

2489 UV/Visible Detector

Overview and Maintenance 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 (GLP), and consult your organization's standard operating procedures as well as your local requirements for safety.

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 2489 UV/Visible Detector

Power cord replacement hazard



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

FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the user's authority to operate the equipment. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Electrical power safety notice

Do not position the 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 the device, 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
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia EMC compliant

Symbol	Definition
	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.
	Serial number
	Part number catalog number

Audience and purpose

This guide is intended for personnel who install, operate, and maintain the Waters 2489 UV/Visible detector.

Intended use of the 2998 Photodiode Array Detector

Waters designed the 2489 UV/Visible detector to analyze and monitor many compounds.

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.

Quality control

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

EMC considerations

Canada spectrum management emissions notice

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

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

ISM Classification: ISM Group 1 Class B

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

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

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

EC authorized representative



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1

Theory and Principles of Operation

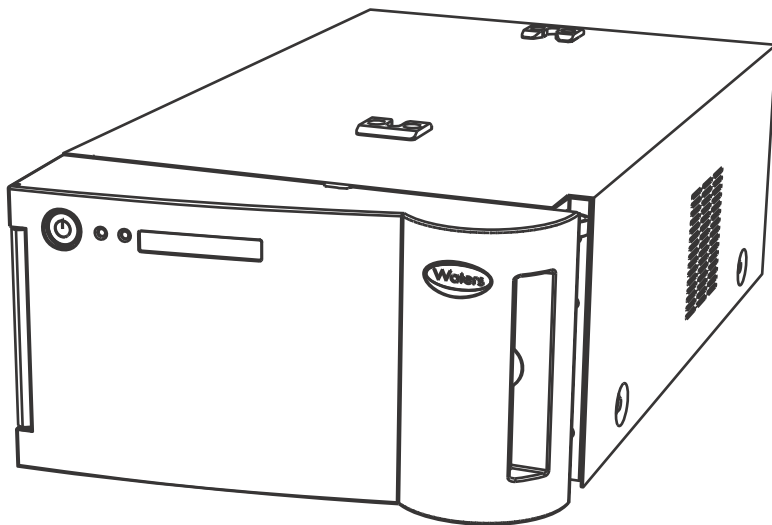
This chapter summarizes the 2489 UV/Visible Detector features and describes the theory and principles of operation.

See also: [Appendix B](#) for system specifications and [Appendix C](#) for information on high-performance liquid chromatography (HPLC) solvent considerations.

1.1 Detector description

The 2489 UV/Vis detector is a two-channel ultraviolet/visible (UV/Vis) detector designed for high-performance liquid chromatography (HPLC) applications.

Figure 1–1: 2489 UV/Vis detector



The detector can operate as a stand-alone unit (with an integrator) or as an integral part of a Waters chromatography system.

The detector can be configured with either Empower[®] or MassLynx[®] chromatography data software.

Table 1–1: Detector capabilities

Capability	Description
Stand-alone programmability	Stores as many as five user-defined programs (or methods) consisting of as many as 50 programmable, timed events and two threshold events each.

Table 1–1: Detector capabilities (continued)

Capability	Description
Single or dual-wavelength	Monitors absorbance at one or two discrete wavelengths.
Wavelength verification reference filter	Ensures wavelength accuracy.
Automatic, second-order filter	Automatically engaged for wavelengths of 370 nm and greater and removed for wavelengths of 369 nm or less.
Spectrum scan and storage	Supports spectrum scan, display, subtraction, storage, and playback, in addition to standard absorbance and UV/Vis functionality.
Method editing and storage	Supports basic method programming, storage, and retrieval from the front panel.
Full diagnostic capability	Supports built-in diagnostic tools to optimize functionality and performance.
Two contact closure outputs	The detector has two configurable switches, each of which can accommodate a maximum of ± 30 Vdc, 1.2-A current carrying capacity, and 0.5-A current switching. The switches (SW1 and SW2) can trigger fraction collectors and other external devices. They can also activate according to time, absorbance threshold, or ratio criteria.
Thermal wander management	The detector's insulation, fans, and baffling are designed to mitigate thermal instability caused by ambient temperature changes.
Median Baseline Filter (MBF)	A variation of the data mode, the MBF decreases the effects of gradient separations on the chromatographic baseline. It enhances the UV detector's baseline stability by decreasing its curvature, making the development of integration methods easier.
If your flow cell requires a cuvette:	
Cuvette qualification	Facilitates qualification of the detector by insertion of a standard in a cuvette without breaking any plumbing connections. Waters qualification kits, available in cuvette form, support this feature, which allows the detector to serve as a benchtop spectrophotometer.
Cuvette sample analysis	Allows recording of the spectrum of any sample placed in the cuvette.

1.2 Principles of operation

To use the detector effectively, become familiar with its optical and electronic design and the theory and principles of its operation.

This section describes the following parts and functions of the detector:

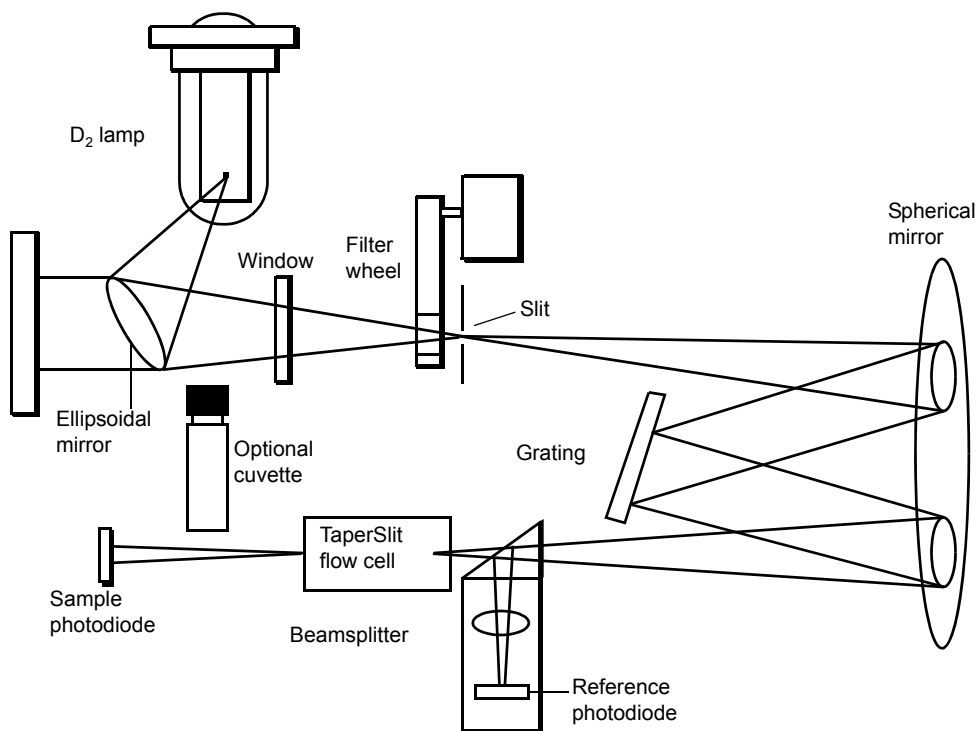
- Optics
- Wavelength verification and test
- Flow cell
- Electronics

1.2.1 Detector optics

The 2489 UV/Vis detector optics are based on a Fastie-Ebert monochromator and include these components:

- High brightness deuterium (D_2) lamp
- Two mirrors: one off-axis, ellipsoidal mirror and one spherical mirror
- Filter wheel
- Shutter, wavelength calibration filter, and second-order filter
- Entrance slit
- Blazed, plane-holographic, diffraction grating
- Beamsplitter
- Sample and reference photodiodes
- Waters TaperSlit™ Flow Cell (its entrance is the exit slit of the monochromator)
- Cuvette holder for flow cells requiring a cuvette.

Figure 1–2: 2489 UV/Vis detector optics assembly



1.2.1.1 Optics assembly light path

The detector provides an extremely efficient design for exceptionally high light throughput. It operates as follows:

1. The ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel and onto the entrance slit. The spherical mirror directs light toward the grating. A different portion of the spherical mirror focuses dispersed light of a particular wavelength band, determined by the grating angle, onto the entrance of the flow cell. Light exiting the flow cell passes through the cuvette location to the sample photodiode.
2. The beamsplitter, located just ahead of the flow cell, diverts a portion of the light to a reference photodiode.
3. When you enter a new wavelength through the detector's front panel (or through Empower or MassLynx software), the detector rotates the grating to the appropriate position.
4. The preamplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer, or integrator.

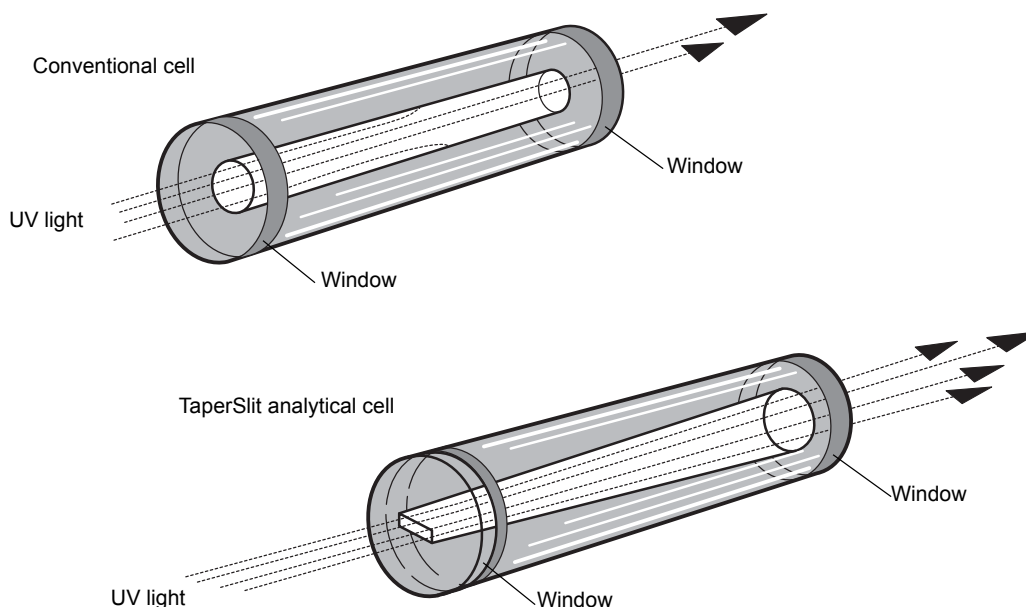
1.2.1.2 Waters TaperSlit flow cell

The Waters TaperSlit flow cell used in this detector renders the detector baseline less sensitive to changes in mobile phase refractive index (RI). RI changes occur during gradient separations or result from temperature or pump-induced pressure fluctuations.

To achieve RI immunity, a combination of a spherical mirror, a lens at the entrance of the flow cell, and a taper to the internal bore of the flow cell prevents light rays from striking the internal walls of the flow cell. An additional feature of the TaperSlit flow cell and the reason for its name is the shape of the flow cell entrance, which matches the shape of the entrance slit. The detector achieves higher light throughput for a given spectral resolution via the TaperSlit cell design, compared to a conventional flow cell with a circular entrance.

As shown in the figure below, in a conventional cell, light bends and hits the wall of the flow cell. Four beams go in, but only two come out. In the Waters TaperSlit analytical cell, the combination of the lens and TaperSlit bore geometry prevents light from hitting the cell walls. Four beams go in, and four beams come out.

Figure 1–3: Comparison of flow cell characteristics



The standard analytical, inert, and LC/MS cells have a path length of 10 mm. The semi-prep and microbore cell path length is 3 mm. The autopurification cell path length is 1.0 mm. A variable path length flow cell (path length 0.15 to 3 mm) is also available.

1.2.1.3 Selecting the appropriate sampling rate

A sufficient number of points must fall across a peak to define its shape. Thus, at very low sampling rates, the definition between peaks is lost. The chromatography data software uses the index of the data point closest to the end time, minus the index of the data point closest to the start time, to calculate a value for each integrated peak in the chromatogram.

Tip: In Empower software, this value is called the Points Across Peak value, and it appears in the Peaks table, at the bottom of the Review Main window. If the Points Across Peak field is not visible, right-click anywhere in the table, and then click Table Properties. Click the Columns tab, and then scroll down to find the Points Across Peak field. Clear the check box, and then click OK.

If the value for the narrowest peak of interest is less than 15, you must specify a higher sampling rate in the instrument method. If the value is greater than 30, specify a lower sampling rate in the instrument method.

Set the sampling rate to the lowest value required to achieve 15 or more points across the narrowest peak. Excessively high sampling rates can slow the system with more data than you need for your analysis.

1.2.1.4 Filtering noise

The detector provides a Hamming filter to minimize noise. The Hamming filter is a digital finite impulse response filter, which creates peak height degradation and enhances the filtering of high frequency noise.

The behavior of the filter depends on the filter time constant you select. You can program a filter time to be Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you do not need to

enter a value. The filter constant is determined by the sampling rate. If you select Other, you can enter a value. However, the value you enter is rounded up or down to a value based on the sampling rate.

The filter time constant adjusts the time window over which the data is filtered, thus controlling the degree of baseline smoothing and the impact on peak height degradation. Optimizing this parameter in the method ensures the highest signal-to-noise ratios are achieved for a particular application.

Decreasing the time-constant settings produces these effects:

- Narrow peaks with minimal peak distortion and time delay.
- Very small peaks become harder to discriminate from baseline noise.
- Less baseline noise is removed.

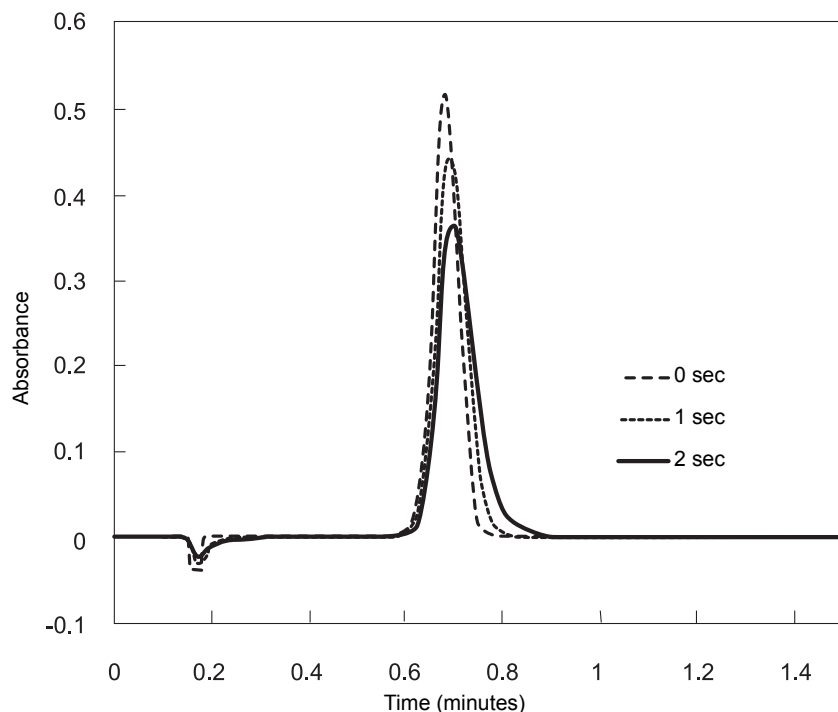
Increasing the time-constant settings produces these effects:

- Greatly decrease baseline noise.
- Shorten and broaden peaks.

The software includes fast or normal filtering constants at each sampling rate that are appropriate for high speed or high sensitivity applications respectively.

The following figure shows the relationship between increased filter time constant and absorbance.

Figure 1-4: Filter time constant comparison



Tip: Although the peak shape shows some distortion and the signal output is delayed with different time constants, the peak area remains the same.

1.2.2 Wavelength verification and test

The detector's deuterium arc lamp and integral erbium filter exhibit peaks in the transmission spectrum at known wavelengths. Upon startup, the detector verifies calibration by comparing the locations of these peaks with expected wavelengths based on calibration data stored in the detector's memory. If the results of this verification differ from the stored calibration by more than 1.0 nm, the detector displays a Wavelength Verification Failure message. The detector verifies, rather than recalibrates, on startup to avoid errors arising from residual materials left in the flow cell or the cuvette, when required.

Requirement: If your flow cell requires a cuvette, always ensure that the cuvette is removed and the cuvette holder and front door are secured during startup verification.

You can initiate a manual wavelength calibration at any time. A manual calibration replaces the previous calibration data with new data.

See ["Wavelength calibration" on page 67](#) for the manual wavelength calibration procedure.

The verification and calibration algorithms are virtually identical. However, the verification algorithm can issue an error message indicating that actual data do not match stored data where the calibration algorithm replaces the stored data with the new.

The detector wavelength verification procedures establish an approximate Home position using a grating homing sensor. Once Home is established, the detector locates and references the 656.1-nm peak in the deuterium lamp emission spectrum.

The integral erbium filter moves into the common light path ahead of the flow cell entrance slit, enabling the detector to locate three additional spectral features at these wavelengths:

- 256.7 nm (UV)
- 379.0 nm
- 521.5 nm

The verification tests for the detector require 5 minutes of lamp warmup time.

If you run the detector continuously, Waters recommends that you perform wavelength verification weekly by cycling power to the detector. See ["Wavelength calibration" on page 67](#).

1.3 Operational modes

The detector operates in single or dual-wavelength mode, allows spectrum scanning using a flow cell or a cuvette, and provides RatioPlot, difference plot, and MaxPlot functions.

Note that these modes are for local control of the detector only. Please check the Empower and MassLynx online help for additional control information in these environments.

1.3.1 Single-wavelength mode

Single-wavelength is the default mode of operation for the detector. The detector supports monitoring of a single wavelength from 190 nm to 700 nm, settable in 1-nm increments on channel A. You can configure the analog outputs for channel B while the detector is operating in

single-wavelength mode, so you can use channel B to obtain additional information about the wavelength selected on channel A.

In single-wavelength mode, the detector automatically engages the second-order filter for wavelengths 370 nm and above and removes it for wavelengths under of 370 nm. The second-order filter is an optical filter that blocks unwanted ultraviolet (UV) light from striking the diffraction grating and interfering with absorbance detection above 370 nm.

You can configure several additional parameters when using the detector in single-wavelength mode.

1.3.1.1 Primary parameters

Table 1–2: Major parameters applicable to single-wavelength mode

Parameter	Description
Wavelength, in nm	Specifies a wavelength for channel A, from 190 nm to 700 nm, settable in 1-nm increments.
Sensitivity, in AU	Specifies the scaling factor for the analog-output channels and corresponds to the AU value where the analog outputs become saturated at full-scale values. Absorbance units full-scale (AUFS) vary, from 0.0001 to 4.000 AUFS. Tip: Changing the sensitivity (AU) setting affects the 2-V output.
Chart polarity (+ or –)	Reverses the polarity of the charted chromatogram. Select + for a normal chromatogram, or select – for an inverted chromatogram. This function changes the direction of the plot on the 2-V output.
Filter time constant	Programs a filter time, in seconds. Options are Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you do not need to enter a value. The filter constant is determined by the sampling rate. If you select Other, you can enter a value, but the value entered is rounded up or down, to a value based on the sampling rate. Selecting Off or Other and entering a value of 0.0 disables all filtering.
Analog rate	Specifies a frequency value as high as 80 Hz.

1.3.1.2 Secondary parameters

Pressing Next on the absorbance (or HOME) screen while in single-wavelength mode, causes several pages of these secondary, or less frequently specified, parameters to appear:

- Absorbance offset (in mV)
- Autozero on inject
- Autozero on λ changes

“Primary and secondary functions” on page 56 and the table on page 49 explain the functions, ranges, and defaults of these parameters.

1.3.2 Dual-wavelength mode

In dual-wavelength mode, the detector can monitor two wavelengths, one on channel A and one on channel B. The sampling frequency is reduced to 1 or 2 Hz, limiting the use of this mode to more standard chromatography where peak widths span at least 20 seconds to enable full characterization of a peak. You can use dual-wavelength mode to obtain additional information about an analyte by running in the RatioPlot or MaxPlot mode.

You can select any two wavelengths from 190 nm to 700 nm.

In dual-wavelength mode, the following conditions apply:

- If both selected wavelengths are greater than 370 nm, the detector applies the second-order filter to block unwanted UV light.
- If both selected wavelengths are less than or equal to 370 nm, the detector removes the second-order filter.
- If the selected wavelengths bracket the 370 nm threshold, the detector does not apply the second-order filter and issues a warning message that any data collected for the wavelength above 370 nm can contain inaccuracies because of possible UV light interference (second-order effects).

1.3.2.1 Chart-out selection modes

When operating in dual-wavelength mode, the detector offers these choices for analog output in addition to the selections offered in single-wavelength mode and explained on page 21. The default selection for dual-wavelength mode is Absorbance.

Table 1–3: Additional options for analog output in dual-wavelength mode

Mode option	Description of mode
Absorbance (A and B)	Standard LC mode where the current absorption is scaled and sent directly out the analog output. The scaling depends on the AU setting and the absorbance offset. The absorbance value is scaled for the 2-V analog output. If a setting of 1 AU/V is desired, you can set an AU of 2.0000 for either the A or B output channels that can be controlled independently, even in single-wavelength mode.
MaxPlot	Results in the output of the larger of the two absorbance values scaled to the selected AU setting. Use this mode when observing, with one data channel, multiple compounds that exhibit absorbancies at two separate wavelengths.

