackets (< >), and the current isocratic conditions (with no timed events) are used for the injection. Choosing a separation method removes the “<direct>” label from the method field and uses any programmed gradients or timed events.

To inject a sample:

1. Place the prepared sample vial(s) in a carousel.
2. Insert the carousel in the sample compartment, as described in [“Load sample vials” on page 67](#).
3. Close the carousel door.
Tip: If the door is ajar, the separations module does not perform the injection.
4. In the Status screen, enter the desired initial conditions, or select the Method field, and then select the separation method that specifies the conditions under which you want to perform the injection.
5. Press the Direct Function screen key.
6. Select the Inject Samples option, and then press Enter.
7. In the Inject Samples dialog box, specify the vial range, which includes the vial positions from which you want to make injections.
Tip: Use the “.” key to separate the entries. For example, to inject samples from vials 1 through 20, enter 1.20, then press Enter.
8. Specify the number of injections from each vial, and then press Enter.
9. Specify the run time, and then press Enter.
10. Specify the volume of each injection, and then press the OK screen key.

5 Automatic Runs

You can set up the separations module to make automatic runs when it operates as follows:

- In System Controller or No Interaction mode
- Controlled by Empower software
- Controlled by MassLynx software

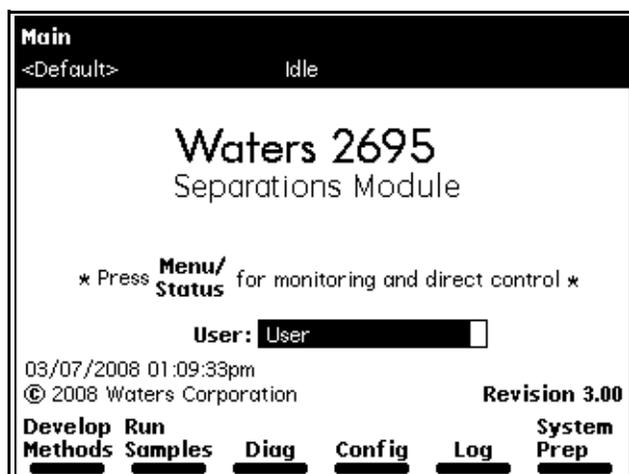
See [“Operating configurations” on page 29](#) for details about separations module control modes.

5.0.1 System Controller or No Interaction mode

Note: Local injections are not available when disabled from within Service Diagnostics.

If your HPLC system uses the separations module in a System Controller or No Interaction mode, you perform an automatic run using a sample set or sample template stored in the separations module. [Chapter 6](#) explains how to create and store separation methods, sample sets, and sample templates. You run existing sample sets and sample templates from the separations module’s Main screen.

Figure 5–1: Main screen



5.0.2 Controlled by Empower software

If your HPLC system is controlled by Empower software, you perform an automatic run using a project, instrument method, configured system, method set, and sample set stored in software. For details on performing runs using Empower software, see the procedures in [“Perform automatic runs under Empower software control” on page 86](#).

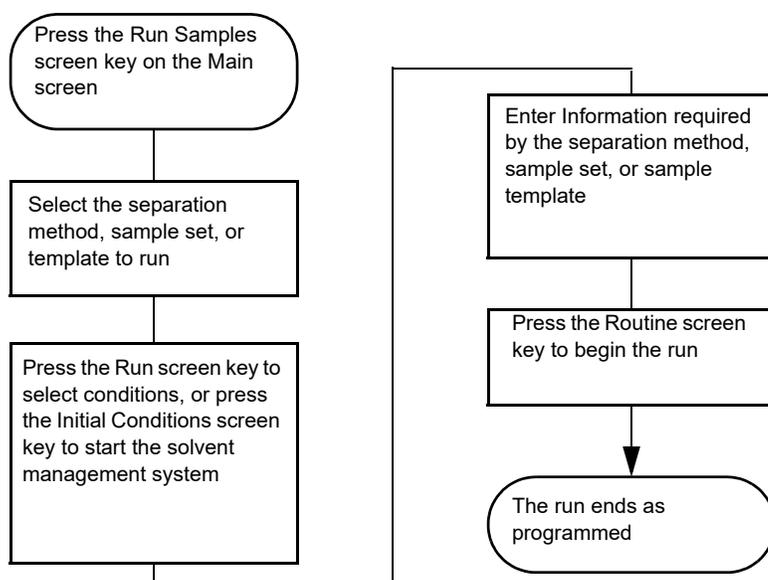
5.0.3 Controlled by MassLynx software

If your HPLC system is controlled by MassLynx software, you must select an LC method that defines the separations module's operating parameters and those of the system's detector (but not the mass spectrometer, if included). For details on performing a run using MassLynx software, see "Perform automatic runs under MassLynx control" on page 87.

5.1 Perform automatic runs in a standalone mode

The following figure shows the steps for setting up and performing an automatic run in a standalone configuration (System Controller or No Interaction mode).

Figure 5–2: Making an automatic run in a standalone mode



5.1.1 Run a sample set

To run a sample set in a standalone mode:

1. Press the Run Samples screen key in the Main screen.

Result: The Run Samples screen appears, listing all available separation methods, sample sets, and sample templates.

The following table describes the icons used in the Run Samples screen.

Table 5–1: Icons in the Run Sample screen

Icon	Description
	Separation method
	Sample set
	Sample template

- Select the sample set you want to run, and then press one of the following screen keys to perform the indicated tasks:

 - Initial Conds – Starts the solvent management system at the initial conditions (specified in the first line of the sample set) and begins equilibrating the temperature in the sample heater/cooler and/or column heater (or column heater/cooler). When the system is equilibrated, press the Run screen key.
 - Run – Displays the selected sample set table (to view or edit). Use the screen keys to modify the table as necessary.
- Load the sample vials into the carousel(s) according to the locations specified in the sample set (see [“Load vials” on page 68](#)).

Tip: For information on adding new sample vials while the instrument is processing a sample set, see [“Add new sample vials during a run” on page 68](#)).
- Press the Start screen key.

Requirement: The Run Samples screen appears with a list of the solvents required for the run. Ensure you prepared the separations module with the indicated solvent(s).
- Press the Routine screen key to start a normal run.

Tip: To make a stat run, or modify a run in progress, see [“Modify a sample set during a run” on page 85](#).

5.1.2 View sample sets

You can display the Sample Set table in one of three views described below. Press the Next View screen key to scroll from one view to another.

- Functional View – Displays the sample set with one function per line. This view gives you a complete overview of the entire run, including vial ranges and any linked rows.
- Injection View – Displays the sample set with one injection per line (see [figure “Sample Set screen – Injection View” on page 84](#)). Press the Injects Only screen key to display only the lines associated with an injection. This list corresponds directly to the chromatograms produced by the run.

- Loading View – Displays the sample set with one vial per line (see [figure “Sample Set screen – Loading View” on page 84](#)). This view shows you how to load samples in the carousel.

Figure 5–3: Sample Set screen – Injection View

	vial	function	method	µL	min
1	*	Equil	Dave Test	*	45.00
2	*	Purge	Dave Test	*	3.00
3	A1	Standard	Dave Test	10.0	22.50
4	A5	Sample	Dave Test	15.0	22.50
5	A5	Sample	Dave Test	15.0	22.50
6	*	Purge	Dave Test	*	3.00
7	A6	Sample	Dave Test	15.0	22.50
8	A6	Sample	Dave Test	15.0	22.50

70 rows total

Scroll Injects Only Next View Start

Figure 5–4: Sample Set screen – Loading View

	vial	function	method	injs	total µL
1	A1	Standard	Dave Test	1	10.0
2	A5	Sample	Dave Test	2	30.0
3	A6	Sample	Dave Test	2	30.0
4	A7	Sample	Dave Test	2	30.0
5	A8	Sample	Dave Test	2	30.0
6	A9	Sample	Dave Test	2	30.0
7	A10	Sample	Dave Test	2	30.0
8	A11	Sample	Dave Test	2	30.0

27 rows total

Scroll Next View Start

5.1.3 Perform a run from a sample template

You can perform an automated run in a standalone mode using a sample template stored in the separations module. See [“Create a sample template” on page 117](#) for the procedure to create and store a sample template. Screen prompts give you step-by-step directions to prepare for the run.

To run a sample template in a standalone mode:

1. In the Main screen, press the Run Samples screen key.
2. Select a sample template, and then press Enter.
3. Specify the number of samples to run, and then press the Continue screen key.
4. Enter the location of the first sample vial, and then press the Continue screen key.

Requirement: You must use consecutive sample vials on one or more carousels.

5. Repeat [step 3](#) and [step 4](#) when the sample template includes multiple separation methods.
6. Enter the number of standards in the run, and then press the Continue screen key.
7. Enter the location of the first standard vial, and then press the Continue screen key.
Requirement: You must use consecutive standard vials.
8. Make sure that the solvents listed in the dialog box are primed and in the appropriate inlet tubes, and then press the Start screen key.

5.1.4 Modify a sample set during a run

You can modify operating conditions during a standalone mode run as follows:

- Via the stat run function
- By editing the separation method

5.1.4.1 Perform a stat run

You can use the stat run function to interrupt a run in progress and perform one or more injections after the current injection is done, then continue with the scheduled run. Performing a stat run is most useful when running separation methods that use identical solvents and temperatures.

To perform a stat run while a sample set is running:

1. Load the sample(s) into the carousel, and then place the carousel in the sample compartment.
2. In the Main screen, press the Run Samples screen key.
3. Select the separation method or sample set you want to use for the stat run, and then press the Run screen key.
4. Enter the carousel position of the vial(s) and other parameters in the appropriate fields in the screen.
5. Press the Start screen key.
Result: A list of the solvents required for the run appears.
6. Confirm that you prepared the separations module with the indicated solvent(s).
7. Press the Stat screen key.

Result: The stat run starts immediately after the current injection is complete. The original sample set resumes after the stat run ends.

5.1.4.2 Edit a separation method

You can modify a separation method while a sample set is running. If the separation method is being used for the current injection, the current injection is unaffected. You must save the changes to the modified separation method before you can apply the method to all subsequent injections that use that separation method. Follow the procedures on [page 90](#) to modify and save the separation method.

5.1.5 Stop a run

To stop a run in progress, press one of the following keys from the Status screen:

- Stop Inject (screen key) – Stops the current injection, and displays a dialog box requesting you to abort or resume the injection.
- Hold Inject (screen key) – Completes the current function, suspends operation of subsequent functions, and displays screen keys that allow you to abort or resume the sample set.
- Hold Gradient (screen key) – Holds the current gradient conditions. A screen key allows you to resume the gradient.

Alternative: On the keypad, press Stop Flow to halt the flow of solvent, suspend operation of the current function, and display screen keys that allow you to abort or resume the function.

If any alarm condition specified in the separation method, sample set, or sample template is set to “Stop Function” or “Stop Flow” when alarm condition occurs, a dialog box appears notifying you of the alarm condition. The following screen keys are in this dialog box:

- Abort – Cancels the current function
- Resume – Resumes the current function

5.2 Perform automatic runs under Empower software control

If your separations module is configured for Empower Chromatography Data Software, you make an automatic run from the Empower software Run Samples window. When the separations module is running under Empower software control, the word “Remote” appears in the banner area.

Configure your separations module for Empower software control as described in [“Configure the operating mode” on page 52](#).

To make an automatic run, perform the following steps at the Empower workstation:

Tip: See the Empower online help for an explanation of how to perform the tasks in these steps.

1. Depending on the type and version of remote control software you are using, create a chromatographic system as follows:
 - For Empower software – Create a system that includes the separations module.
2. Create an instrument method that uses the system you defined in [step 1](#).

Tip: Any parameter values not explicitly set in the Empower instrument method automatically adopt the values in the default separation method in the separations module. These values include:

- Column selection valves (3-column, 6-column, and 2-column regeneration valves) recognized by the e2695 software
- A needle- wash time selection of “normal”, “double”, and “extended”

If you need to modify these values, verify that the term “Idle” appears in the banner area before you edit the default separation method.

You can edit the default separation method in the separations module to set parameter values appropriate for your applications. See [“Edit a separation method” on page 92](#) for details on editing existing methods.

3. Create a method set that uses the instrument method you defined in [step 2](#).
4. Run the samples using the method set you defined in [step 3](#). When you enter run samples, use the HPLC system you defined in [step 1](#).
5. Process and print out the data acquired from the chromatographic run.

5.2.1 Considerations when performing runs controlled by Empower software

- When you start an automatic run, any running sample set or method programmed locally at the separations module automatically terminates.
- When the separations module is running under Empower software control, and you press Abort in the Run Samples window, the instrument continues running the current gradient and event functions.
- When the separations module is running under Empower or MassLynx control, you cannot perform injections from the front panel of the instrument.
- You can operate the separations module from its front panel whenever it is not being run by the Empower software.

Note: When the separations module is running under Empower or MassLynx control, the Stop Flow button remains active on the front panel of the instrument.

! **Notice:** Do not edit the default method in the separations module when the instrument is running under Empower software control. Doing so can cause unexpected behavior.

5.3 Perform automatic runs under MassLynx control

If the separations module is configured for MassLynx software control (v3.5), you perform an automatic run via the MassLynx software. When the separations module is running under MassLynx software control, the term “Remote” appears in the banner area. Configure the separations module for MassLynx software control as described in [“Configure the operating mode” on page 52](#).

5.3.1 Perform an automatic run using MassLynx software

Tip: You can operate the separations module from its front panel whenever it is not being run by the MassLynx software.

To perform an automatic run at the MassLynx workstation:

Tip: See the MassLynx online Help for an explanation of how to perform the tasks in these steps.

1. Configure an LC/MS system that includes the separations module.
2. From the Inlet Editor, create an LC method for the separations module and the detector (but not for the mass spectrometer).
3. Acquire a single sample from the Inlet Editor.
Tip: To acquire multiple samples, create a sample processing list from the MassLynx Top Level window.
4. Process and print the data acquired from the chromatographic run.

6 Create Methods, Sample Sets, and Sample Templates

[Chapter 5](#) explains how to perform an automatic run in a standalone or remote control mode.

You can create and store the separation methods, sample sets, and sample templates with which you make automatic runs when the separations module is in the standalone System Controller mode. You can store up to 60 methods in the internal memory of the separations module. When the number of stored methods approaches 60, the maximum, the time required to create and save methods increases from a few seconds to over 30 seconds. When 60 methods are stored, a warning message appears indicating that memory capacity is reached.

The separations module supports three types of tools for controlling automatic runs in a standalone mode:

- Separation methods
- Sample sets
- Sample templates

6.0.1 Separation methods

The separations module uses and stores programs that can automatically run a separation method, which consists of these time-based and nontime-based conditions that influence the separation:

- Mobile phase composition and flow rate
- Sample temperature
- Column temperature
- I/O parameters

When you run a separation method on another, identically configured separations module in an Alliance system, you can reproduce identical separations.

6.0.2 Sample sets

A sample set applies functions to separation methods. Examples of functions include (inject) sample, purge, prime, and equilibrate. When you combine a function with a separation method, the resulting sample set directs the separations module regarding what to do, when to do it, and under what conditions. You can use multiple separation methods in a sample set.

6.0.3 Sample templates

Using sample templates, you pre-program a sample set so that the operator needs only to enter the number and location of samples and standards. The sample template then prompts you to load and prime the correct solvents for the sample set. As with any sample set, the sample template can use multiple separation methods.

Sample templates are useful in regulatory-compliant laboratories that follow standard operating procedures. Senior personnel can develop, program, and lock the sample template to prevent any unauthorized changes to a sample template. Once you prepare the separations module according to the on-screen directions, the sample template can run unattended.

6.1 Create and edit separation methods

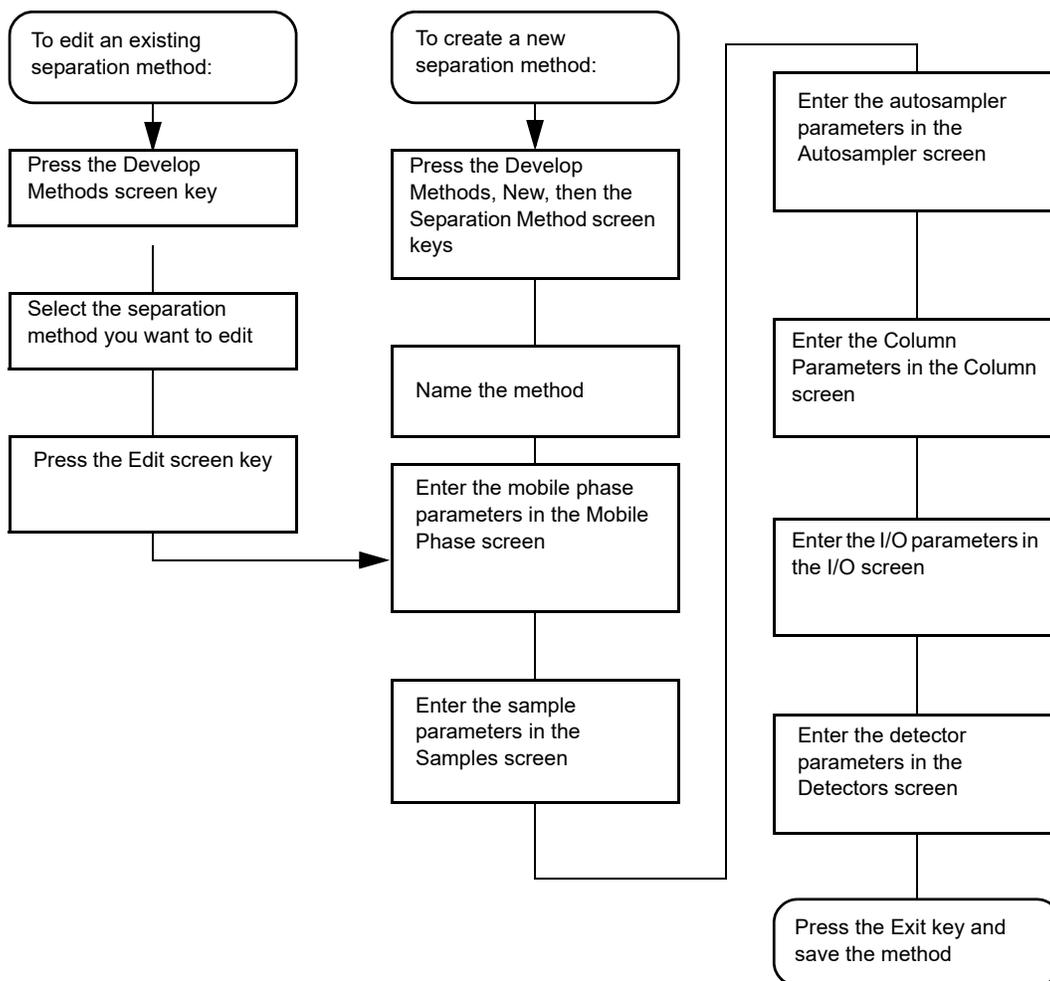
Note: This functionality is not available when **Lock Run Samples** is selected on the Service Diagnostics page.

You create and edit separation methods using the Methods screen and six method parameter screens. From the Methods screen, you can perform these tasks:

- Create a new separation method
- Edit an existing separation method
- Copy and then edit an existing method
- Lock a separation method to prevent changes
- Create a sample template

The following figure shows the steps necessary to create and edit separation methods using the front panel of the separations module. If your HPLC system operates under Empower software control, see the Empower documentation for procedures to create and edit method sets. If your HPLC system operates under MassLynx software control, see the MassLynx online Help for details on using the Inlet Editor to create an LC method.

Figure 6–1: Create or edit a separation method



6.1.1 Create a separation method

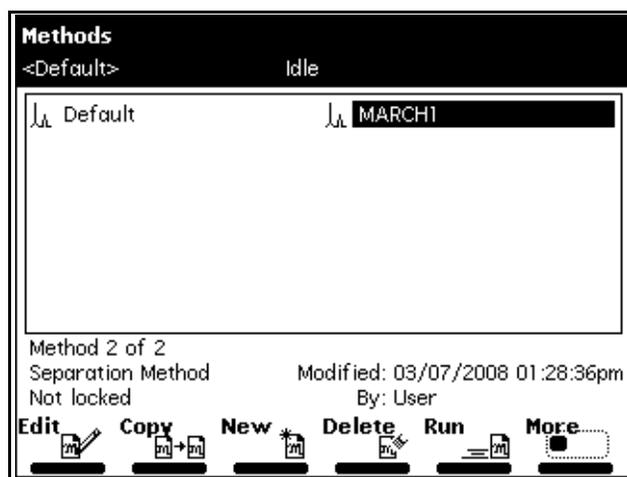
To create a separation method:

1. On the Main screen, press the Develop Methods screen key.

Result: The Methods screen appears, displaying the following information:

- The type of method (Separations Method, Sample Set, Sample Template)
- The date the method was last modified, and by whom
- Whether the method is locked or unlocked

Figure 6–2: Methods screen



2. Press the New screen key.
3. Press the Separation Method screen key.
4. Enter a name for the method, and then press Enter.
5. On the Mobile Phase screen, specify values for all applicable parameters and also in the 5 associated parameter screens.
Tip: Press the Next or Previous screen key to scroll through the parameter screens. For details, see [“Set separation method parameters” on page 94](#).
6. To save the method, press Exit then the Yes screen key.

6.1.2 Edit a separation method

To edit an existing separation method:

1. On the Main screen, press the Develop Methods screen key.
2. In the Methods screen, select the separation method you want to edit.
Tip: If the separation method is locked, the Edit screen key is inactive, and a lock icon appears beside the method name. To unlock a locked separation method, see “Locking and unlocking a separation method”, below.
3. Press the Edit screen key.
4. Enter the new parameter values in the appropriate screens.
Tip: See [“Set separation method parameters” on page 94](#) for details.
5. To save the method, press Exit and then the Yes screen key.

6.1.3 Copy and edit a separation method

To copy and edit an existing separation method, leaving the original method unchanged:

1. On the Main screen, press the Develop Methods screen key.
2. In the Methods screen, select the separation method you want to copy.
3. Press the Copy screen key.
4. Enter the name of the new method in the dialog box that appears, and then press Enter.
Tip: The separations module automatically adds the appropriate method extension.
5. Enter the new parameter values in the appropriate screens.
Tip: See “Set separation method parameters” on page 94 for details.
6. To save the method, press Exit and then the Yes screen key.

6.1.4 Lock and unlock a separation method

To prevent changes to a separation method, you can lock the method. To lock a separation method:

1. On the Main screen, press the Develop Methods screen key.
2. In the Methods screen, select the method you want to lock, and then press the Lock screen key.
3. Enter a key word in the dialog box that appears, and then press Enter.
Tip: You can use as many as 30 alphanumeric characters. The method is locked and cannot be changed, deleted, or renamed until it is unlocked. If necessary, see “Use the keypad” on page 44.
4. Record the key word, and keep it in a safe place.

To unlock the current method:

1. On the Main screen, press the Develop Methods screen key.
2. In the Methods screen, select the method you want to unlock, and then press the Unlock screen key.
3. Enter the key word in the dialog box that appears, and then press Enter.

6.2 Set separation method parameters

You specify separation method parameters in six different screens, which are arranged in the order shown in the following table. Each screen is described in the sections of this manual cited in the Reference column of this table.

Table 6–1: Separation method parameter screens

Screen name	Reference
Mobile Phase	“Set Mobile Phase screen parameter values” on page 94
Sample	“Set sample parameter values” on page 100
Autosampler	“Set autosampler parameter values” on page 101
Column	“Set column parameter values” on page 103
I/O	“Set I/O parameter values” on page 104
Detectors	“Set detector parameters” on page 107

6.2.1 Set Mobile Phase screen parameter values

The Mobile Phase screen appears when you select a separation method to edit. Press the Next or Previous screen keys to move among the six method parameter screens. The icon between the Next and Previous screen keys shows your place in the six-screen sequence. Press Exit to return to the Methods screen. A dialog box prompts you to save the changes to the separation method.

To enter parameters in the Mobile Phase screen:

1. Press the Next or Prev screen key to display the Mobile Phase screen.

Figure 6–3: Mobile Phase screen

The screenshot shows the 'Mobile Phase' screen with the following fields and sections:

- Header:** (1) Mobile Phase, <Default>, Idle
- Edit Separation Method:** Default
- Initial Flow:** 1.100 mL/min
- Initial Composition:**
 - 100.0 % A
 - 0.0 % B
 - 0.0 % C
 - 0.0 % D
- Alarms:**
 - Min: Disable 100 psi
 - Max: Stop flow 4000 psi
 - Bubble detect: Log quietly
- Flow Ramp:** Accelerate to 10.0 mL/min in: 2.00 min (5.0 mL/min/min)
- Isocratic separation:** (Section header)
- Navigation:** Gradient Degas, Labels, Strk Vol Prev Seal Wsh, 6/1, Next

2. Enter values in the fields, as appropriate.

The following table describes the fields and screen keys in the Mobile Phase screen.

Table 6–2: Mobile Phase screen parameters

Parameter	Function	Value range
Initial Flow	Specifies the initial flow rate of the method. For isocratic operation, this is the flow rate for the entire separation.	0.000 and 0.010 to 10.000 in 0.001 mL/min increments
Initial Composition	Specifies the initial composition of the eluents. The sum of the four fields must equal 100%. (You enter solvent names using the Labels screen key.)	0 to 100.0 in 0.1% increments
Alarms: Min	Specifies the system pressure (set in psia, bar, or kPa) below which the alarm condition (selected in the adjacent box) is executed. To enable access to the Pressure fields, set the alarm parameter to anything other than “Disable.”	0 to 4500 in 1 psi increments, 0 to 310 bar in 1 bar increments, or 0 to 31025 kPa in 1 kPa increments See “Alarms” on page 97.
Alarms: Max	Specifies the system pressure (set in psia or bar) above which the alarm condition (selected in the adjacent box) is executed. Use to detect problems with method conditions and to protect your column from overpressure.	0 to 5000 in 1 psi increments, 0 to 344 bar in 1 bar increments, or 0 to 34473 kPa in 1 kPa increments See “Alarms” on page 97.
Alarms: Bubble Detect	Specifies the response that occurs when the solvent management system detects a bubble in the flow path.	See “Alarms” on page 97.

Table 6–2: Mobile Phase screen parameters (continued)

Parameter	Function	Value range
Flow Ramp	<p>Specifies the time (in minutes) for the solvent delivery system to reach the maximum system flow rate. This limits the rate of change of the flow rate to protect the column from potentially damaging sudden changes in pressure.</p> <p>This flow ramp is used for all applications of the separation method and overrides any flow rate time-based changes defined within a gradient table.</p>	0.01 to 30 min in 0.01 min increments
Gradient (screen key)	Displays the Gradient Table screen, which allows you to build a gradient table.	See “Set Gradient table parameter values” on page 98.
Degas (screen key)	<p>Displays the Degasser screen which allows you to set the Degasser Mode. See “Degassing solvents” on page 57.</p> <p>On Degasser Error: Specifies that an alarm response occurs when an in-line vacuum degasser fault is detected. The in-line degasser is disabled on any fault regardless of the alarm setting you select. Waters recommends that you enable either the “Stop Function” or “Stop Flow” alarm setting.</p>	Off On See “Alarms” on page 97.
Labels (screen key)	<p>Displays the Solvents dialog box, which allows you to add, remove, or change the names of solvents used in methods.</p> <p>Use the Add, Remove, and Change screen keys to edit the list of solvents.</p> <p>A, B, C, and D are not valid user-entered solvent names.</p>	

Table 6–2: Mobile Phase screen parameters (continued)

Parameter	Function	Value range
Strk Vol Seal Wsh (screen key)	<p>The Preferred Stroke Volume field sets the volume of solvent delivered with each piston stroke.</p> <p>You can override the default stroke volumes, but do not exceed the flow rate limits displayed on the screen for each of the stroke volume settings. See “Preferred plunger stroke volume” on page 25.</p> <p>The Plunger Seal Wash period sets the time interval between successive plunger seal wash pump cycles.</p>	<p>130 µL (default)</p> <p>100 µL</p> <p>50 µL</p> <p>25 µL</p> <p>Off, 0.50 to 10.00 in 0.01 min increments</p>

6.2.1.1 Alarms

The separations module maintains a log of all enabled errors that occur during operation. Each error can produce a variety of responses, as shown in the following table.

Table 6–3: Alarm responses

Alarm response	Function
Disable	All alarm response reporting is disabled.
Log Quietly	The error is entered into the error log without alerting you.
Alert User	The error is entered into the error log and you are alerted with a dialog box.
Stop Funct	The error is entered into the error log, you are alerted with a dialog box, and the operation is suspended at the end of the current function. You can abort or resume the operation of the sample set by pressing the appropriate screen key.
Stop Flow	The error is entered into the error log, you are alerted with a dialog box, the current function is suspended, and solvent flow is stopped.

6.2.1.2 Set Gradient table parameter values

The Gradient table allows you to make time-based changes to the composition of the mobile phase during a run. You can program up to 25 lines in the Gradient table.

To set the parameters in the Gradient table:

1. On the Mobile Phase screen, press the Gradient screen key to display the Gradient screen.

Figure 6–4: Gradient screen

Gradient							
<Default>			Idle				
Edit Separation Method: Dave Test (modified)							
	time	flow	%A	%B	%C	%D	curve
1	INIT	1.000	100.0	0.0	0.0	0.0	
2	2.00	1.000	55.0	45.0	0.0	0.0	1 0
3	15.00	2.500	45.0	40.0	15.0	0.0	/ 6 0
4							
5							
6							
7							
8							

3 Rows Total

Over-view Insert Row Delete Row Sort by Time Copy Down More

2. In the Gradient screen, specify values in the Gradient table as appropriate.
3. Press Exit to save the Gradient table.

The following table describes the parameters in the Gradient table.

Table 6–4: Gradient table parameters

Parameter	Function	Value range
Time	Specifies the time after the start of the run at which the change is to occur. (INIT is allowed only in the first row of this table.)	INIT, 0.00 to 999.99 in 0.01 minute increments
Flow	Sets the flow rate of the solvent delivery system.	0.000 and 0.010 to 10.000 in 0.001 mL/min increments
%A, %B, %C, %D	Sets the proportion of each solvent in the mobile phase. The sum of these four fields must equal 100%.	0 to 100 in 0.1% increments

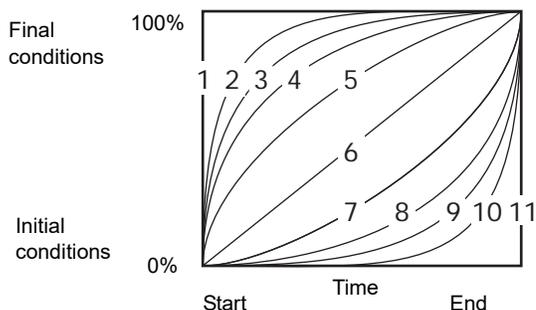
Table 6–4: Gradient table parameters (continued)

Parameter	Function	Value range
Curve	Sets the rate at which the solvent is to change to the new proportions and/or flow rates (see “Gradient curves” on page 99).	Select the desired gradient curve from the list of profiles, or select the curve number (1 – 11) by pressing the appropriate numeric key. Tip: Press “0” for curve 10 and “.” for curve 11.
Overview (screen key)	Displays a time-ordered summary of the events in the Gradient, Detector, and Timed Events tables.	N/A
Insert Row (screen key)	Inserts a row above the current row.	N/A
Delete Row (screen key)	Deletes the current row.	N/A
Sort by Time (screen key)	Sorts the rows based on time.	N/A
Copy Down (screen key)	Copies the contents of the current table cell into subsequent cells in the column.	N/A
Reset Table (screen key)	Clears the table.	N/A
Print (screen key)	Prints the Gradient table.	N/A

6.2.1.3 Gradient curves

The figure, below presents a visual model of the gradient curve for each of the gradient curve values you can specify in the Gradient table.

Figure 6–5: Curve shapes for gradient profiles



6.2.2 Set sample parameter values

To enter parameters in the Sample screen:

1. Press the Next or Prev screen key to display the Sample screen.

Figure 6–6: Sample screen

The screenshot shows the 'Sample' screen with the following parameters:

- Sample Temperature:** Target: 35 °C, On error: Alert user, Range: ± 5 °C
- Syringe Draw:** Rate: Nominal: Normal, Custom: 2.50 µL/sec; Depth: 0 mm from bottom of vial

Navigation buttons 'Prev' and 'Next' are visible at the bottom right.

2. In the Sample screen, enter or select values in the Sample screen parameter fields.

The following table describes the parameters in the Sample screen.

Table 6–5: Sample parameters

Parameter	Function	Value range
Sample Temperature Target	Specifies the temperature of the sample heater/cooler (if installed). To turn off the heater/cooler, press the Clear key.	Ambient, 4 to 40 °C in 1 °C increments
Sample Temperature Range	Specifies the maximum allowable deviation in sample temperature. If the temperature deviation exceeds the range, the alarm condition selected in the adjacent box is triggered.	±10 °C in ±1 °C increments
Sample Temperature On error	Specifies the response that occurs when the sample compartment temperature is out of the specified range during a run.	See table “Alarm responses” on page 97.

Table 6–5: Sample parameters (continued)

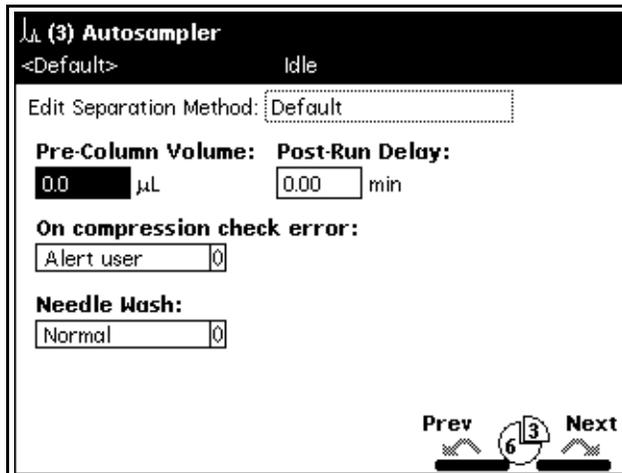
Parameter	Function	Value range
Sample Draw Depth	Adjusts the depth of the needle tip to accommodate for sedimented samples or nonstandard vials. A value of 0 corresponds to the bottom of the vial.	0 to 20 in 1 mm increments

6.2.3 Set autosampler parameter values

To enter parameters in the Autosampler screen:

1. Press the Next or Prev screen key to display the Autosampler screen.

Figure 6–7: Autosampler screen:



2. Enter values in the Autosampler screen as appropriate.

The following table describes the parameters in the Autosampler screen.

Table 6–6: Autosampler parameters

Parameter	Function	Value range
Pre-column volume	The sample management system starts the gradient and delivers this volume before making an injection. Use this parameter when transferring a method from a system that has a delay volume smaller than that of the separations module. This parameter can also be used to reduce the delay volume of the separations module for narrow or microbore columns, because the sample is held in the sample loop (if desired) until the gradient front reaches the sample loop.	0.0 to 10000.0 in 0.1 µL increments
Post Run Delay	Provides time for a data system to process data from the run. During the delay, the separations module draws the next sample into the loop but does not perform an injection.	0 to 999.99 in 0.01 min increments
Needle Wash	Allows you to choose one of three options for needle wash behavior. "Normal" is the default value. In cases where excess carryover occurs, you can choose either "Double" or "Extended". "Normal", the default value, operates the needle wash pump while moving the needle down into the sample vial. "Double" operates the needle wash pump while moving the needle into the sample vial, but moves the needle more slowly than the "Normal" wash option. "Extended" washes the needle while slowly moving into the vial, and then also washes the needle while moving slowly out of the vial to the seal position.	Normal: ~12 sec Double: ~24 sec Extended: ~37 sec

6.2.4 Set column parameter values

To enter parameters in the Column screen:

1. To display the Column screen, press **Next** or **Prev**.

Figure 6–8: Column screen

The screenshot shows the 'Column' screen with the following fields and options:

- Header: (4) Column, <Default>, Idle
- Edit Separation Method: Default
- Column Temperature:
 - Target: OFF °C
 - On error: Disable
 - Range: ± 5 °C
- Column Selection: No Change
- Column Information: (empty field)
- Equilibration: 0.00 min
- Column Info: (bottom left)
- Navigation: Prev, 6, 4, Next (bottom right)

2. Enter values in the Column screen, as appropriate.

The following table describes the parameters in the Column screen.

Table 6–7: Column parameters

Parameter	Function	Value range
Column Temperature Target	Sets the temperature of the column heater or column heater/cooler (if installed). To turn off the column heater or column heater/cooler, press the Clear key.	Column heater: 20 to 65 °C in 1 °C increments (5 °C above ambient) 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 column temperature is out of the specified range.	See table “Alarm responses” on page 97 .
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.	±10 °C in 1 °C increments

Table 6–7: Column parameters (continued)

Parameter	Function	Value range
Column Selection 3-Column Valve	Selects a column position if a column selection valve is installed. Tip: 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 through 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
Equilibration	Allows you to specify an equilibration time that will be applied whenever the application of the separation method involves a change in the column switch position.	≤ 999.99 minutes

6.2.5 Set I/O parameter values

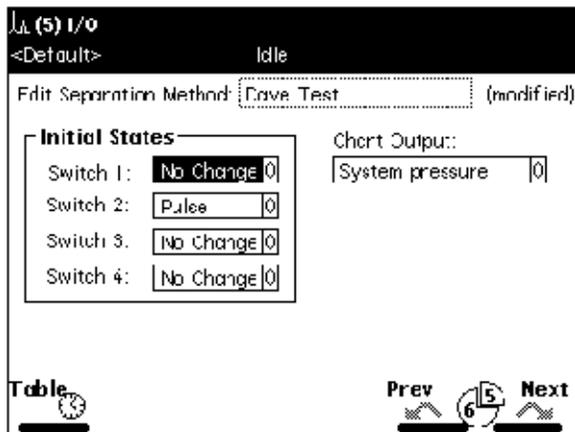
You set the I/O parameter values when you want to use analog signals to notify other instruments of the status of the separations module. You can do any of these things:

- Notify a detector that an injection began.
- Turn on a heating plate or a stirring bar in a solvent reservoir.
- Notify a third-party data system or integrator that an event occurred.
- Activate a switching valve.
- Sound a buzzer.
- Select the system parameter signal sent through the Chart Out terminals.

To enter parameters in the I/O screen:

1. Press the Next or Prev screen key to display the I/O screen.

Figure 6–9: I/O screen



2. Enter values in the I/O screen, as appropriate.

The following table describes the parameters in the I/O screen.

Table 6–8: I/O parameters

Parameter	Function	Value range
Initial States	Defines the initial condition for each of the four event switches. At the beginning of each injection cycle, each switch returns to the state defined in this parameter.	On Off Toggle (Toggle is a one-time change of state.) Pulse (Pulse is a single pulse with a width defined in the Param column of the I/O Events table.) No Change
Chart Output	Defines the signal sent out on the chart out terminals on the rear panel of the separations module.	Flow Rate System Pressure Sample Loop Pressure %A, %B, %C, %D Column Temp Sample Temp Degasser Vacuum Primary Head Pressure
Timed Table (screen key)	Displays the I/O Events table.	See “Edit the I/O Events table” on page 106.

6.2.5.1 Edit the I/O Events table

In the I/O Events table, you set the timing for the following events to occur during a run:

- Changing the state of event switches
- Setting the column temperature
- Setting the sample compartment temperature
- Alerts

You can program up to 25 lines in the I/O Event table.

To enter event in the I/O Events table:

1. Press the Table screen key in the I/O screen to open the I/O Events table screen.

Figure 6–10: I/O Events table

	Time	event type	action	param
1	0.00	Switch 1	Cff	0
2	5.50	Switch 1	Toggle	0
3	10.00	Alert		
4	12.00	Set Temperature	Column	0.25
5				
6				
7				
8				

4 Rows Total

Over-view Insert Row Delete Row Sort by Time Copy Down More....

2. Enter values in the I/O events table as appropriate, and then press Exit.

The following table describes the I/O Events table's parameters.

Table 6–9: I/O Events table parameters

Parameter	Function	Value range
Time	Determines the time after the start of a run during 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 min increments
Event type	Sets the type of event to occur.	Switches 1 through 4 Set Temp Alert

Table 6–9: I/O Events table parameters (continued)

Parameter	Function	Value range
Action	Selects the action to perform with the specified event.	See table “Action parameters” on page 107 .
Parameter	Selects the value for the action.	See table “Action parameters” on page 107 .

Table 6–10: Action parameters

Parameter	Function	Value range
Switches 1-4	On Off Toggle (Changes the state of the switch (open to closed, or closed to open) Pulse (A single pulse with a width defined in the param column of the I/O Events Table) No Change	0.01 to 10.00 in 0.01 min increments (Pulse only)
Set Temperature (if sample heater/cooler, column heater, or column heater/cooler are installed)	Sample Column heater Column heater/cooler	4 to 40 °C in 1 °C increments 20 to 65 °C in 1 °C increments (5 °C above ambient) 4 to 65 °C in 1 °C increments (the greater of 15 °C below ambient or 4 to 65 °C)
Alert	No action	N/A

6.2.6 Set detector parameters

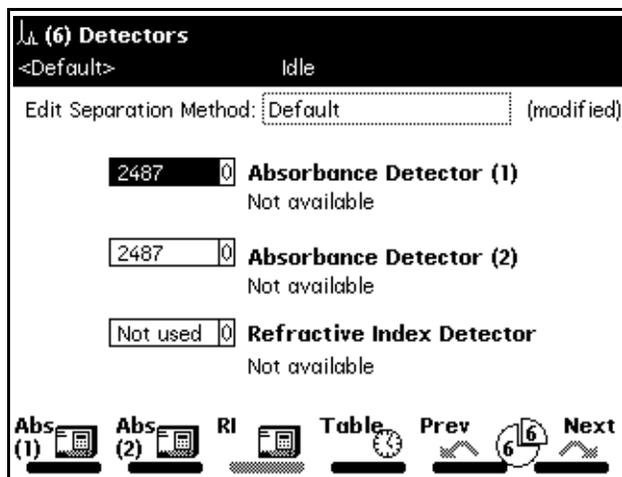
In a standalone mode, System Controller or No Interaction, the separations module can control one Waters 2414 RI, 2410 RI, or 410 dRI detector and as many as two UV/Vis detector channels (Waters 2487 dual wavelength or 486 tunable absorbance detectors). Use the IEEE-488 interface bus to connect the separations module to the detector(s). To make I/O connections to these and other detectors, see [“I/O signal connections” on page 208](#).

Tip: In Ethernet mode, you set up the detector parameters via the chromatography data software (see the detector operator’s guide for specific information).

To enter parameters in the Detectors screen:

1. Press the Next or Prev screen key to display the Detectors screen.

Figure 6–11: Detectors screen



2. In the Detectors screen, use the up/down arrow keys to select the appropriate detector (for example, Absorbance Detector 2), and then press Enter to display the detector selections.
3. Use the up/down arrow to highlight your selection, and then press Enter to accept your selection.

The following table describes the selections in the Detectors screen.

Table 6–11: Detector selections

Parameter	Function	Selections
Absorbance Detector (1)	Enables a Waters 2487 or 486 UV/Vis detector channel	2487, 486, or Not used
Absorbance Detector (2)	Enables a second Waters 2487 or 486 UV/Vis detector channel	2487, 486, or Not used
Refractive Index Detector 410	Enables a Waters 2414 RI, 2410 RI, or 410 dRI detector	410 or Not used
Absorbance (1) (screen key)	Displays the 2487 or 486 TUV screen (see figure “2487(1) UV/Vis detector screen” on page 109).	See table “2414 RI, 2410 RI, and 410 dRI parameters” on page 111 .
Absorbance (2) (screen key)	Displays the second 2487 or second 486 TUV screen (see figure “2487(1) UV/Vis detector screen” on page 109).	See table “2414 RI, 2410 RI, and 410 dRI parameters” on page 111 .

Table 6–11: Detector selections (continued)

Parameter	Function	Selections
RI (screen key)	Displays the 2410/410 RI screen (see figure “410 Detector screen” on page 111).	See table “2414 RI, 2410 RI, and 410 dRI parameters” on page 111.
Table (screen key)	Displays the Detector Table (see figure “410 Detector screen” on page 111).	See table “2487 and 486 Detector Events parameters” on page 112.

- To enter control parameters for Waters detectors, refer to the “Setting absorbance parameters” and “Setting RI parameters” procedures that follow.

6.2.6.1 Set absorbance parameters

To enter control parameters for a 2487 or 486 UV/Vis detector:

- Press the Abs (1) or Abs (2) screen key to display the appropriate absorbance detector screen.

Figure 6–12: 2487(1) UV/Vis detector screen

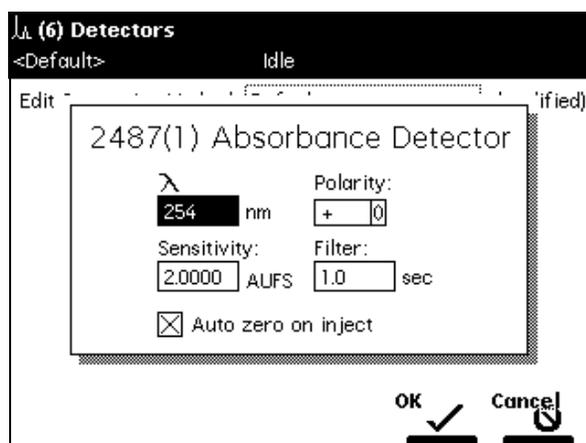
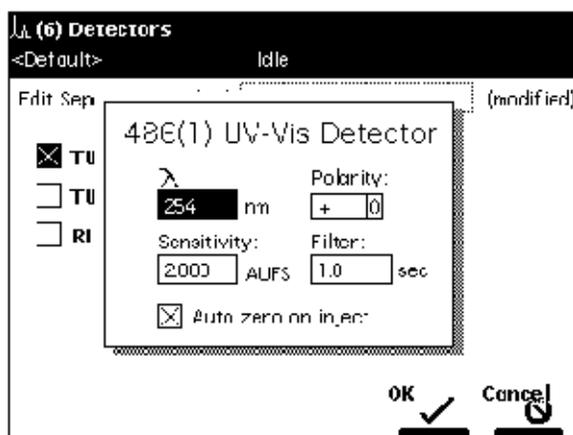


Figure 6–13: 486(1) UV/Vis Detector screen



2. Enter detector parameters as required and click OK.

See also: “Edit the detector table” on page 112 for steps on programming detector events.

The following tables list the parameters for Waters 2487 and 486 detectors.

Table 6–12: 2487 UV/Vis parameters

Parameter	Function	Value range
λ (wavelength)	Sets the wavelength of the detector.	190 to 700 in 1 nm increments
Sensitivity	Sets the sensitivity of the detector.	0.0001 to 4.0000 in 0.0001 AU increments
Filter	Sets the time constant of the filter in the detector.	0.1 to 99.0 in 0.1 sec increments
Polarity	Selects the output of the 2487 to create positive or negative peaks.	+, –

Table 6–13: 486 UV/Vis parameters

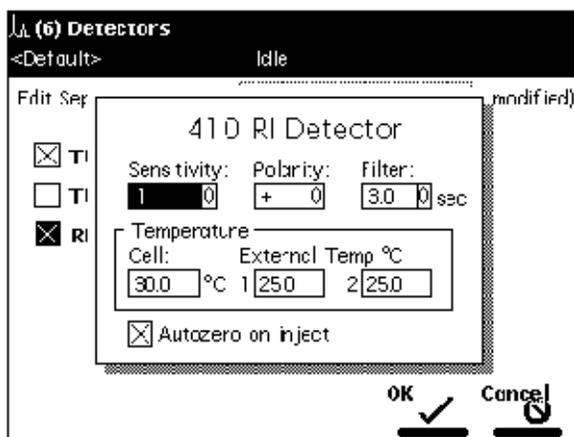
Parameter	Function	Value range
λ (wavelength)	Sets the wavelength of the detector.	190 to 600 in 1 nm increments
Sensitivity	Sets the sensitivity of the detector.	0.001 to 2.000 in 0.001 AU increments
Filter	Sets the time constant of the filter in the detector.	0.1 to 5.0 in 0.1 sec increments
Polarity	Selects the output of the 486 to create positive or negative peaks.	+, –

6.2.6.2 Set RI parameters

To enter control parameters for a 2414 RI, 2410 RI, or 410 dRI detector:

1. In the Detectors screen, press the RI screen key to display the 410 Detector screen.

Figure 6–14: 410 Detector screen



2. Enter detector parameters as required and click OK.

The following table lists the parameters for the Waters 2414 RI, 2410 RI, and 410 dRI detectors.

Table 6–14: 2414 RI, 2410 RI, and 410 dRI parameters

Parameter	Function	Value range
Sensitivity	Sets the initial sensitivity of the detector.	1, 2, 4, ..., 512, 1024
Polarity	Selects the output of the detector to create positive or negative peaks.	+, –
Filter	Sets the time constant of the filter in the detector.	0.2, 1, 3, and 10 sec
Temperature: Cell	Temperature of the flow cell in °C.	30 to 50 °C in 1 °C increments
Temperature: External Temp °C 1	Sets the temperature of external column heater (or column heater/cooler) 1 in °C ¹ .	Ambient to 150 °C in 1 °C increments
Temperature: External Temp °C 2	Sets the temperature of external column heater (or column heater/cooler) 2 in °C ¹ .	Ambient to 150 °C in 1 °C increments
Autozero on inject	Resets the output of the detector to 0 volts when the injection begins.	Selected or deselected

1. The Waters 2414 RI, 2410 RI, and 410 dRI detectors can control the temperature of one or two Waters Column Heater Modules (CHM) or column heater/cooler modules. These devices are separate from the column heater or column heater/cooler attached to the separations module.

6.2.6.3 Edit the detector table

In the detector table, you program 2487 and 486 detector events to occur during a run.

To enter events in the detector table:

1. Press the Table screen key in the Detectors screen. The following figure shows the screen for a Waters 2487 detector using channels 1 and 2.

Figure 6–15: Detector Events screen

time	detector	action	param
1	INIT	2487 (1)	0 Set wavelength 0 254
2	INIT	2487 (1)	0 Set filter 0 1.0
3	INIT	2487 (1)	0 Set AU full scale 0 2.0000
4	INIT	2487 (1)	0 Set chart polarity 0 [+]
5	INIT	2487 (1)	0 Auto zero 0
6	INIT	2487 (2)	0 Set wavelength 0 280
7	INIT	2487 (2)	0 Set filter 0 1.0
8	INIT	2487 (2)	0 Set AU full scale 0 2.0000

10 Rows Total

Over-view Insert Row Delete Row Sort by Time Copy Down More

2. Enter parameters in the Detector Events screen, and then press the Exit key.

The following table describes the parameters in the Detector Events screen.

Table 6–15: 2487 and 486 Detector Events parameters

Parameter	Function	Value range
Time	Indicates the time at which the action is to occur. Press the Clear key to display INIT.	INIT, 0.00 to 999.99 in 0.01 min increments
Detector	Selects the detector on which to perform the action.	2487 (1), 2487 (2) 486 (1), 486 (2)
Action	Selects the action to perform on the selected detector.	See table “2487 and 486 detector actions” on page 113.
Parameter	Defines the action, where appropriate.	See table “2487 and 486 detector actions” on page 113.

The following table describes the actions that you can specify with Waters 2487 and 486 tunable absorbance detectors.

Table 6–16: 2487 and 486 detector actions

Parameter	Function	Value range
Set wavelength	Sets the wavelength of the detector.	2487: 190 to 700 in 1 nm increments 486: 190 to 600 in 1 nm increments
Set filter	Sets the time constant of the filter in the detector.	2487: 0.1 to 99.0 in 0.1 sec increments 486: 0.1 to 5.0 in 0.1 sec increments
Set AU full scale	Sets the absorbance units full scale to adjust the sensitivity of the detector.	2487: 0.0001 to 4.0000 in 0.0001 AU increments 486: 0.001 to 2.000 in 0.001 AU increments
Set chart mark	Sends a chart mark signal to the output terminals.	2487 and 486: (No values)
Set chart polarity	Sets the polarity of the analog output.	2487 and 486: +, –
Auto zero	Performs an autozero on the detector.	2487 and 486: (No values)
Set lamp	Turns the lamp on and off.	2487 and 486: On, Off

6.3 Create and edit a sample set

A sample set is a table of instructions that the separations module uses to perform an automated run in a standalone mode. During a run, the separations module performs the function in each row of the table sequentially.

To create and edit a sample set:

1. On the Main screen, click the Develop Methods screen key.
2. On the Methods screen, click the New screen key.
3. Click the Sample Set screen key.
4. Name the sample set, and then click Enter.
Tip: The separations module automatically adds the appropriate extension.
5. Enter information in the Sample Set table as appropriate.
6. Press Exit to display a dialog box that asks if you want to save the changes.

The following table describes the parameters in the Sample Set table.

Table 6–17: Sample Set table parameters

Parameter	Function	Value range
Vials	Selects the vial(s) from which the injection is to be made. Use the "." key to specify a vial range. For example, press 2.5 to enter vials 2 through 5.	1 to 120
Function	The second column in the sample set table specifies the function you apply to a separation method. The following table describes the functions available with the separations module. You use these functions in the same manner as the direct functions described in "Direct functions" on page 73 .	See table "Sample Set functions" on page 114
Method	Selects the separation method to use. See "Create and edit separation methods" on page 90 for the procedures to create, edit, and store separation methods.	Stored methods
Injs	Number of injections to perform using the current vial(s).	1 to 99
µL	Amount of sample (or standard) to inject from the current vial.	0.1 to 2000 in 0.1 µL increments (You can enter amounts greater than 100 µL if you install larger sample loops.)
Min	Time in minutes before the next line is executed.	0.1 to 999.99 in 0.01 min increments

Table 6–18: Sample Set functions

Function name	Description
Sample	Injects a sample from the selected vial.
Standard	Injects a standard from the selected vial.
AutoStds	Programs the repetition of standards over the course of a run. See "Auto Standards", below.
Prime	Wet primes the solvent management system at the specified rate for the specified period. Press the Row Details screen key to enter additional prime parameters.

Table 6–18: Sample Set functions (continued)

Function name	Description
System Prep	Performs the System Prep function using the parameters from the separation method. For more information on System Prep, see “Run System Prep” on page 75 .
Purge	Purges the sample management system. Press the Row Details screen key to enter additional purge parameters.
Equil	Delivers solvent through the system for the specified period of time using the initial conditions in the specified separation method. Press the Row Details screen key to display the Equilibrate dialog box ¹ to enter additional Equil parameters.
Condition	Delivers to the column the gradient specified in the separation method; conditions the column without injecting samples.
Auto Add	Injects sample from vials in the selected order. See “Auto Add” on page 116 .

1. For typical unattended operation, leave the “Hold when time expires” check box (in the Equilibrate dialog box) unselected. Select the check box and set a time only if you want to maintain (hold) initial conditions once the set time expires (and respond to the two soft keys, “Resume Sample” and “Abort Samples”, that appear in the Status screen). Pressing one of these soft keys performs the desired action. Note that elapsed time continues until you press one of the keys.

6.3.0.1 Auto Standards

Use the AutoStds function to repetitively run standards after a specified number of vials or injections. You can specify multiple standards vials to support large runs.

To use the Auto Standards function:

1. In the Sample Set screen, select AutoStds from the drop-down list of functions, and then press the Row Details screen key to display the Auto Standards dialog box.
2. Enter the frequency with which you want to run standards, and then select either vials or injections.
3. Enter the location of each standard vial and its capacity in the Source Vial(s) table.
When you start the run, the separations module runs the standard. When the current standards vial is empty (based on the number of injections, the size of the injections, and the total volume), the separations module automatically advances to the next standards vial specified in the Source Vial(s) table.

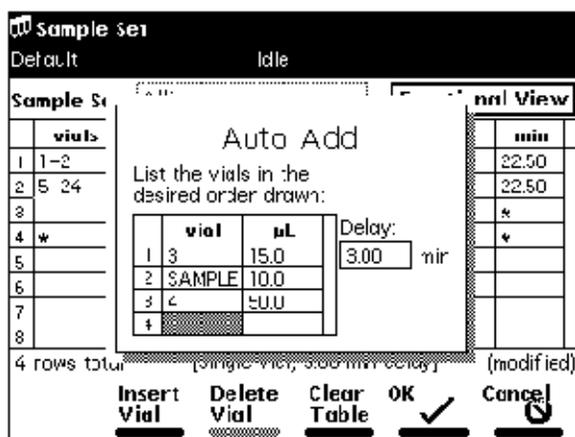
6.3.0.2 Auto Add

Use the Auto Add function to perform an injection composed of aliquots from as many as 10 vials, one of which is the sample vial. The total volume of this injection must be less than the volume of the sample loop. You can specify a time delay to allow the sample to passively mix once sample from all vials is in the sample loop.

To use the Auto Add function:

1. In the Sample Set screen, select AutoAdd from the drop-down list of functions, and then press the Row Details screen key to display the Auto Add dialog box.

Figure 6–16: Auto Add dialog box



In this example, vials 3 and 4 are “reagents” that react with compounds in the sample vial. In the figure “[Linked rows in a sample set](#)” on page 117, vials 25 to 30 are the sample vials used for Auto Add. The separations module draws 15 µL from vial 3, 10 µL from vial 25, 50 µL from vial 4, then holds the three aliquots in the sample loop for the specified 3.00 minutes before making the injection. The process is repeated for vials 26 to 30.

2. Enter the vial from which you want to withdraw the first portion of the sample.
Tip: Press Clear to set the vial to “sample.”
3. Enter the volume (in µL) of the sample you want to withdraw.
4. Repeat [step 2](#) and [step 3](#) for subsequent portions of the sample.
5. Enter the time delay (in minutes) to allow the combined sample to mix.
6. Press OK to return to the Sample Set screen.

6.3.1 Link rows in a sample set

You can link together one or more rows in a sample set so that you can perform multiple functions for the same set of vials. For example, you can link an inject sample function with a purge function, as shown in the following figure. In this example, two 15-µL injections are made from vial 5, followed by a purge. The process is repeated for each vial that you specify.

Tip: When unlinking or deleting linked rows in a sample set, vial numbers in the remaining rows may differ from those originally entered. Make sure that vial numbers for all rows are correct before running the sample set.

Figure 6–17: Linked rows in a sample set

Run Samples						
<Default>			Idle			
Job: Rich Test(002)					Functional View	
	vials	function	method	inj	μL	min
1	*	Equil	Dave Test	0 *	*	45.00
2	*	Purge	Dan Test	0 *	*	*
3	1-2	AutoStds	Dave Test	0 1	10.0	22.50
4	5-24	Sample	Dave Test.dad	0 2	15.0	22.50
5		Purge	Dan Test	0 *	*	*
6	*	Condition	Dave Test	0 *	*	30.00
7	25-30	AutoAde	Dave Test.dad	0 1	10.0	15.00
8						

7 rows total

Insert Row Copy Down Row Details More  Next View Start 

To link rows:

1. Select the row you want to link.
2. Press the More screen key, and then press the Make Link screen key.

Result: The current row is linked with the previous row.

To unlink lines:

1. Select the row you want to unlink.
2. Press the More screen key and then the Break Link screen key.

6.4 Create a sample template

Using a sample template, you define the major parameters of a run and then enter variable parameters, such as number of vials, vial position, and so forth just before you begin the run.

To create a sample template:

1. Create a sample set as described in “Create and edit a sample set” on page 113.
2. On the Main screen, press the Methods screen key.
3. Select the sample set you want to serve as a sample template.
4. Press the Make Tpl screen key.

Tip: To lock the template, use the procedure in “Lock and unlock a separation method” on page 93.

7 Maintenance Procedures

This chapter provides the maintenance guidelines and procedures necessary to maintain the instrument's performance.

Keep to a maintenance schedule, and perform maintenance as required and described in this chapter.

7.1 Maintenance considerations

7.1.1 Proper operating procedures

To ensure your separations module operates as designed, follow the operating procedures and guidelines described in [Chapter 1](#).

7.1.2 Reinitialize the system

It is good practice to reinitialize the system instruments at least once a week. When the separations module and detectors are power-cycled (powered-off for a minimum of one minute, and then powered-on), each instrument undergoes a complete system initialization, including internal diagnostic tests and verifications. This procedure helps ensure that the mechanical and electrical components, as well as internal firmware, synchronize for proper operation.

7.2 Spare parts

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

7.3 Troubleshooting with Connections INSIGHT

Connections INSIGHT is an “intelligent” device management (IDM) Web service that enables Waters to provide proactive service and support for the ACQUITY UPLC system. To use Connections INSIGHT, you must install its service agent software on your workstation. In a client/server system, the service agent must also be installed on the computer from which you control the system. The service agent software automatically and securely captures and sends information about the support needs of your system directly to Waters.

If you encounter a performance issue when using the Instrument Console, you can manually submit a Connections INSIGHT request to Waters customer support. Alternatively, you can use Remote Desktop, a real-time collaboration option that controls the two-way connection with the ACQUITY UPLC system by enabling the Connections INSIGHT iAssist service level.

Consult these sources for more information about Connections INSIGHT and Connections INSIGHT iAssist:

- <http://www.waters.com>
- Connections INSIGHT Quick Start Guide
- *Connections INSIGHT User's Guide*
- *Connections INSIGHT Troubleshooting Notes*
- Your sales representative
- Your local Waters subsidiary
- Waters Customer Support

To submit a Connections Insight request:

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

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

Tip: Saving a service profile or plot file from the Instrument Console can require as much as 150 MB of file space.

7.4 Safety and handling

Bear in mind the following safety considerations when performing maintenance procedures:



Warning: The instrument components can be contaminated with biologically hazardous materials. Always wear chemical-resistant, powder-free gloves while handling the components.



Warning: To prevent injury, always observe Good Laboratory Practice when handling solvents, changing tubing, or operating the instrument. Know the physical and chemical properties of the solvents used (see the Safety Data Sheets for the solvents in use).



- Warning:** To avoid electric shock,
- do not remove the instrument's panels. There are no user-serviceable items inside the instrument.
 - ensure that the instrument is in Standby mode before commencing any maintenance.



Warning: The probe and source can be hot. To avoid burn injuries, take great care while working with these components.



Notice: When performing maintenance inside the source enclosure, ensure that the following criteria are met:

- Instrument is in Standby mode.
- LC flow is diverted to waste or set to off.
- Desolvation gas is turned off.

See [Appendix A](#) for safety advisory information.

7.5 Maintain the solvent management system

Perform the procedures in this section when you discover a problem with a specific component in the solvent management system. For information about isolating problems in the solvent management system, see [Chapter 8](#).