Table 1–3: Additional options for analog output in dual-wavelength mode (continued)

Mode option	Description of mode
RatioPlot (A/B)	Produces the ratio of absorbance from two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak, which results in a nonsquared response. Instead of a programmable AU, the detector provides minimum and maximum ratio values that scale the ratio plot proportionally. In addition, a configurable minimum absorbance threshold activates ratio output scaling only when it reaches the absorbance at both wavelengths.
Difference Plot (A-B)	Plots the arithmetic difference in absorbance for the two monitored wavelengths.

1.3.3 Spectrum scanning

When the detector is operating under the control of the chromatography data software, the scanning function is disabled.

You can use the detector as a spectrophotometer, to acquire spectra from either the flow cell or the cuvette, when required. You can scan and store as many as three spectra (three reference or zero scans, or three sample scans) for playback or to compare with other spectra.

The major difference between the detector and a double-beam spectrophotometer is that the detector uses only one flow cell, or cuvette when required, rather than a sample and a reference pair.

Recommendation: If your flow cell requires a cuvette, use a matched pair of cuvettes for the zero and sample scans.

The detector obtains an absorbance spectrum by performing two types of scans on the flow cell or using the cuvette:

- Zero scan – Characterizes the baseline absorbance spectrum of a solvent.
- Sample scan – Subtracts the zero scan, so the results displayed or charted are of the sample only.

To obtain a spectrum of a sample using the detector, run a zero scan first, followed by a sample scan. Typically, you run the zero scan using pure solvent. The sample scan is a scan of the analyte dissolved in that solvent.

Spectra can be simultaneously charted on the channel A output, or acquired and stored in memory for later playback.

See also: “Scanning using the cuvette” on page 86 and “Scanning using a flow cell and a syringe” on page 88.

1.3.4 Cuvette operations

Note: This section pertains only to flow cells that require a cuvette.

Detector cuvette option is used to measure the absorbance spectrum of a sample in a cuvette.

To generate and store a spectrum:

1. Acquire a zero scan, which measures the absorbance of the contents of the cuvette and flow cell over the desired wavelength range.
2. Acquire a sample (absorbance) scan, which measures the absorbance of the analyte dissolved in mobile phase.

The detector subtracts the zero scan from the sample scan to create a sample spectrum.

Since the cuvette scan is acquired by measuring the absorbance from a light path that includes both the flow cell and the cuvette, the solvent conditions in the flow cell must be identical for both scans. (See [“Scanning using the cuvette” on page 86.](#))

1.3.5 RatioPlot

The detector allows ratio plotting: comparing the absorbances of a compound or analyte at two wavelengths. The RatioPlot divides absorbances at two selected wavelengths and plots the resulting ratio on a data system over one output channel (channel A). Use the RatioPlot when detecting hidden components within individual peaks.

The RatioPlot of a spectrally homogeneous peak appears as a rectangular wave. The RatioPlot of an impure peak appears as a distorted wave. When obtaining a RatioPlot, you must be operating the detector in dual-wavelength mode; the RatioPlot is output on the selected channel. (See [“Obtaining a RatioPlot” on page 70.](#))

1.3.6 MaxPlot

The MaxPlot function monitors absorbance at two selected wavelengths and plots the maximum absorbance value for each sample component. To obtain a MaxPlot, you must operate the detector in dual-wavelength mode. The MaxPlot outputs the greater of the two absorbance values on the selected channel.

For an explanation of the MaxPlot procedure, see the discussion [“Obtaining a MaxPlot” on page 70.](#)

1.3.7 Thermal-wander management

The detector's insulation, fans, and baffling are designed to mitigate thermal instability caused by ambient temperature changes.

2 Installing the Detector

The detector requires connections to electrical power and to sample and waste lines to operate in any standard laboratory environment. This chapter describes how to install the detector, and connect it to the electrical supplies and to other equipment in an HPLC system.

2.1 Before you begin

Requirement: To install the 2489 UV/Vis detector, you should know how, in general, to set up and operate laboratory instruments and computer-controlled devices and also how to handle solvents.

Before installing the detector, ensure that

- the required components are present.
- none of the shipping containers or unpacked items are damaged.

2.2 Unpacking and inspecting



Warning: To avoid injury, Waters recommends that two people lift the 2489 UV/Vis detector.

The detector is packed and shipped in one carton, which contains the following items:

- 2489 UV/Vis detector startup kit
- Power cord

2.2.1 Unpacking the detector

To unpack the detector:

1. Unpack the contents of the shipping carton. As you unpack the carton, check the contents to make sure you have received all items.
2. Check the contents of the startup kit.
3. Save the shipping carton for future transport or shipment.

2.2.2 Inspecting the detector

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

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

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

Tip: Ensure that the instrument serial number, found on the nameplate on the rear panel or inside the front door of the detector, corresponds to the number on the instrument integrity certificate.

2.3 Selecting a laboratory site

Install the detector in an area that meets the requirements listed in the table below.



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


Table 2–1: Installation site requirements


Parameter	Requirement
Operating temperature range	4 to 40 °C (39 to 104 °F)
Operating relative humidity	20 to 80%, non-condensing
Shipping and storage temperature range	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity range	20 to 85%, non-condensing
Bench space	12.7 cm (5 inches) clearance at rear
Vibration	Negligible
Static electricity	Negligible
Power	Grounded ac, 100 to 240 Vac, 50 to 60 Hz. Minimal power transients and fluctuations. Power cord type required: <ul style="list-style-type: none"> • SVT, in the United States • HAR type (or better), in Europe For information regarding the type of cord to use in other countries, contact your local Waters distributor.

Requirement: You must mount the detector on a level surface to allow for proper functioning of the drip management system (drain tube), which you connect to a waste reservoir to divert solvent leaks away from the flow cell.

2.4 Stacking system modules

This procedure applies to system modules that are equipped with interlocking features.

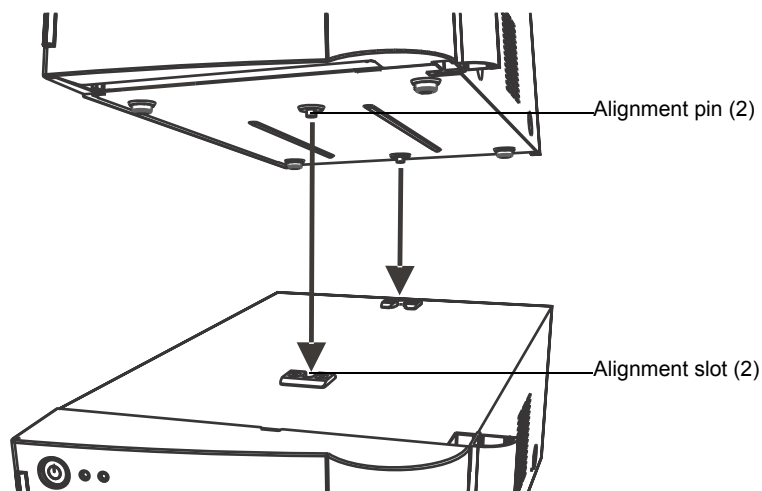
 **Warning:** To avoid spinal and muscular injury, do not attempt to lift a system module without assistance.

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

To stack the modules:

1. Install two alignment pins to the bottom of the module that will be placed on top.
2. Place the rear feet of the module that you are adding atop the previously added module in the system stack, and slide it backward until its rear alignment pin rests in the rear alignment slot on the previously added module.

Figure 2–1: Aligning pins with slots



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

2.5 Connecting to the electricity source

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



Warning: Avoid electrical shock:

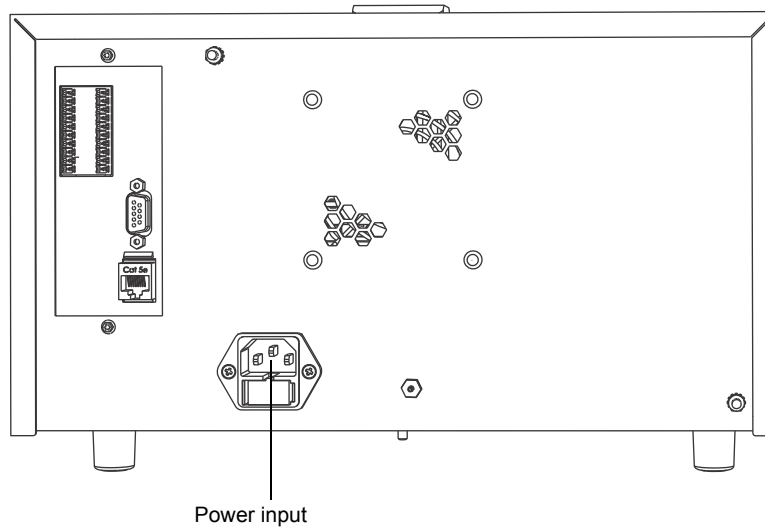
- Use power cord SVT-type in the United States and HAR-type or better in Europe. For other countries, contact your local Waters distributor.
- Power-off and unplug the detector before performing any maintenance on the instrument.
- Connect the detector to a common ground.

To connect to the electricity source:

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

1. Connect the female end of the power cord to the receptacle on the rear panel of the detector.

Figure 2–2: Location of power input on UV/Visible detector rear panel



2. Connect the male end of the power cord to a suitable wall outlet.
3. Press the power switch on the front door to power-on the detector.

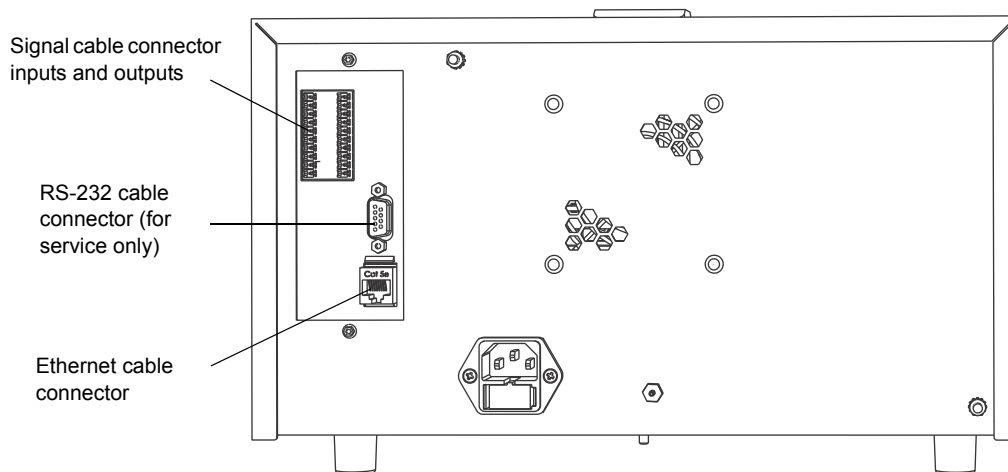
Result: The detector runs a series of startup diagnostic tests while the lamp LED blinks green. The lamp LED shows steady green when the lamp is ignited.

2.6 Connecting signal cables

See also: *Waters Ethernet Instrument Getting Started Guide.*

The following figure shows the rear panel location of the connectors used to operate the detector with external devices.

Figure 2–3: Location of connectors on UV/Visible detector rear panel

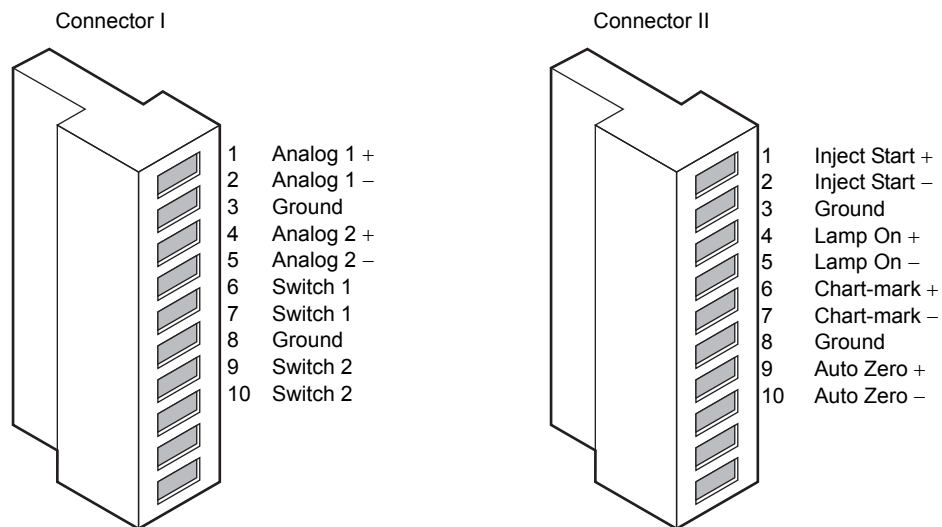


The signal connections necessary for your detector depend on the signal connections available on the other instruments in the HPLC system.

2.6.1 Connecting I/O cables

The rear panel includes two removable connectors that hold the pins for the I/O signals, as shown in the figure below. These connectors are keyed so that you can insert them one way only.

Figure 2–4: I/O signal inputs and outputs



2.6.1.1 I/O signals

The table below describes each of the signals available on the I/O connectors. See [Appendix B](#) for details on the electrical specifications for the signals.

Table 2–2: I/O signals for the detector

Signal	Description
Inject Start ¹	TTL contact closure. Configurable input to initiate sequencing of time-programmed events. Defines the start of a run (typically an injection) and resets and starts the run-time clock to 0.00 minutes. Initial conditions apply immediately.
Lamp On/Off ¹	Configurable input to allow an external device to turn the deuterium lamp off and on.
Chart-mark ¹	Configurable input to add a chart-mark (at 10% of full scale) to either or both analog output channels.
Autozero ¹	Configurable input to autozero either or both channels.
Analog 1 ²	2-V full-scale analog output signal of channel A (scaled to the current AU setting).
Analog 2 ²	2 V full-scale analog output signal of channel B (scaled to the current AU setting).
Switch 1 (2)	Used to connect to a fraction collector. Can be controlled by threshold and timed events.
Switch 2 (2)	

1. Inject Start, Chart-mark, Autozero, and Lamp inputs are configurable from the detector first Configuration screen by setting the appropriate parameter to High. See for more detail.
2. See the discussion of attenuation in the detector analog outputs in ["Connecting to the electricity source" on page 29](#).

Required tools and materials

- Small, flat-blade screwdriver
- Electrical insulation stripping tool

To connect signal cables from other HPLC system devices to the I and II terminals on the rear panel of the detector:

1. Remove terminal I or II (see [page 31](#)).
2. Unscrew the connecting pin terminal.
3. Using the stripping tool, strip the wire about 3 mm (1/8 inch) from the end.
4. Insert the stripped wire into the appropriate connector.
5. Tighten the screw until the wire is held securely in place.
6. Reinsert the terminal.
7. Press firmly to ensure that it is inserted fully.

2.6.2 Connecting signal cables from the rear panel and Ethernet connectors to the detector

The rear panel of the detector provides two analog connectors and an Ethernet communications port for operating the detector with external devices.

Make the signal connections to your detector, considering the following conditions:

- Operating mode you select for the detector (stand-alone or remote control)

- 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 two rear panel connectors and the Ethernet connector.

2.6.3 Making Ethernet connections

The detector's rear panel also includes one Ethernet interface connector for digital signal communications. Use this connector for devices such as these:

- Network adapter card in the Empower workstation
- Solvent manager
- MassLynx v4.1 or later workstation

The Ethernet connector mates with standard Ethernet cable.

! **Notice:** To avoid possible damage to components, power-off all instruments on the Ethernet connector before you connect an Ethernet cable to an instrument.

2.6.3.1 Connecting the Ethernet to chromatography data software

When controlling the detector from chromatography data software (Empower or MassLynx), you can use the Ethernet interface to send and receive information.

When connecting via the Ethernet to chromatography data software, be aware of the following:

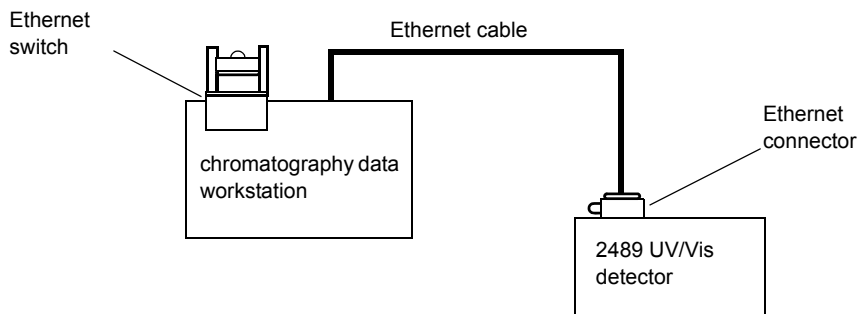
- When in dual-wavelength mode, you must select a sampling rate of 1 point per second in the data system's method editor.
- The maximum range of the detector's time-constant setting depends on the wavelength mode and sampling rate selected. See [page 63](#).
- Empower and MassLynx software allow the detector to operate, in both single and dual-wavelength, modes with a wavelength range of 190 to 700 nm.

To connect Ethernet cables from the detector to chromatography data software:

1. Connect the single receptacle end of the Ethernet cable to your chromatography data workstation by attaching the cable to your network card or Ethernet switch.
Note: The Ethernet cable is supplied with the workstation.
2. Connect the other end of the cable to the Ethernet connector on the rear panel of the detector.

! **Notice:** To avoid intermittent Ethernet communication failures, ensure that the maximum total cable length between Ethernet devices in a system is 20 meters. The maximum recommended cable length between two Ethernet devices is 4 meters.

Figure 2–5: Ethernet connections to the detector in a chromatography data workstation



Tip: When connecting the detector to a chromatography data workstation, all detector parameters not configurable by the chromatography data software in use, defer to local control.

2.6.4 Starting a method

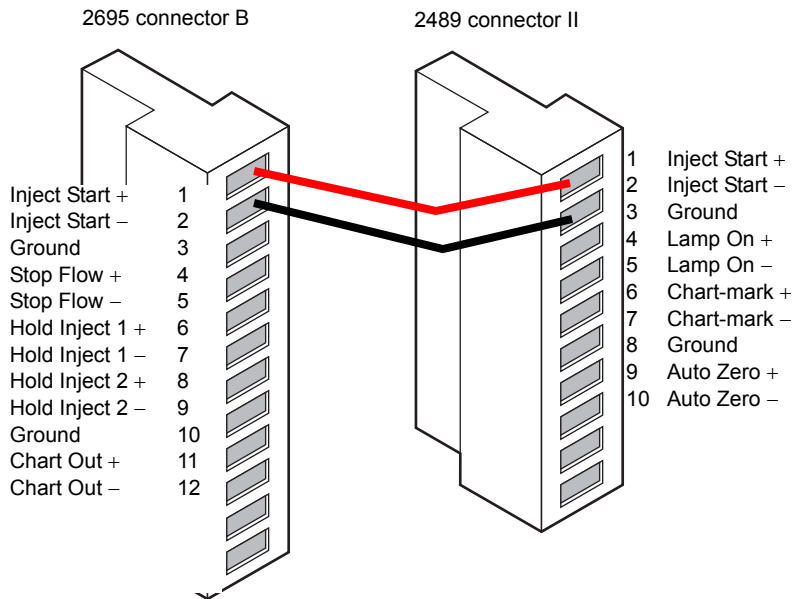
To start a method on the detector at the start of an injection from the 2695 (IEEE) separations module, make the connections summarized in the table and illustrated in the figure below.

Requirement: Connect the inject-start cable is only if the 2695 is configured in IEEE mode. If the 2695 is configured in Ethernet mode, do not connect the inject-start cable.

Table 2–3: Separations module connections to detector for starting a method

2695 separations module (B inputs and outputs)	2489 UV/Vis detector (II)
Pin 1 Inject Start	Pin 1 Inject Start +
Pin 2 Inject Start	Pin 2 Inject Start –

Figure 2–6: 2695 separations module connections to the detector, for starting a method



Note: If the injector is an 2695 separations module running in Ethernet mode, the inject start cable should not be connected. However, if the injector is an 2695 separations module running in IEEE mode, the inject start cable should be connected.

2.6.5 Turning the detector lamp on or off

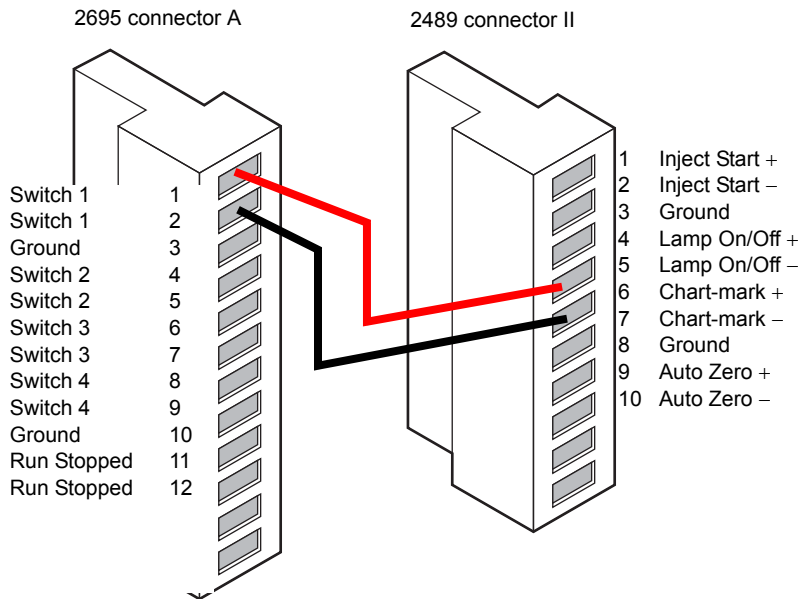
Before you can turn the detector lamp on or off from the 2695 separations module, you need to configure the lamp on/off signal at the front panel. You must change the default lamp configuration parameter setting from Ignore to High or Low. See the discussion “[Configuring event inputs \(contact closures\)](#)” on page 63 for more information.

After configuring the detector lamp’s on/off signal, you can turn the lamp on or off from the 2695 separations module by making the connections shown in the table and figure below.

Table 2–4: 2695 separations module connections to the detector (lamp on or off)

2695 separations module (A outputs)	2489 UV/Vis detector (II)
Pin 1 Switch 1	Pin 4 Lamp On/Off +
Pin 2 Switch 1	Pin 5 Lamp On/Off –

Figure 2–7: 2695 separations module connections to the detector for turning the lamp on or off



2.6.6 Connecting the detector to the 2695 separations module

You can connect the detector to the 2695 separations module to perform the following functions:

- Autozero
- Chart-mark on Inject

2.6.6.1 Generate Autozero

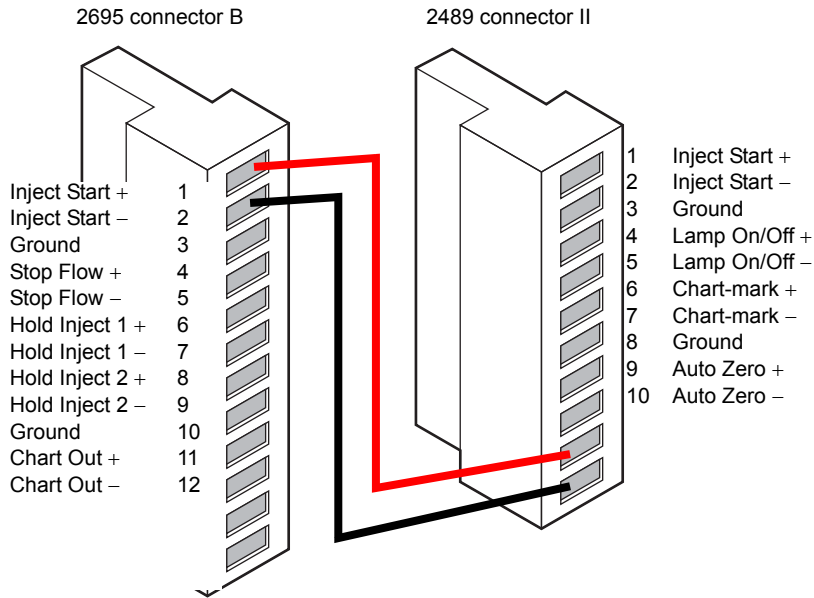
To generate the Autozero function of the detector at the start of an injection, make the connections summarized in the table and illustrated in the figure below.

Table 2–5: 2695 separations module connections to the detector, to generate autozero

2695 separations module (B inputs and outputs)	2489 UV/Vis detector (II)
Pin 1 Inject Start	Pin 9 Auto Zero +
Pin 2 Inject Start	Pin 10 Auto Zero –

Before you can generate an autozero from the 2695 separations module, you need to configure the autozero signal at the detector’s front panel. The default autozero signal is Low. See the discussion “[Configuring event inputs \(contact closures\)](#)” on page 63 for more information.

Figure 2–8: 2695 separations module connections to the detector for autozero on inject



2.6.6.2 Generate a chart-mark on injection

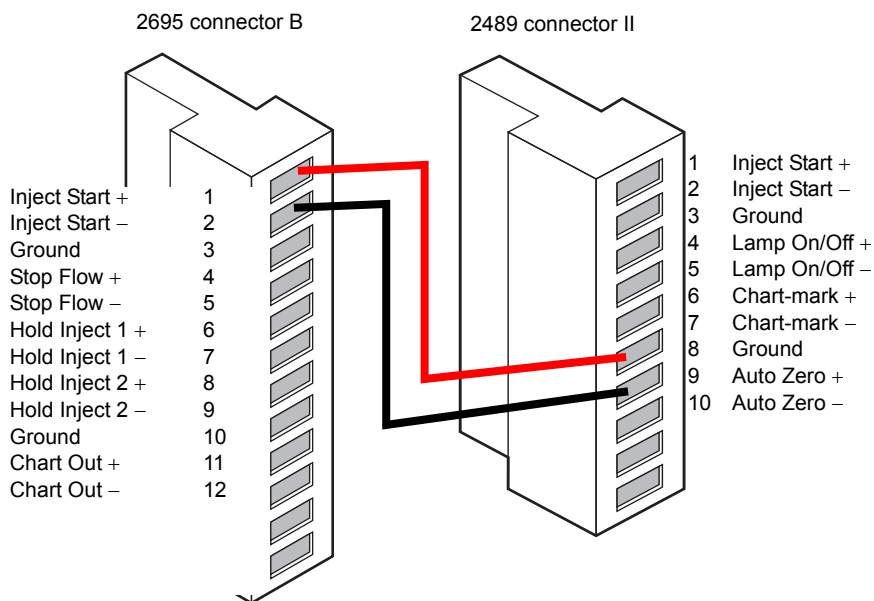
To generate the chart-mark function on the detector at the start of an injection, make the connections summarized in the table and illustrated in the figure below.

Table 2–6: 2695 separations module to the detector to make a chart-mark

2695 separations module (B inputs and outputs)	2489 detector UV/Vis (II)
Pin 1 Inject Start	Pin 6 Chart-mark +
Pin 2 Inject Start	Pin 7 Chart-mark –

Before you can generate a chart-mark from the 2695 separations module, you need to configure the chart-mark signal at the detector’s front panel. The default chart-mark signal setting is Low (see the discussion “Configuring event inputs (contact closures)” on page 63 for more information).

Figure 2–9: 2695 separations module connections to the detector, for making a chart-mark on injection



2.6.7 Connecting the detector to a chromatography data workstation using an e-SAT/IN module

Acquiring data and controlling the detector with Empower software or MassLynx software, by using the e-SAT/IN™ module instead of the Ethernet bus, requires connections between the following hardware:

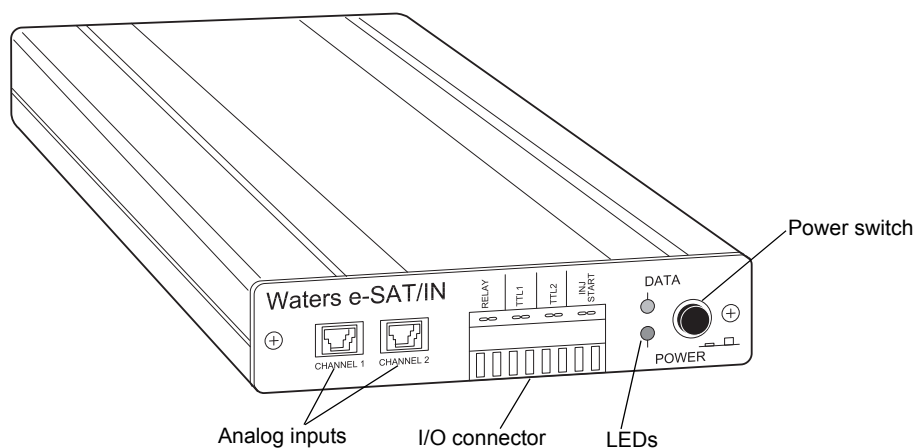
- Ethernet card
- Ethernet satellite interface (e-SAT/IN) module

2.6.7.1 e-SAT/IN module

The Waters e-SAT/IN module shown in the figure below translates analog signals from devices such as the detector into digital form. It then transmits these digital signals using an ethernet card installed in the chromatography data workstation.

Note: The e-SAT/IN module does not have a power switch. Always disconnect the power cord at either the wall outlet or the power supply before attaching or removing the power connection to the e-SAT/IN module.

Figure 2–10: e-SAT/IN module (front panel)



! **Notice:** To avoid damaging the e-SAT/IN module (and thus void its warranty) and to ensure its proper startup, do not power-on the module until you perform all procedures in the *Waters e-SAT/IN Module Installation Guide*.

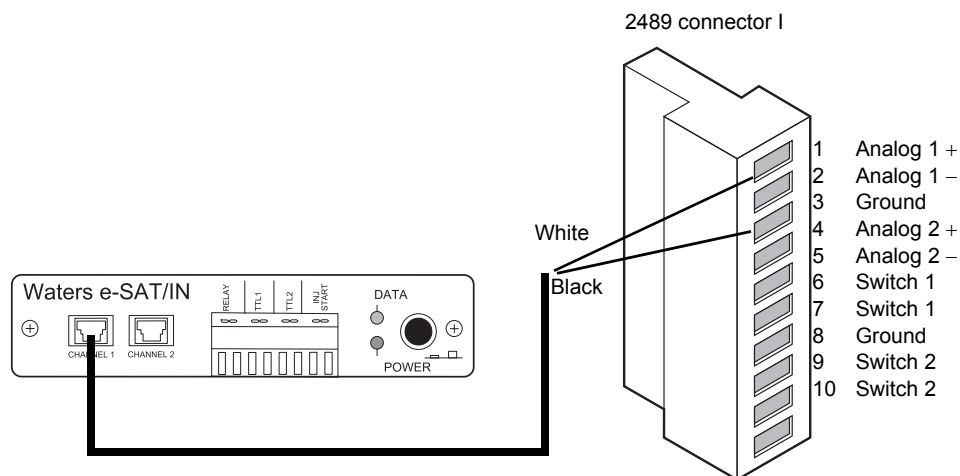
2.6.7.2 Connecting the detector to the e-SAT/IN module

The e-SAT/IN module connects to the detector through the B (inputs and outputs) terminal on the rear panel of the detector, as shown in the figure below.

To connect the detector to the e-SAT/IN module:

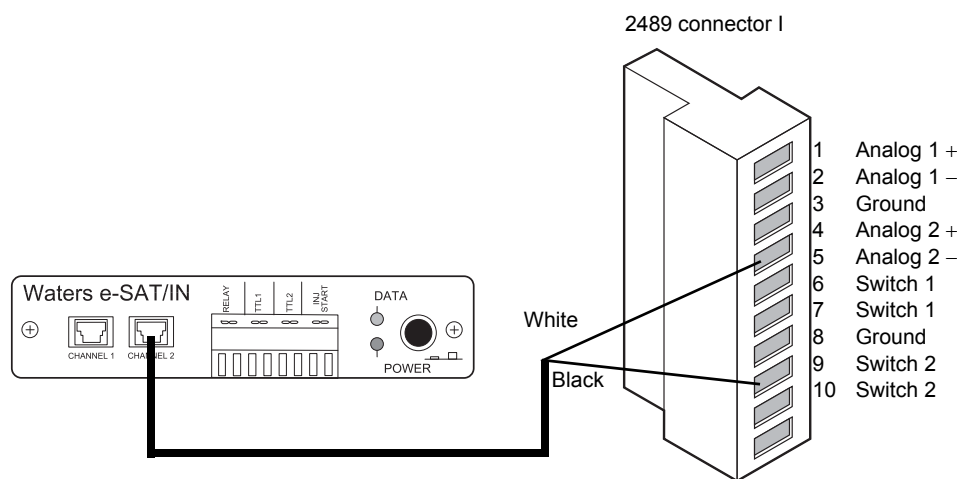
1. Using the electrical insulation stripping tool, strip about 3 mm (1/8 inch) from one end of the e-SAT/IN 9-pin connector, exposing the white and black wires.
2. Connect the other end of the cable to the Channel 1 or Channel 2 connectors on the front panel of the e-SAT/IN module.
3. For Channel 1,
 - connect the white wire to pin 1 on I (Analog 1 +);
 - connect the black wire to pin 3 on I (Ground).

Figure 2–11: e-SAT/IN module channel 1 connection to detector



4. For Channel 2,
 - connect the white wire to pin 4 on I (Analog 2 +);
 - connect the black wire to pin 8 on I (Ground).

Figure 2–12: e-SAT/IN module channel 2 connection to detector



5. Configure the serial port for the e-SAT/IN module as described in the chromatography data software installation and configuration guide.

The following table summarizes the detector connections to the e-SAT/IN module:

Table 2–7: Detector connections to a e-SAT/IN module

2489 UV/Vis detector (I)	e-SAT/IN connector
Pin 1 Analog 1 + (white)	Channel 1 or 2
Pin 3 Ground (black)	
Pin 4 Analog 2 + (white)	Channel 1 or 2
Pin 8 Ground (black)	

2.6.8 Connecting the detector to a 745/745B/746 data module

You can connect the detector to a Waters 745/745B/746 data module using the analog output connector on the rear panel of the detector. The analog connector provides 2-V output that is scaled to the AU sensitivity setting and the voltage offset setting.

Note: To prevent oversaturation of the signal from the detector to the integrator, do not exceed the input voltage rating of the integrator.

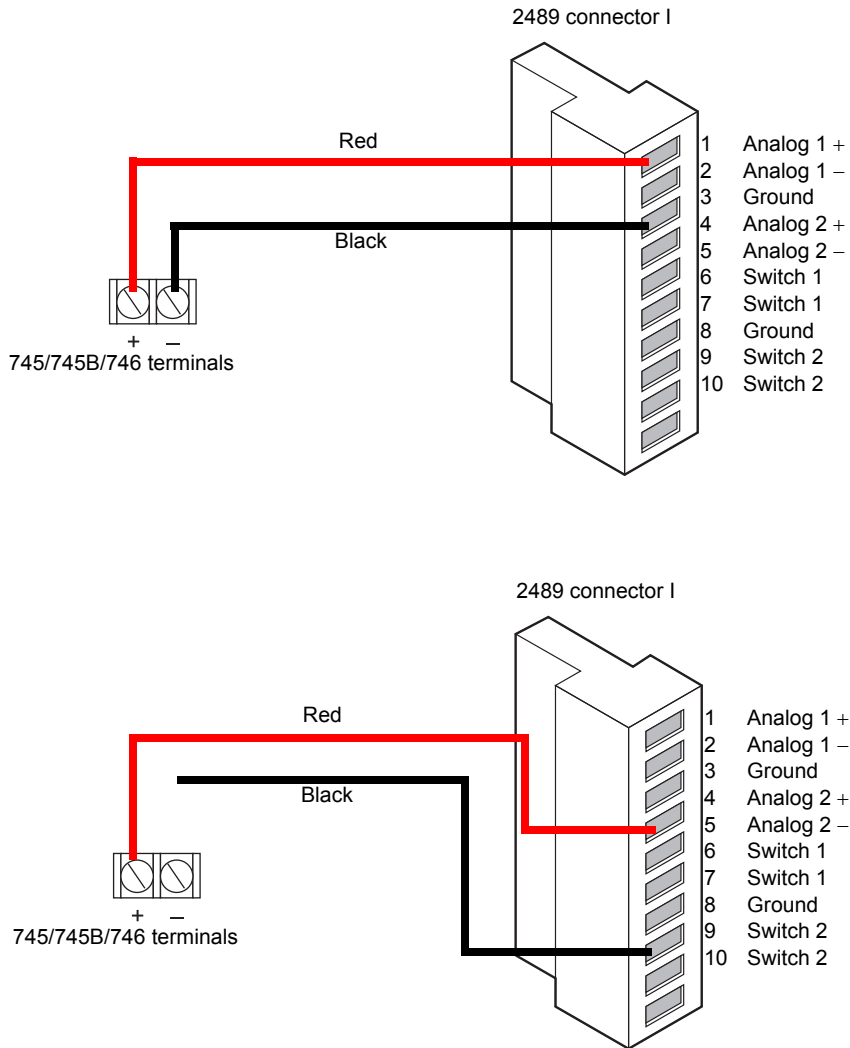
The analog-output signal is transmitted via the cable provided in the 2489 UV/Vis detector startup kit. Make the connections summarized in the table below and illustrated in the figure below:

Table 2–8: Detector connections to a data module

2489 UV/Vis detector (I)	745/745B/746 terminal
Pin 1 Analog 1 + (red)	+
Pin 3 Ground (black)	–
Pin 4 Analog 2 + (red)	+
Pin 8 Ground (black)	–

To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only.

Figure 2–13: Data module connections to detector channels A and B



2.6.9 Connecting the detector to a fraction collector

The detector can trigger a fraction collector based on these things:

- Timed events (see [“Timed events”](#) on page 71)
- Threshold levels (see [“Threshold events”](#) on page 73)

You can connect the fraction collector to one of the detector’s two programmable switches (SW1 or SW2), and program the timed event, threshold, or ratio at the front panel. You can also connect the fraction collector to trigger a chart-mark event input each time a tube is changed at the fraction collector.

The table below indicates the detector-to-fraction collector and autoinjector-to-fraction collector connections.

Table 2–9: Detector connections to the fraction collector

2489 UV/Vis detector connection	Fraction collector
I Pin 3 Ground	Pin 1 Detector In –
II Pin 6 Chart-mark +	Pin 10 Event Marker +
II Pin 7 Chart-mark –	Pin 9 Event Marker –
I Pin 6 SW1	Pin 7 External Count In +
I Pin 8 Ground	Pin 8 External Count In –
2695 separations module	
Inject Start +	External Start In +
Inject Start –	External Start In –

See also: Documentation provided with your fraction collector, for complete instructions.

2.7 Plumbing the detector

Before initially starting the detector:

- Complete the plumbing connections described in this section.
- Complete the electrical connections (see [“Connecting to the electricity source” on page 29](#)).



Warning: To avoid the harmful effects of personal contact with solvents and samples, including inhalation, observe Good Laboratory Practice when you handle them. See the Material Safety Data Sheets for the solvents and samples you use.

Requirement: You must make the following plumbing connections on your detector unit:


- Column connections
- Drip management system connections

Recommendations:

- Before completing the column connections, perform the verification procedures described in [“Verifying that the detector operates properly” on page 66](#).
- Install a waste reservoir connected to the drain tube and located next to the rubber foot on the front, lower, left-hand section of the detector.
- Use Tygon[®] tubing to connect the drain tube to the waste reservoir.

- ! **Notice:** To avoid damaging the flow cell or tubing, do not connect any tubing or device that can cause the backpressure to exceed the pressure rating of those components. The pressure rating of a standard analytical flow cell is 6895 kPa (69 bar, 1000 psi).

2.7.1 Connecting columns in HPLC systems


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

The plumbing connections to the detector are located in the front, left-hand side of the flow cell assembly.

To connect the inlet and outlet tubing in an HPLC system:

1. Attach the stainless steel compression fitting and ferrule (supplied in the startup kit).
2. Connect the inlet tubing to the column outlet.
3. Ensure that the tubing is seated firmly in the column outlet, and then tighten the compression screw.
4. Connect the Tygon tubing to the flow cell outlet tubing, and route the tubing to a waste container.

2.7.2 Connecting columns in ACQUITY Arc systems

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

The plumbing connections to the detector are located in the front, left-hand side of the flow cell assembly.

Note: Tubing assemblies are supplied in the appropriate system fluidic kit.

To connect the inlet and outlet tubing in an ACQUITY Arc[®] system:

1. Connect the inlet tubing to the column outlet, and then to the flow cell inlet.

! **Notice:** To avoid equipment damage caused by spilled solvent, do not place fluid containers directly atop an instrument or device without a solvent tray to contain spills.

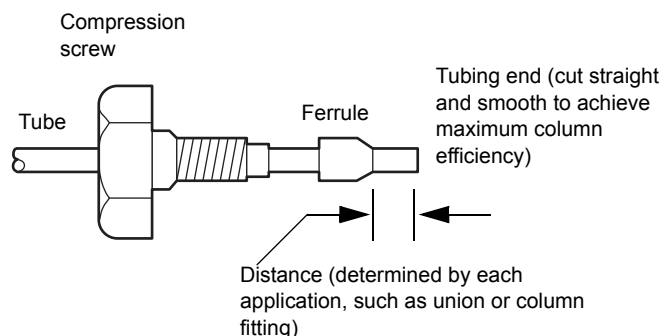
2. Connect the outlet tubing to the flow cell outlet, and route it to a waste container.

2.7.3 Assembling the fittings (HPLC only)

To assemble each fitting:

1. Slide the compression screw over the tubing end, followed by the ferrule.
2. Mount the ferrule with its tapered end facing the end of the tubing.

Figure 2–14: Ferrule and compression screw assembly



2.7.4 Making tubing connections in HPLC systems

To make tubing connections in an HPLC system:

1. Seat each tubing end in the column outlet, detector inlet, or detector outlet fitting.
2. Seat each ferrule by tightening the compression screw 1/2 turn past finger-tight.

Requirement: To ensure accurate verification, power-on the detector before pumping any mobile phase or solvent through the flow cell.

Recommendation: To prevent reabsorption of dissolved oxygen (for systems using vacuum degassers), run the solvent degasser continuously when operating the detector at wavelengths less than 230 nm.

2.7.5 Making tubing connections in ACQUITY Arc systems

To make tubing connections in an ACQUITY Arc system:

1. Bottom each tubing end in the column outlet, detector inlet, or detector outlet fitting.
2. Seat each ferrule by tightening the compression screw finger-tight.

Requirement: To ensure accurate verification, power-on the detector before pumping any mobile phase or solvent through the flow cell.

Recommendation: To prevent reabsorption of dissolved oxygen (for systems using vacuum degassers), run the solvent degasser continuously when operating the detector at wavelengths less than 230 nm.

3

Prepare the Detector

After you install the detector, you can set it up and operate it as a stand-alone instrument or with chromatography data software.