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

7.5.1 Overview

Maintaining the solvent management system involves these tasks:

- Replacing the plunger seals
- Replacing the seal wash seals
- Cleaning and replacing a plunger
- Replacing an inlet check valve
- Replacing the in-line filter

Tip: To ensure proper drip protection, be sure the solvent delivery and solvent conditioning trays are resecured in place with screws before you operate the system.

The following figure illustrates the various solvent management system components (with the solvent delivery tray pulled out). See [table “e2695 solvent management system components and functions” on page 122](#) for a description of the functions of these components.

Figure 7–1: Solvent management system components

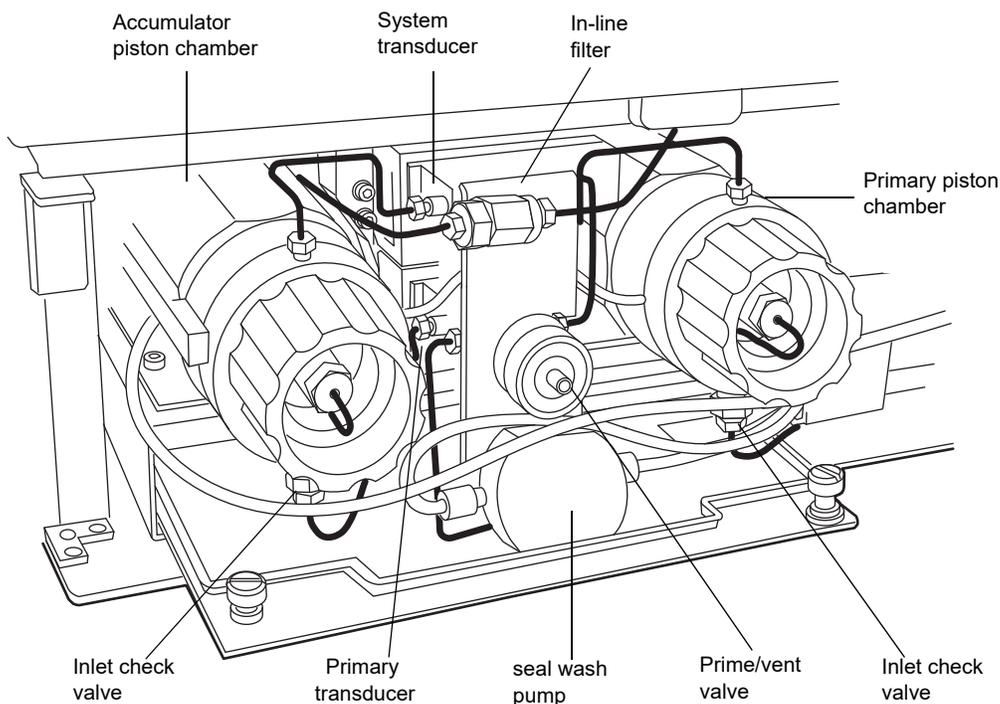


Table 7–1: e2695 solvent management system components and functions

Component	Function
Primary piston chamber	Draws in/expels solvent as part of serial flow design.
Accumulator piston chamber	Draws in/expels solvent as part of serial flow design.
Prime/vent valve	Allows solvent purging, priming, and venting.
seal wash pump	Provides solvent to wash the seals of the primary and accumulator plungers.
Primary inlet check valve	Maintains flow direction in the primary piston chamber by opening in one direction only: opens on the plunger intake stroke; closes on the delivery stroke.
Accumulator inlet check valve	Maintains flow direction in the accumulator piston chamber by opening in one direction only: opens on the plunger intake stroke; closes on the delivery stroke.
Primary transducer	Senses backpressure developed by resistance to solvent flow in the primary piston chamber.
System transducer	Senses backpressure developed by resistance to solvent flow in the HPLC system.

Table 7–1: e2695 solvent management system components and functions (continued)

Component	Function
In-line filter	Provides solvent filtering between the solvent management system and the sample management system.

7.5.2 Remove the head, seal wash assembly, and plunger

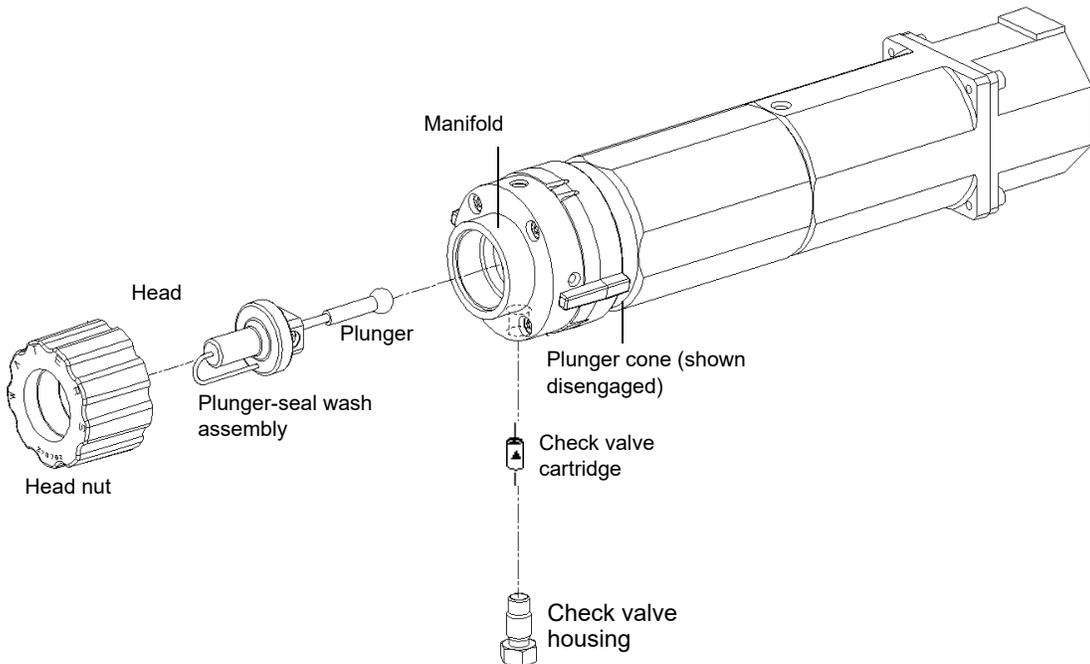
For information about isolating problems in the solvent management system, see [Chapter 8](#).

See [figure “Solvent management system piston chamber components \(exploded view\)” on page 123](#) to view the head, head nut, plunger, seal wash assembly, and check valve assembly of each solvent delivery piston chamber.

Remove the head, seal wash assembly, and plunger whenever you must perform these tasks:

- Replace the plunger seals
- Clean or replace a plunger
- Service the seal wash seals

Figure 7–2: Solvent management system piston chamber components (exploded view)



To remove the head, seal wash assembly, and plunger:

1. On the Main screen, press the Diag screen key.
2. Press the Other Tests screen key in the Diagnostics screen.
3. From the list in the Other Diagnostics screen, select Head Removal and Replacement.

4. Follow the instructions that appear in the Head Removal and Replacement screen.
Requirement: To minimize fluid leakage during the Head removal and replacement procedure, make sure that the GPV valve is set to the Off position (see [“Perform the motors and valves diagnostic test” on page 157](#)) and place the seal wash reservoir on the same level as the separations module.
5. Remove the head, seal wash assembly, and plunger as one unit (see [figure “Plunger, seal wash, and head” on page 128](#)).

For more information about removing the head, see [“Head removal and replacement function” on page 163](#).

7.5.3 Notes on replacing the plunger seals

- For information about isolating problems in the solvent management system, see [Chapter 8](#).
- To ensure optimum performance, replace the plunger seal in a head when you perform service. Replace the face seals after you replace the plunger seal two or three times (see [figure “Plunger and face seal orientation:” on page 125](#)).
- The standard seals in the separations module are manufactured with Fluoroloy-G, an ultra-high molecular weight polyethylene material. Extensive testing has indicated that these seals exhibit excellent lifetimes for the majority of solvents typically used in HPLC separations methods. If you find these seals perform unsatisfactorily for your applications, contact Waters for alternative plunger seals manufactured with graphite-filled PTFE material (WAT271066), or Alliance ClearSeal (700001326).

This procedure involves replacing these parts:

- Plunger seal
- Inlet face seal
- Outlet face seal

Required materials

- Seal insertion tool (start-up kit)
- Seal removal tool (WAT039803)
- Replacement seals
- Squeeze bottle with 100% alcohol (methanol or isopropanol)
- Clean block, such as a detector cell window (WAT097295)
- Syringe (start-up kit)
- Plastic tweezers or can of compressed air

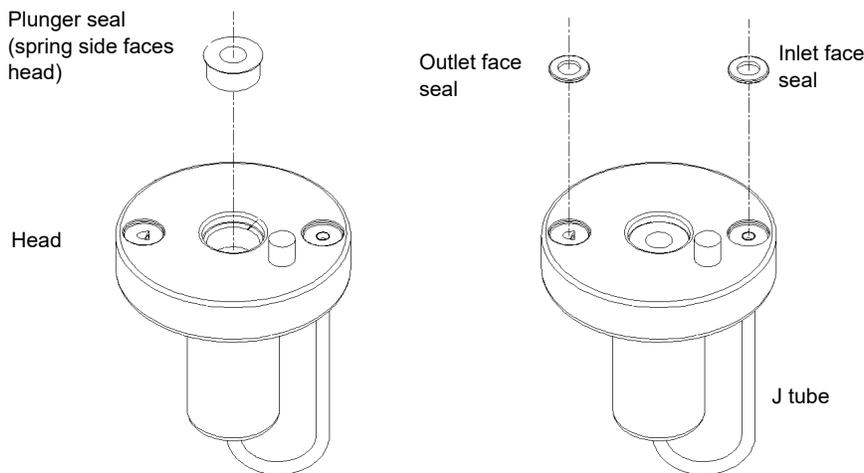
7.5.3.1 Plunger seal replacement

- ! **Notice:** To avoid damaging the sealing surfaces, use the seal removal tool. Do not use a sharp tool to remove or install seals.

To replace the plunger seal:

1. Remove the head (see [“Remove the head, seal wash assembly, and plunger”](#) on page 123).
2. Use the plastic end of the seal removal tool to remove the plunger seal from the head.

Figure 7–3: Plunger and face seal orientation:



3. Wet the insertion tool, seal opening, and new plunger seal with 100% alcohol before you install the seal.
4. Place the new plunger seal on the seal insertion tool with the spring side facing away from the tool. Insert the tip of the tool into the plunger seal opening in the head (see [figure “Plunger and face seal orientation:”](#) on page 125), and then push firmly to install the seal.

7.5.3.2 Face seal replacement

- ! **Notice:** To avoid damaging the sealing surfaces, use the seal removal tool. Do not use a sharp tool to remove or install seals. Replace face seals after every two or three plunger seal replacements.

To replace the face seals:

1. Remove the head (see [“Remove the head, seal wash assembly, and plunger”](#) on page 123).
2. Direct compressed air into the center of the face seal, or use plastic tweezers to remove the inlet and outlet face seals from the seal openings in the head (see [figure “Plunger and face seal orientation:”](#) on page 125).
3. Wet the new seals and seal openings with 100% alcohol.
4. Place the new face seals in the face seal openings of the head.
5. Use an alcohol-cleaned, flat object, such as a detector window, to firmly press the face seals into the openings on the pump head.

6. After you replace the seals, observe the Head Removal and Replacement screen.
7. Follow the on-screen instructions to reinsert the head, seal wash housing, washer, and plunger assemblies.
Requirement: To avoid damaging the tube seals and to properly align the seal wash tubes with the seal wash housing, refer to [step a](#) through [step d](#), below. Also, make sure that the head alignment pin is properly oriented and that the “J” tube faces downward (see [figure “Plunger, seal wash, and head” on page 128](#)).
 - a. After finger tightening the head nut onto the head, loosen (turn counterclockwise) the nut approximately 1/2-turn.
 - b. Inspect the ends of the seal wash tubes for damage.
Tip: If necessary, trim the ends with a razor blade.
 - c. Reinsert the seal wash tubes into the manifold, and then fully tighten the head nut.
 - d. Return the release ring to the run position.
8. If the separations module does not have solvent in the lines, perform a dry prime to draw solvent into the plunger cavity before you perform a wet prime or start delivery of solvents (see [“Prime the solvent management system” on page 58](#)).

7.5.4 Replace the seal wash assembly seals

For information about isolating problems in the solvent management system, see [Chapter 8](#).

This procedure involves replacing these parts:

- seal wash face seal
- Two tube seals
- Plunger wash seal

Required materials

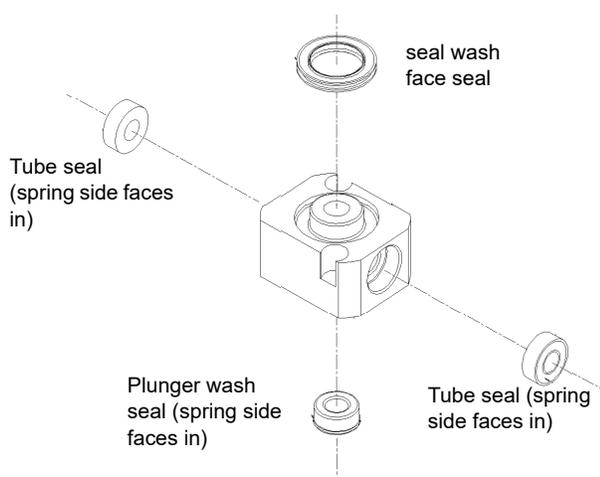
- Seal insertion tool (start-up kit)
- Seal removal tool (part number WAT039803)
- Replacement seals
- Squirt bottle containing 100% alcohol (methanol or isopropanol)
- Syringe (start-up kit)
- Plastic tweezers

! **Notice:** To avoid damaging the sealing surfaces, use the seal removal tool. Do not use a sharp tool to remove or install seals.

To replace the seal wash assembly seals:

1. Remove the seal wash assembly (see [“Remove the head, seal wash assembly, and plunger” on page 123](#)).
2. Use the plastic end of the seal removal tool to remove the plunger wash seal from its seat, repeating the procedure for each of the tube seals (see [figure “Seal wash assembly seals” on page 127](#)).
3. Use the plastic tweezers to remove the seal wash face seal.

Figure 7–4: Seal wash assembly seals



4. Wet the seal insertion tool, each new seal, and seal opening with 100% alcohol.
5. Place the new plunger wash seal on the seal insertion tool with the spring side facing away from the seal insertion tool, and insert the tip of the tool into the seal opening, pushing firmly to install the seal. Repeat this procedure for each of the tube seals.
6. Reinstall the seal wash housing assembly washer (see [figure “Plunger, seal wash, and head” on page 128](#)).
7. Slide the seal wash housing and washer onto the plunger, and then insert the assembly onto the head (see [figure “Plunger, seal wash, and head” on page 128](#)).
8. Follow the instructions in the Head Removal and Replacement screen to reinsert the head, seal wash assembly, and plunger. To avoid damaging the tube seals, and to properly align the seal wash tubes with the seal wash housing, refer to [step a](#) through [step d](#) below. Also, make sure that the head alignment pin is properly oriented and that the “J” tube is facing downward (see [figure “Plunger, seal wash, and head” on page 128](#)).
 - a. After finger tightening the head nut onto the head, loosen the nut approximately 1/2 turn.
 - b. Inspect the ends of the seal wash tubes for damage. If necessary, trim the ends with a razor blade.
 - c. Reinsert the seal wash tubes into the manifold, and then fully tighten the head nut.
 - d. Return the release ring to the run position.

If the separations module’s solvent lines are empty, dry prime the solvent management system before you wet prime it or start delivering solvents (see [“Prime the solvent management system” on page 58](#)).

7.5.5 Clean and replace the plunger

Tip: For information about isolating problems in the solvent management system, see [Chapter 8](#).

Required materials

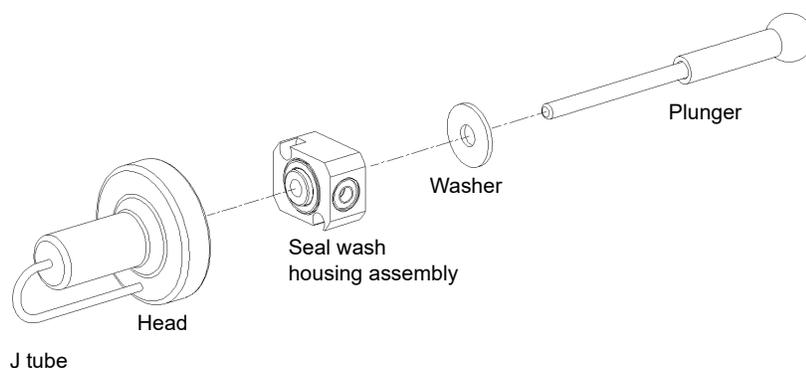
- Pumice, fine
- Cotton swab
- Water, HPLC-grade

To clean the plunger:

1. Remove the head, seal wash assembly, and plunger (see [“Remove the head, seal wash assembly, and plunger”](#) on page 123).
2. Inspect the plunger for damage, excessive wear, or mobile-phase residue without separating it from the pump head and seal wash assembly (see [figure “Plunger, seal wash, and head”](#) on page 128).

Tip: If the plunger or plunger seal has mobile-phase residue, replace with the new plunger.

Figure 7–5: Plunger, seal wash, and head



3. Reinspect the plunger. If the plunger shows excessive wear, replace it.
If necessary, replace the plunger seal and face seals (see [“Notes on replacing the plunger seals”](#) on page 124).

To replace the plunger:

1. Slide the seal wash housing with washer onto the new plunger, and then insert the assembly into the head (see [figure “Solvent management system piston chamber components \(exploded view\)”](#) on page 123).
2. Follow the directions in the Head Removal and Replacement screen to reinsert the head, seal wash assembly, and plunger.

Requirement: To avoid damaging the tube seals and to properly align the seal wash tubes with the seal wash housing, refer to [step a](#) through [step d](#) below. Also, make sure that the head alignment pin is properly oriented and that the “J” tube is facing downward.

- a. After finger tightening the head nut onto the head, loosen the nut approximately 1/2 turn.

- b. Inspect the ends of the seal wash tubes for damage. If necessary, use a razor blade to trim the ends.
- c. Reinsert the seal wash tubes into the manifold, and then fully tighten the head nut.
- d. Return the release ring to the run position.

If the separations module's solvent lines are empty, dry prime the solvent management system before you wet prime it or start delivering solvents (see ["Dry prime the system" on page 74](#)).

7.5.6 Replace an inlet check valve cartridge

Tip: For information about isolating check valve problems, see [Chapter 8](#).

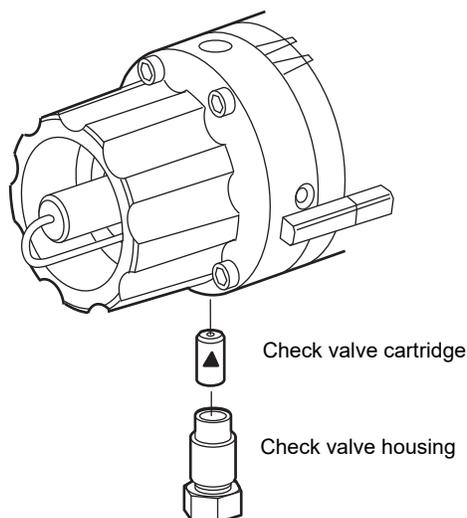
Required materials

- Wrench, 1/2-inch, open-end
- Wrench, 5/16-inch, open-end
- Replacement check valve cartridge
- Squirt bottle with 100% alcohol (methanol or isopropanol)
- Syringe (in start-up kit)

To replace an inlet check valve on either piston chamber:

1. Turn off the GPV by selecting "Diag" menu from Alliance Home screen, select "Other test", and scroll down to "Turn Off GPV".
2. Loosen the screws that secure the solvent delivery and conditioning trays, and pull the trays out a few inches to access the check valves (see [figure "Solvent management system components" on page 122](#)).
3. Use the 1/2-inch wrench to hold the inlet check valve housing while you disconnect the inlet check valve compression screw with the 5/16-inch wrench (see [figure "Inlet check valve" on page 130](#)).
4. Use the 1/2-inch wrench to disconnect the check valve housing from the manifold.

Figure 7–6: Inlet check valve



5. Tip the inlet check valve housing upside down to dislodge the old check valve cartridge.
6. Inspect the inlet check valve housing, clean it, if necessary, and wet it with 100% alcohol.
7. Wet the new check valve cartridge with 100% alcohol.
8. Insert the replacement check valve cartridge into the inlet check valve housing.
Tip: Make sure the arrow on the check valve cartridge points toward the piston chamber.
9. Insert the inlet check valve housing into the piston chamber housing, and then finger-tighten the check valve housing.
10. Use the 1/2-inch wrench to tighten the inlet check valve housing 1/8 of a turn.
11. Use the 1/2-inch wrench to hold the check valve housing while you reinstall and tighten the compression screw into the check valve housing with the 5/16-inch wrench.
12. Select Motors and Valves from the Other Diagnostics screen.
Requirement: Set the GPV valve to the Solvent A position (see [“Perform the motors and valves diagnostic test” on page 157](#)).
13. If the separations module’s solvent lines are empty, dry prime the solvent management system before you wet prime or start delivering solvents (see [“Dry prime the system” on page 74](#)).

7.5.7 Replace the in-line filter

The in-line filter provides solvent filtering between the solvent management system and sample management system. Clean and replace the in-line filter element when it is the source of elevated backpressure.

! **Notice:** It is important to maintain proper functioning of the in-line filter. Damage to downstream components can occur if you replace the in-line filter with a union, or remove the filter element.

Tip: For information about isolating problems in the solvent management system, see [Chapter 8](#).

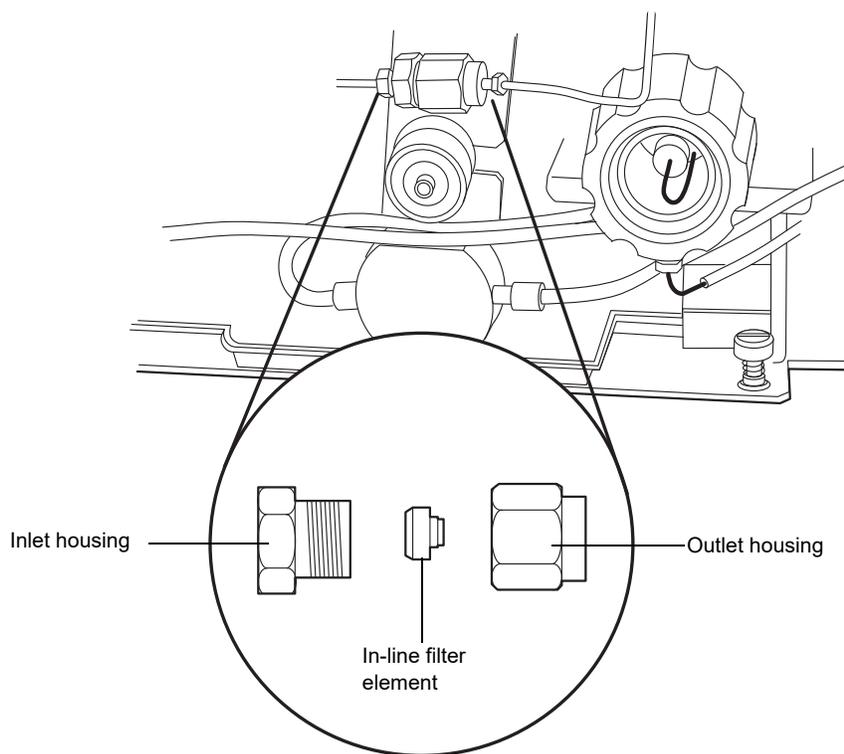
Required materials

- Wrenches, 5/8-inch, open-end, two
- Wrench, 5/16-inch, open-end
- Squirt bottle with 100% alcohol (methanol or isopropanol)
- Absorbent tissue
- Replacement in-line filter element

To replace the in-line filter:

1. Use a 5/8-inch wrench to disconnect the compression screw on the left side of the in-line filter from the inlet housing (see [figure “Replacing the in-line filter” on page 132](#)). Use an absorbent tissue to absorb the small amount of solvent that is likely to leak.
2. Use a 5/8-inch wrench to hold the filter outlet housing while you loosen the inlet housing with another 5/8-inch wrench.
3. Tip the inlet housing upside down to dislodge the in-line filter element.
4. Wet the replacement in-line filter element with alcohol.
5. Insert the replacement in-line filter element into the in-line filter housing (see [figure “Replacing the in-line filter” on page 132](#)).

Figure 7–7: Replacing the in-line filter



6. Reconnect the in-line filter inlet and outlet housings.
7. Re-tighten the compression screw into the inlet housing.
8. Flush the solvent management system with mobile phase at 1 mL/min for 10 minutes (see [“Power-off the separations module” on page 64](#)).
9. Inspect the in-line filter for leaks, and tighten the fittings as required.

7.6 Maintain the sample management system

Perform the procedures in this section when you determine there is a problem with a sample management system component.

Tip: For information about isolating problems in the sample management system, see [Chapter 8](#).

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

Maintaining the sample management system involves these tasks:

- Replacing the lower needle wash frit (if so equipped)
- Replacing the syringe
- Replacing the needle and seal pack
- Cleaning the sample compartment

The following two figures illustrate the various assemblies and components of the sample management system. The [table “e2695 sample management system assemblies/components” on page 134](#) describes the functions of each assembly and component.

Figure 7–8: Sample management system components (front view)

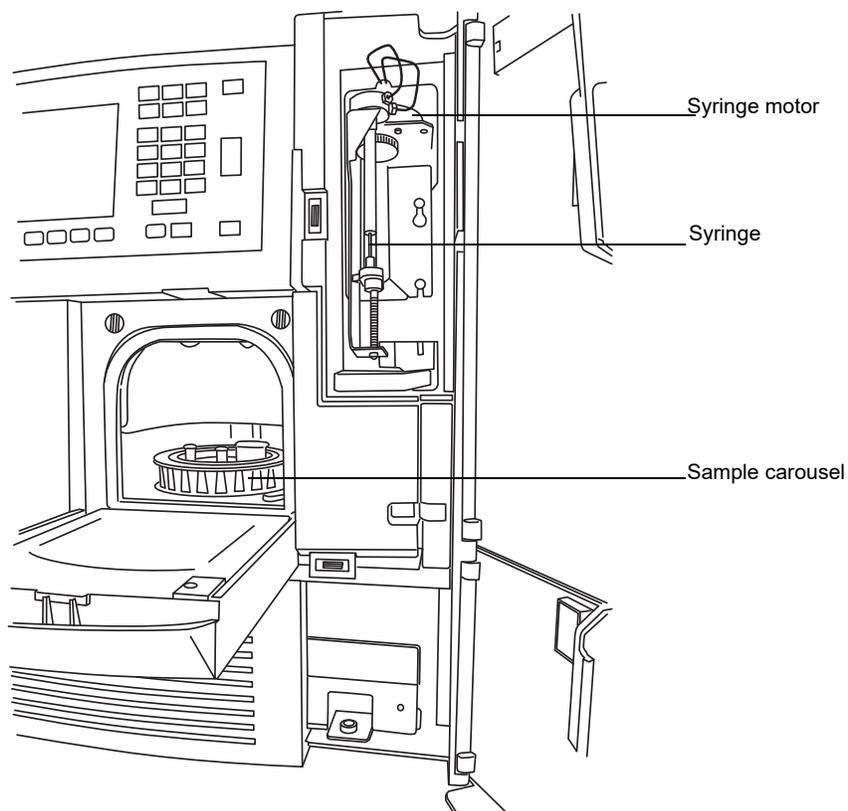


Figure 7–9: Sample management system components (right-side view)

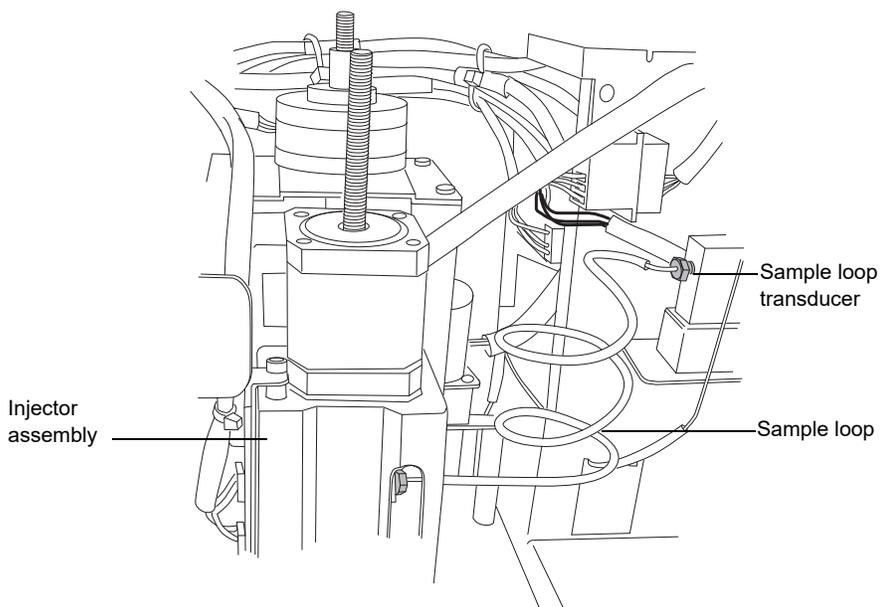


Table 7–2: e2695 sample management system assemblies/components

Assembly/component	Function
Syringe	Draws sample from the sample vial into the sample loop.
Sample carousels and carousel carrier	Five, color-coded carousels. Each carousel holds up to 24 sample vials for a total capacity of 120 sample vials.
Injector motor	Controls injector needle movement.
Sample loop transducer	Senses backpressure developed by resistance to solvent flow in the sample loop.
Sample loop	Holds up to one sample-loop volume (standard 100 μ L) of sample.
Needle tee	Holds the injector needle and provides the connector for sample loop.
Needle (see figure “Remove the injector assembly” on page 143)	Perforates the sample vial caps, draws sample, and injects the sample into the flow path.
Seal pack (see figure “Remove the injector assembly” on page 143)	Contains high-pressure seals and the needle wash system.

7.6.1 Remove the side panel of separations module

Some maintenance procedures, such as sample loop and needle and seal-pack replacement, require that you remove the side panel of the separations module to access internal components.

Note: This section only applies if you are using the column heater or column heater/cooler option and need to remove that unit to access the side panel on the separations module. If you are not using this option, consult the appropriate maintenance item for side panel removal instructions.

Required materials

Screwdriver, T-20 TORX

To access and remove the side panel:

1. Power-off the separations module.
2. Disconnect these cables:
 - Ethernet and power cord from rear of detector
 - External control cable and power cord from rear of column heater or column heater/cooler
 - Power cord from rear of separations module
3. Remove the detector from its base and set it aside.
4. Remove the following tubing from the detector drip tray:
 - Release the tubing from the routing clip in the drip tray and move the tubing free of the detector base.
 - Disconnect the convoluted tubing from the waste drain outlet of the drip tray.
5. Lift the detector base off of the two feet on the right side of the solvent bottle tray and set the base aside.
6. Open the column heater or column heater/cooler door and release the column tubing from the clip on the front of the unit.
7. Move the column tubing to the left until it is positioned outside of the column heater or column heater/cooler door.
8. Slide the drip tray assembly and tube out from the bottom of the column heater or column heater/cooler compartment and set it aside.
9. Lift up the column heater or column heater/cooler from the 3 keyed ports on the separations module, then remove the unit and set it aside.
10. Remove the two T-20 screws that secure the separations module's right-hand side panel, then slide the panel back until it clears the posts on the rear panel.
11. Remove the side panel from the separations module.