Table 3–1: Detector configuration

Configuration	Programming required for operation
The detector is a stand-alone instrument within a system, or used with any fluid-handling unit, injector, integrator, or data system	Program the detector using the front panel (see page 65), except when the detector is in remote mode.
The detector is controlled by Empower or MassLynx chromatography data software	Program the detector using the data system, to control and collect digital data, following the instructions in the data system’s online Help.

Requirement: To ensure accurate operation, and before pumping mobile phase or solvent through the flow cell, be sure to perform the procedures on [page 66](#).

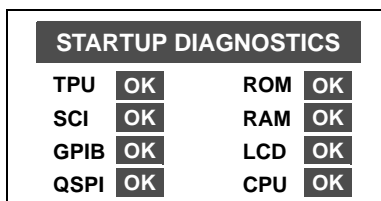
3.1 Initializing the detector

Before you power-on the detector, be sure the connector from the rear panel to the power source is properly installed.

To power-on the detector, press the On/Off switch located on the top left-hand side of the instrument.

At startup, the detector beeps three times and runs a series of startup diagnostic tests. If it passes all startup diagnostic tests, the Startup Diagnostics screen appears, with “OK” following each test.

Figure 3–1: Startup Diagnostics screen



After it displays the Startup Diagnostics screen, the detector displays the following series of messages in sequence—a process that takes about five minutes:

- Initializing grating
- Initializing system
- Lighting lamp

- Warmup time left: *n* minutes
- Homing optical filter
- Searching for 656 nm
- Optimizing system performance
- Finding calibration peaks
- Restoring last setup
- Completing initialization

When initialization ends, the detector displays the absorbance screen shown (see [page 49](#)). The sections “[Using the keypad](#)” on [page 51](#) and “[Navigating the user interface](#)” on [page 55](#) provide more information on this and subsequent screens.

Tip: For normal use, allow the detector to warm up for at least 30 minutes before operating.

3.1.1 Diagnostic test failure

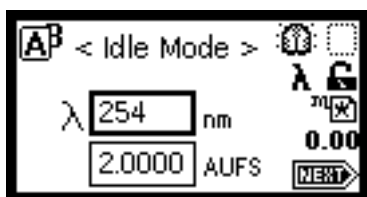
If one or more of the internal startup diagnostic tests show a failing result, the detector beeps and displays an error message. For serious errors, it displays the word “Error”, in brackets (<Error>), in place of the run-time absorbance on the absorbance screen.

See [page 111](#) for a list of startup diagnostic test failures, error messages, and recommended recovery actions. See [page 119](#) for hardware-related causes of startup diagnostic test failure and corrective actions.

3.1.2 Idle mode

When the detector is successfully started, it defaults to idle mode (see the [figure “2489 UV/Vis detector idle mode screen”](#) on [page 48](#)). When it is not performing any function requiring the shutter to be open (local methods, scans, noise test, and so on), the shutter is closed, and the detector remains in idle mode with the lamp lit. The Closed Shutter limits unnecessary UV light from reaching the detector’s optics bench.

Figure 3–2: 2489 UV/Vis detector idle mode screen

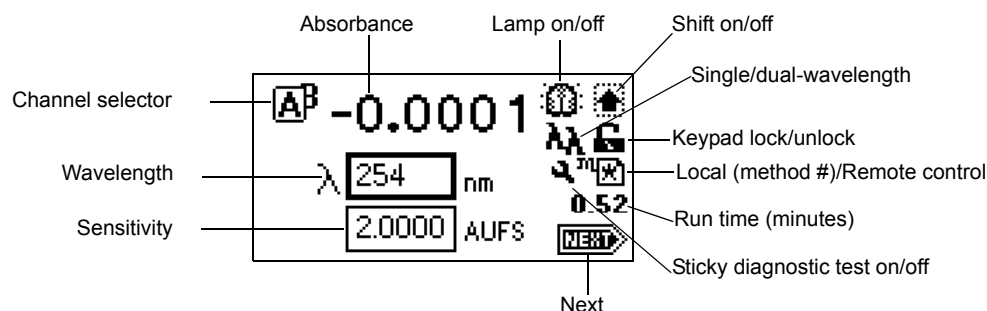


3.2 Using the operator interface

3.2.1 Using the display

The detector employs a 128 × 64-bitmap graphic display and a 24-key membrane keypad for the operator interface. After the startup diagnostic tests are run successfully, the detector displays the absorbance, or HOME, screen.

Figure 3–3: Detector absorbance (HOME) screen



You can recall the absorbance screen at any time by pressing the HOME key. When you first use the detector, the absorbance screen shows the factory-set defaults. Afterward, the absorbance screen shows the settings displayed before the detector was last powered-off. The absorbance screen continues to change as the run continues.

The detector monitors absorbance of one or two wavelengths in real time while allowing you to modify all the parameters discussed in the table below. You can use the A/B key to toggle between absorbance screens for channels A and B.

3.2.1.1 Absorbance and message icons

The absorbance screens and message screens in the detector program display the icons or fields shown in the figure on [page 49](#) and described in the table below. For a list of ranges and defaults for the function icons, AUs, and fields described in this table, see the [table titled “Primary and secondary function \(method\) parameters” on page 60](#).

Note: Changing the sensitivity (AU) setting affects the 2-V output. For example, 1 AU gives 0.5 AU/V, and 2 AU gives 1 AU/V.

Table 3–2: Absorbance and message screen icons

Icon or field	Icon/field name	Function
Field requiring entry	Sensitivity or AU	Selects the chart sensitivity, in absorbance units (AU), for the selected channel (Ethernet signal is not affected).
Field requiring entry	Wavelength	Selects the wavelength monitored on the selected channel. In single-wavelength mode, you cannot independently control wavelength on channel B.

Table 3–2: Absorbance and message screen icons (continued)





















Icon or field	Icon/field name	Function
	Channel selector	Changes the channel when you press A/B. The icon of the selected channel overlaps that of the other channel.
	Channel On	Displays ON A or ON B for the channel a timed or threshold event is programmed on.
	Channel trace	When you press TRACE, displays only the channel being viewed.
Numerical field	Absorbance	Displays current absorbance for the selected channel.
	Lamp on	Indicates the lamp is turned on.
	Lamp off	Indicates the lamp is turned off.
	Shift off	Blank = Shift off.
	Shift on	Indicates the shift is on for one key press.
	Single-wavelength	Indicates the detector is operating in single-channel mode.
	Multiwavelength	Indicates the detector is operating in dual-channel mode.
	Keypad unlock	Indicates unrestricted keypad entry.
	Keypad lock	Indicates changes to parameter values are not allowed. The keypad locks when the instrument is under control of an external data system (remote mode only).
	Sticky diagnostic on	Indicates a sticky diagnostic setting is active. (See page 111 for an explanation of sticky diagnostic settings.)
	Local method number	Indicates the detector is not controlled by a data system. The icon's cursive "m" and the current method number or an asterisk (*) indicate current conditions are not stored as a method.
	Ethernet control	Indicates that the detector is controlled by a chromatography data system.

Table 3–2: Absorbance and message screen icons (continued)

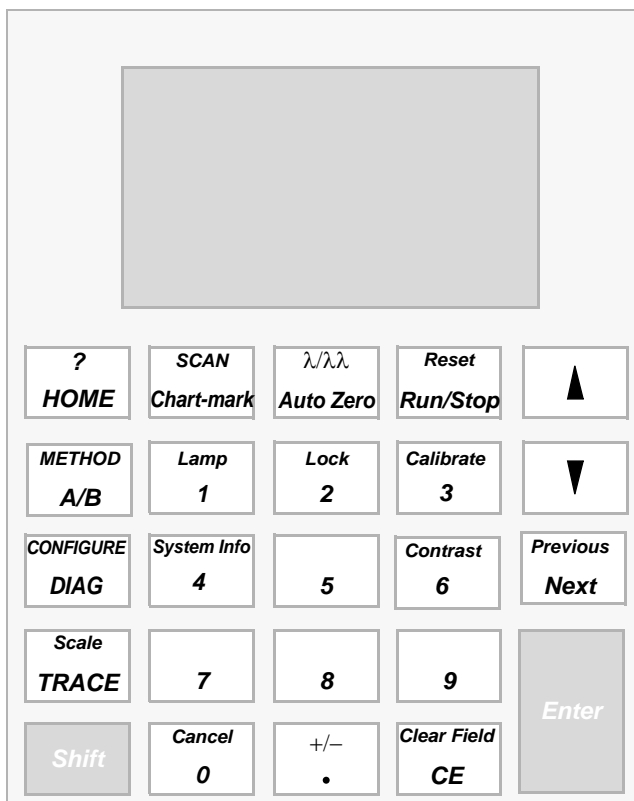
Icon or field	Icon/field name	Function
Numerical field	Run time (minutes)	Displays the time elapsed since you pressed Run, or since an inject-start signal was received.
	Next	Indicates that pressing Next displays additional screens.
	Message screen icon.	Indicates an error message.
	Message screen icon.	Indicates a question.
	Message screen icon.	Indicates a warning message.
	Message screen icon.	Indicates information is being displayed.
	Message screen icon.	Indicates that you must standby.

3.2.2 Using the keypad

The detector's keypad (shown below) consists of 24 keys providing

- full numeric entry (10 digits plus a decimal point);
- Enter, Shift, CE (Clear Entry), Next, and Help functions;
- s and t (used for navigation only; pressing s can also move the highlighted area to the left, t to the right);
- A/B for channel selection;
- navigation to specific screens (HOME, or absorbance; DIAGNOSTICS; TRACE; CONFIGURE; METHOD; and SCAN);
- primary function keys (Chart-mark, Autozero, and Run/Stop);
- secondary function keys (Scale, Single- or Dual-wavelength, Reset Clock, Lamp, Lock, Calibrate, System Information, Contrast, Previous, Cancel, +/-, and Clear Field).

Figure 3–4: Keypad:



Primary function keys take effect immediately, with no further entry required. Secondary function keys require you to enter information into parameter fields and to press Enter for the functions to take effect.

Keys that appear in all caps (HOME, DIAG, TRACE, METHOD, CONFIGURE, and SCAN) directly invoke a function from most screens.

For numerical entries from 1 to 9 on choice lists or menus, enter the number corresponding to the desired item, and then press Enter. For the number 10, select 0, and then press Enter. To go to the end of a choice list, select •. For entries numbered 11 or 12, scroll to the desired item on the choice list, and then press Enter.

The table below presents the functions of the primary and secondary keys.

Table 3–3: Detector keypad description

Key	Unshifted	Shifted
<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: auto;"> <p style="text-align: center;">?</p> <p style="text-align: center;">HOME</p> </div>	HOME – Displays the absorbance screen containing the icons, and the Wavelength and AU fields.	? – Displays context-sensitive help when available.

Table 3-3: Detector keypad description (continued)

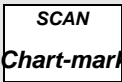
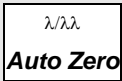

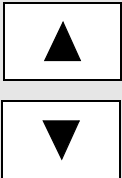
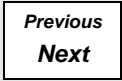
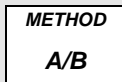

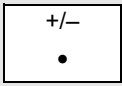
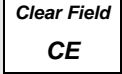
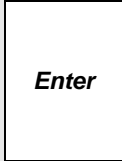
Key	Unshifted	Shifted
	Chart-mark – Causes a momentary pulse to the analog output (A, B, or both depending upon the current settings). This key has no effect if the chart-mark function is disabled on both channels.	SCAN – Displays the list of options for generating and manipulating spectra.
	Auto Zero – Sets the absorbance offset so that the analog output (A, B, or both depending on the current settings), reads 0 AU. You can enable or disable the autozero function from the third absorbance screen (see the figure on page 59).	$\lambda/\lambda\lambda$ – From the absorbance screen, use this key to toggle between single- and dual-wavelength modes. The current mode is indicated by an icon on the display.
	Run/Stop – Starts or stops (freezes) the run clock. (The elapsed time appears near the lower right-hand corner of the absorbance screen.) Initiates scans.	Reset – Resets the detector run clock to 0 minutes. Returns the detector to initial conditions for the current method.
	Arrow keys – On screens with entry fields (edit, check box, or choice list), the border of the active field is thickened (highlighted). Using the arrow keys, you move the highlighted board to the field you want to activate. (Up moves up or left; Down moves down or right.) On screens with scrollable lists, these keys move the highlighted border up (toward the beginning of the list) or down (toward the end). For other screens, special instructions apply to the use of the Up and Down arrow keys (for example, the Display Contrast screen).	
	Next – Displays a screen with additional options related to a current screen. Repeatedly pressing this key brings back the screen on which you started. On most screens where this key is active, the NEXT arrow appears in the lower, right-hand corner of the display.	Previous – When the Next key is available, use Previous to navigate through the screens in the reverse order.
	A/B – On screens with the A/B icon in the upper, left-hand corner, this key toggles between channel A and channel B parameters.	METHOD – Displays the list of options for creating and clearing timed and threshold events, storing, retrieving, and resetting methods.
	DIAG – Displays the choice list of diagnostic tests.	CONFIGURE – Displays the first Configuration screen.

Table 3-3: Detector keypad description (continued)

Key	Unshifted	Shifted
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Scale</i> TRACE </div>	TRACE – Displays the absorbance monitor trace for channel A or B.	Scale – When the wavelength trace or spectrum screen is visible, this function permits modification of the display range in the x (time or wavelength) and y (absorbance) dimensions.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> Shift </div>	Shift – Enables the shifted functions (identified by the text at the top of most keys). The shifted state is temporary and resets after the next keystroke.	
<div style="border: 1px solid black; padding: 2px; text-align: center;"> 0-9 </div>	0-9 – Enters a numeral, from 0 through 9, into a current field. Also positions the cursor at the corresponding entry in a list (0 = tenth item). Selects the corresponding number from a choice list.	0-9 – See descriptions for specific shifted numeric keys.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Lamp</i> 1 </div>	1 – See 0-9, above.	Lamp – Displays a lamp use statistics for the currently installed lamp and provides the means for you to turn the lamp on or off. The current state of the lamp is indicated by an icon on the absorbance screen.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Lock</i> 2 </div>	2 – See 0-9, above.	Lock – Use from the absorbance screen, to lock or unlock the keypad. The lock prevents inadvertent changes to detector settings. The current lock state is indicated by an icon on the absorbance screen.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Calibrate</i> 3 </div>	3 – See 0-9, above.	Calibrate – Initiates wavelength calibration.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>System Info</i> 4 </div>	4 – See 0-9, above.	System Info – Displays system information including firmware version and instrument serial number.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Contrast</i> 6 </div>	6 – See 0-9, above.	Contrast – Permits contrast adjustment (viewing angle) of the liquid crystal display.
<div style="border: 1px solid black; padding: 2px; text-align: center;"> <i>Cancel</i> 0 </div>	0 – See 0-9, above.	Cancel – In some modes, backs out of a prompt without completing the task. The word “Cancel” appears as a cue in the lower, right-hand border of the message text.

Table 3–3: Detector keypad description (continued)

Key	Unshifted	Shifted
	<ul style="list-style-type: none"> • – Enters a decimal point. Also positions the cursor at the last entry in a list. 	+/- – Some edit fields accept negative-number entries. Use this function to invert the sign of the number in an active field.
	CE – Clears an editing change, and returns the contents of a field to its previous value. Sets the value to a unique word for some fields. For example, in the voltage offset diagnostic display, you can enter either a numeric offset value or press CE to change it to OFF.	Clear Field – Clears the current entry field before you enter the desired values.
	Enter – Completes the entry in an edit field. Also advances the active field, as if the Down arrow had been pressed (except after editing the wavelength on the absorbance screen). Press Enter to acknowledge error messages and other prompts. In these cases, the word “Enter” appears as a cue in the lower, right-hand border of the message text.	

3.2.3 Navigating the user interface

To operate the detector:

1. Press the Enter key or the up and down arrow keys to navigate among editable fields on a display.

Tip: A thickened border highlights the active field.
2. Press Enter to advance the active field once you complete an entry.
3. When you make an error, press CE (Clear Entry) to undo any changes and return to the active entry field.

Tip: An active field containing a choice list is indicated by a numeral to the right of the field and within the thickened border.
4. To display a choice list, press Enter. Then take one of these actions:
 - Press the corresponding number key to select an item immediately.
 - Use the up and down arrow keys to scroll through the list, and then press Enter.

Tip: You can press the numeral that corresponds to a desired choice without first pressing Enter.

Rule: The Up and Down arrow keys do not incrementally increase or decrease numerical field entries. Use the numerical keypad for that purpose.

3.2.3.1 Navigating to and from the absorbance screen

Pressing HOME from most screens causes the absorbance screen to appear. From the absorbance screen, you can access several secondary functions. To move to the secondary function screens of the absorbance screen, press Next. These are the secondary functions:

- Analog output specifications
- Time constant
- Absorbance offset
- Voltage offset
- Chart polarity
- Enable/disable inputs
- Enable/disable external events

Tip: The parameters you enter into the secondary function fields become part of the current method conditions and are stored when you store the method (see [page 70](#)).

In single-wavelength mode, the detector displays three additional screens, labeled 2 of 4, 3 of 4, and so on. In dual-wavelength mode, the detector displays four additional screens, labeled 2 of 5, 3 of 5, and so on (see [page 59](#)).

3.2.3.2 Setting up a run

Once you press HOME, causing the absorbance screen to reappear, and you select a wavelength mode (λ or $\lambda\lambda$), you can set up the detector for a run. In addition to wavelength mode, you must program these parameters before beginning a run:

- Operating wavelength
- Sensitivity
- Time constant
- Analog output sensitivity

Depending on the other functions that you can perform during a run, several other parameters can require programming. See [page 56](#) and the table on for the function descriptions, fields, screen number, type of function, display units, allowable ranges, and the default settings for the absorbance screen and the secondary function screens.

3.2.4 Primary and secondary functions

You can access the following functions directly from the absorbance screen or by pressing the Next key on that screen.

Table 3–4: Detector functions

Function	Description
Wavelength	Defines the operating wavelength for the channel.

Table 3–4: Detector functions (continued)

Function	Description
AUFS (absorbance units full-scale)	<p>Defines the relationship between the absorbance and the output voltage. The output voltage reaches full scale (2 V) when the current absorbance attains the AUFS value.</p> <p>Caution: Changing the sensitivity (AUFS) setting affects the 2-V output.</p>
Analog out (single λ)	<p>This function charts the current absorbance of a selected channel's analog output, 2-V connector (scaled to the AUFS setting for that channel) and adjusts the absorbance value according to the voltage and absorbance offsets for that channel.</p> <p>Example: For the 2-V output with 0 used for both the voltage and absorbance offsets;</p> $\text{Volts out} = \text{Absorbance} \times 2 \text{ V/AUFS}$
Analog out (dual λ)	<p>In addition to the selections for single λ above, you can chart the same parameters on the other channel, at a different wavelength, and you can chart the following parameters:</p> <ul style="list-style-type: none"> • MaxPlot – Charts the absorbance of multiple compounds with different absorbances at two different wavelengths on a single data channel. Scaling for MaxPlot is the same as for Absorbance (above) except that the charted absorbance is the larger of the two absorbances as measured on channel A and channel B. The detector uses the AUFS, absorbance offset, and voltage offset of the selected channel regardless of which absorbance (channel A or channel B) is larger. $\text{Volts out} = \text{Larger absorbance (A or B)} \times 2 \text{ V/AUFS (of selected channel)}$ • RatioPlot (A/B) – Charts the ratio of absorbances at two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak. This mode applies the three ratio parameters on screen 5 of 5 (see the figure below). <ul style="list-style-type: none"> – Minimum AU: This setting defines the minimum AU value for either wavelength (A or B) before the actual ratio is calculated. Both absorbance values (A and B) must be above this value or 0 V is charted. If both absorbances are above this value, the absorbances are divided (A/B) and charted. The output voltage is scaled proportionally to the ratio depending on the selected channel minimum ratio and maximum ratio settings. – Minimum ratio: An actual ratio equal to the minimum ratio value results in 0 V being charted.

Table 3–4: Detector functions (continued)

Function	Description
	<p>– Maximum ratio: An actual ratio equal to the maximum ratio results in a full-scale output of 2 V. Absorbance offset is ignored with this selection.</p> <p>For a RatioPlot, the actual voltage charted is</p> <p>Volts out = 0 V if Absorbance A and B < minimum AU</p> <p>Volts out = (Absorbance ratio – minimum ratio) × 2 V/(maximum ratio – minimum ratio)</p> <p>To ensure proper operation of the RatioPlot function, be sure the selected time constants for both channels are set to the same value.</p> <ul style="list-style-type: none"> • Difference Plot (A-B) – Charts the difference in absorbances at two different wavelengths. The scaling for the difference plot is identical to the Absorbance selection above, except that the charted absorbance is the difference (subtracted) in value of the two absorbances measured on the A and B wavelengths. The detector uses the AUFS, absorbance offset, and voltage offset of the selected channel for scaling. <p>Volts out = Absorbance difference (A – B) × 2 V/AUFS (of selected channel)</p>
Filter time constant	Adjusts the noise filter (time constant) to achieve the optimum signal-to-noise ratio without changing the sensitivity setting. See the discussion “Filtering noise” on page 19 for more information.
Voltage offset	Adjusts the charted analog output signal. Entered in millivolts, this function adjusts the 2-V signal by the entered value. This function is useful for making minor adjustments and for rendering null any offset between the detector and a connected external data system.
Chart polarity	Inverts the charted chromatogram. Entering the plus sign (+) produces a normal chromatogram; entering the minus sign (–) produces an inverted chromatogram.
Autozero on inject	Selected by default, this function results in an autozero each time the detector receives an inject-start signal via contact closure, Ethernet, or through the front panel. You can disable this parameter by pressing any numerical key.
Autozero on λ changes	This function results in an autozero each time a wavelength change is requested. If you disable this function, significant changes in measured absorbance can occur after each wavelength change. Selecting “to zero” sets the signal level to zero. Selecting “to baseline” maintains the previous baseline level when the wavelength change is made. The default is “to zero.”