To re-assemble the system when maintenance is complete:

1. Slide the side panel back onto on the separations module and secure it, using the 2 screws that you removed earlier. Make sure the column tubing is routed through the slot in the side panel.
2. Align the 3 slotted holes on the side of the column heater or column heater/cooler with the 3 keyed ports on the side panel of the separations module, then carefully lower the unit into place.

3. Open the column heater or column heater/cooler door and route the column tubing through the clip on the front of the unit.
4. Slide the drip tray back into the bottom of the column heater or column heater/cooler compartment and close the door on the unit.
5. Align the left side of the detector base with the two feet on the right side of the solvent bottle tray and lower the right side of the base into position.
6. Connect the free end of the convoluted tubing near the detector base to the waste drain outlet of the detector drip tray.
7. Route the remaining bundle of tubing near the detector base through the routing clip in the detector drip tray.
8. Align the feet on the detector with the indentations on the detector base, then carefully lower the detector into position on the base.
9. Reconnect the cables:
 - Ethernet and power cord on rear of detector
 - External control cable and power cord on rear of column heater or column heater/cooler
 - Power cord on rear of separations module
10. Power-on the separations module.

7.6.2 Replace the lower needle wash frit (if so equipped)

Replace the lower needle wash frit when these conditions apply:

- Ghost peaks appear in the chromatogram
- Sample carryover is evident
- Scheduled preventive maintenance is required (every 6 months or 5000 injections)

Replacement parts

The one-piece needle wash frit is available as a spare part.

Required materials

- Squirt bottle with 100% alcohol (methanol or isopropanol)
- Vial of needle wash solvent being used or alternatively 100% alcohol (methanol or isopropanol)
- Replacement one-piece needle wash frit

Tip: Immerse the replacement frit for several minutes in the vial of the needle wash solvent being used, or alternatively in alcohol before beginning the replacement procedure.

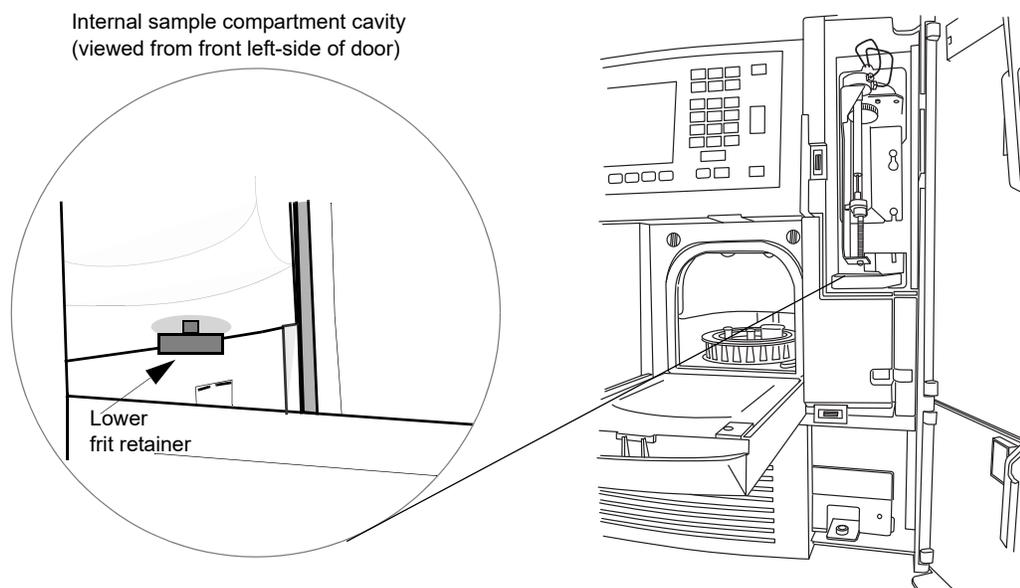
To replace the lower frit:

1. Open the sample carousel door, as shown below in the figure.
2. Remove all carousels that restrict access to the lower retainer.

Tip: Use the Door is Open dialog box to move the carousel carrier to the “A” position. See [“Load carousels” on page 62](#) for details.
3. Power-off the separations module, and disconnect it from the electrical outlet.

4. Find the lower frit retainer, which is inside the sample compartment on the top right side, approximately 4 inches within the cavity.

Figure 7–10: Locate the lower frit retainer



5. Unscrew the lower frit retainer by hand.
6. Remove the frit from the retainer by inserting a small-diameter pick (or paper clip) through the hole in the retainer cap, and discard it.
7. Install the new frit, which has been immersed and wetted; seat it in the retainer.
8. Reinstall the lower retainer using a clockwise motion, tightening it by hand until it seats.
9. Reconnect the separations module to the electrical outlet, and power-on the unit.
10. Reinstall the sample carousels, and close the sample carousel door.
11. Prime the needle wash pump (see [“Prime the needle wash pump” on page 60](#)).

7.6.2.1 Replace the syringe

Tip: For information about isolating problems in the sample management system, see [Chapter 8](#).

Replace the syringe when these conditions apply:

- The plunger tip becomes worn
- You want to change to the optional 25- μ L or 2500- μ L syringe size

Tip: To ensure accurate results, use only syringes approved by Waters for use in the sample management system.

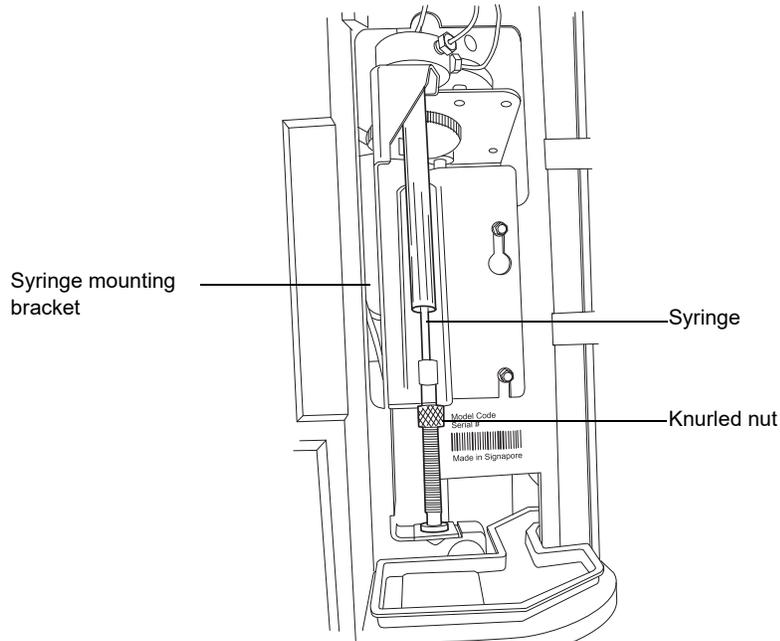
Required material

Replacement syringe

To remove the syringe:

1. Set the flow rate to 0.000 mL/min.
2. Open the syringe compartment door.
3. Remove the knurled nut that holds the syringe barrel to the syringe mounting bracket.

Figure 7–11: Syringe assembly components



4. On the Main screen, press the Diag screen key.
5. On the Diagnostics screen, press the Other Tests screen key.
6. In the Other Tests screen, select Motors and Valves.
7. In the Motors and valves screen, verify valve V3 is open.
Tip: If V3 is closed, select the V3 valve and enter any number on the keypad to toggle the valve to the open position.
8. Press Enter to save the change.
9. Select the Syringe Position field.
 - a. Press Enter to display the drop-down list.
 - b. Select the Full field.
Result: With the knurled nut removed, the plunger tip remains pointed upward as the mounting bracket is lowered.
10. When the mounting bracket is fully lowered, unscrew the syringe assembly from the mounting bracket as follows:
 - a. Grasp the syringe barrel near the mounting bracket.
 - b. Turn the syringe counterclockwise until it is released from the mounting bracket.

7.6.2.2 Install a new syringe

If you need to install a syringe different in size from the original syringe, refer to the following table for available syringe sizes. Be sure to enter the new syringe size in the Configuration screen. (See [page 140](#).)

! **Notice:** It is very important not to bottom the plunger in the syringe barrel, because this will damage the Teflon plunger tip and negatively affect syringe lifetime.

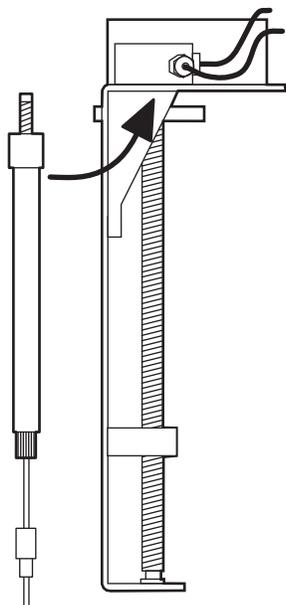
Table 7–3: Syringe sizes

Standard size	Optional sizes
250- μ L	25- μ L, 100- μ L, 2500- μ L

To connect and initialize the syringe:

1. The syringe package contains the unassembled plunger and spare syringe barrel. Remove each component from the package and wet the Teflon tip of the plunger with mobile phase or methanol.
2. Gently insert the plunger into the syringe barrel, about 3/4s down the length of the barrel.
3. Dip the end of the syringe into mobile phase or MeOH, and draw the liquid until the syringe is partially filled.
4. Hold the syringe vertically and gently tap the barrel so that the liquid settles on the plunger tip and any air rises to the top.
5. Gently push the plunger to expel the air, without bottoming the plunger in the syringe barrel.
6. Screw the new syringe partially into the mounting bracket. Do not tighten.

Figure 7–12: Syringe and mounting bracket



7. Push the syringe plunger down so that the threaded end slides through the syringe guide mounting bracket.
8. Hand tighten the syringe.
9. On the Diagnostics screen, press Other Tests.
10. In the Other Tests screen, select the Syringe Position field, and then press Enter to display the drop-down list.
11. Select Empty, and then press Enter.
Result: The mounting bracket moves upward so that you can install the knurled nut.
12. Press the Exit key to exit the Other Tests screen.
13. Install and finger tighten the knurled nut.

7.6.2.3 Modify syringe configuration parameters

If the replacement syringe differs in size from the original syringe, you must configure the separations module for a new syringe size.

To configure the separations module for a new syringe size:

1. On the Main screen, press the Configure screen key.
2. In the Configuration screen, select the Syringe Size field.
3. In the drop-down menu, select the appropriate syringe size, and then press Enter.
4. Press the Exit key.

Tip: For more information about changing the syringe size, see [“Configure the separations module” on page 47](#).

7.6.2.4 Purge the sample management system

Once you installed a new syringe, purge the sample management system with at least six sample loop volumes to remove any bubbles from the system.

To purge the sample management system:

1. Press the Menu/Status key.
2. Set the pump to deliver 1 mL/min of degassed, 100% methanol.
3. Select the purge cycle parameters:
 - a. Press the Direct Function screen key.
 - b. Select Purge Injector, and then press Enter.
 - c. Enter 6 in the Number of Sample Loop Volumes field, and then press Enter.
 - d. Select the Compression Test check box to perform a compression test (to detect air bubbles in the syringe).
4. Press **OK** to start the purge cycle.

Tip: For more information about purging the sample management system, see [“Purge the system” on page 59](#).

7.6.3 Replace the injector needle and seal pack

Replace the injector needle and seal pack when these conditions apply:

- The needle is bent.
- The tip of the needle is damaged.
- Contaminant peaks appearing in the chromatogram cannot be corrected by changing the wash solvent in the needle wash system.

Tip: For information about isolating problems in the sample management system, see [Chapter 8](#).

The procedure for replacing the needle and seal pack involves these tasks:

- Removing the injector
- Removing the seal pack and needle
- Removing and replacing the needle
- Installing the new seal pack and/or needle
- Installing the injector
- Adjusting the seal pack

Required materials

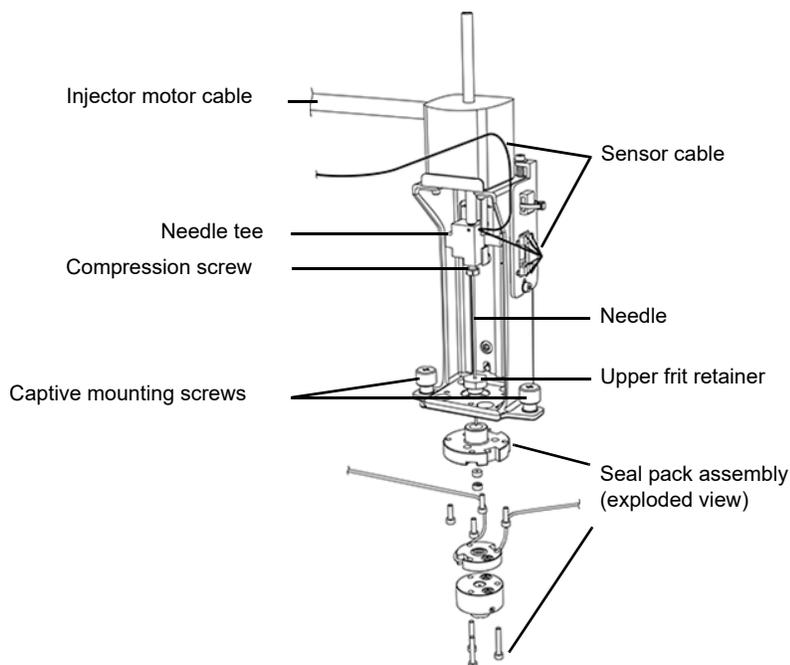
- Screwdrivers, T-10 and T-20 TORX
- Wrenches, 5/16-inch, open-end, two
- Squirt bottle with 100% alcohol (methanol or isopropanol)
- Screwdriver, #2 cross-head

Required kits

To replace the seal pack, you need one of the following kits:

- Seal Pack Assembly and Needle Kit (includes an assembled seal pack, replacement needle, and instructions)
- Seal Pack Seal Replacement Kit (includes seal pack seals, frits, replacement needle, and instructions)

Figure 7–13: Injector assembly components

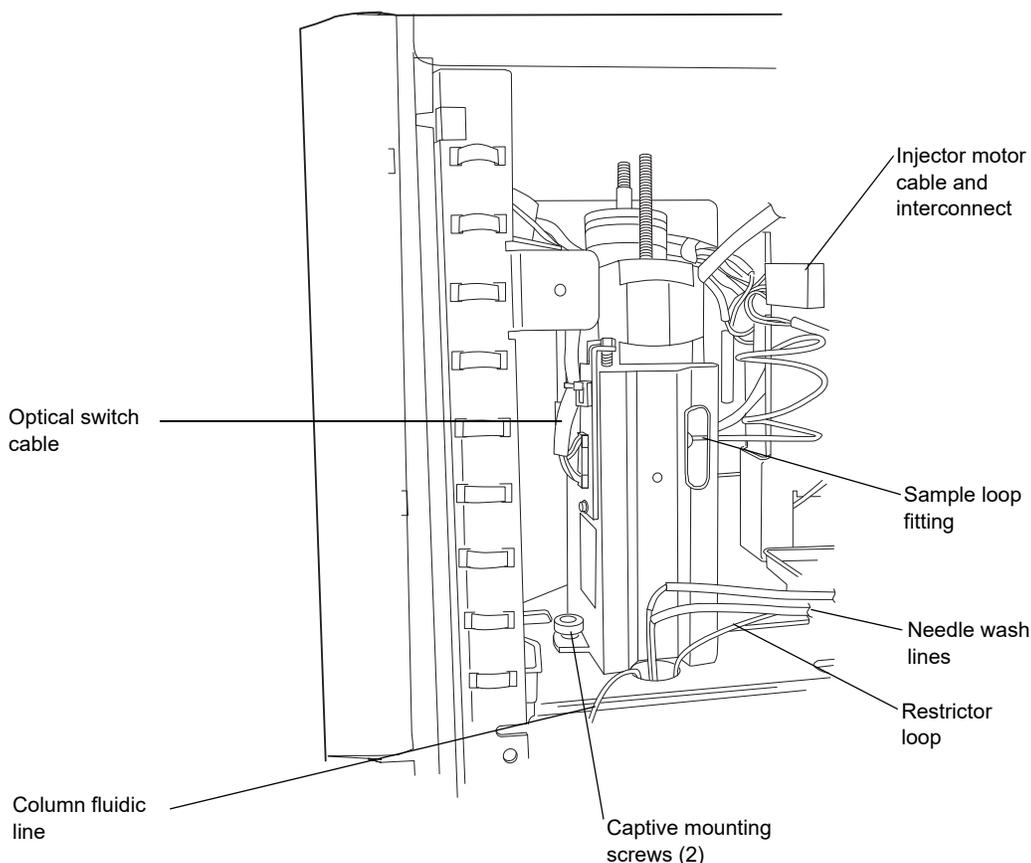


To access the injector assembly:

Note: This procedure applies only to a separations module with no column heater or column heater/cooler option installed. If the column heater or column heater/cooler option is installed, see [“Remove the side panel of separations module” on page 135](#) for instructions on how to remove the side panel of the separations module.

1. Power-off the separations module, and disconnect it from the electrical outlet.
2. Remove the 2 screws that secure the separations module’s right-hand side panel.
3. Slide the side panel to the rear, off the rear panel posts, to access the injector assembly.

Figure 7–14: Remove the injector assembly



To remove the injector:

1. Cut and remove the tie wraps securing the needle wash lines and restrictor loop.
2. Disconnect the column fluid line between the injector assembly and the column.
3. Insert a 5/16-inch open-end wrench through the slot in the injector, and remove the sample loop (yellow tubing) fitting from the needle tee.
4. Use a 5/16-inch open-end wrench to remove the restrictor loop fitting that is attached to the shorter of the two stainless steel tubes, exiting the bottom of the injector.
5. Disconnect the injector motor cable from the interconnect on the chassis-mounted bracket.
6. Disconnect the optical switch cable from the driver circuit board.
7. Remove the two needle wash lines from the stainless steel connecting lines at the bottom of the injector.

Tip: The two needle wash lines are color-coded. The needle wash in tubing is green. The needle wash out tubing is yellow or clear.

8. Loosen the two captive mounting screws that secure the injector to the compartment floor.
9. Lift the injector assembly a few inches, and then remove it from the instrument.
10. Carefully lay the injector on a clean surface on its side.

To remove the seal pack with needle from the injector assembly:

1. With the injector assembly removed from the separations module, use the two 5/16-inch open-end wrenches to disconnect the injector needle from the needle tee.

Tip: Use one 5/16-inch wrench to hold the tee, while you use the second one to loosen the compression screw that secures the needle to the tee (see [figure “Injector assembly components” on page 142](#)).

2. Loosen and remove the four M3 screws that secure the seal pack to the base of the injector (see [figure “Align the needle port” on page 145](#)).
3. Remove the seal pack with needle, and place it on a clean surface.

To remove the needle from the seal pack with needle assembly:

! **Notice:** To prevent damaging the needle, hold the needle only by its end opposite the tip (needle port end). Use a clean wipe with 100% alcohol to clean the needle after installation.

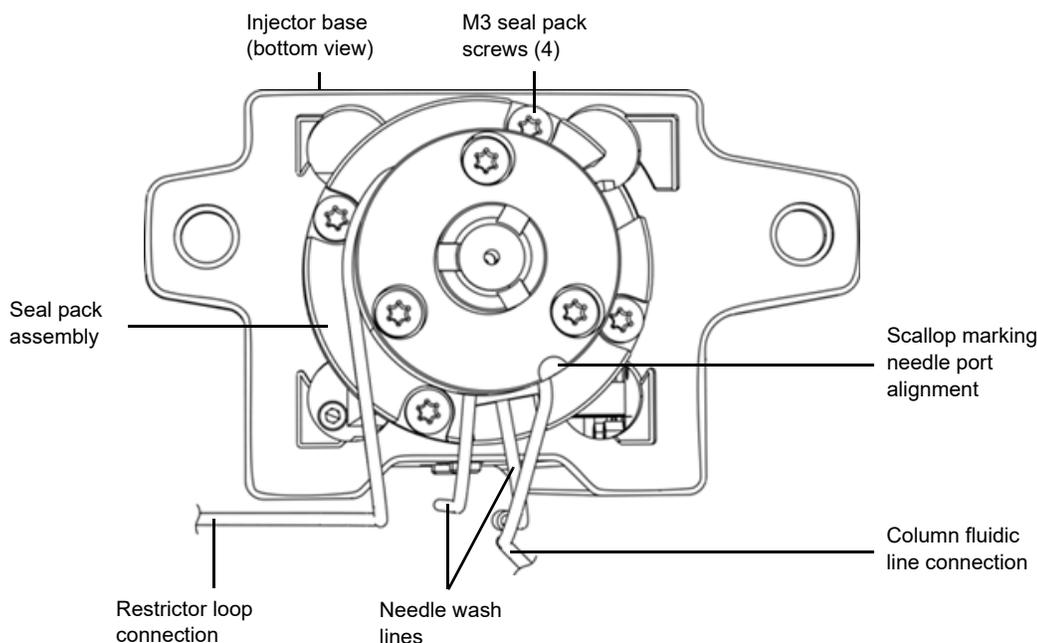
1. With the seal pack removed from the injector, loosen the seal pack's upper and lower (if so equipped) frit retainers. (See [figure “Injector assembly components” on page 142](#).)
2. Grasp the upper end of the needle and carefully withdraw the needle from the seal pack.

To replace the needle in the seal pack:

1. With the seal pack removed from the injector, lubricate the tip of the injector needle with 100% alcohol.
2. Align the needle port with the scallop located on the underside of the seal pack, before sliding the seal pack onto the needle (see [figure “Align the needle port” on page 145](#)).

Tip: If you orient the needle port 1/8 inch to the left of the scallop, it should align with the scallop when you tighten the compression screw.

Figure 7–15: Align the needle port



To install the seal pack with needle:

1. With the seal pack with needle removed from the injector, rotate the injector's motor shaft until the needle tee is furthest from the injector base (see [figure "Injector assembly components" on page 142](#)).
2. Place the needle compression screw and ferrule onto the upper end of the needle.
3. Position the seal pack with needle so that the stainless steel needle wash lines face the channel in the back of the injector.
4. Align the four holes in the seal pack and injector base, and insert the seal pack with needle into the injector base, guiding the needle and ferrule into the needle tee until it seats, and then hand tighten the compression screw.
5. Secure the seal pack to the injector base using the four M3 screws.
6. Use the 5/16 open-end wrench to tighten the needle compression screw.
7. Verify the needle port is aligned with the scallop in the seal pack (see [figure "Align the needle port" on page 145](#)).

Requirement: To prevent chromatographic inconsistencies, ensure the orientation of the needle port is correct. To verify needle port orientation, rotate the motor drive shaft to push the needle through the seal pack until the needle port is visible, and then verify that the needle port is aligned with the scallop on the seal pack. If you need to readjust the needle port orientation, use the procedures, above, to remove the seal pack with needle and to replace the needle.

To reinstall the injector assembly:

! **Notice:** To avoid damaging the injector and/or needle wash tubing, ensure that the tubing is not pinched between the injector and the compartment floor when you reinstall the injector assembly.

1. Lower the injector so that its base seats in the compartment floor, and then secure it with the two captive screws (see [figure “Remove the injector assembly” on page 143](#)).
2. Reconnect the two needle wash lines to the stainless steel connecting lines at the bottom of the injector.

Tip: The two needle wash lines are color-coded. The needle wash in tubing is green. The needle wash out tubing is yellow or clear.

3. Reconnect the optical switch cable to the driver circuit board.
4. Reconnect the injector motor cable to the interconnect on the chassis-mounted bracket.
5. Reconnect the restrictor loop to the fitting attached to the shorter of the two stainless steel tubes, exiting the injector bottom.
6. Insert a 5/16-inch open-end wrench through the slot in the injector and reconnect the sample loop (yellow tubing) to its fitting on the needle tee.
7. Reconnect the column fluidic line between the injector assembly and the column.
8. Use tie wraps to resecure the needle wash lines and the restrictor loop.

To re-assemble the system when maintenance is complete:

Note: This procedure applies only to a separations module with no column heater or column heater/cooler option. If the column heater or column heater/cooler option is installed, see [“Remove the side panel of separations module” on page 135](#) for instructions on how to reinstall the side panel on the separations module.

1. Mount the side panel on the rear panel posts.
2. Slide the side panel forward, aligning the red outlet tube with the slot in the side panel.
3. Install the 2 screws on the side panel.

Tip: You must adjust the seal pack after you reinstall the injector.

7.6.3.1 Adjusting the seal pack

Seal pack adjustment involves these tasks:

- Purging the sample management system (see [“Purge the system” on page 59](#)).
- Adjusting the seal pack (see [“Adjust the seal pack” on page 61](#)).

7.6.4 Clean the sample compartment

Clean the sample compartment when these conditions apply:

- The sample carousels do not turn properly.
- The carousel carrier does not engage.

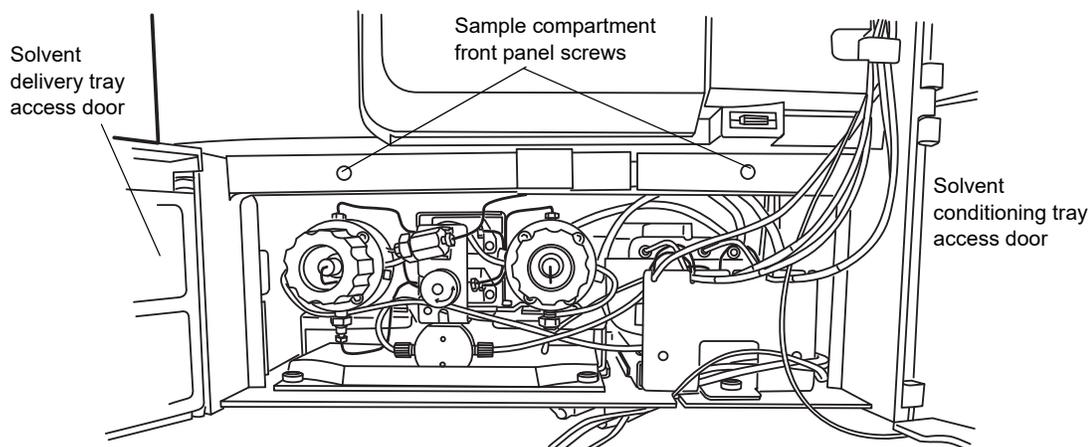
Required materials

- Wrench, hex, 3-mm
- Nonabrasive cleaner

To access the sample compartment:

1. Open the sample compartment and syringe cover doors (see [figure “Sample management system components \(front view\)” on page 133](#)).
2. Remove the five carousels.
Tip: Use the Door is Open dialog box to move the carousel carrier to the “A” position (see [“Load carousels” on page 62](#)).
3. Open the solvent delivery tray and solvent conditioning tray access doors (see [figure “Access the sample compartment” on page 147](#)).
4. Use the hex wrench to loosen the two sample compartment front panel screws.

Figure 7–16: Access the sample compartment

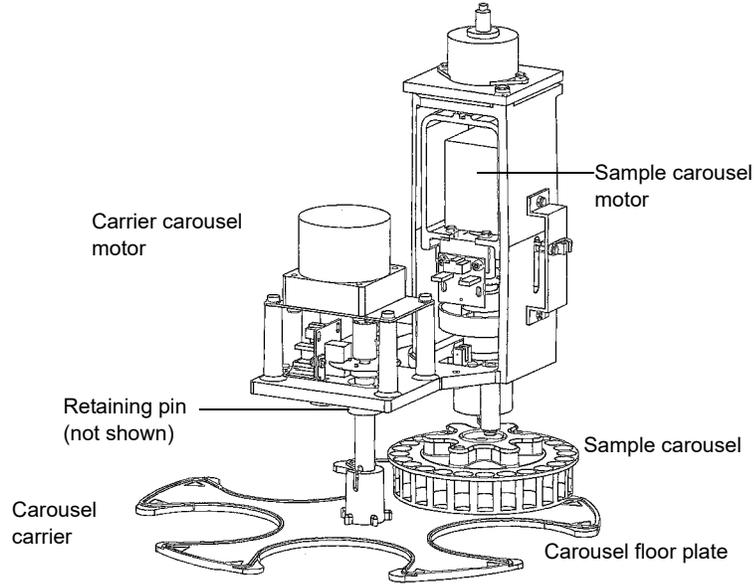


5. Pull the sample compartment front panel towards you to remove the panel.

To clean the sample compartment:

1. Push down about 1/4-inch on the spring-retaining pin in the center of the carousel carrier to loosen the carousel carrier (see [figure "Sample compartment components" on page 148](#)).

Figure 7-17: Sample compartment components



2. Slide the carousel carrier toward the front of the unit, and then remove the carousel carrier.
3. Remove the carousel floor plate, and clean it with a non-abrasive cleaner.
4. Reinstall the carousel floor plate.
5. Push down about 1/4-inch on the spring-retaining pin in the center of the carousel carrier to reinstall the carousel carrier.
6. Reinstall the sample compartment front panel with the two sample compartment front panel screws.

8

Diagnostic Testing and Troubleshooting

You troubleshoot the separations module using diagnostic tests and functions, and the troubleshooting procedures described in this chapter.

Tip: For information about start-up diagnostic tests, see “Start-up diagnostic tests” on page 41.

8.0.1 Proper operation

To keep your separations module running optimally, follow the operating procedures and guidelines in [Chapter 3](#).

8.0.1.1 Spare parts

To ensure that your instrument operates as intended, use only Waters Quality Parts (WQP). Visit www.waters.com/wqp for more information about WQP, including how to order them.

8.1 Safety and handling

When you troubleshoot the separations module, keep the following safety considerations in mind:



Warning: To avoid electric shock,

- never disconnect an electrical assembly while power is applied to the separations module. Once power is turned off, wait approximately ten seconds before you disconnect an assembly.
- do not open the power supply cover. The power supply does not contain user-serviceable parts.



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



Notice: To prevent circuit damage due to static charges, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.

8.2 Use the error log

In the error log, view the status messages and warnings that the separations module generated. You can view or print the information to help track possible problems that occurred during system operation.

Requirement: To print the error log, connect a serial printer to the RS-232 serial port on the separations module's rear panel (see ["RS-232 connections" on page 211](#)).

To display the Error Log screen, press the **Log** screen key in the Main screen.

The Error Log screen displays the last 10 messages in chronological order with the most recent message at the bottom of the screen. Use the **Page** key or the up arrow key to view older messages. The error log can save as many as 100 messages.

To display any messages logged since the screen was last accessed, press the **Refresh** screen key.

To print the error log to a printer configured for the separations module (if printing is configured), press the **Print** screen key.