Figure 3-5: Secondary functions of the absorbance screen

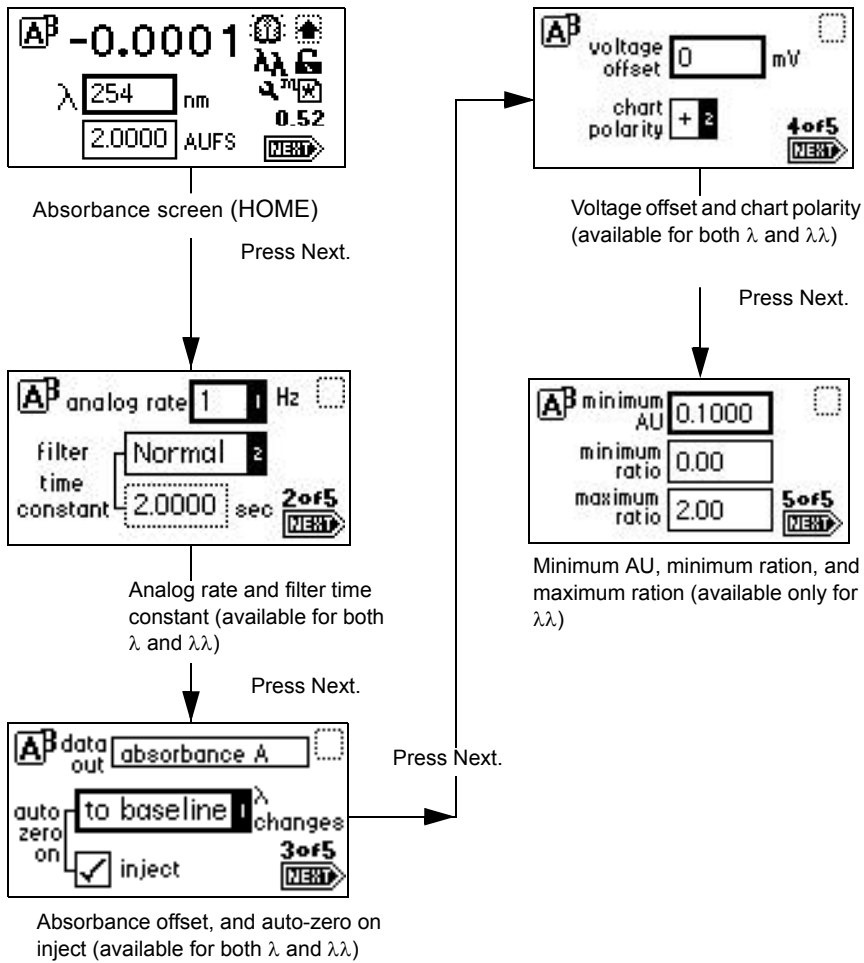


Table 3–5: Primary and secondary function (method) parameters

Function	Screen	Type	Units	Range	Default
λ (Wavelength)	1 (Absorbance screen)	Numeric	nm	Integer 190 nm to 700 nm	254 nm
AUFS	1	Numeric	AUFS	0.0001 to 4.0000	2.0000
Analog rate	2 (of 4) or 2 (of 5)	Choice	Hz	(λ): 10, 20, 40, 80 ($\lambda\lambda$): 1 or 2	10 1
Filter time constant	2 (of 4) or 2 (of 5)	Numeric	Sec	Slow Normal Fast Other: (λ): 0.0125 to 5.0 ($\lambda\lambda$): 0.5 to 5.0 Off to disable filtering	Normal (for Other:) 1.000
Data out (single λ)	3 (of 4)	Choice	None	Absorbance A	Absorbance A
Data out (dual λ)	3 (of 5)	Choice	None	Absorbance A Absorbance B Maxplot A, B Difference A-B Ratio A/B	Absorbance A
Autozero on inject	3 (of 4) or 3 (of 5)	Check box	None	Selected/ Not selected	Checked
Autozero on λ changes	3 (of 4) or 3 (of 5)	Choice	None	To baseline To zero Disable	To zero
Voltage offset	4 (of 4) or 4 (of 5)	Numeric	mV	Integer –2000 to 2000	0
Chart polarity	4 (of 4) or 4 (of 5)	Choice	None	+ –	+
Minimum AU	5 (of 5)	Numeric	AU	0.0001 to 4.0000	0.1000
Minimum ratio	5 (of 5)	Numeric	None	0.00 to 999.99	0.00
Maximum ratio	5 (of 5)	Numeric	None	0.00 to 999.99	2.00

3.2.4.1 Operating the Trace and Scale functions

Using the Trace function, you can display an absorbance trace for the last n minutes (as many as 60) of detector operation.

- When you press the TRACE key, the detector displays the absorbance acquired over the last 30 minutes by default. It updates the trace once every 20 seconds.
- When you press the Scale key (Shift TRACE), the detector displays the scaled trace with T1 (ending time) displayed (–30 for the last 30 minutes) by default.

You can change the ending time parameter to any number from 1 to 60. You can use the Scale function to “zoom” in on a particular section of the trace.

To display the Scale parameters after you press the Scale key:

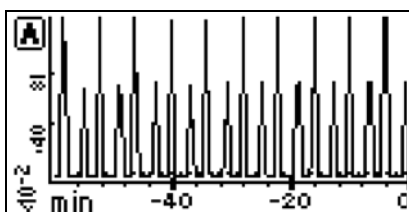
1. Press Next to display T2 (starting time).
Tip: The default is 0.
2. Press Next again to display AU1 (starting or low absorbance).
Tip: The default is Auto.
3. Press Next again to display AU2 (ending or high absorbance).
Tip: The default is Auto.

By entering appropriate times and absorbance numbers in the four scaling parameter boxes, you can zoom in on one section of the current absorbance trace:

- For AU1 and AU2, press CE to reset to auto.
- T1 represents the left-hand side of the trace, or ending time to be displayed. The default is -30.
- T2 represents the right-hand side of the trace, or starting time. The default is 0.

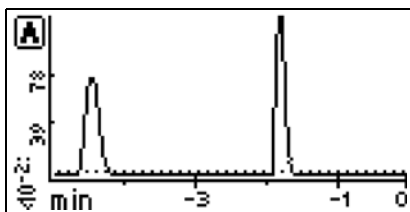
The figure below shows a 60-minute trace of continuous injections of caffeine and propyl paraben in 1:1 methanol/water.

Figure 3-6: Scaled trace of continuous injections, with T1 changed to -60



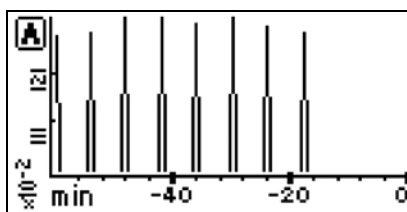
The figure below shows a 5-minute, scaled trace (or an enlarged, zoomed, image) of the 60 minutes of continuous injections shown in the previous figure. T1 is changed to -5. T2 is changed to 0. AU1, and AU2 remain as "auto."

Figure 3-7: Scaled trace for 5 minutes of continuous injections, with T1 changed to -5



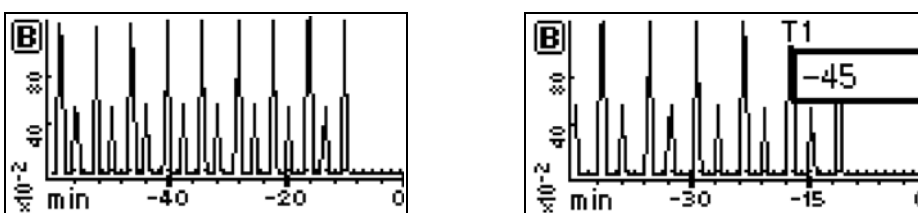
The figure below shows a 60-minute scaled trace similar to that shown in the preceding figure, with the starting absorbance or AU1 changed from auto to 1. T1 remains at 60. T2 remains at 0.

Figure 3–8: Scaled trace for 60 minutes of continuous injections, with AU1 changed to 1



The figure below shows a 60-minute trace on channel B scaled to the last 45 minutes. T1 is changed to -45.

Figure 3–9: Scaled trace changing T1 to -45



As you modify the output using the Scale function, the Trace function continues to display the detector output in real time on either or both channels.

3.2.5 Operating other detector functions

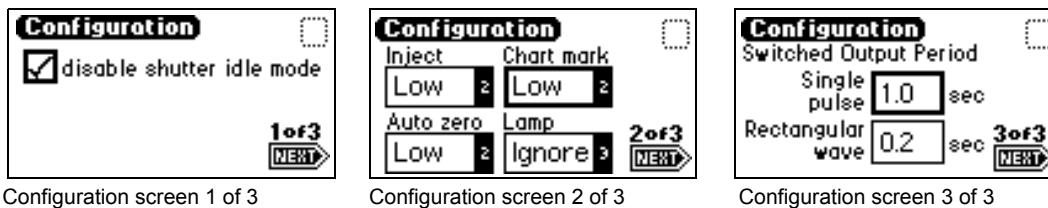
3.2.5.1 Configuring the detector

To change default configurations, use the Configuration screens.

Press the CONFIGURE key (Shift DIAG). The first of three Configuration screens appears.

Tip: Other functions, such as specifying event inputs and enabling pulse periods, are activated from the Configuration screens.

Figure 3–10: Configuration screens



Requirement: To prevent acquisition of incorrect data when operating the detector in dual-wavelength mode under the control of Empower software or MassLynx software, you must select a data sampling rate of 1 point per second.

3.2.5.2 Configuring event inputs (contact closures)

You use the CONFIGURE key to edit event input settings and specify switched output settings.

Using the Enter key and the numeric keypad (or the s and t keys) to select the appropriate entry, you can edit four fields on the second Configuration screen.

The default for Inject, Chart-mark, and Autozero is Low; the default for Lamp is Ignore.

- Inject – You can specify the inject input to signal the start of a run. This event resets the run-time clock and applies initial method conditions immediately:
 - High – Start run when contact closure changes from off (open) to on (closed).
 - Low – Start run when contact closure changes from on (closed) to off (open).
 - Ignore – Do not respond to inject-start input.
- Chart-mark – You can specify the chart-mark input to create a chart-mark on channel A, channel B, or both. You determine the response of the channel using the enable chart-mark function explained in the table on [page 60](#) and shown in the figure on [page 59](#).
 - High – Create chart-mark(s) when contact closure changes from Off (open) to On (closed).
 - Low – Create chart-mark(s) when contact closure changes from On (closed) to Off (open).
 - Ignore – Do not respond to chart-mark input.
- Autozero – You can configure the autozero input to autozero absorbance readings on channel A, channel B, or both. You determine the response of the channel using the enable autozero function explained in the table on [page 60](#) and shown in the figure on [page 59](#).
 - High – Autozero the channel when contact closure changes from Off (open) to On (closed).
 - Low – Autozero the channel when contact closure changes from On (closed) to Off (open).
 - Ignore – Do not respond to autozero input.
- Lamp – You can configure the lamp input level to turn the deuterium lamp on or off from an external device as follows:
 - High – Turn lamp off when contact closure is On (closed).
 - Low – Turn lamp off when contact closure is Off (open).
 - Ignore – Do not respond to Lamp input.

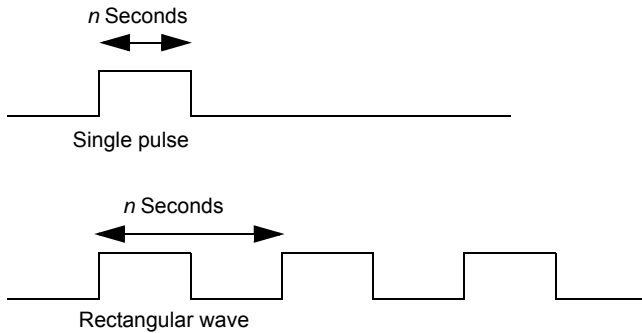
3.2.5.3 Setting pulse periods

You use the third Configuration screen (see [page 62](#)) to set pulse or signal width or to activate a pulse or rectangular wave on SW1 or SW2.

- Single pulse (in seconds) – If SW1 or SW2 is programmed to generate a pulse as a timed or threshold event, then the period of the signal (single pulse width) is as specified in this field (range is 0.1 to 60 seconds).
- Rectangular wave (in seconds) – If SW1 or SW2 is programmed to initiate a rectangular wave as a timed or threshold event, then the period of the signal (the width of one pulse period in a rectangular wave or pulse train) is as specified in this field (range is 0.2 to 60 seconds).

The figure below shows the difference between a single pulse and a rectangular wave.

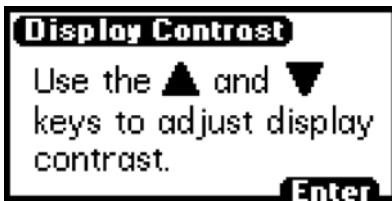
Figure 3–11: Set the pulse period or signal width using SW1 or SW2



3.2.5.4 Setting the display contrast

The Contrast function allows you to adjust the contrast of the detector's display screen. When you press the Contrast key (Shift 6), the Display Contrast screen appears.

Figure 3–12: Display Contrast screen



Use the *s* and *t* keys to adjust the contrast of the display.

3.2.5.5 Displaying system information

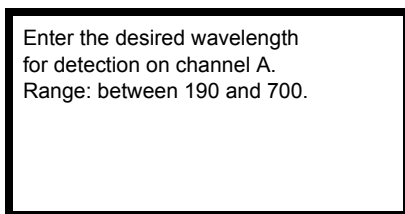
The System Info key (Shift 4) displays information about the detector including the serial number and the firmware version number.

Tip: Use the scroll bar to see all of the message. The Firmware Rev and ChkSm values shown here are examples only. They do not indicate the released version's information.

3.2.5.6 Using Help

The detector has limited context-sensitive Help. When you press ? (Shift HOME) from a point in the program that has a Help screen associated with it, the appropriate screen appears.

Figure 3–13: Help screen



To exit the Help screen, press Enter. If online Help is not available for the function you are working on, pressing ? gets no response.

3.2.6 Operating the detector

3.2.6.1 Overview of detector operation:

Tip: If you are operating the detector under the control of an external system, you can program any parameters not controlled by the external data system at the front panel of the detector before the external system takes control.

Recommendation: To prevent reabsorption of dissolved oxygen, run the solvent degasser continuously when operating the detector at wavelengths less than 230 nm.

Requirement: To maintain optimum system performance, be sure to close the front door before resuming normal operation of the detector.

3.2.6.2 Operate modes

You can use the detector in either single- or dual-wavelength mode over a range of 190 to 700 nm. The detector defaults to the mode of operation in effect when the instrument was last powered-off.

3.2.6.3 Stand-alone operation

When using the detector as a stand-alone instrument, you can store as many as five methods containing as many as 50 timed and 2 threshold events each. An asterisk in the method number field on the absorbance screen indicates current conditions, not a stored method. See [page 70](#) for information on how to store a method.

3.2.6.4 Remote control

To operate under Empower or MassLynx control, the detector uses the Ethernet bus connector (see [page 33](#)).

To connect the detector to your HPLC system, see [page 43](#).

When under the control of an external data system, the detector is operating under remote control conditions. The Remote Control icon, incorporating the letter “E”, appears on the absorbance screen (see the table on [page 49](#)).

See [page 32](#) for more information on connecting the detector to an external system.

3.2.7 Verifying that the detector operates properly

After you have installed the detector, verify that it is operating properly by performing the procedures in this section.

Note: If your flow cell does not require a cuvette, you can obtain an analytical flow cell to perform the validation procedures. See the Waters Quality Parts[®] Locator on the Services and Support page of the Waters' web site.

Requirements:

- For complete validation procedures, you must obtain the Waters accuracy and linearity cuvette kits and system qualification tool for the detector.
- For spare parts details, see the Waters Quality Parts Locator on the Services & Support page of the Waters' web site.

Tip: Before you pump solvent or mobile phase through the system:

1. Flush the lines with filtered, degassed, and sparged HPLC-grade methanol.
2. Pump your mobile phase, provided that there are no miscibility problems, at 1 mL/min for 15 minutes minimum.

3.2.7.1 Before you begin

Because the detector is shipped dry, you must pump fresh, filtered and degassed clear solvent through the unit before its initial use.

Requirement: To ensure accurate verification, be sure to start up the detector and follow steps 1 to 4 in this section and 1 to 5 in “Recording sample and reference beam energies” on page 66 before pumping any mobile phase or solvent through the flow cell.

To start up the instrument:

1. Connect the detector to a data system or integrator (see [Chapter 2](#) for complete information on connecting the detector to external devices).
2. Power-on the detector.
Result: The front panel displays a series of initialization messages for about 5 minutes. See [page 47](#).
3. When initialization ends, the detector displays the absorbance screen.
4. Allow the detector to warm up for at least 30 minutes before operating.

Tip: If the startup verification diagnostic tests fail, note the error message to determine the corrective action, and refer to [Chapter 5](#).

3.2.7.2 Recording sample and reference beam energies

To determine baseline values on your detector for future reference, and to monitor lamp aging (decreased lamp output energy), you must record the baseline sample and reference beam energies to compare against future readings. Use these baseline values to troubleshoot the detector to determine whether

- the solvent is contaminated;
- the flow cell is contaminated;

- the lamp needs to be replaced;
- an air bubble is in the flow cell.

To record the sample and reference beam energies:

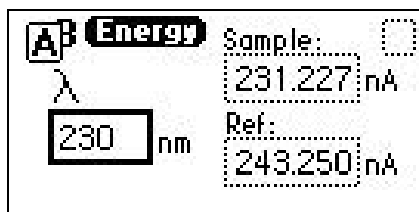
1. Ensure that the detector is connected to the workstation.
2. Flush the system tubing using degassed, HPLC-grade acetonitrile at the recommended flow rate.
3. Pump mobile phase for 15 minutes or more.
4. Ensure the detector cell is filled with solvent and free of air bubbles.
5. When both LEDs show constant green, initialization is complete. Start the data application.
6. On the absorbance screen, use the arrow keys to highlight the λ field.
7. Enter 230 into the λ field, and then press Enter.

Figure 3–14: Absorbance screen diagnostic display



8. Press DIAG and then press 2, Sample & ref energy. The sample and reference energy diagnostic display appears.

Figure 3–15: Sample and reference energy diagnostic display



9. Record the numbers for later comparison.

Rule: Run this procedure each time you change the detector lamp.
10. Flush the flow cell with approximately 30 to 60 mL of HPLC-grade methanol at 1 mL/min for 15 minutes, minimum, to clean the cell.

Make the plumbing connections described in “Plumbing the detector” on page 43, before beginning the peak response test.

3.2.8 Wavelength calibration

You can calibrate the detector, manually, from the keypad at any time during operation or if calibration errors arise during startup.

To calibrate the detector manually:

1. Press Calibrate (Shift 3) from the detector keypad.
Result: A message appears asking whether you removed the cuvette and flushed the flow cell with a transparent solvent (Waters recommends methanol or water).
2. Press Enter, to continue the calibration cycle, or Cancel, to invoke the absorbance screen without calibrating the detector.
Result: After you press Enter, the detector cycles through the calibration procedure and briefly displays a series of initialization messages similar to those you saw at startup (see [page 47](#)).
If calibration is successful, the detector beeps three times and displays the maximum error, in nanometers, of the calibration shift farthest from the last calibration.
3. Press Enter to complete the calibration.
Result: A “Calibration complete” message appears momentarily. Other messages such as “Optimizing system performance” and “Restoring last setup” can appear before the display returns to the absorbance screen.
If calibration is successful, the error message (<Error>) that was displayed on the absorbance screen before you recalibrated the detector disappears.
4. If calibration is unsuccessful, retry, cycle power to the detector, or see [Chapter 5](#).

3.2.9 Operating the detector in single-wavelength mode

The detector is optimized for single-wavelength operation, the default operating mode.

To invoke single-wavelength mode:

1. If the detector is in dual-wavelength (the wavelength icon shows $\lambda\lambda$) mode, from the absorbance (or HOME) screen, press the $\lambda/\lambda\lambda$ key (Shift Auto Zero).
Result: The detector displays a message indicating it is switching to single-wavelength operation.
2. Enter the wavelength and sensitivity on the absorbance screen, as well as any secondary parameters and timed or threshold events.
Note: Changing the sensitivity (AUFS) setting affects the 2-V output.
3. Select a second sensitivity setting while in single-wavelength mode, by pressing A/B and specifying the appropriate AUFS values on the channel B screen.
Result: A single-wavelength is tracked on channel A so that you can use channel B to monitor absorbance with an alternate AUFS setting, or to make the primary absorbance measurement using a specific AUFS value on channel A.
Example: While operating in single-wavelength mode, specifying an AUFS value of 2.0000 on the second channel provides 1.000 V/AU on the channel B, 2-V output.
The detector automatically engages the second-order filter for all wavelengths greater than 370 nm.

3.2.10 Operating the detector in dual-wavelength mode

You can operate the detector with expanded chart-out selections using dual-wavelength mode. In addition to absorbance offered with single-wavelength mode, dual-wavelength mode offers the following functions:

- Absorbance (A and B)
- MaxPlot
- RatioPlot (A/B)
- Difference plot (A-B)

To change from single to dual-wavelength mode:

1. From the absorbance (or HOME) screen, when the detector is in single-wavelength mode (the wavelength icon shows λ), press the $\lambda/\lambda\lambda$ key (Shift Auto Zero).

Result: A momentary message “Setting up dual-wavelength mode” appears.

2. Enter the wavelength to be monitored in the λ field, and then press Enter.
3. Enter the other operating parameters and timed or threshold events, if desired.
4. Press the A/B key to switch channels.

Result: The absorbance screen for the other channel appears.

5. Enter the operating parameters for monitoring the second wavelength, including timed and threshold events, if desired.

Tips:

- If both selected wavelengths are greater than 370 nm (+/- 1 nm), the detector applies the second-order filter to block unwanted UV light.
- If both selected wavelengths are less than 370 nm (+/- 1 nm), the detector removes the second-order filter.
- If the selected wavelengths bracket the 370 nm (+/- 1 nm) threshold, the detector does not apply the second-order filter and issues a warning message stating that any data collected for the wavelength above 370 nm can contain inaccuracies because of possible UV light interference (second-order effects).

Recommendation: In dual-wavelength mode, select a wavelength pair below or above 370-nm. If one or both of the selected wavelengths spans the 370 nm threshold, the detector beeps three times, and the warning message shown below appears. Because of possible UV light interference (second-order effects), you can observe additional peaks and inaccurate peak areas.

Figure 3–16: Dual-wavelength, 370-nm, threshold warning message



3.2.10.1 Obtaining a RatioPlot

The RatioPlot output, on one channel only (channel A), depends on the values you specify on absorbance screen 5 for minimum and maximum ratios. You must be operating the detector in dual-wavelength mode to obtain a ratio plot. The RatioPlot provides a plot of the ratio of the absorbances of two wavelengths from 0 V to 2 V. The minimum and maximum ratio parameters are measured ratio units, not absorbances. (See [page 56](#).)

To obtain a RatioPlot:

1. Be sure the detector is operating in dual-wavelength mode (see the previous discussion).
2. From the absorbance screen, press Next to reach screen 3 of 5.
3. In the Data out field, press 8, ratio A/B.
4. Press Enter to select the ratio plot.
5. Press Next until screen 5 of 5 appears.
6. Enter the minimum AU, and then press Enter.

Tip: The minimum AU field contains a threshold value. The RatioPlot function is not plotted if either wavelength does not exceed the minimum AU threshold.

7. Enter the minimum ratio for the RatioPlot, and then press Enter.
8. Enter the maximum ratio for the RatioPlot, and then press Enter.
9. Return to the absorbance screen by pressing the HOME key.

3.2.10.2 Obtaining a MaxPlot

The detector obtains a MaxPlot by monitoring absorbance at two selected wavelengths while plotting the maximum absorbance for each sample component.

To run a scan using the MaxPlot function:

1. Be sure the detector is operating in dual-wavelength mode (see [“Operating the detector in dual-wavelength mode” on page 69](#)).
2. From the absorbance screen, press Next to reach screen 3 of 5.
3. In the Data out field, press 5, MaxPlot A,B.
4. Press Enter to select the MaxPlot function.
5. Return to the absorbance screen by pressing the HOME key.

3.2.10.3 Programming timed events, threshold events, and methods

You can retrieve as many as five methods, which the detector references as numbers 1 to 5. An asterisk in the method number icon (see the table on [page 49](#)) tells you the current conditions are not stored. If you are using a stored method, the method number appears on the absorbance screen.

If you edit a parameter such as wavelength or AUFS, you are editing the current conditions (Method *), which can be stored as a method. You can store the method in one of the 10 available method storage slots, or you can replace the current method with one of the methods previously stored. When you recall a previously stored method, you replace the existing method conditions with those of the stored method.

The method number displayed in the absorbance screen is that of the retrieved method until you make a change. Any parameter change (for example, wavelength or AUFS) alters the current conditions so that the original recalled method is no longer in effect, causing the method number to change to an asterisk.

The operating parameters at the time of system shutdown are restored; however, any timed events or thresholds associated with the method are deactivated when power is restored. On startup, you always see an asterisk inside the method icon on the absorbance screen.

When the detector is operating under remote control—that is, control by Empower or MassLynx software—the remote icon incorporating the letter “E” (for Ethernet) appears (see the table on [page 49](#)).

3.2.10.4 Timed events

You can program as many as 50 timed events, to the nearest 0.01 minutes. As you enter timed events, each new event appends to the end of the timed-event list. If you enter a time that is not in sequence with the events entered previously, the timed-event list sorts automatically when you press Next. The detector allows programming of the timed events shown in the table below.

Note: Changing the sensitivity (AUFS) setting affects the 2-V output. For example, 1 AU gives 0.5 AU/V, and 2 AU gives 1 AU/V.

Table 3–6: Timed event parameters

Number	Event	Units	Range or default	Specify channel
1	Wavelength	nm	190 to 700	Yes
2	Filter time constant	Seconds	0: Disable filter λ: 0.0125 to 5.00 λλ: 0.5 to 5.0	Yes
3	Sensitivity	AUFS	0.0001 to 4.0000	Yes
4	Chart-mark (10% of full scale)	Does not apply	Does not apply	Yes
5	Polarity	1. + 2. –	+	Yes
6	Auto-zero	Does not apply	Does not apply	Yes
7	Lamp	1. Off 2. On	Off	No
8	Switch 1	1. High 2. Low 3. Pulse 4. Rect wave	High	No
9	Switch 2	1. High 2. Low 3. Pulse 4. Rect wave	High	No

Table 3–6: Timed event parameters (continued)

Number	Event	Units	Range or default	Specify channel
10	Threshold	AU	–4.0000 to 4.0000 or variable, depending on output selection	Yes

To program a new timed event:

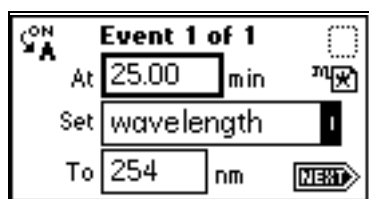
1. Press the METHOD (Shift A/B) key on the detector keypad.

Figure 3–17: Method choice list



2. Press 1, Timed events.
3. Enter the time for the event.

Figure 3–18: Timed events screen



4. Press Enter to enter the time.
5. Press Enter again to display the choice list, or, if you know the event number, press the number for the event you are programming.
6. If the To field appears, enter the appropriate wavelength, in nanometers.
7. Press A/B to set the threshold on the other channel.

Tips:

- ON A or ON B indicates the channel the event is programmed on. You can program all events on channel A, all on channel B, or some on channel A and some on channel B.
- Event programming is time-based, not channel-specific.

8. Press Next to advance to a new timed event.
9. To delete a timed event, press CE when the time field is active.
10. Press HOME to return to the absorbance screen, and then press Run/Stop.

- Press Reset.

Tips:

- If the detector is under the control of an external device, the Inject Start programmed from that device runs the method.
- If you are working in real time under current conditions (method *), when a power failure or shutdown occurs, you lose all timed or threshold events if you do not store them as a method. (See [page 74](#).)

3.2.10.5 Threshold events

You can program threshold events on channel A and channel B to control the switch contact closure outputs: for example, when using a fraction collector. You can program the switch to change when the programmed output (absorbance, ratio, energy, etc.) on the detector channel A or B is above a specified threshold. Below the specified threshold, the switch is set as shown in the table.

Table 3–7: Threshold events “Set” parameters

Number	Event
1	Set switch 1
2	Set switch 2

Table 3–8: Threshold events “To” parameters

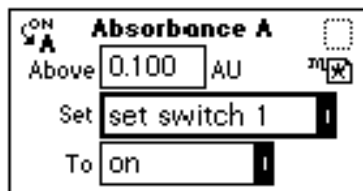
Number	Set to	Below threshold switch state
1	On	Off
2	Off	On
3	Pulse	Off
4	Rect wave (rectangular wave)	Off

To define the pulse period, or the frequency of a wave, see [“Configuring the detector” on page 62](#).

To program a threshold event:

- Press the METHOD (Shift A/B) key on the detector keypad.
- Press 2, Threshold events.

Figure 3–19: Threshold events screen



- Press Enter to advance to the next (Set) field, or press the s and t keys to move among the three fields on the threshold events screen.

- When the Set field is active, press Enter to display the threshold events choice list, or press the number corresponding to the event you are programming (see the table above).
- When the To field is active, press Enter to display the options in the table shown above, or press the number corresponding to the threshold parameter you are programming.
- To set the threshold on the other channel, press A/B.

3.2.10.6 Storing a method

A method consists of all programmable parameters on the absorbance and associated screens as well as timed and threshold events. You can store the current method by selecting a location from 1 to 5.

To store a method:

- Press METHOD (Shift A/B) to return to the Method choice list (see the figure on [page 72](#)).
- Press 4, Store method *.

Result: A method number field appears.

Figure 3–20: Store a method, method number field



Note: No warning message appears when the method number you select is already assigned to a previously stored method. Pressing Enter stores the current method conditions, overwriting any previous method stored in the same slot.

- Enter a number from 1 to 5 and press Enter.

Result: A brief message (“Storing * as method *n*”) appears.

When the display returns to the Method choice list, the method number you selected appears within the method icon. That method remains active until you retrieve another method or reset the detector to default conditions (Method *).

To retrieve a previously stored method:

- Return to the Method choice list by pressing METHOD (Shift A/B).
- Press 3, Retrieve a method.

Result: The last method number stored or retrieved appears in the method number slot box.

- Enter the number of the method you want to retrieve, and press Enter.

Result: A brief message (“Retrieving method *n*”) appears.

When the Method choice list reappears, the method number icon incorporates the method number you specified (see the table on [page 49](#)).

To view timed and threshold events that make up a stored method:

- Retrieve the method (see “[To retrieve a previously stored method:](#)” on [page 74](#)).

Result: Once you specify a method number to retrieve, the method choice list appears, and the method number appears within the method number icon.

2. Press 1, to view the timed events, or 2, to view threshold events within the displayed method.

Tip: If you change to a timed or threshold event within a method, the asterisk appears (Method *), indicating that the method (*) is no longer the same as the stored method you retrieved in step 1. You can then store the method containing the altered event(s) in the same storage slot.

3.2.10.7 Resetting a method

Resetting a stored method is a two-step process. First, you reset the current conditions to the defaults. Then you save the defaults in one of the storage locations. (See [page 60](#) for the parameter default settings.)

To clear one or more methods:

1. Return to the Method choice list by pressing METHOD (Shift A/B).
2. Press 5, Reset method *.

Result: A message appears, asking whether you approve of setting current conditions to factory defaults.

Tip: If you press Enter, these events take place:

- All timed events are deleted.
- All threshold events are disabled.
- All other operating parameters of the method (λ , AUFS, etc.) are set to defaults.

If you press Cancel (Shift 0), the display returns to the Method choice list.

Recommendation: To prevent loss of the current conditions, store them in one of the available storage slots before you clear the method. After clearing the storage slots, you can restore the previous conditions.

3. Press 4, Store method, and enter a storage location number.

Tip: To clear other stored methods, repeat this step until you clear all desired methods.

When you press HOME, the absorbance screen's method number icon displays an asterisk.

3.2.10.8 Clearing events

You can clear only timed or threshold events without resetting any other operating parameters.

To clear all active timed or threshold events:

1. Return to the Method choice list by pressing METHOD (Shift A/B).
2. Press 6, Clear events.

Result: A message appears asking whether you approve of clearing all active events.

Tip: If you press Enter,

- all timed and threshold events in the method are cleared;
- all other operating parameters of the method (λ , AUFS, etc.) are unaffected.

If you press Cancel (Shift 0), the Method choice list appears.

- When you press HOME, the absorbance screen's method number icon displays an asterisk.

3.3 Scanning spectra

Note: If your flow cell does not require a cuvette, you can obtain an analytical flow cell to perform the spectral scanning. See the Waters Quality Parts Locator on the Services and Support page of the Waters' Web site.

The detector must make two scans to produce an absorbance spectrum, as shown in the table below.

Table 3–9: Absorbance spectrum scans

Scan	Description
Zero scan	A reference scan that characterizes the absorbance spectrum of the solvent in the cuvette or the flow cell.
Sample scan	An absorbance scan of the analyte in solvent (after subtracting out the zero scan of the solvent) to provide the actual spectrum of the sample.

The detector can measure the spectrum of a sample using the cuvette or the flow cell. See [page 86](#) and [page 88](#) for scanning procedures.

Requirement: When using the cuvette, if the contents of the flow cell change, you must rerun the zero scan.

3.3.1 Before you begin

Before you run a spectrum scan, specify values for the following parameters:

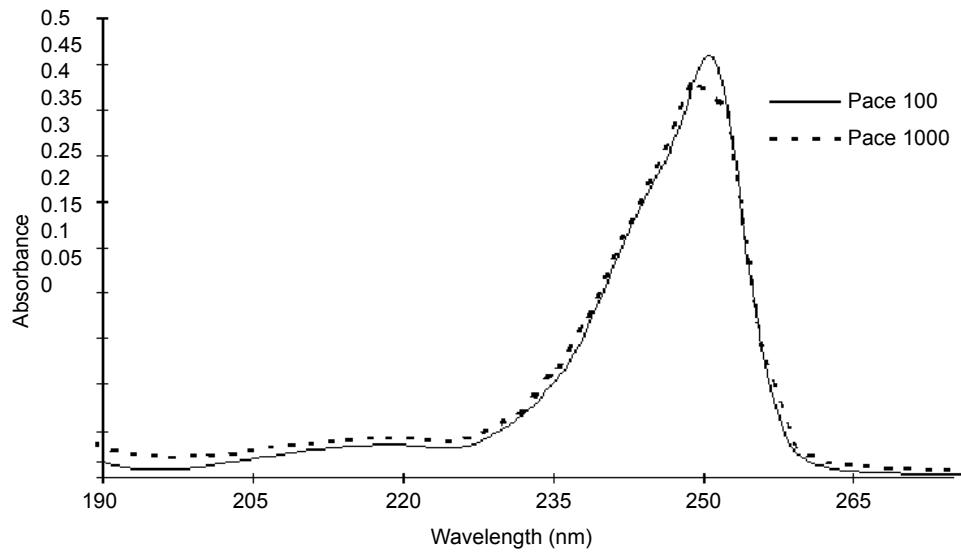
- λ_1 – Starting wavelength. Scanning begins at this wavelength.
- λ_2 – Ending wavelength. Scanning ends at this wavelength.
- Pace – Rate of scanning, in nanometers/min. Determines how fast the scan is output and data are acquired. The scan data are acquired at the highest possible resolution for the specified pace. Specifying a very high pace reduces resolution.

Pace and sampling resolution examples

Pace (nm/min)	Sampling resolution (nm)
100 and less	0.5
200	1.0
400	2.0

The figure below shows two scans of anthracene, one overlaying the other. At a pace of 1000 nm/min, the overlaid scan (dotted line) shows a reduced number of points scanned, lowering the resolution relative to the original scan, done at a pace of 100 nm/min.

Figure 3–21: Scan of anthracene at 100 nm/min and 1000 nm/min



Tip: The higher the number you enter in the Pace field, the lower the resolution of the scan.

- Tick marks – This value allows tick marks (check marks) to be generated at the specified wavelength increment help interpret charted data.

The figures below show a 190-nm to 600-nm scan of erbium standard in a cuvette at 200 nm/min, with tick marks specified every 20 nm, and the same scan without tick marks.

- AUFS – The sensitivity setting used for scaling the charted spectrum.

Figure 3–22: Scan of erbium standard in a cuvette from 190 nm to 600 nm at 200 nm/min, with tick marks every 20 nm

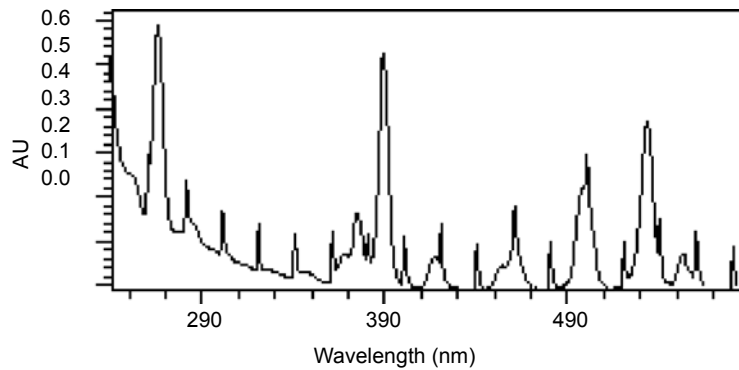
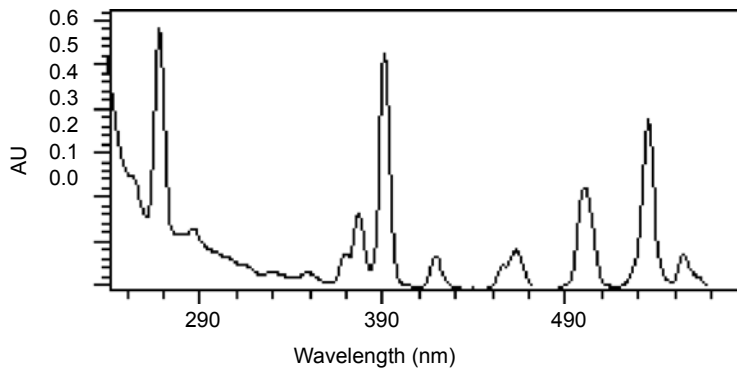


Figure 3–23: Scan of erbium standard in a cuvette from 190 nm to 600 nm at 200 nm/min without tick marks



Specify these parameter values when you select a scan: zero or sample.

When you select a zero scan, the detector displays three additional screens, labeled 2 of 4, 3 of 4, and 4 of 4. You can change all parameters on these screens, including starting and ending wavelength, and the pace parameter.

When you select a sample scan, the detector displays two additional screens, labeled 2 of 3 and 3 of 3 (see the figure on [page 81](#)). You cannot change the starting and ending wavelength values or the pace parameter.

When you run a zero scan, you establish starting and ending wavelengths, pace, tick marks, and sensitivity for both the zero scan and subsequent sample scans. Run sample scans within 15 minutes of the baseline zero scan.

The most recent zero scan performed or retrieved remains current until you perform or retrieve another scan. The zero scan should be appropriate for the subsequent sample scans you perform. The sample scan adopts the starting and ending wavelengths and the pace from the most recent zero scan. Only when those values are identical for both the zero and sample scans can the zero scan be subtracted.

You use the SCAN key (Shift Chart-mark) to run a new zero or sample scan, store, review, subtract and review, and replay stored or existing scans.

During a sample scan, data are charted via analog channel A using the specified AUFS setting. At the same time, sample energy at 150 nA/V is charted via channel B.

During a zero scan, data are charted via analog channel A. At the same time, reference energy, at 150 nA/V, is charted via channel A, at the AU specified on channel A.

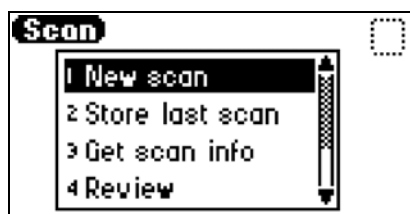
3.3.2 Scanning new spectra

To specify a new spectrum:

1. Press SCAN (Shift Chart-mark).

Result: A Scan choice list appears.

Figure 3–24: Scan choice list



2. Press 1, New scan, or use the s and t keys to move through the Scan choice list.

Result: The detector displays the first of three parameter screens for a sample scan or four parameter screens for a zero scan (figure “Zero and sample scan screen” on page 81).

3. Press Next to advance through the New scan parameter screens.
4. On the first New scan screen, specify the type of scan:
 - Press 1 for sample scan, or press Enter to display the choice list. The detector displays two additional screens.
 - Press 2 for zero scan, or press Enter to display the choice list. The detector displays three additional screens.

All parameters appear on the first New scan screen for both a zero and a sample scan. You can return to screen 1 to review the parameters for either scan type by pressing Next from the Run screen (screen 3 of 3 for sample scan, or 4 of 4 for zero scan).

Tip: You can press Run from any New scan screen.

For both zero and sample scans, the table below provides the defaults and ranges for all parameters.

Table 3–10: Sample and zero scan parameters

Parameter	Screen	Scan type	Units	Range or default
Type	1	Sample and zero	n/a	Sample scan: 1 Zero scan: 2 Default: 1
λ range	2	Zero scan only	nm	Range: 190 to 700 nm Default: 190 to 700 nm
Pace	2	Zero scan only	nm/min	Range: 30 to 1000 nm/min Default: 100 nm/min
AUFS	2 or 3	Sample and zero	AU	Range: 0.0001 to 4.0000 Default: Last number entered
Tick mark (Mark each nm)	2 or 3	Sample and zero	nm	Range: 10 to 100 Default: Last number entered

To program a zero scan:

1. Press SCAN, then 1, New scan, and press 2, Zero scan.
2. Press Next to advance to the second Zero scan parameter screen.
3. Enter the starting wavelength for the zero scan, and then press Enter.

4. Enter the ending wavelength for the zero scan, and then press Enter.
5. Enter a value in the Pace field for the rate at which the detector scans the specified wavelength range.

Tips:

- The default is 100 nm/min. The allowable range is from 30 to 1000 nm. Refer to the figure on [page 77](#), which shows two scans of anthracene overlaid, one at 100 nm/min and one at 1000 nm/min.
- The higher the value you enter in the Pace field, the lower the resolution of the scan.