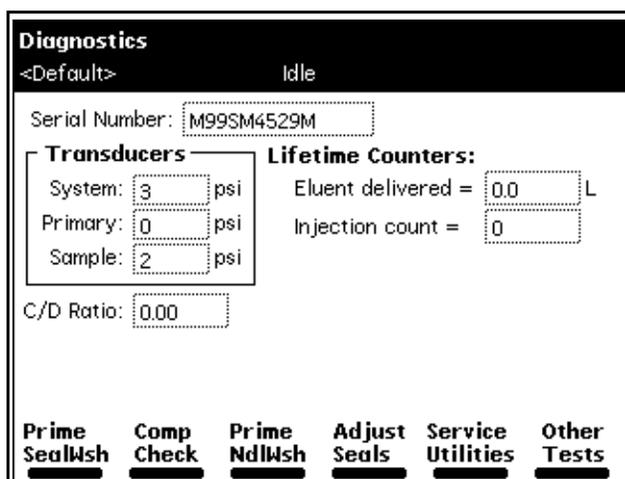
8.3 Perform main diagnostic tests

You can perform many diagnostic operations from two diagnostics screens:

- Diagnostics screen – Accesses certain system status information and diagnostic and performance tests for the solvent management system and the sample management system; also accesses utilities for Waters Technical Service representatives.
- Other Diagnostics screen – Accesses diagnostic tests and functions that allow the user to perform maintenance procedures and control optional devices (such as the column heater, column heater/cooler, and sample heater/cooler).

To access the Diagnostics screen, press the Diag screen key in the Main screen. The Diagnostics screen appears.

Figure 8–1: Diagnostics screen:



8.3.1 Exit the Diagnostics screens

To avoid unexpected behavior, ensure that you exit from all diagnostic screens before you allow external control of the separations module via Empower, MassLynx, or other third-party system control.

8.3.1.1 Diagnostics screen fields

The fields in the Diagnostics screen include:

- Transducers fields – Display the current pressure readings for the separations module's three transducers.
- Lifetime Counters fields – Display total values for the indicated parameters.

8.3.1.2 Diagnostics screen keys

The following table describes the functions of the screen keys in the main Diagnostics screen.

Table 8–1: Diagnostics screen keys

Screen key	Function	Reference
Prime SealWsh	Primes the plunger seal wash	“Prime the plunger seal wash pump” on page 152
Comp Check	Starts a compression check of the sample management system	“Perform the compression check” on page 152
Prime Ndl Wash	Primes the needle wash and homes the needle to the Stream position	“Prime the needle wash pump” on page 154
Adjust Seals	Starts the adjust-seal-pack procedure, which optimizes the needle port position in the lower seal	“Adjust the seals” on page 154

Table 8–1: Diagnostics screen keys (continued)

Screen key	Function	Reference
Service Utilities	Displays a password-protected list of service diagnostics	“Service utilities diagnostic tests” on page 156
Other Tests	Displays the Other Diagnostics screen, which displays more diagnostic tests	“Perform other diagnostic tests and functions” on page 156

The following sections describe the diagnostics accessed from the Diagnostics screen keys.

8.3.2 Prime the plunger seal wash pump

The prime plunger seal wash test automatically primes the solvent management system plunger seal wash pump. Perform this test if you discern a lack of flow in the plunger seal wash system or if you are changing the seal wash solvent. If required, use an intermediate solvent to prevent miscibility or precipitation problems when changing solvents.

8.3.2.1 Required materials

- Seal wash solution
- Tubing adapter (start-up kit)
- Syringe (use only if no solvent is in the seal wash pump)

To prime the plunger seal wash:

1. Ensure that the plunger seal wash supply line (labeled “Inlet”) is in the seal wash solvent bottle and that the plunger seal wash waste line (clear) is in a suitable waste container.
2. Attach the tubing adapter (start-up kit) to the syringe.
3. Press the **Prime SealWsh** screen key in the Diagnostics screen.
4. In the Prime Seal Wash dialog box, press **Start** to begin the seal wash procedure.
5. Pull on the syringe plunger to draw seal wash solvent through the system.
6. When seal wash solvent flows out the seal wash waste tube, press the **Halt** screen key.
Result: The seal wash pump prime will time-out after five minutes if the test is not halted.
7. To return to the Diagnostics screen, press the **Close** screen key.

For more information about priming the plunger seal wash pump, see [“Prime the plunger seal wash pump” on page 58](#).

8.3.3 Perform the compression check

The compression check serves these functions:

- Automatically compresses the solvent in the sample loop and syringe
- Checks for leaks
- Determines the compressibility of the solvent in the system

Perform a compression check if you suspect seal pack leakage. Symptoms of seal pack leakage include:

- Dripping in the sample chamber
- Abnormally low system pressure

To perform the Compression Check diagnostic test:

1. Install on the red outlet line a restrictor that generates at least 3447.4 kPa (34.5 bar, 500 psi) of backpressure when the solvent flow is 3.0 mL/min or less.

Tip: If a column is installed in the flow path during this test, you do not need a restrictor.

2. Connect the restrictor outlet to a waste line.
3. In the Diagnostics screen, press the Comp Chk screen key.

Result: The Compression Check dialog box appears.

These are the fields in the Compression Check dialog box:

- Compressed Volume field – Reports the compressed volume of the sample loop.
- Delta Decay Pressure field – Reports the change in the decay pressure.
- Final Pressure field – Reports sample loop pressure after the test is completed.

See [page 52](#) for the correct parameter settings.

4. Press the Start screen key to begin the compression check test.

The test is completed, the Compression Check screen either displays values in the Compressed Volume, Delta Decay, and Final Pressure fields (indicating a successful test) or a Fail status.

8.3.3.1 Failing result

If the compression check indicates a failing result, perform these actions:

- Verify that the parameters you entered for the test are correct (see [page 52](#)).
- Confirm that the appropriate solvent is used and that the solvent was properly conditioned.
- Visually check the syringe for bubbles. Confirm that the syringe is secure.
- Adjust the seals (see [“Adjust the seals” on page 154](#)).
- Inspect the sample chamber for dripping.
- Confirm that no leaks appear in the flow path (inspect compression/ferrule connections, valves, tubing).
- Purge the sample management system (see [“Purge the system” on page 59](#)).

If a failing result persists, replace the syringe.

Note: Replace the syringe only as a last resort, after all other recommended actions have been performed without resolution.

8.3.4 Prime the needle wash pump

The prime needle wash pump test procedure automatically primes the needle wash system and homes the needle into the stream position. Perform this procedure if these conditions apply:

- A lack of flow in the needle wash system
- You are changing the seal wash solvent
- A needle sensor malfunctions

To prime the needle wash:

1. Ensure that the needle wash supply line (“Needle Wash”) is in the solvent bottle and that the waste line (yellow) is in an appropriate waste container.
2. On the Diagnostics screen, press the **Prime NdlWash** screen key.
3. In the Prime Needle Wash dialog box, press the Start screen key to begin the needle wash.
4. If the solvent does not flow out of the waste line within 30 seconds, press **Start** again. If the solvent still does not flow out of the waste line:
 - In the sample chamber, inspect for solvent dripping from the injector.
 - Confirm that fluid is properly drawn from the needle wash reservoir.

8.3.5 Adjust the seals

The adjust seals test procedure automatically adjusts and optimizes the vertical needle port position in the seal of the autoinjector.

8.3.5.1 Required materials

- Restrictor or union with pin plug (start-up kit)
- Methanol, 100%, degassed

Before you run the function, confirm these things:

- The sample management system is purged at least twice.
- The solvent is degassed 100% methanol (or another compatible solvent).
- You removed the column to avoid damaging it with a pressure pulse of approximately 6894 kPa (69 bar, 1000 psi).
- You installed on the red outlet line a restrictor that generates at least 6894 kPa (69 bar, 1000 psi) of backpressure at < 3.0 mL/min.

Tips:

- To attain sufficient backpressure without a column, you can partially cap the red line with a union and fitting plug assembly. Collect solvent runoff in an appropriate waste container.
- High-resistance restrictors (> 13788 kPa (138 bar, 2000 psi) @ 1 mL/min) can cause this test to take longer, and result in a missing restrictor error.

To adjust the seals:

1. On the Diagnostics screen, press the Adjust Seals screen key.
2. On the Adjust Seals dialog box, press the Start screen key.

At the end of the test, the screen displays this information:

- Pass or fail status
- Current and previous seal data

Confirm the seal data values—top, bottom, and width—fall within the acceptable ranges shown in the table, below. A seal geometry error can appear if these conditions apply:

- The adjust seals parameter values are not in the acceptable range.
- Air bubbles occur in the sample management system. (If so, purge the sample management system and try again.)
- Solvent is not properly degassed (even though air bubbles are not visible).

Table 8–2: Acceptable ranges for the adjust seals diagnostic test

Parameter	Acceptable range	Typical range
Top	30 to 80	30 to 60
Bottom	80 to 200	120 to 150
Width (Bottom–Top)	50 to 120	60 to 90

8.3.5.2 Adjust seals failure

To correct for a failing result on the adjust seals test:

1. Confirm that the flow rate for the adjust seals test was set to 1.0 to 1.5 mL/min, the 100% methanol was properly degassed and filtered, and that degassing was enabled.
2. If all the conditions in [step 1](#) are satisfied, proceed to [step 3](#).
If any conditions in [step 1](#) are not satisfied, correct it (or them), and then repeat the test.
Requirement: If the test fails again, proceed to [step 3](#).
3. Replace the restrictor in the red outlet line with a pin plug.
4. Ramp system pressure up to 31026.4 kPa (310.3 bar, 4500 psi); hold this pressure for 2 minutes.
5. Repeat the adjust seals test.
6. If the test fails again:
 - a. Stop solvent flow.
 - b. Monitor the displayed pressure value (which must be stable and approaching zero).
 - c. Contact Waters Technical Service.

8.3.6 Service utilities diagnostic tests

Service utilities diagnostic tests are for use by Waters Technical Service representatives only. These utilities are password-protected. Local injections can be disabled on the Service Utilities page by selecting **Lock Run Samples**, which will require a service call.

8.4 Perform other diagnostic tests and functions

To display the Other Diagnostics screen, on the Diagnostics screen, press the Other Tests screen key. Some of the tests and maintenance functions that appear in the screen's scroll box correspond to options installed on your separations module.

The following table describes the tests and maintenance functions run from the Other Diagnostics screen.

Table 8–3: Tests and functions run from the Other Diagnostics screen

Test or function name	Purpose	Reference
Motors and valves	Ensures parameter values specified fall within acceptable limits for protecting the system and column.	“Perform the motors and valves diagnostic test” on page 157
Sensors	Allows you to view the current status of instrument sensors.	“Sensors diagnostic function” on page 159
Static leak	A ramp and decay test of the high pressure part of the solvent management system. Tests for leaks of all seals and fittings in the solvent management system.	“Static leak test” on page 161
Valve leak	Tests for pressure leaks in valves of the sample management system and the upper and lower sides of the needle seal pack.	“Valve leak test” on page 162
Head removal and replacement	Moves the plunger into its most forward position so that you can remove the head and replace the plunger and seal wash assembly.	“Head removal and replacement function” on page 163
Inputs and outputs	Displays the current status of the I/O ports.	“Inputs and outputs test” on page 164
Keypad	Tests the keys on the front panel keypad.	“Keypad test” on page 165
Display	Tests the display screen.	“Display test” on page 165
Carousel	Test the sample carousel drive.	“Carousel test” on page 165

Table 8–3: Tests and functions run from the Other Diagnostics screen (continued)

Test or function name	Purpose	Reference
Sample heater/cooler	Tests the heater/cooler module.	“Sample heater/cooler test” on page 166
Defrost sample cooler	Defrosts the sample heater/cooler module.	“Defrost sample heater/cooler function” on page 166
Column heater or column heater/cooler	Tests the column heater or column heater/cooler module.	“Column heater or column heater/cooler test” on page 167
Defrost column cooler	Defrosts the column heater/cooler module.	<i>Waters Alliance Series Column Heater and Column Heater/Cooler Operator’s Guide</i>
Rebuild injector valves	Tests valves V1 and V2.	“Rebuild injector valves function” on page 168
Turn off GPV	Turns off the GPV.	“Verify the firmware checksum” on page 168
Create “GPV Test” Method	Use to create a GPV test separation method for the operational qualification of the separations module.	“Create a GPV Test Method” on page 168
Firmware Checksum	Use to verify the checksum value to ensure that firmware upgrades were loaded correctly.	“Verify the firmware checksum” on page 168

8.4.1 Perform the motors and valves diagnostic test

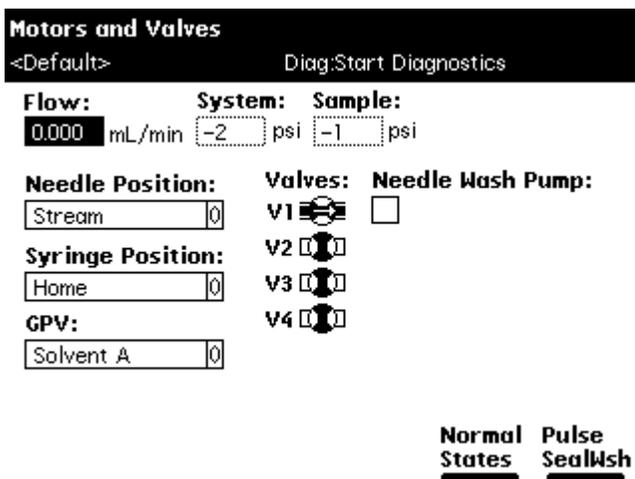
The motors and valves test results inform you whether the parameter values you assign to motors and valves fall within limits acceptable for protecting the system and column.

The test checks these components:

- Motors:
 - Injector (needle) drive
 - Syringe drive
 - Needle wash pump
- Valves:
 - Gradient proportioning valve (GPV)
 - V1 (Sample loop valve)
 - V2 (Syringe valve)
 - V3 (Waste valve)
 - V4 (Needle wash valve)

To perform the motors and valves test, select Motors and valves in the Other Diagnostics screen, and then press the OK screen key. The Motors and Valves diagnostic screen appears.

Figure 8–2: Motors and Valves diagnostic screen



To control the needle or syringe drive:

1. Select the motor position parameter (needle position or syringe position) that you want to change in the Motors and Valves screen.
2. Press Enter to display a drop-down list of parameter values.
3. Select the value you want, and then press Enter to save your selection. The following two tables describe the position selections available in the drop-down lists for the injector (needle) and the syringe motors.

Table 8–4: Needle position parameters

Needle position	Description
Stream	Needle side port is in the high pressure stream (default position).
Seal	Needle side port is in the lower seal position.
Wet	Needle is in the needle wash position.
Vial	Needle is in the sample vial.

Table 8–5: Syringe position parameters

Syringe position	Description
Empty	Syringe is in the highest position to purge the syringe.
Home	Syringe is in the home (default) position (approximately 20% full).
Full	Syringe is in the lowest permitted position (80% full) to draw in sample.

8.4.1.1 Control the GPV valve

The following table describes the valve positions available in the GPV drop-down list.

Table 8–6: GPV position parameters

Solvent A	GPV selecting solvent from reservoir A
Solvent B	GPV selecting solvent from reservoir B
Solvent C	GPV selecting solvent from reservoir C
Solvent D	GPV selecting solvent from reservoir D

8.4.1.2 Control valves V1-V4

To control a valve:

1. In the Motors and Valves screen, select the valve whose position you want to change.
2. Press any numeric key to toggle the valve between the open and closed position.
Tip: In figure “Motors and Valves diagnostic screen” on page 158, valve V1 is in the open position, as indicated by the valve symbol.
3. Press the Enter key to save your changes.

8.4.2 Sensors diagnostic function

The sensors diagnostic function monitors and displays the current position of all the digital sensors in the separations module.

To perform the Sensors diagnostic function, select Sensors in the Other Diagnostics screen, and then press the **OK** screen key.

Result: The Sensor Diagnostic screen appears.

The following table describes the function of each sensor when it is selected (enabled).

Table 8–7: Sensors diagnostic parameters

Component	Sensor	Description
Carousel	Home	The carousel that is under the injector is in the home position.
	ID	The carousel that is under the injector is identified during the injection sequence.
	Position	A vial position is detected in the carousel. (The Vial Detected sensor determines whether a vial is present at the vial position.)
	Engaged	The sample carousel rotary drive mechanism (z axis) engages the selected carousel (see figure “Sample compartment components” on page 148).
	Vertical Home	The sample carousel rotary drive mechanism (z axis) is in the topmost position.
Carrier	Position	The selected carousel is in the “load” position.
	Home	Carousel A is in the “load” position.
Injector	Bottom	The needle mounting block is at the bottom sensor position (Wet).
	Top	The needle mounting block is at the top sensor position (Stream).
Miscellaneous	Door Open	The sample compartment door is open.
	Syringe Home	The syringe is in the “home” position (approximately 20% full).
	Vial Detected	A vial is present in the selected vial position. (Works in conjunction with the carousel position sensor).

8.4.3 Static leak test

The static leak test performs a solvent pressure ramp up, and monitors the pressure decay in the solvent delivery system to determine whether the inlet check valves, tubing, fittings, or plunger seals are faulty.

Perform the Static Leak test when these conditions apply:

- You suspect leakage in the inlet check valves or plunger seals.
- You performed maintenance on the solvent management system.
- You replaced fittings in the flow path.

8.4.3.1 Required materials

- Pin plug
- Wrenches, 5/16-inch, open-end, two
- Wrench, 5/8-inch, open-end
- Waste container

To ensure a successful static leak test, precondition the solvent management system.

To precondition the solvent management system:

1. Ensure that the solvent line that you choose for the static leak test is in 100% methanol.
2. Place a restrictor at the in-line filter outlet to produce 27579 kPa (275.8 bar, 4000 psi) at a flow rate of 1.0 to 1.5 mL/min. for 10 minutes.

Requirement: Keep the in-line degasser in On mode throughout the preconditioning phase.

To perform the static leak test:

1. In the Other Diagnostic screen, select Static Leak Test in the Other Diagnostics screen, and then press the OK screen key.
2. Follow the instructions in the Static Leak Test screen.
3. Disconnect the tubing at the outlet of the in-line filter, and loosely insert the pin plug into the in-line filter outlet.
4. Collect the solvent runoff in an appropriate waste container.
5. Press Continue after you have inserted the pin plug.
6. Follow the instructions in the subsequent Static Leak Test screens to complete the test.

When the test ends, the Static Leak Test Results screen appears.

8.4.3.2 Failing result

If the static leak test indicates a failing result, confirm these things:

- No obvious leaks exist.
- The prime/vent valve is closed.
- The degasser is set to On.

- Compression fittings and head nuts are tight.
- The appropriate solvent (methanol or water is recommended) was used.

If necessary, ensure a good seal around the plunger and head surface by flowing at high pressure before rerunning the test. For example, install a restrictor sufficient to achieve 27579 to 31026 kPa (4000 to 4500 psi) backpressure at a flow rate of 0.5mL/min.

Finally, repeat the static leak test.

8.4.3.3 Continued failing result

If the static leak test continues to fail, perform these actions:

1. Ensure that the degasser is set to **On**.
2. Perform a wet prime for 10 minutes, at 1.0 mL/min, using methanol and an in-line restrictor to achieve 27579 to 31026 kPa (4000 to 4500 psi) backpressure. Performing a wet prime increases the stroke to its maximum length.
3. After 7 to 8 minutes of wet priming, inspect the plumbing and fittings for leaks. Use thermal paper (if available), which turns dark on contact with methanol, to detect leaks. Repair any leaks.
4. Remove the resistor, replacing it with a pin plug, and rerun the static leak test.

If a failing result persists replace the check valves, plunger seals, and plungers.

Note: Replace the check valves, plunger seals, and plungers only as a last resort, after all other recommended actions have been performed without resolution.

8.4.4 Valve leak test

The valve leak test serves these purposes:

- It tests the sample inlet valve (V1) and syringe valve (V2) of the sample management system (see [figure “Flow path through the solvent management system.” on page 23](#)).
- It tests the upper and lower side of the needle seal pack for pressure leaks.

Perform the valve leak test on these occasions:

- When you suspect leakage in the sample management valves or upper and lower needle seal pack.
- When you perform maintenance on the sample management system.

8.4.4.1 Required materials

- Pin plug
- Wrenches, 5/16-inch, open-end, two
- Waste container

To perform the valve leak test:

1. In the Other Diagnostic screen, select Valve leak test, and then press the OK screen key.
Tip: You can send the system pressure ramp and decay to an external device using the System Pressure Chart Output parameter. See [“Set I/O parameter values” on page 104](#) for details.
2. Disconnect the red outlet line from the sample management system, and use the pin plug to plug the red outlet line.
3. Collect the solvent runoff in an appropriate waste container.
4. In the Valve Leak dialog box, press Enter to display the test location selections, and then select the valve to be tested (V1 or V2) or the needle seal pack position (Above seal or Below seal).
5. Select the test pressure, usually 31026 kPa (310 bar, 4500 psi), and then press Continue.
6. Follow the instructions in the subsequent Valve Leak Test screens to complete the test.

When the valve leak test is finished, the results are displayed in the Valve Leak Test screen.

8.4.5 Head removal and replacement function

The head removal and replacement function moves the plunger to its most forward position. Use this function whenever you want to remove and replace a head, seal wash assembly, plunger seals, or plunger.

To run the head removal and replacement function:

1. In the Other Diagnostics screen, select Head Removal & Replacement, and then press the OK screen key.
2. In the Head Removal & Replacement screen, follow the instructions to remove and reinstall the head, seal wash assembly, plunger seals, and plunger. For more information about removing the head, see [“Remove the head, seal wash assembly, and plunger” on page 123](#).

8.4.6 Inputs and outputs test

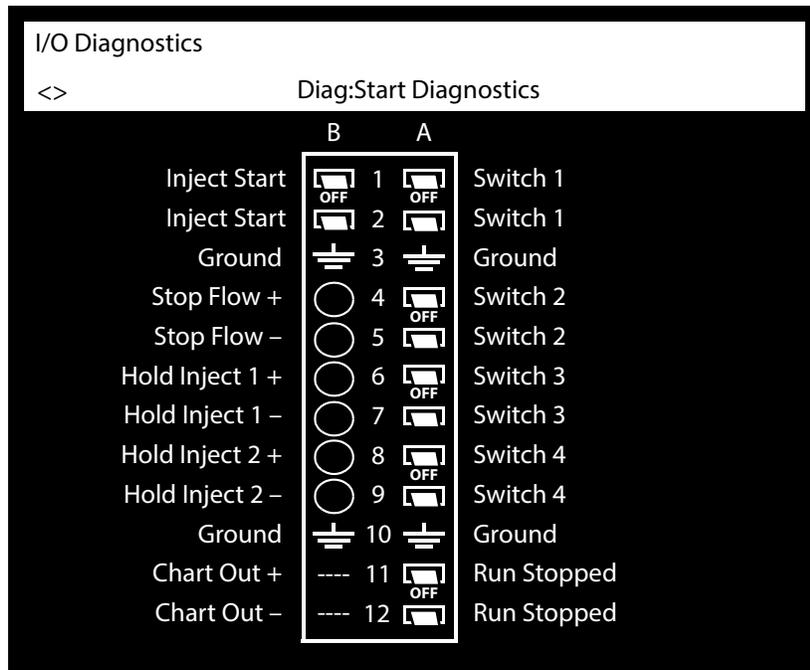
The inputs and outputs test allows you to view the status of the input and output connections on the I/O connectors of the separations module.

To perform the inputs and outputs test:

1. In the Other diagnostics screen, select **Inputs and outputs**, and then press the **OK** screen key. The I/O Diagnostics screen appears.

Note: The image below is a graphic representation of the screen.

Figure 8–3: I/O Diagnostics screen:



2. Connect two jumper wires between any one of the paired switch outputs that you want to test and any of the inputs.

Table 8–8: Connecting I/O switches and inputs

Paired switch outputs	Inputs
Switch 1	Stop Flow
Switch 2	Hold Inject 1
Switch 3	Hold Inject 2
Switch 4	
Inject Start	
Run Stopped	

3. In the I/O Diagnostics screen, select the paired switch to which the jumper wires are connected, and then use any numeric key to toggle the switch between the On and Off positions.

4. Confirm that the input to which the jumper wires are connected changes state (circles change from empty to full) as the switch is toggled.

8.4.7 Keypad test

The keypad test tests the keypad keys. You press the key on the keypad and observe the key as it changes appearance in the Keypad diagnostic screen. Run this test if you experience problems with the keypad.

To perform the keypad test:

1. In the Other Diagnostics screen, select Keypad, and then press OK. The Keypad diagnostic screen appears.
2. Follow the on-screen instructions to perform the test.

8.4.8 Display test

The display test tests the display screen by projecting test patterns on it. Run this test if you experience problems with the separations module's display.

To perform the display test, select Display in the Other Diagnostics screen, and then press OK.

8.4.9 Carousel test

The Carousel test tests these components:

- Vial sensor
- Carousel ID sensor
- Carousel drive

Run this test if you experience problems with the carousel carrier system.

To perform the carousel test:

1. In the Other Diagnostics screen, select Carousel, then press OK.
2. follow the instructions in the Carousel test screen.

8.4.10 Sample heater/cooler test

The sample heater/cooler test tests the sample heater/cooler for proper functioning. It ramps down the temperature in the sample heater/cooler and then measures the rate at which the temperature rises.

You can monitor the sample heater/cooler temperature by connecting a chart recorder to the Chart Out terminals on the rear panel of the separations module.

Complete these tasks before you perform the test:

- Remove all carousels and vials from the sample compartment.
- Confirm that the column heater or column heater/cooler (if installed) is not in use.
- Ensure that the sample compartment temperature is between 18 and 28 °C.

Requirement: To ensure this test's accuracy, do not open the sample compartment door during the test.

To perform the sample heater/cooler test:

1. In the Other Diagnostics screen, select Sample Heater/Cooler, and then press OK.
2. In the Sample Heater/Cooler screen, press Start to begin the test.

Result: The sample heater/cooler's temperature behaves as follows:

- Equilibrates
- Ramps down (for approximately 10 minutes)
- Stabilizes
- Returns to ambient temperature

When the test ends, the Sample Heater/Cooler screen displays the pass/fail status and the unit's current temperature.

8.4.11 Defrost sample heater/cooler function

Run the defrost sample heater/cooler function to defrost the sample heater/cooler and monitor the temperature change as the unit warms up.

Tip: The build-up of frost degrades the cooling efficiency of the sample heater/cooler. You should defrost the sample heater/cooler if it fails to reach the lowest recommended setpoint at ambient conditions.

You can monitor the sample heater/cooler's temperature by connecting a chart recorder to the Chart Out terminals on the rear panel of the separations module.

Complete these tasks before you perform the test:

- Remove all carousels and vials from the sample compartment.
- Confirm that the column heater or column heater/cooler (if installed) is not in use.

To run the defrost sample heater/cooler function:

1. In the Other Diagnostics screen, select **Defrost**, and then press **OK**.
2. Press **Start**.
3. Open the sample compartment door when you are prompted.

Requirement: To complete this procedure, the sample compartment door must remain open during the entire procedure.

The Defrost Sample Heater/Cooler screen indicates when the procedure ends and prompts to close the sample compartment door.

Note: A log entry results if the Sample Heater/Cooler Defrost diagnostic is interrupted or terminated prematurely.

8.4.12 Column heater or column heater/cooler test

The column heater (or column heater/cooler) test tests the column heater or column heater/cooler for proper functioning by increasing the temperature in the column heater.

You can monitor the column heater or column heater/cooler temperature by connecting a chart recorder to the Chart Out terminals on the rear panel of the separations module.

Before you perform the column heater (or column heater/cooler) test, confirm these things:

- The sample heater/cooler is not in use.
- The sample compartment temperature is between 18 and 28 °C.

Requirement: To ensure the accuracy of this test, do not open the column heater or column heater/cooler door during the test.

To perform the column heater or column heater/cooler test:

1. In the Other Diagnostics screen, select Column heater (or Column heater/cooler), and then press **OK**.
2. On the Column Heater or Column Heater/Cooler Diagnostic screen, press **Start**.

Result: These events occur during the test:

- The software prompts you to close the column heater module door.
- The column heater test begins at ambient temperature.
- The column heater temperature ramps up (for about 8 minutes).
- The system measures the increase in temperature.

When the test ends, the Column Heater or Column Heater/cooler Diagnostic screen displays the pass/fail status and the change in temperature.

8.4.13 Rebuild injector valves function

Run the rebuild injector valves function to rebuild and test the sample loop and syringe valves, V1 and V2, respectively.

The function causes the valve motor to move forward and reverse so that you can remove and replace valve components. The Injector Valve Rebuild Kit includes information on rebuilding the injector valves.

8.4.13.1 Required material

Injector Valve Rebuild Kit

To rebuild and test the injector valves:

1. In the Other Diagnostics screen, select Rebuild Injector Valves, and then press OK.
2. Follow the directions in the rebuilding kit, to rebuild the valve or valves.
3. Follow the on-screen instructions.

8.4.14 Create a GPV Test Method

Use the Create GPV Test Method function to create a gradient proportioning valve test method for use when qualifying the operation of the separations module for compliance purposes. You can delete this method when you complete the operational qualification and then rerun the utility if you need to requalify.

To create a GPV test method, select Create GPV Test in the Other Diagnostics screen, and then press OK. The screen informs you about the GPV test separation method.

8.4.14.1 Close the gradient proportioning valve

Closing the GPV is useful when you replace an inlet check valve cartridge (see [“Replace an inlet check valve cartridge” on page 129](#)).

To close the GPV, select **Turn Off GPV** in the Other Diagnostics screen, and then press **OK**. The screen displays the current state of the GPV as closed.

8.4.15 Verify the firmware checksum

Run the firmware checksum function to test the installation of the firmware by displaying and checking the checksum value.

To view the firmware checksum, select **Firmware checksum** in the Other Diagnostics screen. The checksum and its accuracy are displayed.

8.5 Troubleshoot

This section provides information for troubleshooting the separations module. It addresses these topics:

- General troubleshooting hints
- Chromatography troubleshooting
- Hardware troubleshooting for the:
 - System
 - Solvent management system
 - Sample management system

Tip: See “[Safety and handling](#)” on page 149, for information on safety and handling considerations while you troubleshoot the separations module.

8.5.1 General troubleshooting hints

To perform basic troubleshooting steps:

1. Examine the system, first considering the likely causes of a problem. Unresponsive instruments can mean that power or signal cables are disconnected or improperly connected. A fluid or vacuum leak can indicate defective tubing and valve connections.
2. Compare the current system operation with the way the system operated before the problem arose. To identify normal operating conditions, maintain a daily log noting overall system performance. Specifically, measure the performance of individual instruments using samples whose identity you know, preferably the ones used for instrument acceptance.
3. Tracking system parameters and performance during normal operation is a necessary precondition of effective troubleshooting. You must know, for example, when system-tuning parameter values approximate those specified at the time a test sample was previously run or that the lens settings needed to achieve optimum sensitivity are higher than those specified before. A need to specify extreme values to attain good results implies that some part of the system requires attention.

When your system is installed, and each time you develop a new method, fill out a checklist to record system conditions during normal operation. Methodically investigate and eliminate possible causes of a problem to identify the atypical system parameter. To do so, identify, in the order listed below, the symptom that varies from normal system operation:

- System pressure (high, low, erratic)
 - Baseline (flow-path-related or detector-electronics-related)
 - Changes in peak retention time (incorrect or changing over time)
 - Loss of peak resolution
 - Abnormal peak shape (smaller than expected, broad, tailing, and so on)
 - Incorrect qualitative/quantitative results
4. Run performance tests for each chromatographic instrument to quickly determine whether a problem exists with a particular instrument.
 5. Consult the troubleshooting information contained in tables in this chapter. The tables identify possible causes of a symptom and suggest corrective actions. They are organized according to the parameters in [step 3](#), so you can narrow the possible causes of a symptom

and find suggested corrective actions. If you trace a problem to a system instrument other than the separations module (for example the detector), consult the operator's guide for that instrument.