6. Press Next.
7. On the third Zero scan parameter screen, enter the AUFS value, and then press Enter.

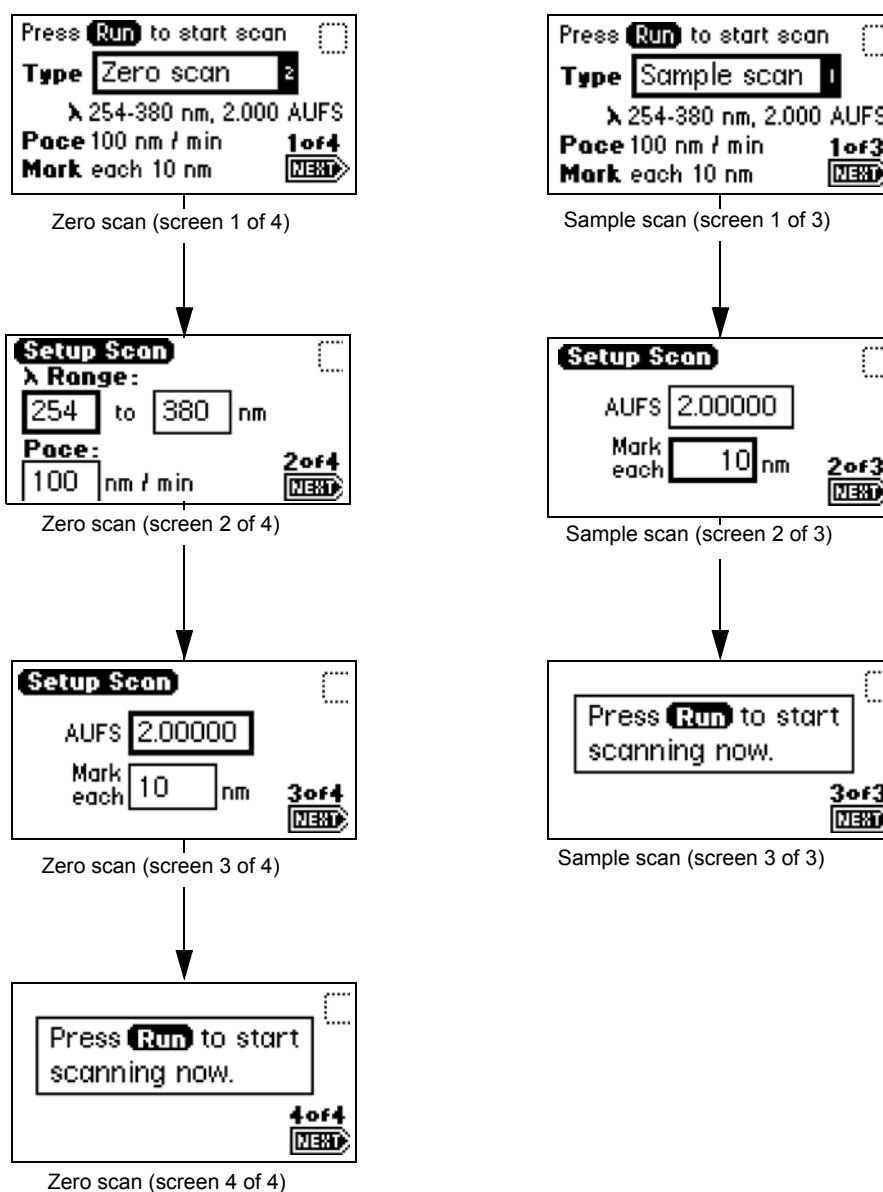
Tips:

- To specify tick marks, enter a number from 10 nm to 100 nm, and then press Enter.
- To clear tick marks, press CE. See [page 77](#) for examples of scans made with and without tick marks.

8. Press Run to start the zero scan, or press Next to return to the first Zero scan parameter screen to verify parameter values, and then press Run.

Result: After the detector runs the zero scan, it returns to the Scan choice list.

Figure 3–25: Zero and sample scan screen



3.3.2.1 Running the sample scan

Run the zero scan before running the sample scan. To ensure identical flow cell and solvent conditions, run the sample scan for the corresponding zero scan within 15 minutes of running the zero scan.

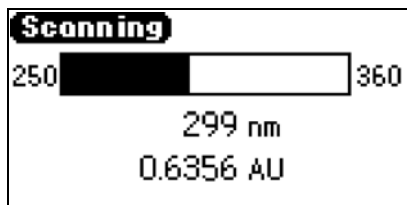
To run a sample scan:

1. Set up the zero (or reference) scan by following the steps in the zero scan procedure on “To run a sample scan:” on page 81.
2. On the first New scan screen, press 1, Sample Scan.

Result: The parameters for wavelength range, AUFS, Pace, and Mark (tick mark) you entered for the corresponding zero scan appear.

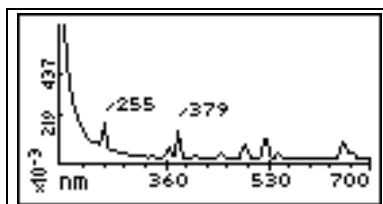
- Press Next to advance to the second sample scan screen.
Tip: You can change the entries in the AUFS and Mark each fields.
- Press Next to advance to the third sample scan screen, and then press Run.
Result: A brief message (“Initializing”) appears. The Scanning screen shows the progress of the scan, in nanometers, across a progress bar.

Figure 3–26: Scanning progress bar



After a brief pause, the detector displays a sample scan graphically.

Figure 3–27: Sample erbium scan graphical display

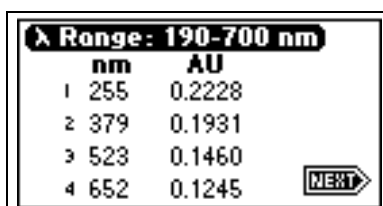


Tip: To return to the Scan choice list, at the end of the scan, press SCAN (Shift Chart-mark).

- Press Next.
Result: Doing so displays as many as four of the highest peaks scanned within the specified range.

The figure below shows the four highest peaks for the erbium scan shown in the graphical display (above).

Figure 3–28: Sample erbium scan, four highest peaks



- Press Next.
- On the graphical display, press Scale (Shift TRACE) to change the scale (enlarge, or zoom, a part) of the display.
Tip: You can alter the following four scaling parameters:
 - λ1 – minimum wavelength displayed.
 - λ2 – maximum wavelength displayed.
 - AU1 – minimum absorbance displayed. The default is Auto.

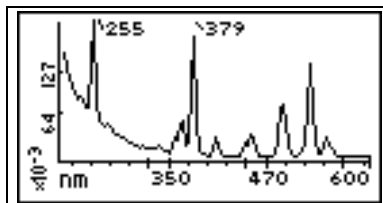
- AU2 – maximum absorbance displayed. The default is Auto.

Using this function, you zoom in on various sections (artifacts) of the spectrum.

The scale of the spectrum is affected by the AUFS setting.

8. Press Next to advance through the four scaling parameters. The figure below shows the sample scanned in the figure on [page 82](#). The sample has been scaled by changing the wavelength parameters to 225 and 600 nm.

Figure 3–29: Sample erbium scan with λ_1 changed to 225 nm and λ_2 changed to 600 nm



9. Once you change one or more scaling parameters, press Enter to reformat the graphical display.
10. Once the scan reappears, press Next to display the four highest peaks of the scaled scan.

Figure 3–30: Four highest peaks from the scaled sample erbium scan

Range: 220-600 nm	
nm	AU
1 255	0.2228
2 379	0.1931
3 523	0.1460
4 241	0.0949

NEXT →

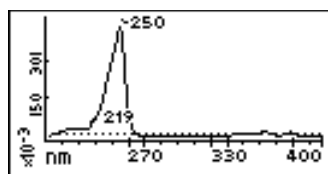
11. Press Next again to return to the sample scan display.
12. When you finish manipulating the sample scan graphical display, press SCAN (Shift Chart-mark) to return to the Scan choice list.

Tip: To store the scan, see [“Storing a spectrum” on page 84](#).

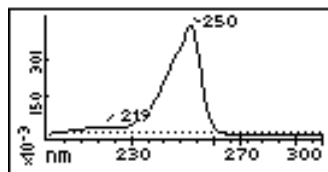
The figure below shows a series of scans of anthracene dissolved in acetonitrile to display the use of the Scale function. The zero scan is not displayed.

For the scaling parameters AU1 and AU2, the default is Auto. You can change the AU parameter based on the absorbance of the spectrum. To return the default to auto, press CE.

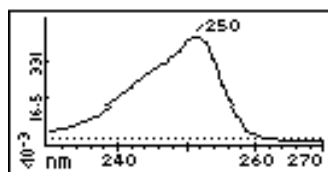
Figure 3–31: Series of scans of anthracene in acetonitrile



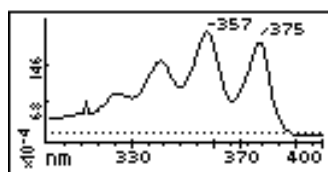
Sample scan
200 nm to 400 nm
–0.001 AU to 0.5 AU
anthracene



Zoom of sample scan
200 nm to 300 nm
–0.001 AU to 0.5 AU
anthracene, 230 nm to 270 nm
 λ_2 changed to 300 nm



Zoom of sample scan
230 nm to 270 nm
–0.001 AU to 0.5 AU
anthracene, 250 nm
 λ_1 changed to 230 nm
 λ_2 changed to 270 nm
AU1 and AU2 on auto



Zoom of sample scan
300 nm to 400 nm
–0.001 AU to 0.025 AU
anthracene, 330 nm to 400 nm
 λ_1 changed to 300 nm
 λ_2 changed to 400 nm
AU1 and AU2 on auto

3.3.3 Storing a spectrum

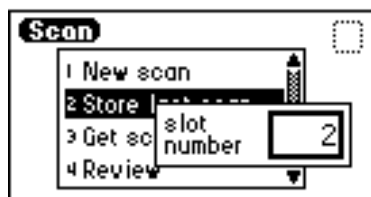
Once you run a spectrum, you can store it for later review, subtraction, or playback. You can store as many as three spectra.

To store a spectrum:

1. From the graphic display of a sample scan, return to the first Scan screen by pressing SCAN (Shift Chart-mark).
2. Press 2, Store last scan.

Tip: When you select Store last scan, you are storing the zero scan and the sample scan as a pair.

Figure 3–32: Storage slot number box



3. In the slot number box, enter a number from 1 to 3.
4. Press Enter to store the last sample scan, paired with its zero scan.

To obtain information about a stored spectrum:

1. Press SCAN (Shift Chart-mark) to view the Scan choice list.
2. Press 3, Get scan info.
Result: A slot number box appears with a default of “Last” (the most recently stored spectrum).
3. Press Enter to get information about the last stored spectrum, or type the number (1 to 3) of the stored spectrum for which you want information, and then press Enter.
Result: A screen containing the following information appears:
 - Storage slot number of the selected scan (or “Last scan”)
 - λ range – Displays the wavelength range for the selected spectrum
 - Pace – Displays the pace for the selected spectrum
4. Press Enter to exit the information screen and return to the Scan choice list.

3.3.4 Reviewing a stored spectrum

Once you store a spectrum, you can retrieve it for review from one of the three available storage slots by selecting the Review option from the Scan choice list.

To review a spectrum:

1. Press SCAN (Shift Chart-mark) to view the Scan choice list.
2. Press 4, Review.
Tip: When you select Review, you actually are retrieving the zero scan and the sample scan that were stored as a pair.
3. Enter the storage slot number (1 to 3) of the spectrum you want to review.
4. Press Enter. The message “Retrieving spectrum *n*” appears.

Once you retrieve a spectrum for review, you can view it graphically and adjust the wavelength and AU ranges, if necessary. You can also run a new sample scan based on the retrieved zero scan.

3.3.5 Subtracting a spectrum

Once you store more than one spectrum, you can create a difference spectrum.

Tip: The current spectrum is the spectrum that the stored spectrum (whose slot number you enter) is subtracted from.

To subtract and review the difference spectrum:

Rule: To subtract the stored spectrum from the current spectrum, the starting and ending wavelengths (λ_1 and λ_2) and the pace of both spectra must be identical.

1. Press SCAN (Shift Chart-mark).
2. Press 5, Subtract & review.
3. Enter the storage slot number (1 to 3) of the spectrum you want to subtract from the current (or retrieved) spectrum.
4. Press Enter.

Once the difference spectrum appears, you can store the results in one of the three storage slots.

3.3.6 Replaying a spectrum

You can play back the current spectrum or a stored spectrum, in real time, using the Real-time replay function on the Scan choice list. The detector plays back the selected spectrum in real time both on the detector display and out the analog connector to the chart or A/D device in a data collection system. Once you retrieve a spectrum for playback, the detector displays it graphically, and you can adjust the AUFS. Sample energy is not charted during a replay.

Tip: If you adjust the AUFS on a spectrum for replay, the adjusted spectrum displays only on the charted output, not on the detector's graphical display.

To replay a spectrum:

1. Press SCAN (Shift Chart-mark).
2. Press 6, Real-time replay.
3. Enter the storage slot number (1 to 3) of the spectrum you want to play back.
Tip: The default is the last spectrum acquired.
4. Press Enter.

Result: After a pause, to retrieve the selected spectrum, the detector begins to play back the spectrum on the analog connection. Then the spectrum appears graphically.

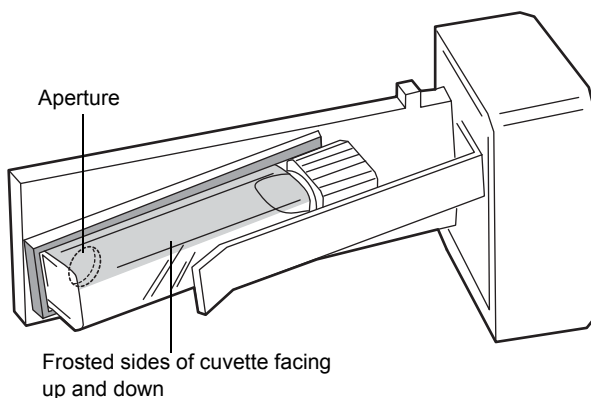
3.3.7 Scanning using the cuvette

Note: If your flow cell does not require a cuvette, you can obtain an analytical flow cell to perform the validation procedures. See the Waters Quality Parts Locator on the Services and Support page of the Waters' Web site.

Using the cuvette option facilitates sample handling and verifying and qualifying the instrument.

The detector uses a standard, 10-mm path length, spectrophotometric cell (quartz cuvette). You insert the cuvette with one of the two frosted sides facing up in the cuvette holder and place it in the detector flow cell assembly.

Figure 3–33: Detector cuvette holder with the cuvette inserted



Restriction: Because the scan is actually a composite of both the contents of the cuvette and the flow cell, you need to perform cuvette scans under identical flow cell conditions. If you store spectra and acquire new spectra for subtraction, beware the differences, if any, in flow cell conditions.

Ideally, you perform both the zero and sample scans using the cuvette when the HPLC instruments are in the idle or static state, under identical flow cell conditions.

! **Notice:** To avoid compromising the integrity of cuvette scanning operations, handle the cuvette gently and on the frosted sides only. Fingerprints on the clear quartz interfere with the light path.

3.3.7.1 Before you begin

Recommendation: To ensure accurate results, use a 10-mm path length, quartz cuvette and matched pairs (from the same manufacturing lot) of quartz cuvettes for your zero and sample scans.

Before beginning a scan using the cuvette:

1. Flush the flow cell with the eluent you are going to scan.
2. Wipe the clear portion of the cuvette with low-lint, nonabrasive tissue wipes.

To begin a cuvette scan:

1. Open the detector's front door.
2. Remove the cuvette holder, sliding it toward you.
3. With the spring guide facing you, gently insert the cuvette (containing eluent) up and under the guide, with the cap facing upward (into the holder) and a frosted side of the cuvette facing up. (See the figure on [page 87](#).)

Recommendations:

- Ensure that you have enough liquid (3 mL) in the cuvette so that when it is inserted into the holder, you can see liquid through the holder's aperture. That is, the liquid must completely cover the aperture.

- Because the cuvette holder is angled, use your thumb or forefinger to ensure the cuvette is secure in the slot and does not slide forward. Ensure that it does not become dislodged when you are replacing the cuvette holder.
4. Gently guide the cuvette holder back into the flow cell assembly until it bottoms in the space.
 5. Close the door.

Rules:

- To prevent invalid chromatographic results, remove the cuvette from the detector and replace the empty holder after running cuvette scans.
 - To maintain optimum system performance, close the front door before resuming normal operation of the detector.
6. Insert a reference cuvette containing the mobile phase standard, and run a zero scan.
 7. Replace the reference cuvette with a cuvette containing your analyte dissolved in mobile phase solvent, and run a sample scan.
 8. Use the storage, review, subtract and review, and replay functions to analyze the data obtained.

3.3.8 Scanning using a flow cell and a syringe

If you do not have a cuvette, you can scan using a manually filled flow cell.

Requirement: Before you scan using a flow cell, make sure no cuvette is in the cuvette holder.

To run a spectrum using a flow cell:

1. With a syringe, fill the flow cell with the mobile phase or solvent in which your sample is dissolved.
2. Run a zero scan according to the procedure on [page 78](#).
3. With a syringe, fill the flow cell with the analyte, and then run a sample scan according to the procedure on [page 78](#).

Use the storage, review, subtract and review, and replay functions of the detector to compare the scanned data.

3.3.9 Conserving lamp life

You can conserve the lamp without powering-off the detector by leaving the instrument on and turning off only the deuterium lamp.

Tip: If the detector is operating under remote control, you can program the controller to turn the lamp off and on without using the front panel.

Recommendation: Program the lamp to shut off or turn the lamp off manually only if the value of the “Lamp off” parameter is more than 4 hours.

Without powering off the system, you can conserve lamp life by,

- turning power to the lamp off and on again manually;
- programming a timed event to turn the lamp off and on again;
- programming the lamp to turn off and on again using the external contact closure.

To turn power to the lamp on and off manually, use the Lamp keypad function (Shift 1). When the lamp is turned off, the absorbance screen displays the words “Lamp off”, and the lamp icon appears with an “X” through it.

Use the Lamp key (Shift 1) to,

- shut the lamp off or turn the lamp on manually;
- display the number of times the lamp has ignited;
- display the hours and minutes the lamp has been turned on during the current run, since its installation, or both.

To turn off the lamp manually from the detector front panel:

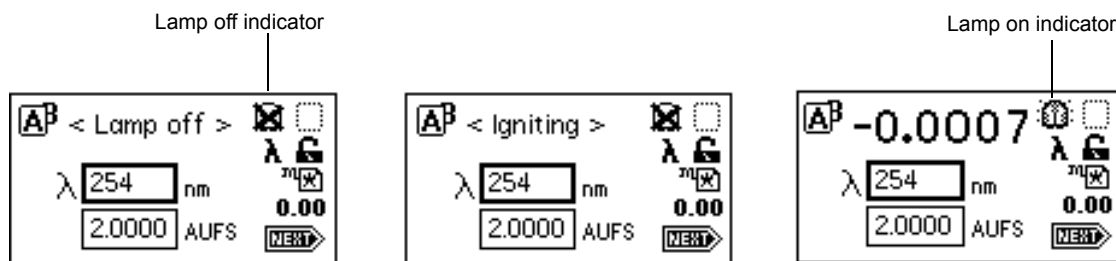
1. Press Lamp (Shift 1) from the keypad.
Result: The lamp control screen appears.

Figure 3–34: Lamp control screen



2. Press Lamp (Shift 1) again to turn the lamp off.

Figure 3–35: Lamp off/on sequence



To ignite the lamp manually:

1. When there is an X through the lamp icon on the absorbance screen, press Lamp (Shift 1).
2. Press Lamp (Shift 1) again to turn the lamp on.

Result: The absorbance screen appears with the message “Igniting.” The lamp can take as much as 1 minute to illuminate. Once the lamp illuminates, the display returns to the absorbance screen, and the X is removed from the lamp icon.

You can conserve lamp life by programatically igniting and extinguishing it: for example, overnight, using a timed event method.

To ignite or extinguish the lamp programatically, select the Timed events option in the Method choice list, or program the lamp through one of the external contact closures.

- See “Programming timed events, threshold events, and methods” on page 70, and the table on page 71 for more information on programming the lamp to turn on or off using a timed event.
- See “Configuring event inputs (contact closures)” on page 63 for more information on programming the lamp through the external contact closure.

3.3.10 Shutting down the detector

If you need to power-off the detector for an extended length of time, you must remove any buffered mobile phase from the fluid path.

! **Notice:** To avoid damage to the column, remove it before you perform the following procedure. Before you remove the column, see your column care and use guide.

To remove mobile phase from the fluid path of the detector:

1. Replace the buffered mobile phase with 100% HPLC-quality water, and flush the system for 10 minutes at 3 mL/min.
2. Replace the 100% water mobile phase with a solution of 90:10 methanol/water, and flush the system for 10 minutes at 3 mL/min.

Follow the recommended procedures for injector purging and priming for the pump in use with your HPLC.

To shut down the detector:

Press the On/Off switch.

4 Maintaining the Detector

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

4.1 Contacting Waters technical service

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800 252-4752). Otherwise, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Our Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Go to www.waters.com.

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

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

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

4.2 Maintenance considerations

Perform the procedures in this chapter when you discover a problem with a 2489 UV/Vis detector component or during preventive maintenance.