8.5.2 When to call Waters Technical Service

You can correct many problems with a Alliance e2695 Separations Module relatively easy. However, if you cannot correct a condition, contact Waters Technical Service. When you do, have the following information available:

- Completed normal operation checklist for the method you are using
- Nature of the symptom
- Separations module serial number (located behind the syringe access door)
- Detector model number and serial number
- Flow rate
- Operating pressure
- Mobile phase(s)
- Detector settings (sensitivity and wavelength)
- Type and serial number of column
- Sample type
- Control mode (Empower Chromatography Data Software, System Controller, No interaction, other)
- Software version and serial number

8.5.3 Troubleshoot chromatography problems



Warning: Always observe good laboratory practices when you handle solvents, change tubing, or operate the separations module. Know the physical and chemical properties of the solvents you use. Refer to the Safety Data Sheets for the solvents in use.

The following table lists chromatographic symptoms, possible causes, and suggested corrective actions for the separations module.

Recommendation: Before you use this table, read [“General troubleshooting hints” on page 169](#), and follow the basic troubleshooting steps to isolate the cause of the chromatographic symptom.

Table 8–9: Chromatography troubleshooting

Symptom	Possible cause	Corrective action
Erratic retention times	Air bubble in plunger head	Degas all solvents, prime solvent management system. Evaluate the degasser performance. Set the bubble detect alarm in the Mobile Phase screen. (See “Set Mobile Phase screen parameter values” on page 94.)
	Malfunctioning check valves	Clean/replace inlet check valve cartridges. (See “Replace an inlet check valve cartridge” on page 129.)
	Leaking plunger seals	Replace seals. (See “Notes on replacing the plunger seals” on page 124.)
	Separation chemistry	Check mobile phase, column, GPV.
	Clogged solvent filters	Replace filters.
Increased retention times	Incorrect flow rate	Change flow rate.
	Incorrect solvent composition	Change solvent composition, check GPV.
	Column heater (or column heater/cooler) not turned on; temperature low	Turn column heater (or column heater/cooler) on, determine temperature.
	Incorrect mobile phase	Use correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
	Leak in solvent management system, loss of solvent	Inspect fittings for leaks. Perform Static Leak Test. (See “Static leak test” on page 161.)

Table 8–9: Chromatography troubleshooting (continued)

Symptom	Possible cause	Corrective action
Decreased retention times	Incorrect flow rate	Change flow rate.
	Incorrect solvent composition	Change composition, evaluate GPV performance.
	High column temperature	Reduce column temperature.
	Incorrect mobile phase	Replace with correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
	Solvent not properly degassed	Degas solvent.
Reproducibility errors	Solvent not properly degassed	Degas solvent.
	Incorrect chemistry/integration	Evaluate chemistry/integration.
	Sample management system problem	Troubleshoot sample management system.
Baseline drift, rapid	Column not equilibrated	Equilibrate column.
	Detector not allowed to warm up	Allow detector to warm up until baseline is stable. Time varies with wavelength and sensitivity.
	Solvent contaminated	Use fresh solvent.
	Solvent not properly degassed (rapid or slow drift)	Degas solvent.
	Flow fluctuations (rapid or slow drift)	Prime solvent management system, replace pump seals, check valves (see “Erratic Retention Times” symptom).
	Incorrect wavelength for solvent	Check that solvent does not have absorbance at the wavelength used.
Baseline drift, slow	Solvent contaminated	Use fresh solvent.
	Decreased UV lamp energy	Determine lamp energy using detector diagnostics.
	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration.
	UV detector flow cell leaking (internal, cross-port)	Inspect flow cell, tighten connections.
	Dirty flow cell	Clean flow cell.

Table 8–9: Chromatography troubleshooting (continued)

Symptom	Possible cause	Corrective action	
Baseline noise cycling, short term (30 to 60 sec)	Flow fluctuating	Stabilize flow by checking for leaks and for proper degassing (see “Erratic retention times” symptom).	
	Solvent not well mixed (short- or long-term cycling)	Stir solvent.	
	Radio frequency noise (short- or long-term cycling)	Eliminate interference.	
Baseline noise cycling, long-term (approximately 10 min to 1 hour)	Ambient temperature fluctuations	Stabilize ambient temperature.	
	Faulty Integrator or recorder	Evaluate integrator or recorder performance.	
	Solvent time in the degasser is not sufficient to remove UV-absorbing gas (e.g., O ₂)	Maintain ≤ 1 mL/min. maximum flow rate.	
Baseline noise, random	Air in detector	Purge detector to remove air.	
	Solvents not properly degassed	Degas solvents.	
	Bubble detected	Reprime the solvent management system. Remove and vacuum filter solvents.	
	Solvents contaminated	Use fresh solvent.	
	Column contaminated	Clean/replace column.	
	Dirty flow cell	Clean flow cell.	
	Analog output cable not properly connected between the separations module and data system, recorder, or integrator	Properly connect cable.	
	System improperly grounded		Plug into outlet on different electrical circuit.
			Use power conditioner.
	Incorrect voltage setting: • Recorder • Analog-to-digital converter		Adjust voltage setting.
	Unit not cooling properly	Operate unit with covers in place. Confirm prescribed clearance at the rear of device. Run the defrost sample heater/cooler function (see “Defrost sample heater/cooler function” on page 166).	
Radio frequency noise	Eliminate interference.		
Defective detector	Troubleshoot detector.		

Table 8–9: Chromatography troubleshooting (continued)

Symptom	Possible cause	Corrective action
Carryover is seen from previous injection	Previous injection is exceedingly high in concentration	Calculate mass injected. Try re-injecting a larger volume of less concentrated solution while keeping injected mass constant.
	Choice of needle wash solvent is insufficient to solubilize all sample components.	Choose a needle wash solvent that solubilizes all components.
	Lower needle wash frit (if so equipped) is contaminated	Replace lower needle wash frit
Flat baseline, no peaks	No solvent flow	Determine flow rate.
	Lamp not ignited	Use detector diagnostics to determine reference/sample energy. Zero energy indicates lamp not ignited.
		Ignite lamp. If this does not resolve the problem, replace the lamp.
	Detector not zeroed	Zero the detector baseline.
	Improper connection between detector and recorder or data system	Examine cabling between the detector and the recorder.
	Incorrect wavelength	Ensure correct wavelength setting.
	Leak in solvent path	Inspect fittings.
Flat-topped peaks	Detector not zeroed	Zero the detector baseline.
	Incorrect input voltage setting: <ul style="list-style-type: none"> • Recorder • Analog-to-digital converter 	Adjust input voltage setting or detector output cable to correct position.
	Sensitivity too high	Select a less sensitive detection range.
	Sample concentration or injection volume exceeds voltage output of detector	Decrease sample concentration or injection volume.

Table 8–9: Chromatography troubleshooting (continued)

Symptom	Possible cause	Corrective action
Sensitivity loss	Leak in sample management system	Troubleshoot sample management system.
	Degraded, contaminated, or improperly prepared sample	Use fresh sample.
	Column contaminated	Clean/replace column.
	Loss of column efficiency	Clean/replace column.
	Peaks wider than expected	Troubleshoot sample management system. Inspect fittings for leaks and proper ferrule setting. Confirm tubing inner diameter is correct.
	Incorrect detector filter time constant	Set correct detector filter time constant.
	Change in mobile phase composition	Correct mobile phase pH or ionic composition.
	Leak in flow cell	Tighten cell seal.
	Incorrect flow rate	Change flow rate.
Sample energy decreased, reference energy not decreased	Contaminated mobile phase	Use fresh mobile phase.
	Dirty flow cell	Clean outside of flow cell windows.
		Flush system with water. If necessary, remove the column, and then flush the system with stronger solvent to remove particulate or filmy matter from flow cell windows.

8.5.4 Troubleshooting hardware problems

This section contains troubleshooting tables that address these issues:

- System problems
- Solvent management system problems
- Sample management system problems

Before you use these tables, read [“General troubleshooting hints” on page 169](#), and isolate the cause of the hardware symptom.

8.5.4.1 System troubleshooting

The following table provides troubleshooting information for system problems that are not related to a particular system module.

Table 8–10: General system troubleshooting

Symptom	Possible cause	Corrective action
Unit does not power-on	Power cord not connected	Establish line voltage is present.
	No power at outlet	
No response from keypad.	Keypad broken	Call Waters Technical Service.
Screen is blank	Unit not powered-on	Power-on unit.
	Start-up diagnostic test failure	Power-off and power-on again. If the failure is not corrected, call Waters Technical Service.
Start-up diagnostics indicate failing result	Internal problem with controller board, solvent management system, sample management system	Power-off and power-on again. If failure not corrected, call Waters Technical Service.
Start-up failure with error message “Plunger homing over pressure (0 or 1)”	Clogged in-line filter	Clean or replace filter element.
	Blockage in sample management waste line	Remove blockage or replace line.
	Restriction between solvent and sample management system	Remove restriction.
Front panel software “lock up”	Software problem, power line problem	Power-off and power-on again. If the failure is not corrected, call 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.
The separations module is not fully functional in remote configuration (controlled via RS-232 by a remote data system)	Data system is not programmed to control all separations module functions	Disconnect the separations module from the remote data system, then run the unit in a standalone mode to test functionality.

8.5.4.2 Solvent management system troubleshooting

The following table provides troubleshooting suggestions for solvent management system problems in the separations module.

Table 8–11: Solvent management system troubleshooting

Symptom	Possible cause	Corrective action
Plunger head leaks solvent	Worn plunger seals	Replace plunger seals (see “Notes on replacing the plunger seals” on page 124).
	Worn seal wash seals	Replace seal wash seals (see “Replace the seal wash assembly seals” on page 126).
	Loose head	Hand-tighten head nut.
	Loose inlet check valve	Tighten the check valve.
	Defective face seals	Replace face seals.
	Seal wash tubes not properly installed	Install tubes properly.
Erratic flow/pressure pulsations	Gas dissolved in mobile phase	Degas solvents.
	Air bubble in head	Prime solvent management system to remove bubble. Increase the degassing time before use. Set the bubble detect alarm in the Mobile Phase screen (see “Set Mobile Phase screen parameter values” on page 94).
	Dirty inlet check valves	Loosen the check valve for a few strokes and see if it starts flowing properly. You can also try cleaning the check valves by running wet prime. To replace inlet check valves (see “Replace an inlet check valve cartridge” on page 129).
	Prime/vent valve open or leaking	Close or rebuild prime/vent valve.
	Plugged solvent reservoir diffuser	Clean or replace diffuser.
	Plunger seal leaking	Make sure that seal wash is primed and operating properly. Replace plunger seal assembly (see “Notes on replacing the plunger seals” on page 124).

8.5.4.3 Sample management system troubleshooting

The following table provides troubleshooting suggestions for resolving sample management system problems in the separations module.

Table 8–12: Sample management system troubleshooting

Symptom	Possible cause	Corrective action
Compression check failed	Syringe contains an air bubble (usually seen at top or bottom of syringe)	Remove air bubble (see “Replace the syringe” on page 137).
	Mobile phase not properly degassed	Degas mobile phase.
	Incorrect syringe used for test or syringe data incorrectly entered in the Configuration screen	Install a syringe of the correct size, consulting the syringe data in the Configuration screen (see “Replace the syringe” on page 137).
	Leaks at tubing connections	Inspect for leakage at the connections with a tissue, and tighten connection slightly if the tissue is moistened.
	Seal pack failure	Adjust the seals (see “Adjust the seals” on page 154). Replace the seal pack (see “Replace the injector needle and seal pack” on page 141).
	Parameters used for compression check unacceptable for solvent	Set the appropriate parameters, then repeat the compression check in the Diagnostics screen.
	Valve V3 (waste valve) faulty	Call Waters Technical Service.
Leak at seal pack	Damaged needle, seal pack	Replace needle and seal pack (see “Replace the injector needle and seal pack” on page 141).
Cannot maintain high or low pressure	Leak at needle, seal pack	Adjust seals, or replace needle and seal pack (see “Replace the injector needle and seal pack” on page 141).

Table 8–12: Sample management system troubleshooting (continued)

Symptom	Possible cause	Corrective action
Reproducibility errors	Syringe contains an air bubble (usually seen at top or bottom of syringe)	Remove air bubble (see “Replace the syringe” on page 137).
	Leaks at tubing connections	Inspect for leakage at the connections using a tissue, and then tighten connection slightly if the tissue is moistened.
	Seal pack failure	Replace seal pack (see “Replace the injector needle and seal pack” on page 141).
Carrier malfunction	Carousels not properly positioned	Reposition carousels in sample compartment.
	Vials not properly seated	Reseat vials. (see “Load carousels” on page 62).
	Sample compartment tray contaminated	Clean sample compartment (see “Clean the sample compartment” on page 147).
Carousel malfunction	Carousels do not turn properly	Clean/replace the carousel.
	Defective carousel sensor	Call Waters Technical Service.
	Carousel carrier obstructed or contaminated	Check for obstructions. Clean sample compartment (see “Clean the sample compartment” on page 147).
	Sample compartment tray contaminated	Clean sample compartment (see “Clean the sample compartment” on page 147).
Carousel malfunction	Carousels do not turn properly	Clean/replace the carousel.
Seal pack adjustment failed	System not purged before seal pack adjustment	Purge system and repeat adjustment.
	Solvent not properly degassed	Degas mobile phase.
	Syringe contains an air bubble (usually seen at top or bottom of syringe)	Remove air bubble (see “Replace the syringe” on page 137).
	Seal pack failure	Replace seal pack (see “Replace the injector needle and seal pack” on page 141).
	Leaks at the tubing connection	Check for leakage at the connections with a tissue, then tighten connections slightly if the tissue is moistened.
	Damaged needle	Replace needle (see “Replace the injector needle and seal pack” on page 141).

Table 8–12: Sample management system troubleshooting (continued)

Symptom	Possible cause	Corrective action
Needle bottom sensor alarm	Bent needle. Needle hitting bottom of vial	Replace needle (see “Replace the injector needle and seal pack” on page 141). Adjust the syringe draw depth (see “Set sample parameter values” on page 100).

A Safety Advisories

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

A.1 Warning symbols

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

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appear in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.

 **Warning:** (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

 **Warning:** (Risk of burn injury from contacting hot surfaces.)

 **Warning:** (Risk of electric shock.)

 **Warning:** (Risk of fire.)

 **Warning:** (Risk of sharp-point puncture injury.)

 **Warning:** (Risk of hand crush injury.)

 **Warning:** (Risk of injury caused by moving machinery.)

 **Warning:** (Risk of exposure to ultraviolet radiation.)

 **Warning:** (Risk of contacting corrosive substances.)



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



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



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



Warning: (Risk of tipping.)



Warning: (Risk of explosion.)



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

A.1.1 Specific warnings

The following warnings (both symbols and text) can appear in the user manuals of particular instruments and devices and on labels affixed to them or their component parts.

A.1.1.1 Burst warning

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



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

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

A.1.1.2 Biohazard warning

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



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

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the biohazard safety representative for your organization regarding the proper use and handling of infectious substances.

A.1.1.3 Biohazard and chemical hazard warning

These warnings apply to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.



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

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

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

A.2 Notices

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

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

A.3 Bottles Prohibited symbol

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



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

A.4 Required protection

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



Requirement: Use eye protection when refilling or replacing solvent bottles.



Requirement: Wear clean, chemical-resistant, powder-free gloves when handling samples.

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

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



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



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



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



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



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



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



注意: 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。



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



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



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

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



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

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- 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.
- Spegnerle tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.



Advertencia: se recomienda precaución cuando se trabaja 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。
- 不要使用已經被壓癢或嚴重彎曲管線。
- 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 디메틸설폭사이드 (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ます、その場合、チューブは極めて低い圧力で破裂します。



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



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



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



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



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



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



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



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

A.6 Warnings that address the replacing of fuses

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

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



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



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



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



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



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



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



警告：為了避免火災，應更換與儀器保險絲蓋旁邊面板上印刷的類型和規格相同的保險絲。



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



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

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



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



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



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



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



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



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



警告：為了避免火災，應更換“維護步驟”一章的“更換保險絲”一節中介绍的相同类型和规格的保险丝。



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



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

A.7 Electrical and handling symbols

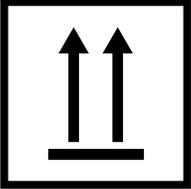
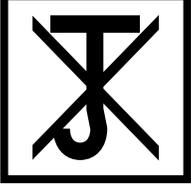
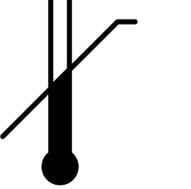
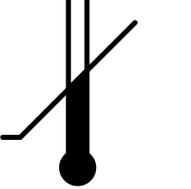
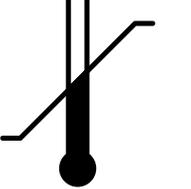
A.7.1 Electrical symbols

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

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

A.7.2 Handling symbols

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

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

B External Connections

This section provides instructions for making all external connections to the Alliance e2695 Separations Module.

B.1 Connect to the electrical power supply

Proper operation of the separations module requires that the AC power supply be grounded and that has no abrupt voltage fluctuations.

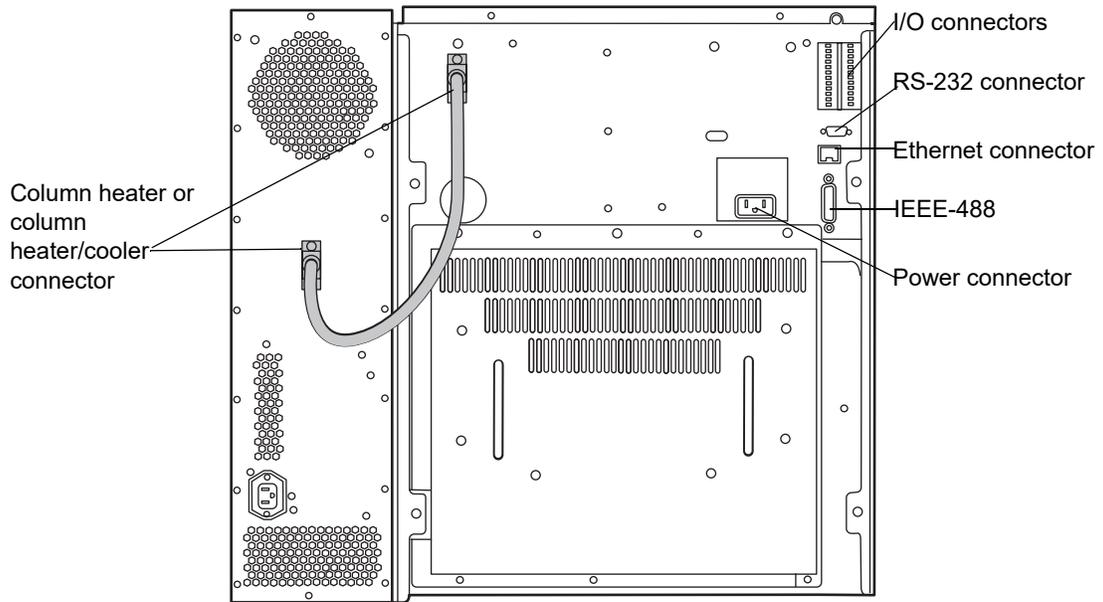
The separations module automatically adjusts for input voltages in the range of 100 – 240 Vac, 50 – 60 Hz.

To connect to the electrical supply:

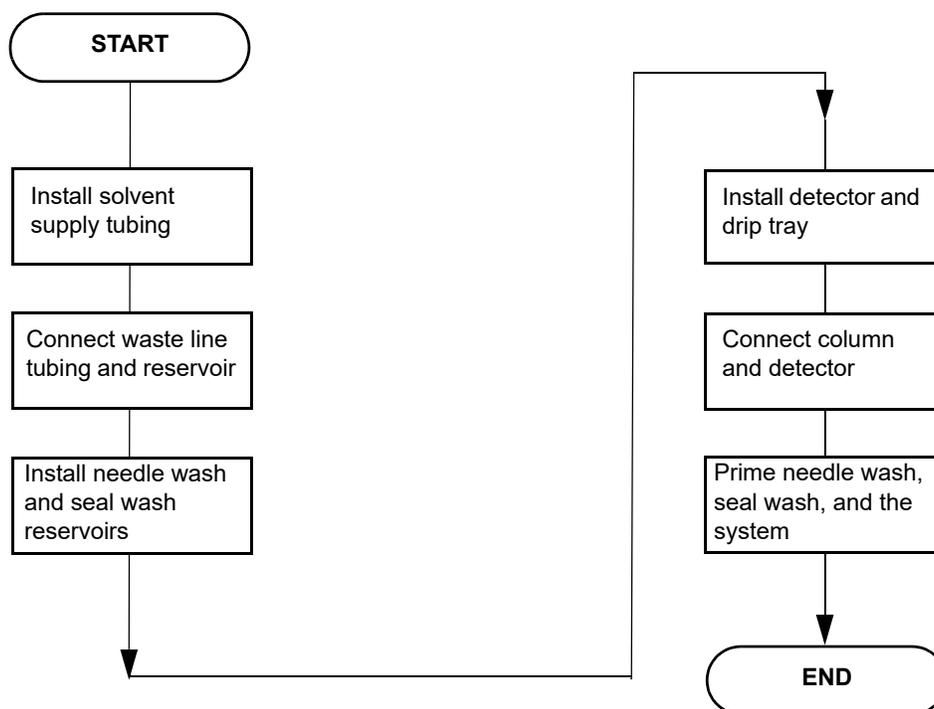
1. Ensure that the separations module is powered-off.
2. Connect the power cord to the connector on the rear panel.
3. Connect the other end of the power cord to a properly grounded AC power supply.

Requirement: Do not power on the separations module until you complete all plumbing and signal connections.

Figure B-1: Rear panel of the separations module



B.2 Connect tubing and attachments



! **Notice:** To ensure proper drip protection, be sure that the retaining screws in the solvent delivery and solvent conditioning trays are tightened before you operate the system.

B.2.1 Install the B-series drip tray

In the Alliance HPLC B-series configuration, the solvent bottle tray and detector base kit (176003104) consists of a solvent bottle tray and adjacent detachable detector base. The detector drip tray is part of the detector base. (See [figure "B-series solvent bottle tray configuration \(only single detector shown\)" on page 21.](#))

There are two steps to this procedure:

1. Replace the feet on the detector and then install the bottle tray and detector base.

Important: You should replace the feet on the bottom of the detector so that they correctly fit into the detector base. Failure to do so may result in the feet becoming wedged or stuck in the detector base.

2. Install the detector drip tray tubing.

Required materials

- Diagonal cutters
- Screwdriver, T-20 TORX

To replace the feet and install the bottle tray/detector base:

1. Disconnect all cabling and other connections from the detector.
2. Tip the detector so that it rests on its side.
3. Using a T-20 TORX screwdriver, remove the securing screws and rubber feet from the chassis.
4. Secure the new plastic feet (410004802) to the chassis using the provided T-20 screws (WAT034346), and set the detector aside temporarily.
5. Place the bottle tray on top of the separations module as shown in [figure “B-series solvent bottle tray configuration \(only single detector shown\)” on page 21](#).
6. Position the detector base to the right of the bottle tray, ensuring that the base is seated correctly on the two locating guides on the right side of the bottle tray assembly. These locating guides fit into slots on the underside of the detector base.
7. Ensure that the drip tray is seated in the correct position at the front of the detector base.

To install the detector drip tray tubing:

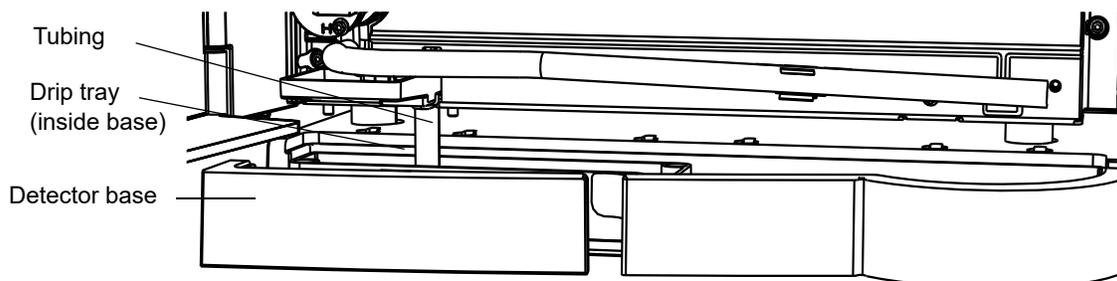
1. Prepare and fit the drip tray tubing for your specific model of detector. The drain fitting for all detectors is located on the underside of the detector, on the front left-hand side. You must install the drain fitting for the 2998; the rest are pre-installed.

Table B–1: Drip tray preparation steps, by detector model

Detector model	Drip tray tubing and fitting preparation steps
2414	<ol style="list-style-type: none">1. Cut a 3” length of the 1/4” ID convoluted tubing (WAT241095) supplied in the kit.2. Attach the tubing to the fitting on the underside of the detector.
2424	<ol style="list-style-type: none">1. Cut a 1-1/4” length of the Tygon tubing (430000604) supplied in the kit.2. Attach the tubing to the barbed fitting on the underside of the detector.
2475	Tygon tubing is supplied pre-cut and already connected to the fitting on the detector. No preparation is needed.
2489	<ol style="list-style-type: none">1. Cut a 2” length of the 5/16” ID convoluted tubing (430003945) supplied in the kit.2. Attach the tubing to the elbow fitting on the underside of the detector.
2998	<ol style="list-style-type: none">1. Press the stainless steel barbed fitting (405016495) supplied in the kit into the matching hole located on the front left-hand side, on the underside of the detector.2. Cut a 1” length of the 3/8” ID convoluted tubing (430001556) supplied in the kit.3. Attach the tubing to the barbed fitting.

2. Seat the detector on the detector base.
3. Make sure the end of the detector drip tray tubing protrudes from the detector drain fitting into the black-colored drip tray, as shown in the figure below. In detectors with longer drip tray tubing, you may need to bend the tubing slightly to the right or left to ensure that the end of the tubing lays inside the drip tray.

Figure B–2: B-series detector to drip tray tubing (2424 shown)



Caution: To allow the system to drain properly, ensure that the end of the tubing does not sit flush on the bottom of the drip tray.

B.2.2 Install A- and C-series drip tray and support bracket

In the Alliance HPLC A- and C-series configurations, the detector drip tray is not included with the solvent bottle tray kit, but is part of the auxiliary tray and support bracket kit (205001128). In these configurations, the detector is stacked atop the separations module on a support bracket. The bracket provides an extended platform on which the feet in the rear of the detector can securely sit.

Important: For A- and C-series configurations, only the 2424, 2489, and 2998 detectors require installation of the drip tray. The remaining detectors drain directly into a small drip tray in the syringe compartment via a length of tubing.

The following is an overview of the procedure:

- Install the drip tray on the detector (2424, 2489, and 2998 detectors only).
- Install the detector support bracket and detector on the separations module.
- Route the drip tray or detector drain tubing into the separations module.

Required materials

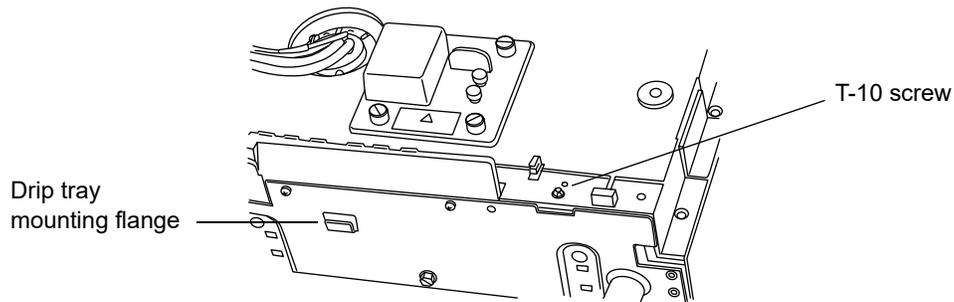
- Screwdriver, T-10 TORX
- Screwdriver, T-20 TORX
- Wrench, 7 mm

To install the drip tray on the detector (applies to 2424, 2489, and 2998 only):

1. Disconnect all cabling and other connections from the detector.
2. Open the front door of the detector.
3. Rest the detector on its rear panel, so that the front panel is facing up.

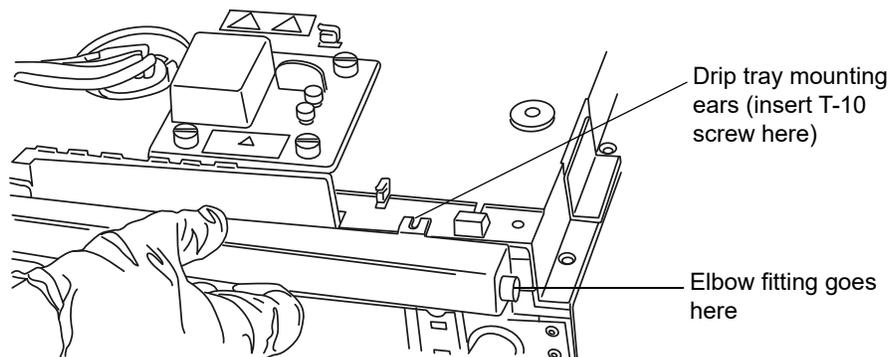
4. If the detector is a 2424, remove the barbed fitting from the underside of the detector, as this interferes with the mounting of the drip tray.
5. Remove the T-10 screw located on the bottom right corner of the detector front panel and set it aside.

Figure B–3: A- and C-series drip tray mounting location on detector



6. Position the drip tray so that the mounting hole for the T-10 screw you removed is aligned between the mounting ears on the right end of the drip tray.
7. Push the mounting slot on the opposite side of the drip tray into the mounting flange on the detector until the drip tray locks into place.
8. Insert the T-10 screw you removed earlier in the hole between the drip tray mounting ears and secure the drip tray to the detector.

Figure B–4: A- and C-series drip tray installed

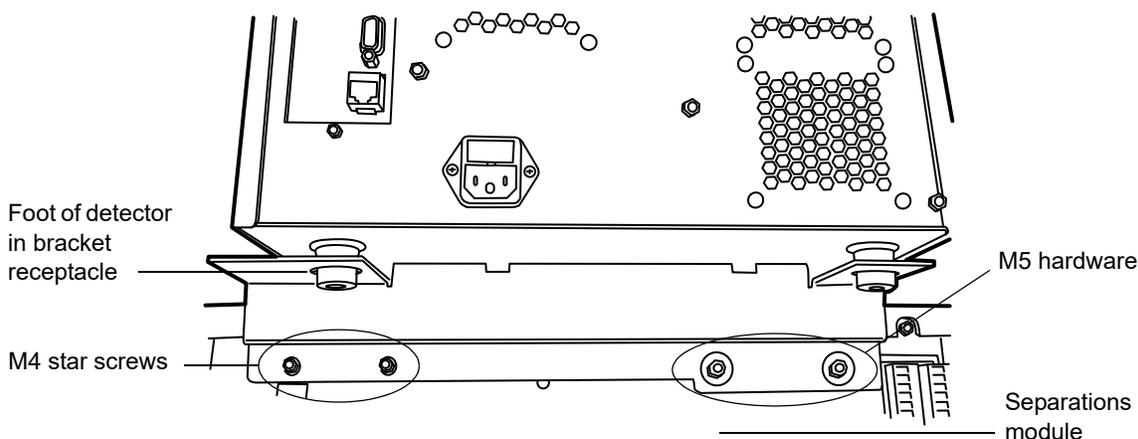


9. Install the elbow fitting on the right end of the drip tray.

To install the detector support bracket and detector:

1. Power-off the separations module and disconnect the column module cable from the rear panel of the separations module. Refer to the figure below for locations referenced in the rest of this procedure.

Figure B–5: Detector support bracket installed on rear of separations module



2. Remove the left most M4 star screw that is located just above the column module connector on the rear panel.
3. Using the hardware provided with the support bracket, secure the bracket to the top of the rear panel on the separations module:
 - a. On the left side of the separations module, fasten the two M4 star screws.
 - b. On the right side of the separations module, fasten the two flat washers with M5 nuts.
4. Place the detector on the detector support bracket. Make sure that the rear feet on the bottom of the detector are seated in the large receptacles in the tabs on either end of the detector support bracket.

To route the drip tray or detector drain tubing into the separations module:

1. For detectors with a drip tray installed (2424, 2489, and 2998 only), connect one end of a length of convoluted tubing supplied in the kit to the elbow fitting on the right side of the drip tray.

For detectors without a drip tray, connect one end of the convoluted tubing to the drain fitting located on the front left-hand side of the underside of the detector.
2. Route and secure the tubing in the clips in the syringe compartment so that the end of the tubing is positioned just above the small drip tray under the syringe, as shown in the figures below.

Note: Pull the tubing tight in the clips, to ensure there are no upward bends that can cause traps when solvent is draining.

Figure B-6: A- and C-series detector drain routing without drip tray

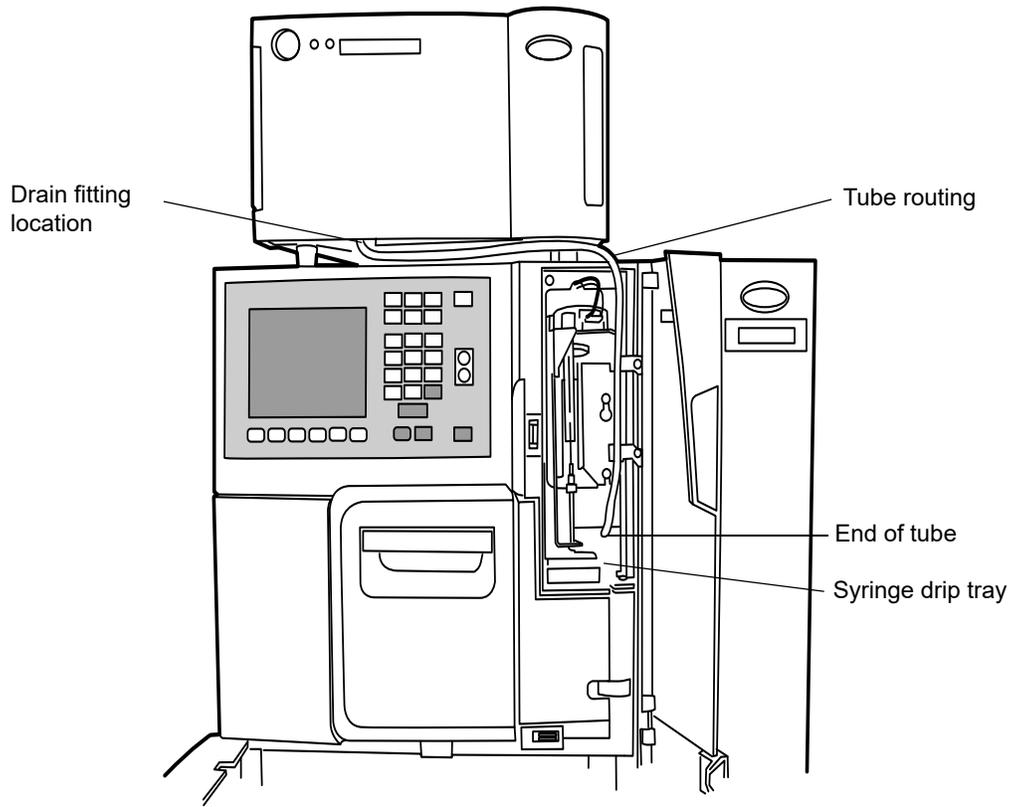
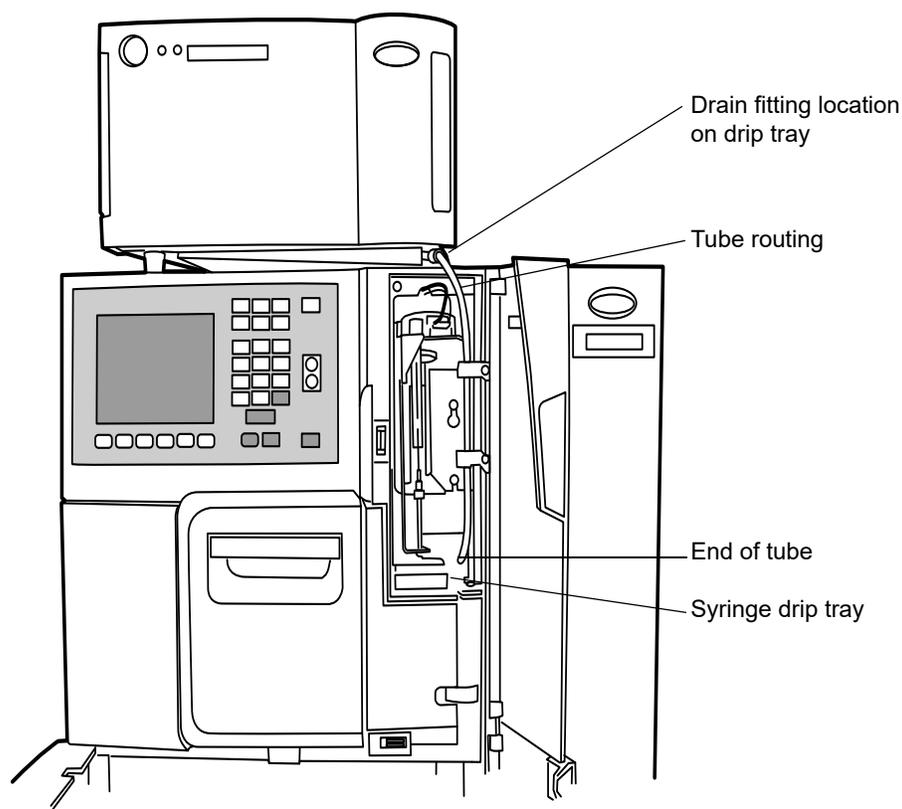


Figure B–7: A- and C-series detector drain routing with drip tray



B.2.3 Install solvent supply tubing

The tubing from the separations module to the solvent reservoirs is coiled and stored in front of the separations module, near the syringe compartment. One end of the solvent tubing is connected (at the factory) to the in-line vacuum degasser, which is connected to the GPV.

Each tube is marked with a letter (corresponding to each solvent reservoir) and a color-coded label.

Table B–2: Solvent supply tubing identification

Solvent line label	Solvent line color
A	Yellow
B	Blue
C	Red
D	Green

To install the solvent lines:

1. Uncoil the solvent lines stored in front of the separations module, near the syringe compartment.
2. Remove the filter from each solvent line and insert the free end of each solvent line through a reservoir cap (start-up kit).
3. Reinstall a filter on the end of each solvent line.
4. Install each reservoir cap on the appropriate reservoir.

Requirement: Make sure the solvent line extends to the bottom of the reservoir.

B.2.4 Make waste line connections

You must make waste line connections for spill tray and condensation waste.

Ensure all drain lines inside and outside the instrument are properly connected and unrestricted by obstructions or bends. The two main drain lines exiting toward the front of the instrument must lay flat on the bench top without allowing any upward travel. You must port these drain lines to an appropriate waste container of a volume equal to or greater than that of the combined volume of all solvent reservoirs. The waste container must be at a level lower than the instrument. Always check the drain lines after moving the instrument or performing maintenance on the instrument.



Caution: Failure to properly perform these waste line connections can result in damage to the bench or adjacent instruments in the event of a solvent leak.

B.2.4.1 Spill tray waste line

The spill tray waste outlet is located under the front panel of the separations module, below the solvent conditioning tray access door.

To install the spill tray waste line:

1. Cut a length of 1/4-inch ID tubing (start-up kit) long enough to reach from the spill tray waste outlet to the front of the bench.
2. Connect one end of the tubing you cut in [step 1](#) to the spill tray waste outlet.
3. Install a 90° elbow (start-up kit) on the other end of the tubing.
4. Cut another length of 1/4-inch ID tubing long enough to reach from the elbow fitting to the waste reservoir.
5. Connect the tubing you cut in [step 4](#) between the elbow and the waste reservoir.

B.2.4.2 Condensation waste line

The condensation waste outlet from the sample compartment is also located under the front panel of the separations module, to the right of the spill tray waste outlet. In addition to condensate from the sample heater/cooler, the condensation waste can contain spills from leaking sample vials.

To install the condensation waste line:

1. Cut a length of 1/4-inch ID tubing (start-up kit) long enough to reach from the condensation waste outlet to the front of the bench.
2. Connect one end of the tubing you cut in [step 1](#) to the condensation waste outlet.
3. Install a 90° elbow (start-up kit) on the other end of the tubing.
4. Cut another length of 1/4-inch ID tubing long enough to reach from the elbow fitting to the waste reservoir.
5. Connect the tubing you cut in [step 4](#) between the elbow and the waste reservoir.

B.2.5 Install needle wash and plunger seal wash reservoirs

The tubing for the needle wash and plunger seal wash reservoirs emanates from the syringe compartment. Each wash line is marked with a label and/or is color-coded. The following table lists the color code and size of each line. The plunger seal wash inlet line is labeled “Pump Wash In”, and has a diffuser attached to one end.



Caution: To prevent damage to the separations module, be sure to use needle wash and plunger seal wash solutions that are miscible with the mobile phase you are using.

Use separate solutions and containers for plunger seal wash and needle wash. Because the functions of these solutions differ, the use of one solution for both functions can compromise the effectiveness of either needle washing or plunger seal washing.

Table B–3: Needle wash and plunger seal wash line color codes

Wash line	Color code	Inside diameter (inches)
Needle wash inlet	White/clear (labeled “Needle Wash”)	1/16
Needle wash outlet	Yellow or clear	1/16
Sample loop waste	Clear	1/16
Plunger seal wash inlet	Clear (labeled “Pump Wash In”)	1/8
Plunger seal wash waste	Clear	1/8

To install the needle wash and plunger seal wash reservoirs:

1. Place the needle wash reservoir and plunger seal wash reservoir in the drip tray atop the separations module.
2. Remove the diffuser from the needle wash inlet line (clear line, white Needle Wash label), insert the line through a reservoir cap, reinstall the diffuser on the end of the line, and install the cap on the needle wash reservoir.
3. Place the needle wash outlet line (yellow or clear) and the sample loop waste line (clear) in a suitable waste container.

4. Remove the diffuser from the plunger seal wash inlet line (clear, labeled “Pump Wash In”), insert the line through a reservoir cap, reinstall the diffuser on the end of the line, and install the cap on the plunger seal wash reservoir.
5. Place the plunger seal wash waste line (clear) in a suitable waste container.

B.2.6 Connect the column

The stainless steel outlet tubing of the sample management system, which connects to the column inlet, is included in the start-up kit. This tube connects to the injector outlet tube that passes through the right panel of the instrument.

When you are connecting the separations module to a column switching valve, connect the outlet tubing to the column switching valve inlet. When you are connecting the separations module to an external autosampler and configuring for operating in Operate Gradient by Event In mode, connect the outlet tubing to the inlet of the autosampler.

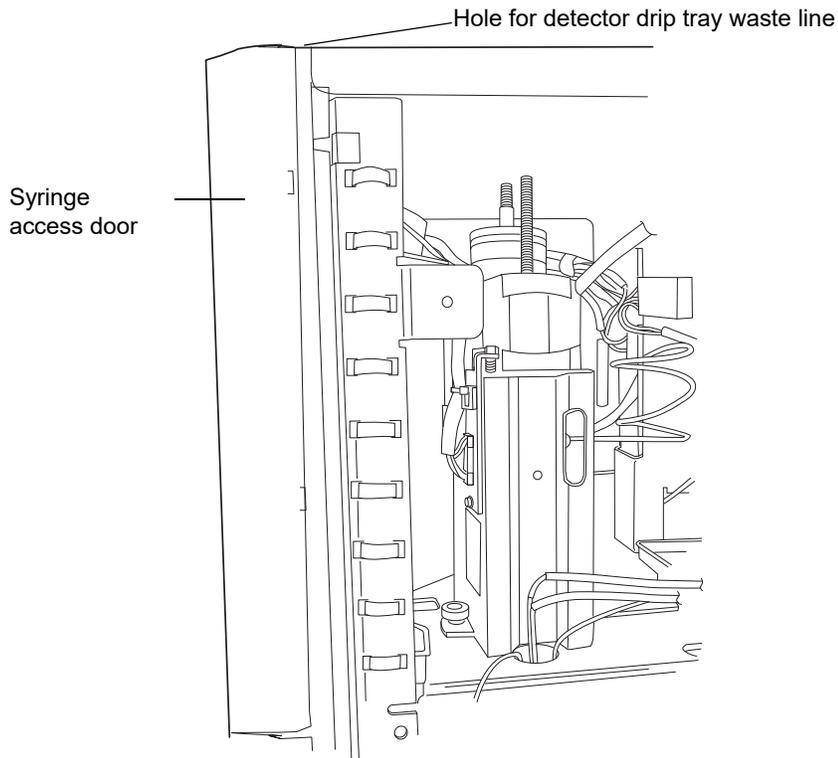
To connect the column:

1. Pass the outlet tubing through the opening in the middle of the right-hand side panel.
2. Connect the outlet tubing to the column inlet, and tighten the fitting.

Two sets of column clips and M4 x 10 screws are included in the start-up kit. Using the shorter clips, you can mount columns of 7.9 mm OD or less and 15 cm long in the compartment behind the syringe door. For longer columns, mount the longer column clips so that they protrude from the side of the separations module, through the slots shown in the [figure “Side view of frame openings for tubing connections \(side panel removed\)” on page 203](#).

If the optional column heater (or column heater/cooler) is installed. (See [“Install the column heater and column heater/cooler” on page 216](#) for the procedure to connect the outlet tubing to the column heater [or column heater/cooler]).

Figure B–8: Side view of frame openings for tubing connections (side panel removed)



B.2.7 Connect the column selection valve

These optional column selection valves switch the flow path among multiple columns:

- 3-column selection valve
- 6-column selection valve
- 2-column regeneration valve

B.2.7.1 Two-column selection valve

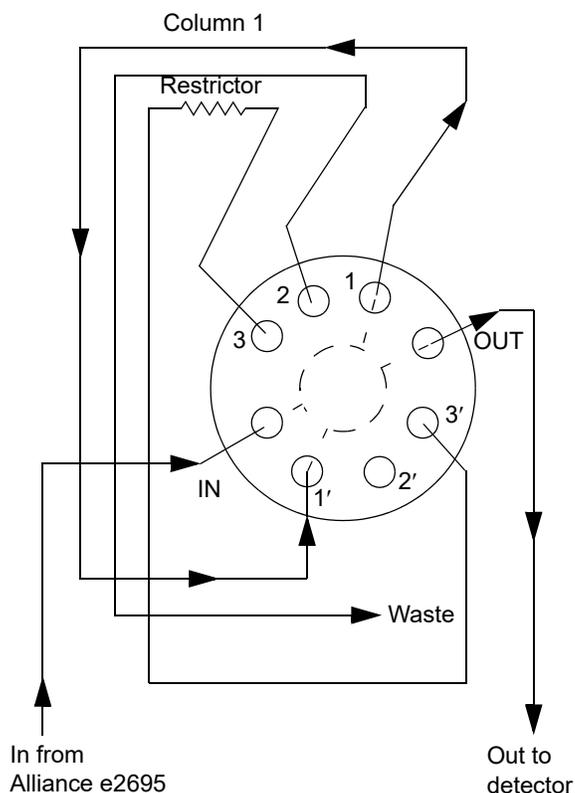
The 2-column (6 port, 2 position) selection valve is a standalone option, which you can order for field installation.

B.2.7.2 Three-column selection valve

The 3-column selection valve is an eight-port, three-position valve preconfigured at the factory as follows:

- Port 1 – Routed to the column (a 2.1 mm × 50 mm column is supplied)
- Port 2 – Routed to waste
- Port 3 – Routed through a restrictor bypass to the detector

Figure B-9: Three-column selection valve configuration (as shipped)



Install the 3-column selection valve after installing the column heater or column heater/cooler. (See [“Install the column heater and column heater/cooler”](#) on page 216.)

Required material

Open-end wrench, 5/16-inch

To connect the 3-column selection valve to the fluid path of the sample management system:

1. Use the 5/16-inch wrench to connect the red line (from the e2695 injector) to the IN port on the column selection valve.
2. Use the tubing and fittings supplied with the 3-column selection valve to connect the OUT port of the column selection valve to the detector inlet.
3. Replace the stainless steel union shipped inside the column heater or column heater/cooler with the column.

Tip: The column can be the one supplied with the valve, or it can be a different one.

4. Route the waste line (installed) from port 2 of the column selection valve through the hole in the bottom of the column heater or column heater/cooler to a suitable waste reservoir.

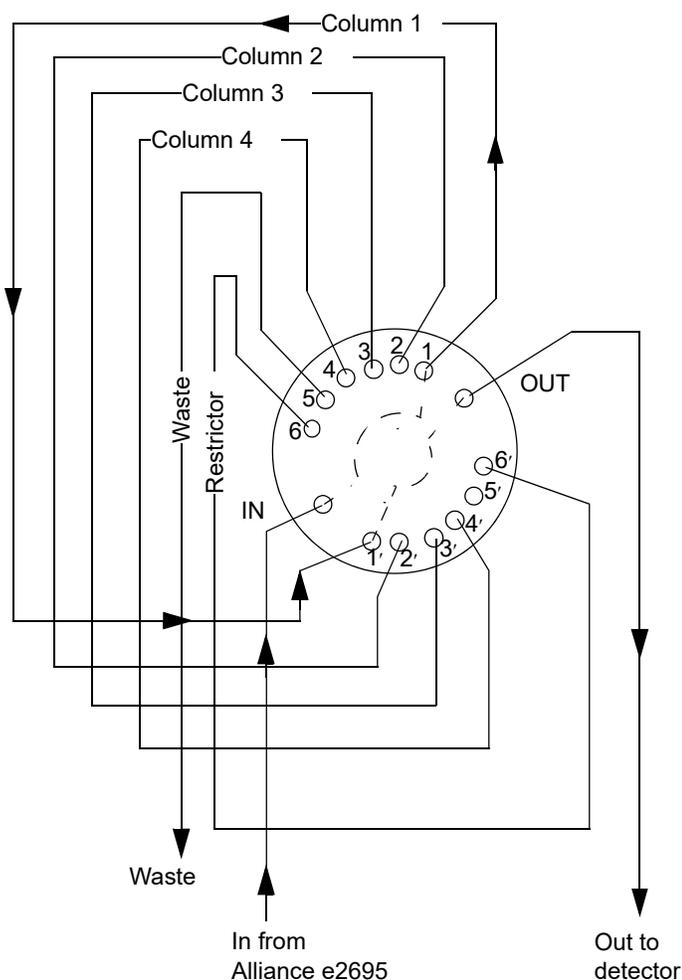
Reconfigure the 3-column selection valve as appropriate for your application.

B.2.7.3 Six-column selection valve

The 6-column selection valve is shipped separately with a coil of 0.010-in PEEK tubing, a 0.0025-inch ID restrictor tube, and SealTight fittings. Connect as appropriate for your application. (See figure “Six-column selection valve connections (example)” on page 65.)

Tip: Any of the six column positions can be used for a restrictor or for waste, as in the three-column selection valve. (See figure “Three-column selection valve configuration (as shipped)” on page 204.)

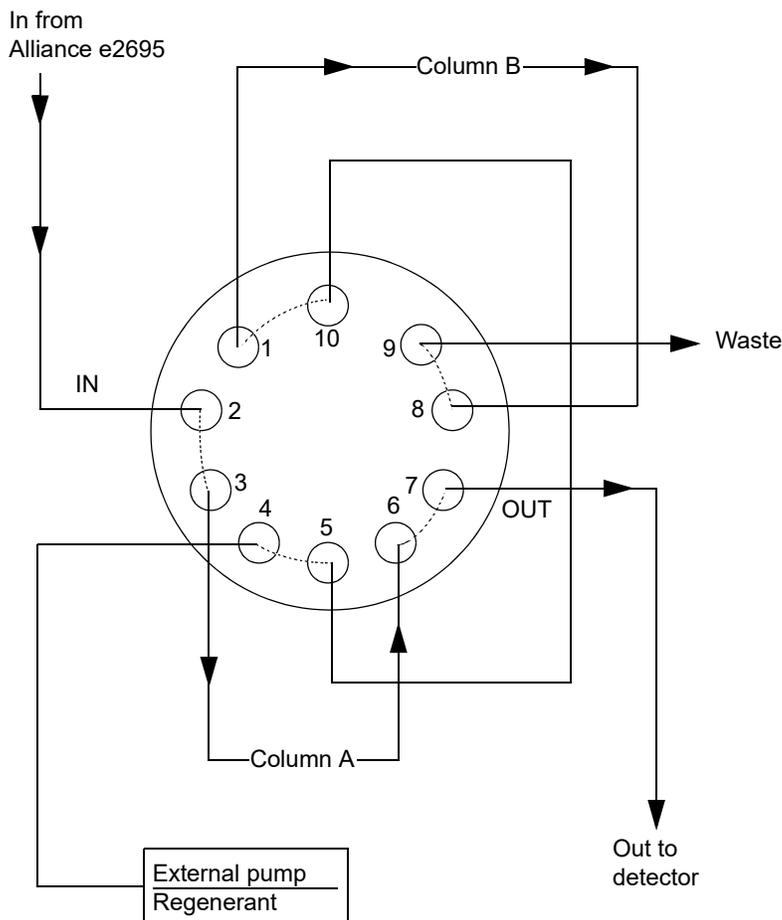
Figure B–10: Six-column selection valve connections (example)



B.2.7.4 Two-column regeneration valve

The 2-column regeneration valve is shipped separately with a coil of 0.010-inch PEEK tubing, a 0.0025-inch ID restrictor tube, and SealTight fittings. Connect as appropriate for your application. (See [figure “Column regeneration valve connections \(example\)” on page 206.](#)) Contact Waters Technical Service for more information.

Figure B–11: Column regeneration valve connections (example)



B.2.8 Connect the detector

The following provides general instructions for connecting the detector to the Alliance HPLC system. For more detailed information on connecting your specific detector, see the detector's documentation.

To connect the detector:

1. Connect the outlet of the column to the inlet of the detector.
2. Connect a waste line from the detector outlet to a suitable waste container.

Tip: To avoid spill damage, install a drip tray below the detector's tubing connections. See [“Install the B-series drip tray” on page 193](#) and [“Install A- and C-series drip tray and support bracket” on page 195](#) for drip tray installation instructions.

B.3 Signal connections

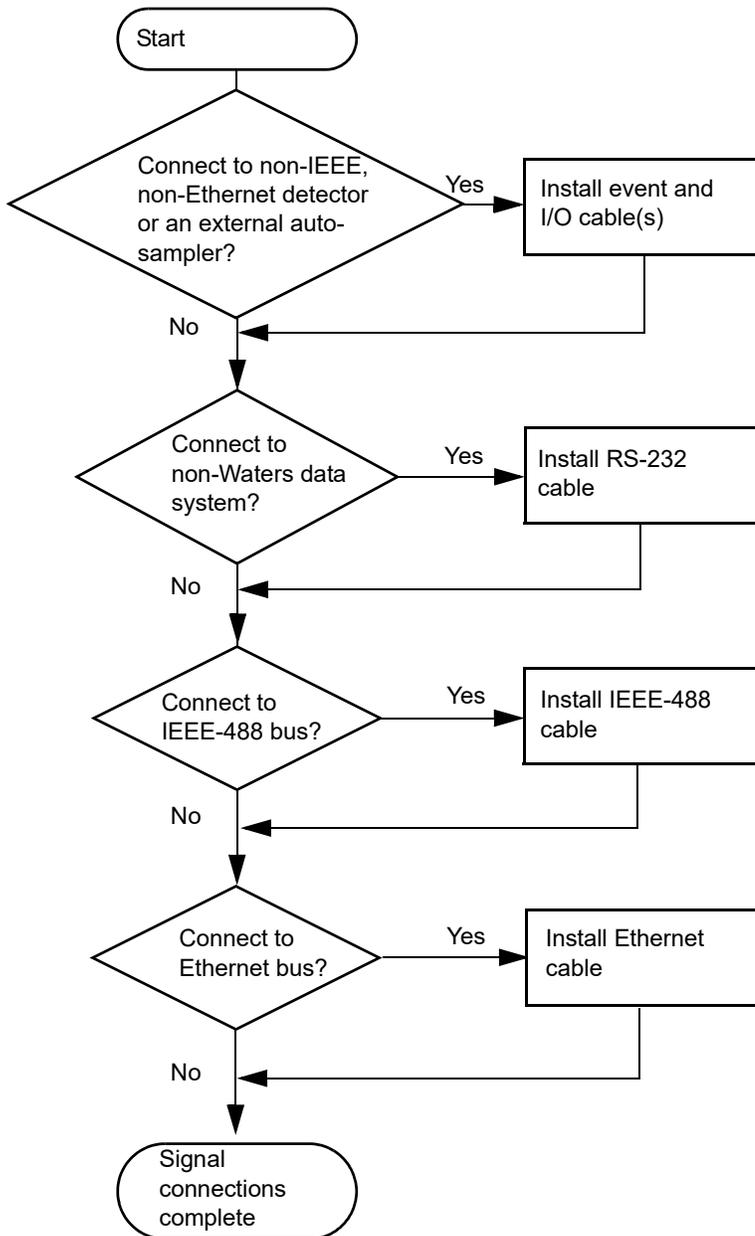
The rear panel of the separations module provides connection terminals and communication ports for operation with external devices. (See [figure “Rear panel of the separations module” on page 192.](#))

The signal connections you need to make to your separations module depend on these considerations:

- The operating mode you select for the separations module (System Controller, No Interaction, or one of the Remote Control modes)
- Types of instruments that make up your HPLC system

This section describes the input/output (I/O) and digital signal connections that you can make from the terminal strips, RS-232 connectors, IEEE-488 bus connector, and Ethernet interface on the rear panel of the separations module.

Figure B–12: Connect signal cables



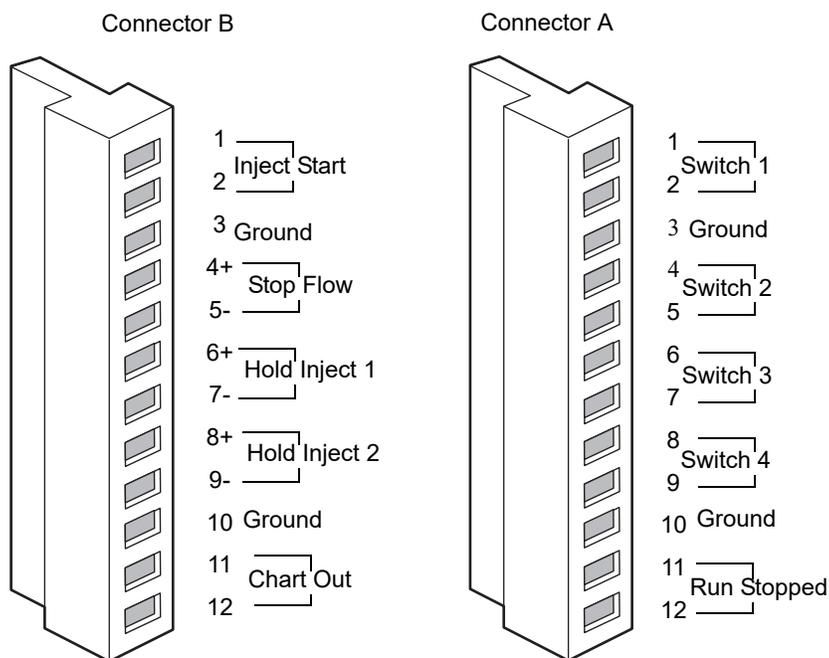
B.3.1 I/O signal connections

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



Caution: To avoid the risk of damaging other components, do not remove the keys in the A and B connectors. These keys ensure that the A connector fits only in the right-hand slot and the B connector only in the left-hand slot (when you face the rear panel).

Figure B–13: I/O signal connectors



B.3.1.1 I/O signals

The following table describes the signals sent via the I/O connectors of the separations module. See [Appendix C](#) for information about the electrical specifications of each signal.

Table B–4: I/O Signals

Signal	Description
Inject start	An output (contact closure) to indicate that an injection began. Connect a wire pair to the two Inject start terminals.
Stop flow	An input that enables other LC components to stop the solvent flow from the separations module. You specify the signal conditions that stop the flow using the Events In screen key in the Configuration screen. Connect the positive input wire to the “Stop Flow +” terminal and the negative input wire to the “Stop Flow -” terminal.
Hold inject 1 and 2	Inputs that enable other LC components to delay the injection (for example, an external autosampler used in the Operate Gradient by Event In mode or a fraction collector). You can specify the signal conditions (using the Events In screen key in the Configuration screen) that prevent the separations module from making an injection. Connect the positive input wire to the appropriate “Hold Inject +” terminal and the negative input wire to the appropriate “Hold Inject -” terminal.

Table B–4: I/O Signals (continued)

Signal	Description
Chart out	<p>Outputs the analog signal (specified in the current separation method, or default separation method) to a device such as an integrator or strip chart recorder. (See “Set I/O parameter values” on page 104.) You can select one of the following signals as the chart out signal:</p> <ul style="list-style-type: none"> • Flow rate • System pressure • Sample loop pressure • Primary head pressure • Composition (%A, %B, %C, %D) • Sample temperature • Column temperature • Degasser vacuum <p>The table, below, lists the output signal conditions for each of these parameters.</p>
Switch 1 - Switch 4	See “Event switches” on page 211.
Run stopped	An output indicating that the separations module ceased operating because of an error condition or operator request (pressing the Stop Flow button, for example).

The following table describes the parameter voltages for the chart out signals.

Table B–5: Chart out signal conditions

Signal	Parameter setting at 0 volts (minimum)	Parameter setting at 2.000 volts (maximum)
Flow rate	0.000 mL/min	10.000 mL/min
System loop pressure	-344.7 kPa (-3.45 bar, -50 psi)	37920 kPa (380 bar, 5500 psi)
Sample loop pressure	-0.69 kPa (-0.007 bar, -0.1 psi)	3454.3 kPa (34.5 bar, 501 psi)
Primary head pressure	-344.7 kPa (-3.45 bar, -50 psi)	34473.8 kPa (344.7 bar, 5000 ^a psi)
Composition	0.0 percent	100.0 percent
Sample temperature	4 °C	40 °C
Column temperature	20 °C	60 °C
Degasser vacuum (absolute)	0.0 psi	68.9 kPa (0.7 bar, 10.0 psi)

a. Although the chart out signal for pressure is scaled (and displayed) to 5500 psi, the maximum operating pressure of the separations module is 34473.8 kPa (344.7 bar, 5000 psi).

B.3.1.2 Event switches

Switches 1 through 4 on connector A can serve as contact-closure switches to control column selection valves, fraction collectors, or similar external devices. Each pair of switch terminals (the two switch 1 terminals, for example) connects to an external device. You can operate the switches in either of two ways:

- Manually, from the Status screen
- Automatically, in a separation method using the timed table in the I/O screen

See [“Set I/O parameter values” on page 104](#) for information on using switches in an automated run.

The following table describes the functions associated with each position of an event switch.

Table B–6: Event switch positions and functions

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

B.3.2 Digital signal connections

The rear panel includes one RS-232 interface connector, one IEEE-488 interface connector, and one Ethernet connector for digital signal communications. (See [figure “Rear panel of the separations module” on page 192.](#))

B.3.2.1 RS-232 connections

The separations module is equipped with a DB-9 connector for RS-232-port communications. (See [figure “Rear panel of the separations module” on page 192.](#)) The RS-232 port supports both binary and ASCII communications.

For remote operation, you use the RS-232 to connect to a non-Waters data system that uses RS-232 communications to control the separations module. To enable this communication, you choose either Controlled via RS232 (ASCII) or Controlled via RS232 (Binary) in the Configuration screen depending on your data system requirements. For more information on the Configuration screen, see [“Set configuration parameters” on page 47.](#)

Alternatively, when the separations module is in the System Controller mode, you can use the RS-232 port to transmit data to external devices (a Waters 746 Data Module or serial printer, for example). You can transmit two types of data over this interface:

- Print data – When you press the Print screen key
- Report data – When a report is generated

The following table lists the values of the RS-232 output data parameters that you can select in the Configuration screen. For more information on the Configuration screen, see [“Set configuration parameters” on page 47.](#)

Table B–7: RS-232 output parameters displayed in the configuration screen

Parameter	Value
Device	None, 746 Integrator, Serial Printer, BCD output
Baud rate	1200, 2400, 9600

B.3.2.2 IEEE-488 connections

Use the IEEE-488 interface connector to connect the separations module to these IEEE-488 devices: (See [figure “Rear panel of the separations module” on page 192.](#))

- Waters 2487, 486, 2414, 2410, and 410 detectors
- BusLAC/E card in an Empower software system, a network LAC/E module, or a LAC/E32 Acquisition Server (Empower software)
- General purpose instrument board of a MassLynx computer

Requirements:

- You cannot use the separations module’s IEEE-488 interface at the same time you are using its Ethernet port for communications.
- For remote control, you can only configure the separations module for IEEE-488 communications if it belongs to one of these configurations:
 - Where the separations module and detector(s) communicate with the data system via IEEE-488
 - Where the separations module and data system communicate via IEEE-488, and include one or more detectors that can only communicate with the data system via Ethernet (for example, Waters 2998, 2489, 2475, 2424, e-SAT/IN module-connected, or 3100 Mass Spectrometer Detector. See [“Supported IEEE-488 and Ethernet configurations” on page 31.](#))

The IEEE-488 connector in the separations module mates with a standard IEEE-488 cable.

! **Notice:** To avoid damaging components, power-off all instruments connected to the IEEE-488 bus before you connect an IEEE-488 interface cable to an instrument.

See [figure “Digital signal control of an HPLC system” on page 30](#) for the signal connections (IEEE-488 and RS-232) in a typical HPLC system where the separations module functions as the system controller. See [“Empower control” on page 32](#) for a description of the signal connections used when the separations module functions under Empower software control.

B.3.2.3 Set the IEEE-488 address

From the Configuration screen, you can view or change the IEEE-488 address of the separations module. (See [“Configure the operating mode” on page 52.](#))

B.3.2.4 Ethernet connections

The separations module is equipped with a RJ-45 connector for Ethernet port communications. (See [figure “Rear panel of the separations module” on page 192.](#)) The Ethernet port, a 10/100 Base-T interface, is used with the separations module configured for remote control, which is the case when Empower controls its operation. See [figure “Typical e2695 Separations Module HPLC system under Empower 3 software control” on page 32](#) for the signal connections in a typical HPLC system with a separations module and Empower Chromatography Data System.

Requirements:

- You cannot use the separations module's Ethernet port at the same time you are using its IEEE-488 interface for communications.
- For remote control, you can only configure the separations module for Ethernet communications if it belongs to a configuration where both the separations module and detector(s) communicate with the data system via Ethernet. (See [“Supported IEEE-488 and Ethernet configurations” on page 31.](#))

Tip: For help with HPLC Ethernet connections, you can order from Waters the Ethernet Switch Communications Kit (700004123). The kit includes an 8-port Ethernet switch, cables, and a mounting bracket, for mounting the switch on the rear panel of the separations module or 1500 series HPLC pump. For additional Ethernet information, see the *Waters Ethernet Instrument Getting Started Guide* (71500074403).

As with IEEE-488 control, triggering of Waters Ethernet-controlled devices, such as 2489, 2998, 2414, and 2424 detectors, occurs over the Ethernet cable, and so requires no external I/O cables.

Exception: Waters 2475 Fluorescence Detector, v2.01 requires an external I/O trigger cable under Ethernet control.

B.3.2.5 Configure the Ethernet port

On the Configuration screen, select Auto/Ethernet for the separations module. (See [“Configure the operating mode” on page 52](#) for information about configuring the Ethernet port.)

B.4 Add hardware options

Depending on your applications, you can install the following options on the separations module:

- Larger sample loop
- Column heater
- Column heater/cooler
- Larger or smaller syringe
- Column selection valve

B.4.1 Install an optional sample loop

To inject sample volumes greater than 100 μL , install an optional sample loop. To do so, you must perform these tasks:

- Remove the right-hand side panel
- Install the sample loop
- Refit the right-hand side panel

Required materials

- Screwdriver, #2 cross-head
- Wrenches, 5/16-inch, two
- Wrench, adjustable
- Sample Loop Option Kit
- Union (WAT097334)

To remove the side panel:

Note: This procedure applies only to a separations module with no column heater or column heater/cooler option installed. If the column heater or column heater/cooler option is installed (See [“Remove the side panel of separations module” on page 135](#) for instructions on how to remove the side panel of the separations module.)

1. Power-off the separations module, and disconnect it from the electrical outlet.
2. Remove the 2 screws that secure the separations module’s right-hand side panel.
3. Slide the side panel to the rear, off the rear panel posts.



To avoid having your fingers pinched by movement of the sample carousel, do not remove the cover plate on the side of the sample compartment.

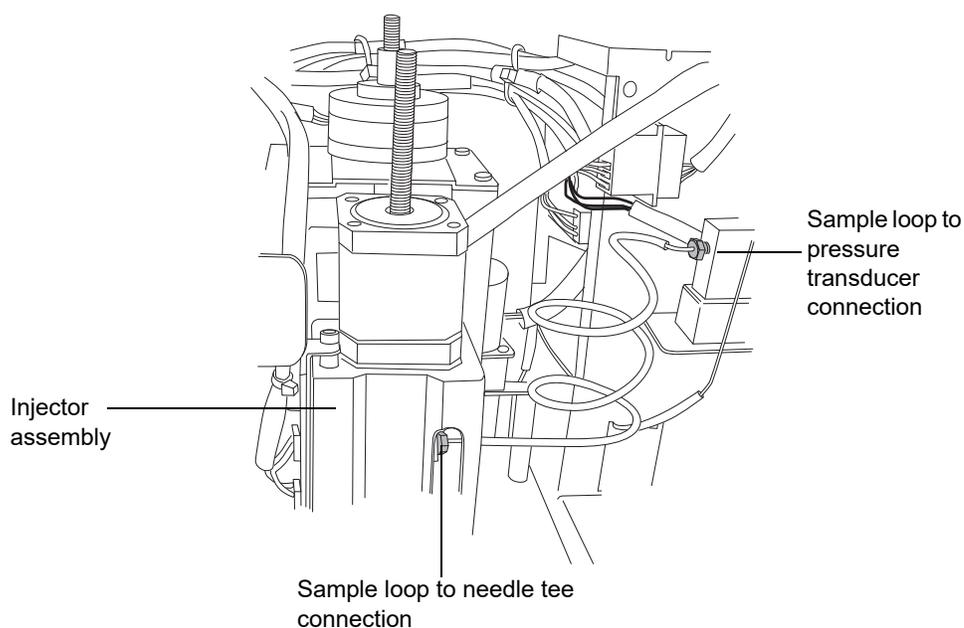
To add a sample loop to the factory-installed 100- μL loop:

1. Use the 5/16-inch wrench to disconnect the sample loop from the pressure transducer. (See [figure “Disconnect the sample loop” on page 215](#).)
2. Connect the open end of the existing sample loop to one end of the union.
3. Connect the other end of the union to the new sample loop.
4. Connect the open end of the new sample loop to the pressure transducer.
5. Use the wrenches to tighten the connections.
6. Connect the power cable, and power-on the separations module.
7. In the Configuration screen, enter the new sample loop volume. (See [“Configure the separations module” on page 47](#).)
8. Perform a purge and compression check, and check for leaks. (See [“Purge the system” on page 59](#) for the purge procedure.)

B.4.1.1 To install a sample loop:

1. Use the 5/16-inch wrench to disconnect the sample loop from the pressure transducer, and the needle tee on the injector assembly. (See [figure “Disconnect the sample loop” on page 215.](#))
2. Connect one end of the sample loop to the needle tee.
3. Connect the open end of the new sample loop to the pressure transducer.
4. Use the wrenches to tighten the connections.
5. Connect the power cable, and power-on the separations module.
6. Enter the new sample loop volume in the Configuration screen. (See [“Configure the separations module” on page 47.](#))
7. Perform a purge and compression check, and check for leaks. (See [“Purge the system” on page 59](#) for the purge procedure.)

Figure B–14: Disconnect the sample loop



B.4.1.2 Reinstall the right-hand side panel

This procedure applies only to a separations module with no column heater or column heater/cooler option. If the column heater or column heater/cooler option is installed, see [“Remove the side panel of separations module” on page 135](#) for instructions on how to reinstall the side panel on the separations module.

To reinstall the panel:

1. Mount the side panel on the rear panel posts.
2. Slide the side panel forward, aligning the outlet tube with the slot in the side panel.
3. Install the screws on the side panel.
4. Reconnect AC power to the separations module, and then power-on the separations module.

B.4.2 Install the column heater and column heater/cooler

The column heater or column heater/cooler mounts onto the right-hand side panel with three screws. A connector on the rear panel of the separations module provides power and signal connections to the column heater or column heater/cooler. See the *Waters Alliance Series Column Heater and Column Heater/Cooler Operator's Guide* for procedures explaining the installation, operation, maintenance, and basic troubleshooting of the Waters Alliance series column heater and column heater/cooler instruments.

B.4.3 Install a syringe

See [“Replace the syringe” on page 137](#) for the procedure to install a syringe.

C Specifications

The specifications presented here depend on the conditions in individual laboratories. Refer to the *Alliance e2695 Separations Module Site Preparation Guide*, or contact the Waters Technical Service organization for additional information about specifications.

C.1 Physical specifications

The following table lists the physical specifications for the Alliance e2695 Separations Module.

Table C–1: Physical specifications

Attribute	Specification
Height	57.1 cm (22.5 inches)
Depth	57.1 cm (22.5 inches) 64.8 cm (25.5 inches) with optional sample heater/cooler
Width	45.7 cm (18 inches) 58.4 cm (23.0 inches) with optional column heater or column heater/cooler
Weight	48.5 kg (107 lb) 62.1 kg (137 lb) with optional sample heater/cooler and column heater (or heater/cooler) module
Wetted surface materials	316 stainless steel, ruby, sapphire, MP35N, PEEK, PPS, UHMWPE, Tefzel (ETFE), Teflon® (FEP and PTFE), Teflon AF, Fluoroloy G, Fluoroloy-08R

C.2 Environmental specifications

The following table lists the environmental specifications for the Alliance e2695 Separations Module.

Table C–2: Environmental specifications

Attribute	Specification
Operating temperature	4 to 40° C (39 to 104 °F)
Relative humidity	20 to 80%, noncondensing
Acoustic noise	< 65 dB(A)
Solvent compatibility (see Appendix D)	Solvents consistent with materials of construction. Salts and buffers can reduce seal life, especially at pressures in excess of 3000 psi.

C.3 Electrical specifications

The following table lists the electrical specifications for the Alliance e2695 Separations Module.

Table C–3: Electrical specifications

Attribute	Specification
Power requirements	950 VA (maximum)
Line voltage	100 - 240 Vac
Frequency	50 - 60 Hz
Time or user-controllable switch closures S1 – S4	<p>Four controllable contact closures (two terminals per closure)</p> <p>Maximum allowable current = 0.5 A per contact</p> <p>Maximum allowable voltage = 30 Vdc</p> <p>Contact resistance = 0.2 ohms</p> <p>Outputs can be controlled from the I/O events table or directly from the front panel.</p> <p>Two modes available from the front panel and I/O events table: On = contact closed; Off = contact open</p> <p>Additional modes available in the I/O events table: Pulse = single contact closure for programmable period; Toggle = changes the current state</p>
Stop flow (input)	<p>Two terminals (+, -) that allow other LC devices to immediately stop solvent flow</p> <p>User-selectable to halt flow on high or low signal</p> <p>Input voltage range: ± 30 Vdc</p> <p>Logic high = >3.0 Vdc $\pm 10\%$, logic low = <1.9 Vdc $\pm 10\%$</p> <p>Minimum pulse width = 10 msec</p>

Table C-3: Electrical specifications (continued)

Attribute	Specification
Hold inject (input)	Two sets of (+, -) terminals that allow other LC devices to delay an injection Boolean operator selects whether one or both inputs delay the injection Input voltage range: ± 30 Vdc Logic high = > 3.0 Vdc $\pm 10\%$, logic low = < 1.9 Vdc $\pm 10\%$ Minimum pulse width = 10 msec
Chart output	Two terminals (+, -) for recording the following user-selectable outputs: <ul style="list-style-type: none"> • Programmed flow rate • Sample loop pressure • System pressure • Primary head pressure • Programmed composition (%A, %B, %C, %D) • Sample temperature • Column temperature • Degasser vacuum
Run stopped	Contact closure indicating: <ul style="list-style-type: none"> • Sample set is suspended • Current function is suspended Maximum allowable current = 0.5 A Maximum allowable voltage = 30 Vdc Contact resistance = 0.2 ohms
Inject start	Contact closure (across terminals 1 and 2) for 1 second on injection Maximum allowable current = 0.5A Maximum allowable voltage = 30 Vdc Contact resistance = 0.2 ohms
Ground terminals	Connected to signal ground and used as reference for outputs

C.4 Solvent management specifications

The following table lists the solvent management specifications for the Alliance e2695 Separations Module.

Table C–4: Solvent management system specifications – e2695 XE configuration

Attribute	Specification
Number of solvents	One to four
Solvent conditioning	Vacuum degas, four chambers ≈ < 500 uL internal volume per chamber
Programmable flow rate range	0.000 and 0.010 to 10.000 mL/min in 0.001 mL/min increments
Typical operating flow rate range	0.050 to 5.000 mL/min in 0.001 mL/min increments
Compressibility compensation	Automatic and continuous
Effective system delay volume	< 650 µL, independent of back-pressure at 1 mL/min
Plunger seal wash	Integral, active, programmable
Gradient profiles	11 gradient curves, including: <ul style="list-style-type: none"> • Linear • Step (2 curves) • Concave (4 curves) • Convex (4 curves)
Dry prime/wet prime	Automatic, front panel control
Flow ramping	Time (0.01 to 30.00 min in 0.01 min increments) to reach maximum flow rate
Maximum operating pressure	345 bar (5000 psi) (0.010 to 3.000 mL/min) Programmable upper and lower limits Pressure fall-off at > 3.000 mL/min
Pressure ripple	≤ 2.5% (1 mL/min, degassed methanol, at 1700 psi back-pressure)
Composition range	0.0 - 100.0 in 0.1% increments
Composition accuracy	± 0.5% absolute, independent of back-pressure (Proportioning valve pair test [degassed methanol (or methanol/propylparaben), 2 mL/min, 254 nm])
Composition precision	≤ 0.15% RSD or ≤ 0.02 min SD, whichever is greater, based on retention time (Degassed methanol/water 60:40 Dial-a-Mix, 1 mL/min, six replicates, phenone mix, 254 nm)
Flow precision	≤ 0.075% RSD or ≤ 0.02 min SD, based on retention time (N = 6) or volumetric measures (0.200 to 5.000 mL/min), isocratic premix
Flow accuracy	±1% or 10 µL/min, whichever is greater, (0.200 to 5.000 mL/min), degassed methanol, at 600 psi back-pressure

C.5 Sample management specifications

The following table lists the sample management specifications for the Alliance e2695 Separations Module.

Table C–5: Sample management specifications

Item	Specification
Number of sample vials	120 vials configured in 5 carousels of 24 vials each
Number of sample injections	1 to 99 injections per sample vial
Sample delivery precision	Typically < 0.5% RSD, 5 to 80 μ L (Degassed methanol/water 60:40 Dial-a-Mix, 1 mL/min, six replicates, phenone mix, 254 nm)
Injector needle wash	Integral, active, programmable
Sample carryover ¹	\leq 0.0025% (based on the following chromatographic conditions): <ul style="list-style-type: none"> • Column – XBridge C18, 4.6 x 50 mm, 3.5 μm • Mobile phase – 70% water/30% methanol • Needle wash – 100% Methanol • Challenge sample – 4.0 mg/mL caffeine (in mobile phase) • Carryover standard – 0.4 mg/mL caffeine (in mobile phase) • Injection volume – 10 μL
Injection accuracy	\pm 1 μ L (\pm 2%), 50 μ L, N = 6 Sample is degassed water, analytical solvent is degassed methanol
Standard sample vial	2 mL
Sample temperature control (optional)	Ambient minus 25 $^{\circ}$ C or 4 $^{\circ}$ C (whichever is greater) to 40 $^{\circ}$ C, in 1 $^{\circ}$ C increments <ul style="list-style-type: none"> • \pm 3 $^{\circ}$C temperature accuracy • 60-minute time limit from lab ambient to heating set-point • 90-minute time limit from lab ambient to cooling set-point
Advanced operations	Stat runs, auto additions, auto standards
Injection volume range	0.1 to 100 μ L, standard 0.1 to 2000 μ L, with optional sample loop
Injector linearity	> 0.999 coefficient of deviation (1 to 100 μ L)

a.

D Solvent Considerations



Warning: To avoid chemical hazards, always observe safe laboratory practices.

D.1 Introduction

D.1.1 Clean solvents

Clean solvents provide these benefits:

- Reproducible results
- Operation with minimal instrument maintenance

A dirty solvent can cause these conditions:

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

D.1.2 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 generally maintain their purity from lot to lot; use them to ensure the best possible results.

D.1.3 Solvent reservoirs

The following recommendations apply to the solvent reservoirs used with the separations module:

- When using the instrument for general chromatography (that is, reverse and/or normal phase and gel permeation [GPC]), Waters recommends the use of high-quality lab glassware made of borosilicate glass for all reservoirs (solvent, seal wash & needle wash).
- When using techniques such as ion chromatography, where glass containers can contribute ionic contamination (sodium and/or chloride ions), it is acceptable to use laboratory-grade polypropylene or polyethylene containers as reservoirs.
- When using the instrument in combination with a mass spectrometric detector, please refer to recommendations within the most recent version of “Controlling Contamination in UPLC/MS and HPLC/MS Systems” at the Waters’ website (www.waters.com) in the Support Library under Waters Division/Services & Support.

D.1.4 Preparation checklist

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

- Filter solvents with a 0.45- μm filter.
- Degas the solvent.
- Stir the solvent.
- Keep solvents in a location that is free from drafts and shock.

D.1.5 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.

D.1.6 Buffers

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

D.1.7 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.

D.1.8 GPC solvents and seal choices

For GPC separations, see the following table for plunger seal and seal wash solution recommendations. If required, use an intermediate solution to prevent immiscibility or precipitation problems when you switch between GPC and reversed-phase analytical solvents.

Table D–1: Recommended plunger seal and seal wash solution to be used for GPC solvents:

GPC solvent	With standard seal ^a Fluoroloy G (yellow)	With optional seal ^b Alliance Clear Seal (opaque)	Seal wash solution
Chloroform	Recommended	Recommended	100% methanol
Dimethylsulfoxide (DMSO)	Recommended	Recommended	100% methanol
Dimethylacetamide (DMAC) ^c	Do not use	Strongly recommended	100% methanol
Hexafluoroisopropanol (HFIP) ^d	Recommended	Strongly recommended	50:50 methanol/water
Methylene chloride	Recommended	Recommended	100% methanol

Table D–1: Recommended plunger seal and seal wash solution to be used for GPC solvents: (continued)

GPC solvent	With standard seal ^a Fluoroloy G (yellow)	With optional seal ^b Alliance Clear Seal (opaque)	Seal wash solution
N,N-dimethylformamide (DMF) ^c	Do not use	Strongly recommended	50:50 methanol/water
N-methyl-2-pyrrolidone (NMP) ^c	Do not use	Strongly recommended	50:50 methanol/water
Ortho-dichlorobenzene (o-DCB)	Not recommended	Recommended	None
Tetrahydrofuran (THF)	Strongly recommended	Strongly recommended	50:50 methanol/water
Toluene	Not recommended	Recommended	None
1,2,4 Trichlorobenzene (TCB) ^e	Not recommended	Recommended	None
Water ^f	Recommended	Recommended	20:80 methanol/water

a. Package contains two seals, PN: WAT270938.

b. Package contains two seals, PN: 700001326.

c. This solvent is typically used with an additive of 0.05M lithium chloride or lithium bromide.

d. HFIP is typically used with an additive of 0.05M sodium trifluoroacetate.

e. Remove installed seal wash seals (if any) to reduce risk of premature plunger-seal failure.

f. Most aqueous GPC separations use a salt or buffer.

To prevent premature plunger seal failure when using toluene, o-dichlorobenzene (o-DCB), or 1,2,4 trichlorobenzene (TCB), Waters recommends the use of the optional Alliance Clear Seals, the removal of installed seal wash seals, and no seal wash solvent. The use of graphite-filled PTFE (black) seals is not recommended when any salts are present in the solvent.

D.2 Solvent compatibility

The separations module is constructed of high-quality (316) stainless steel components that, with some minor restrictions, can be used with all solvents. This section lists the solvents that have and have not been approved for use with the separations module.

D.2.1 Solvents to avoid

The following solvents attack or dissolve the Teflon AF tubing in the in-line vacuum degasser:

- All perfluorosolvents produced by 3M under the trade name Fluorinert
- All perfluorosolvents produced by Ausimont under the trade names Galden and Fomblin

! **Notice:** Damage to the degassing tubing from exposure to perfluorosolvents is immediate and irreversible.

Long-term static exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) will cause pitting and corrosion of stainless steel parts. When using these salts, flush your system thoroughly with water if the pump will be idle for more than two days. (See “Wet prime the system” on page 76).

D.2.2 Solvents to use

Materials of construction used in the separations module are nonreactive with most acids, bases, salts, and organic solvents.

The solvents listed in the following four tables have been approved for use with the separations module. These include salts, acids and bases in concentrations up to 1 M (unless otherwise noted), and organic solvents in concentrations of up to 100% (unless otherwise noted). Higher concentrations can be used in many instances.

Information on the use of a specific solvent or concentration that is not listed in this manual can be obtained by contacting Waters.

Table D–2: Aqueous buffers for use with the separations module

Aqueous buffers			
Acetate	K ₂ SO ₄	Na ₂ S	Perfluorobutyric acid
Al ₂ SO ₄	K ₃ Fe(CN) ₆	Na ₂ CO ₃	NH ₄ Cl
Ca(OCl) ₂	K ₄ Fe(CN) ₆	Na ₂ SO ₄	Phosphate
CaCl ₂	KBr	NaCl	Tartrate
Citrate	KCl	Sodium acetate	Trilithium citrate
H ₂ O ₂ up to 10%	KHCO ₃	NaH ₂ BO ₃	Tris
HIBA	KMnO ₄	NaHCO ₃	4-(2-pyridylazo) resorcinol monosodium salt monohydrate
K ₂ CO ₃	KNO ₃	NaHSO ₄	
K ₂ Cr ₂ O ₃	LiClO ₄	NaNO ₃	
K ₂ S	Na ₂ B ₄ O ₇	NaOCl	

Table D–3: Acids for use with the separations module

Acids			
Acetic acid, glacial	Hydrochloric acid	Perchloric acid	Acetic acid, glacial
Benzoic acid	Lactic acid	Phosphoric acid	Benzoic acid
Chromic acid	Methanesulphonic acid	Pyridine-2,6-dicarboxylic acid	Chromic acid
Citric acid	Nitric acid, up to 37.5% (6 N)	Sulfuric acid, up to 0.20 M	Citric acid

Table D-3: Acids for use with the separations module

Acids			
Formic acid	Octanesulphonic acid	Trifluoroacetic acid (TFA), up to 10%	Formic acid
Glyceric acid	Oxalic acid		Glyceric acid

Table D-4: Bases for use with the separations module

Bases	
Ba(OH) ₂	NaOH, up to 10 M
KOH	NH ₄ OH, up to 3 M
LiOH	Tetramethylammonium hydroxide pentahydrate

Table D-5: Organic solvents for use with the separations module

Organic solvents			
4-cyanophenol	Chloroform	Ethylene glycol	Methylene chloride
Acetone	Cyclohexane	Formaldehyde	<i>n</i> -Propanol
Acetonitrile	Cyclohexanone	Heptane	Phenol
Amyl acetate	Dibutyl phthalate	Hexane	Tetrahydrofuran (THF)
Benzaldehyde	Dimethyl formamide	iso-Octane	Toluene
Benzene	Dimethyl sulfoxide	iso-Propanol	Waters PIC Reagents
Benzyl alcohol	Ethanol	Lysine hydrochloride	Xylene
Butanol	Ethyl acetate	Methanol	
Carbon tetrachloride	Ethylene dichloride	Methyl ethyl ketone	

D.3 Solvent miscibility

Before you change solvents, refer to the following table to determine the miscibility of the solvents to be used. When you change solvents, be aware of these issues:

- Changes involving two miscible solvents are made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as methanol).
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- Buffers dissolved in water can precipitate when mixed with organic solvents.

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

Table D–6: Solvent miscibility

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (@1 atm)	Miscibility number (M)	λ cut-off (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	<i>P</i> -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	Butyl alcohol	3.00	117.7	—	—
3.9	Butanol	3.01	177.7	15	—
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—

Table D–6: Solvent miscibility

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (@1 atm)	Miscibility number (M)	λ cut-off (nm)
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	Dimethylformamide	0.90	153.0	12	—
6.5	Dimethylsulfoxide	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
7.3	Formamide	3.76	210.5	3	—
9.0	Water	1.00	100.0	—	—

D.3.1 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 between 25 and 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 cut-off point for miscibility.

D.4 Buffered solvents

When using a buffer, use a good quality reagent and filter it through a 0.45- μ m filter.

Do not leave the buffer stored in the system after use. Flush all flow 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). Flush using a minimum of 45 mL.

D.5 Head height

Position the solvent reservoirs at a level above the separations module or on top of the separations module (with adequate spill protection).

D.6 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 twice the pressure of either water or methanol alone.

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

D.7 Mobile phase solvent degassing

Mobile phase difficulties account for 70% or more of all liquid chromatographic problems. Using degassed solvents is important, especially at wavelengths below 220 nm. Degassing provides these benefits:

- Stable baselines and enhanced sensitivity
- Reproducible retention times for eluting peaks
- Reproducible injection volumes for quantitation
- Stable pump operation

D.7.1 Gas solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount depends on these factors:

- The chemical affinity of the gas for the liquid.
- The temperature of the liquid.
- The pressure applied to the liquid.

Changes in the composition, temperature, or pressure of the mobile phase can lead to out-gassing.

D.7.1.1 Effects of intermolecular forces

Non-polar gases (N₂, O₂, CO₂, He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent whose intermolecular attractive forces are similar to those in the gas—like dissolves like.

D.7.1.2 Effects of temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H₂O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

D.7.1.3 Effects of partial pressure

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

D.7.2 Vacuum degassing

The in-line vacuum degasser operates on the principle of Henry's Law to remove dissolved gases from the solvent. According to Henry's Law, the mole fraction of a gas dissolved in a liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced, for example, by evacuation, then a proportional amount of that gas comes out of solution. (See "[Vacuum degassing](#)" on page 231 and "[Degasser considerations](#)" on page 25.)

Tip: Vacuum degassing can change the composition of mixed solvents.

D.7.2.1 Vacuum degassing considerations

The longer a solvent is exposed to a vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- Flow rate – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- Surface area of the degassing membrane – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

D.8 Wavelength selection

The tables in this section provide UV cut-off values for these items:

- Common solvents
- Common mixed mobile phases
- Chromophores

D.8.1 UV cut-offs for common solvents

The following table shows the UV cut-off (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 cut-off increases baseline noise due to the absorbance of the solvent.

Table D-7: UV cut-off wavelengths for common chromatographic solvents

Solvent	UV cut-off (nm)	Solvent	UV cut-off (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Isooctane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	<i>n</i> -Pentane	190
Cyclopentane	200	<i>n</i> -Propanol	210
Diethyl amine	275	<i>n</i> -Propyl chloride	225
Dioxane	215	Nitromethane	380
Ethanol	210	Petroleum ether	210
Ethyl acetate	256	Pyridine	330

Table D-7: UV cut-off wavelengths for common chromatographic solvents (continued)

Solvent	UV cut-off (nm)	Solvent	UV cut-off (nm)
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

D.8.2 Mixed mobile phases

The following table provides approximate wavelength cut-offs 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.

Table D-8: Wavelength cut-offs for different mobile phases

Mobile phase	UV cut-off (nm)	Mobile phase	UV cut-off (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoroacetic 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	190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Potassium phosphate, dibasic, 10 mM	190		
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

D.8.3 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.

Table D–9: Refractive indices for common chromatographic solvents

Solvent	RI	Solvent	RI
Fluoroalkanes	1.25	Tetrahydrofuran (THF)	1.408
Hexafluoroisopropanol (HFIP)	1.2752	Amyl alcohol	1.410
Methanol	1.329	Diisobutylene	1.411
Hexafluoroisopropanol (HFIP)	1.2752	Amyl alcohol	1.410
Water	1.33	<i>n</i> -Decane	1.412
Acetonitrile	1.344	Amyl chloride	1.413
Ethyl ether	1.353	Dioxane	1.422
<i>n</i> -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	<i>N,N</i> -Dimethyl Formamide (DMF)	1.428
Ethyl acetate	1.370	<i>N,N</i> -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
<i>n</i> -Propanol	1.38	Dimethyl sulfoxide (DMSO)	1.477
Methylethylketone	1.381	Toluene	1.496
Diethyl amine	1.387	Xylene	~1.50
<i>n</i> -Propyl chloride	1.389	Benzene	1.501
Methylisobutylketone	1.394	Pyridine	1.510
Nitromethane	1.394	Chlorobenzene	1.525
1-Nitropropane	1.400	<i>o</i> -Chlorophenol	1.547
Isooctane	1.404	Aniline	1.586
Cyclopentane	1.406	Carbon disulfide	1.626