4.2.1 Safety and handling

Observe these warnings when you perform maintenance operations on a 2489 UV/Vis detector.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the 2489 UV/Vis detector. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



Warning: To avoid electric shock:

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



Notice: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the detector. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the ac wall outlet. Wait 10 seconds after discontinuing power before attempting to disconnect the assembly.

4.2.2 Spare parts

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

4.3 Proper operating procedures

Note:

- The detector contains no user-serviceable parts. Do not remove the top cover.
- To maintain optimum performance, close the detector's front door before resuming normal operation.

4.3.1 Routine maintenance

The detector requires minimal routine maintenance.

To achieve optimal performance,

- replace solvent reservoir filters in your HPLC system regularly;
- filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise;
- flush buffered mobile phases out of the detector with HPLC-grade water followed by a 5 to 10% methanol solution each time the detector is powered-off. This process prevents,
 - plugging of the solvent lines and flow cell.
 - damage to the components.
 - microbial growth.

4.4 Maintaining the flow cell

Note: To ensure proper initialization of the detector and the long serviceability of flow cells, use well-degassed eluents, making sure they are flowing before you power-on the detector.

A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems with operation of the detector.

There are two phases in cleaning the flow cell:

- Flushing
- Removing and cleaning

If flushing is ineffective, remove and clean the flow cell. Replace flow cell components, as required.

Note: Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

4.4.1 Flushing the flow cell

Flush the flow cell when it becomes contaminated with the residues of previous runs and after each detector shutdown. A dirty flow cell can cause baseline noise, decreased energy levels, calibration failure, and other problems. Always flush and purge the flow cell as an initial attempt to correct these problems.

You should flush the flow cell whenever,

- noise is higher than expected;
- noise test results are not meeting specifications;
- the detector fails to normalize.

! **Notice:** To avoid damaging the flow cell during reverse flushing, do not overpressure the cell.

If you use buffered mobile phase, flush it from the detector before powering-off.

! **Notice:** If the flow cell will not be used for several days, flush it with clean mobile phase, such as a water/acetonitrile or water/methanol, and then cap the flow ports or dry the flow cell with pure nitrogen or pure helium.

! **Notice:** To prevent flow cell failure, do not connect any tubing or device that can create backpressure exceeding the flow cell's maximum rating of 6895 kPa (69 bar, 1000 psi).

Requirement: Always use well-degassed eluents.

To flush the flow cell:

1. Stop the solvent flow, and remove the column.
2. Replace the column with a union or piece of tubing.

Note: If the mobile phase is not compatible with water, flush with an intermediate solvent first.

3. Flush the detector with HPLC-quality water.
4. Pump 100% methanol through the flow cell to clean it internally. Do not exceed 6895 kPa (69 bar, 1000 psi).
5. Pump a strong cleaning solvent, such as isopropanol, through the flow cell (optional). Do not exceed 6895 kPa (69 bar, 1000 psi).

Note: If the mobile phase is not miscible in water, first use an intermediary solvent.

6. Resume pumping mobile phase.
7. Reattach the column.
8. If the flow cell remains dirty or blocked, reverse flush it.

To perform optimal cleaning:

1. Extinguish the detector lamp.
2. Stop the solvent flow, and then disconnect the column inlet and flow cell inlet tubing.
Tip: For more efficient flushing, replace the column with a union or piece of tubing.
3. Connect the column inlet tubing to the flow cell inlet.
4. If another instrument is downstream of the flow cell outlet, break the connection at the other instrument, and route the outlet tubing to waste while flushing.
5. Flush the detector using a miscible solvent and HPLC-quality water at the recommended flow rate.
6. Flush the detector using HPLC-quality water at the recommended flow rate. If the mobile phase is not compatible with water, flush using an intermediate solvent first.
7. Pump 100% acetonitrile through the flow cell at the recommended flow rate to clean it internally.
8. Pump a strong cleaning solvent, such as isopropanol, through the flow cell at the recommended flow rate (optional).

If the flow cell is dirty, remove any other active detectors from the system, and then pump 1% weak concentration acid solution (such as formic acid solution) through the flow cell at the recommended flow rate.



Warning: To prevent injury, always wear eye protection and gloves when handling strong acids or bases.

9. Flush using HPLC-quality water at the recommended flow rate until the pH of the effluent is neutral.
10. Reattach the column, and then resume pumping mobile phase.

Requirement: If the mobile phase is not miscible in water, first use an intermediary solvent.

To reverse flush the flow cell:

If directly flushing the flow cell does not improve flow cell performance, reverse flush it at the recommended flow rate.

1. Reverse the inlet and outlet tubing connections to the flow cell.

2. Flush the flow cell for approximately 15 minutes at the recommended flow rate. Decreasing system pressure indicates the flow cell is clean.
3. If the flow cell remains dirty or blocked, remove and replace it.

4.4.2 Removing and cleaning the flow cell

If flushing the flow cell is not effective, follow the procedures to remove the flow cell and inspect for dirty or broken windows or dirty gaskets. Clean and replace parts if necessary.

Before removing the flow cell assembly, purge the flow cell with nitrogen, and allow it to dry.

To purge the flow cell:

1. Connect the nitrogen supply line to the sample inlet, routing the sample line to waste.
2. Purge the cell for 25 to 30 minutes at 103 to 138 kPa (1 to 1.4 bar, 15 to 20 psi).
3. Allow the flow cell to dry completely.
4. Disconnect the detector's inlet and outlet tubing from the main column connection and cap.

4.4.3 Disassembling and reassembling the flow cell

4.4.3.1 Before you begin

Take the following precautions when disassembling and reassembling the flow cell:

- To prevent contamination, wear clean, chemical-resistant, powder-free gloves when touching the flow cell lenses or window.
- Take care to prevent scratching of the flow cell parts.
- Use a clean, lint-free cloth or a similar clean surface in the work area where you are removing, rebuilding, and replacing the flow cell.

! **Notice:** Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

Required tools and materials

- Chemical-resistant, powder-free gloves
- 1/4-inch flat-blade screwdriver
- Plastic tweezers
- Torque wrench set to 0.9 N•m (128 in-oz, or 8 in-lb) with 1/4-inch flat-blade screwdriver bit
- Ethyl alcohol or methanol
- Flow cell rebuild kit
- Lint-free swab
- Nitrogen

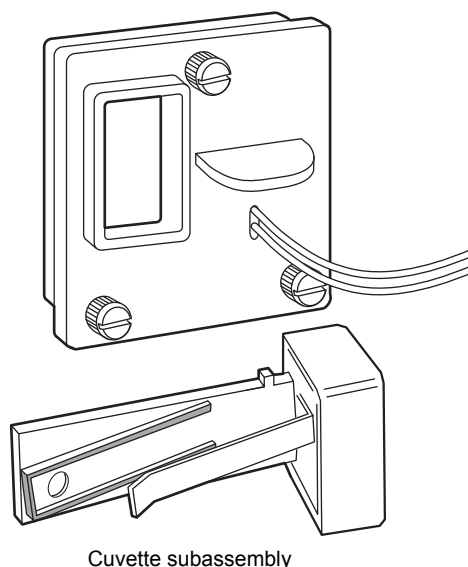
Save all parts after removal unless otherwise indicated. Most of the removed parts are needed when you reinstall a flow cell.

4.4.3.2 Removing the flow cell

To remove the flow cell assembly:

1. Power-off the detector and disconnect the power cord.
2. Open the door.
3. After flushing and drying the flow cell (see [page 93](#)), disconnect and cap the inlet and outlet LC tubing to the detector.
4. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the flow cell assembly front plate.
5. Gently pull the assembly toward you.
6. If the detector has an analytical flow cell (standard in the Alliance HPLC 2489 UV-Vis), once you remove the flow cell assembly from the detector chassis, remove the cuvette subassembly from the flow cell assembly.

Figure 4–1: Analytical flow cell assembly with the cuvette subassembly removed



7. Place the flow cell assembly on a flat clean surface.
8. If the flow cell will not be used for a period of time, flush it using clean mobile phase, such as a water/acetonitrile mix, and either cap the flow ports or dry the flow cell using pure lab gases such as helium, nitrogen, or air for 5 to 10 minutes.

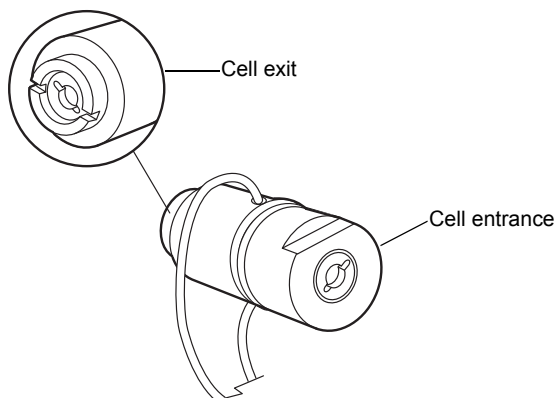
4.4.3.3 Disassembling the flow cell

! **Notice:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly.

The TaperSlit flow cell consists of these components:

- Flow cell body
- Cuvette lens
- Split ring (cuvette lens holder)
- Cuvette lens screw
- Lens mount screw
- Exit window
- Exit window mount
- Entrance lens holder
- Entrance lens
- Entrance lens mount
- Two gaskets

Figure 4–2: Waters TaperSlit flow cell



For replacement parts for the TaperSlit flow cell, use the Flow Cell Rebuild Kit.

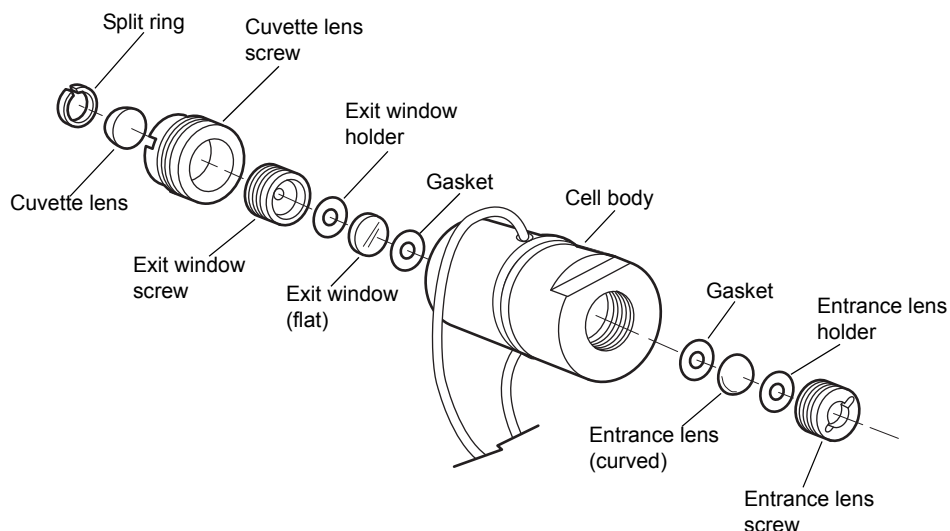
Tip: Use nitrogen to clean the flow cell. Use ethanol or methanol to clean the lenses and window.

To remove the individual parts of the flow cell for cleaning or replacement:

1. With the notches on the flow cell cuvette lens end facing you, use the flat-blade screwdriver or a coin to remove the cuvette lens screw.
2. Insert the cotton end of the lint-free swab into the cuvette end of the flow cell assembly, and remove the split ring and the cuvette lens.
3. Using the screwdriver, remove the exit lens mount screw.
4. Using plastic tweezers, pull out the exit window from inside the exit lens mount screw.
5. Using the plastic tweezers or the lint-free swab, remove the clear plastic gasket.
6. Flip the flow cell over to the entrance lens side.
7. Using the flat-blade screwdriver, remove the entrance lens screw.
8. Using the plastic tweezers, remove the entrance lens from inside the entrance lens screw.
9. Using the plastic tweezers or the lint-free swab, tap and remove the clear plastic gasket.

The following figure shows an exploded view of all parts of the flow cell within the flow cell assembly.

Figure 4–3: Waters TaperSlit flow cell, exploded view



4.4.3.4 Inspecting, cleaning, and replacing damaged flow cell components

! **Notice:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly. Work on a clean flat surface, such as a lint-free cloth or similar surface.

To inspect or clean the parts of the flow cell or to replace damaged parts of the flow cell such as the lenses, exit window, or gaskets, follow this procedure and then rebuild the flow cell.

Recommendation: Replace the clear plastic gaskets each time you inspect and clean the flow cell.

To inspect and clean the flow cell:

1. Inspect each part of the removed flow cell for dirt.
2. Using ethanol or methanol, clean the contaminated part, and dry it under a stream of nitrogen.
3. Using the Flow Cell Rebuild Kit, replace any flow cell parts that are scratched, burred, damaged, or do not come clean using nitrogen.
4. Rebuild the flow cell by following the procedure in the next section.

4.4.3.5 Rebuilding the flow cell

After cleaning or replacing other flow cell parts, rebuild the flow cell.

To rebuild the flow cell:

1. Using the plastic tweezers, remove the new clear plastic gaskets from the Flow Cell Rebuild Kit, and inspect them for dirt.
2. Drop one clear, plastic gasket into the groove at the bottom of the entrance lens end of the flow cell body.
3. Inspect the entrance lens, and use nitrogen to blow dust off, if necessary.
4. Using plastic tweezers, drop the entrance lens into the flow cell body, curved-side facing up.
5. With the tan-colored entrance lens holder curve facing down, use the torque wrench to tighten the entrance lens screw into the flow cell body to 0.9 N•m (128 in-oz, or 8 in-lb).
6. Flip the flow cell body over to the exit window side.
7. Using plastic tweezers, inspect the second new gasket for cleanliness.
8. Drop the clear plastic gasket into the groove at the bottom of the cuvette lens end of the flow cell body.
9. Inspect the exit window for cleanliness. Using nitrogen, clean the exit window, if necessary.
10. Using plastic tweezers, place the exit window into the flow cell body.
11. With the tan-colored exit window holder facing down, use the torque wrench to tighten the exit window screw into the flow cell body to 0.9 N•m (128 in-oz, or 8 in-lb).
Tip: To ensure complete compression of the gaskets, after replacing the exit window and torquing the exit window screw, and after replacing the cuvette lens and torquing the cuvette lens screw, you must flip the flow cell body and torque the entrance lens screw to 0.9 N•m (128 in-oz, or 8 in-lb) a second time.
12. Flip the flow cell body again, and torque the entrance lens screw to 0.9 N•m (128 in-oz, or 8 in-lb).
13. Flip the flow cell body again to the cuvette lens end, and torque the exit lens screw again to 0.9 N•m (128 in-oz, or 8 in-lb).
14. With the curved side of the cuvette lens facing up, replace the lens in its screw mount.
15. With gloved fingers, place the split ring onto the cuvette lens, and press it firmly until it is flush on all sides.
16. Using the cuvette tool, turn the cuvette lens screw into the exit-lens- mount end of the flow cell body.
17. Torque the cuvette lens screw to 0.9 N•m (128 in-oz, or 8 in-lb).
18. Follow the procedures in the next section to refit the flow cell in the flow cell assembly.

4.4.3.6 Replacing the flow cell

- !** **Notice:** To prevent contamination, wear clean, chemical-resistant, powder-free gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters patented TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly.

The detector is shipped with a standard analytical flow cell installed. Replace the flow cell when

- the flow cell becomes damaged;
- you want to use one of the optional flow cells (see the table on [page 134](#)).

To prepare for replacing the flow cell:

1. Flush columns using at least 10 column-volumes of clean mobile phase before connecting them to the flow cell.
Example: flush a 4.6 × 50 mm HPLC column for 10 minutes at the recommended flow rate.
2. Unpack and inspect the new flow cell.
3. Power-off the detector and disconnect the power cord.
4. Open the front door.
5. Disconnect the detector's inlet and outlet tubing from the main column connection and cap.

To replace the flow cell:

1. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the front plate of the flow cell assembly.
2. Gently pull the assembly toward you.
3. Insert the new flow cell assembly into the detector.
4. Tighten the captive screws.
5. Reconnect the inlet/outlet tubing to your LC system.
6. Reconnect the power cord, and then power-on the detector.

4.5 Replacing the lamp

Recommendation: Waters recommends that you change the detector lamp when it repeatedly fails to ignite, or when the detector fails to calibrate.

Recommendation: Invoke the lamp optimization software routine at least once a week by powering-off the detector, waiting 10 seconds, and then powering-on the detector.

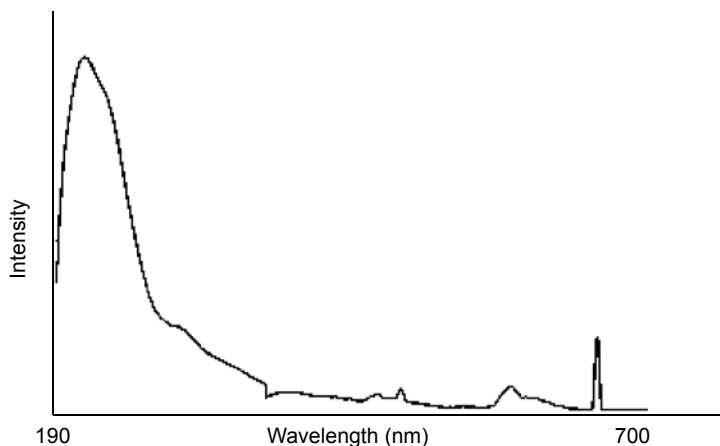
See also: Online Help, for alarm messages and recommended actions you can take to correct them and [“Using the diagnostic tests” on page 113](#).

This section describes the procedure for removing and replacing the detector deuterium lamp.

4.5.1 Lamp characteristics

The intensity of the deuterium source lamp varies by wavelength, as shown in the figure below.

Figure 4–4: Deuterium lamp sample beam intensity profile



4.5.2 Lamp energy and performance

As the lamps used in traditional detectors age, the signal-to-noise performance of the instruments degrade. Determining the longevity of useful lamp life can be difficult because of varying user requirements and individual lamp behavior.

The detector's design also compensates for the lamp energy changes that occur across the deuterium spectrum as well as lamp aging. Thus the detector can operate at the same, high signal-to-noise performance at longer wavelengths of the visible range without the use of a second lamp, such as tungsten.

The detector performs several self-diagnostic tests when powered-on or when the Calibrate key is selected. One of these tests is the lamp optimization software routine. After the detector verifies the calibration of the monochromator, it evaluates energy levels in several characteristic regions across the spectrum. The integration time of the front-end electronics is adjusted to maximize the signal within these regions. The effect is to maintain a high signal-to-noise ratio and operate with a clean signal.

When operating the detector, run the lamp optimization software routine at least once a week.

Eventually, the lamp's signal becomes low enough that replacement becomes necessary. You should replace the lamp when the energy value approaches 15 nanoamps (nA), which corresponds to the cutoff adopted in the detector's diagnostic tests. Ultimately, however, the detector's performance is a function of its application so replace lamps whenever you determine doing so is appropriate for your method.

The detector's on-board diagnostic tests allow the recording of lamp usage and reporting of the lamp serial number.

4.5.2.1 When to replace the lamp

Requirement: Always run the Change Lamp diagnostic test after you install a new lamp (see [page 116](#)).

Replace the lamp when

- it fails to ignite at startup;
- its energy level causes a decrease in sensitivity to the point where the baseline is too noisy for your LC application.

Rule: Always run the procedure in “Recording sample and reference beam energies” on page 66 when you change the detector lamp.

Waters warrants the lamp to ignite and pass startup diagnostic tests for 2000 hours or one year from the date of purchase, whichever comes first.

4.5.3 Removing the lamp



Warning: The lamp housing gets extremely hot during operation. To prevent burn injuries,

- allow the lamp to cool for 30 minutes before removing it;
- keep the lamp in the housing when handling the lamp.



Warning: To avoid eye injury from ultraviolet radiation exposure,

- power-off the detector before changing the lamp;
- wear eye protection that filters ultraviolet light;
- keep the lamp in the housing during operation.

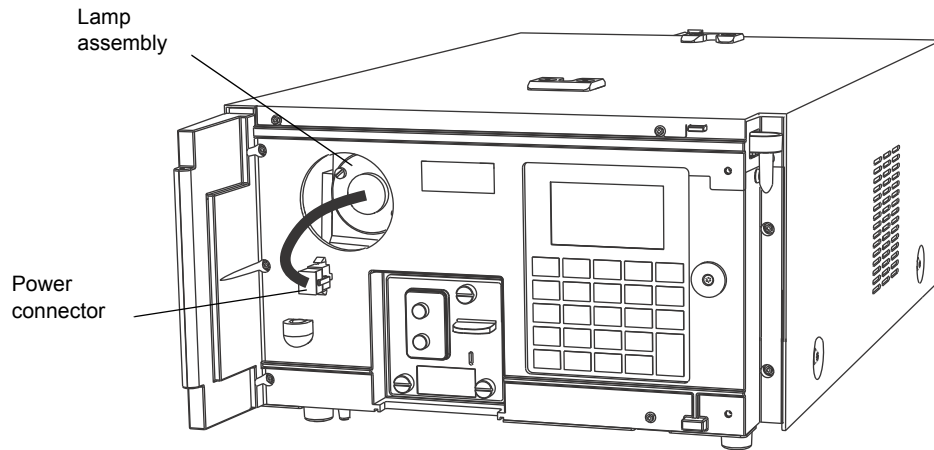
To remove the lamp:

1. Extinguish the lamp using the keypad by pressing <Shift> <Lamp> and then pressing <Shift> <Lamp> again.

Tip: Using the keypad to extinguish the lamp allows the lamp to cool faster because of the operation of the instrument’s cooling fan. To power-off the lamp using a timed event, see the instructions in the Empower or MassLynx online Help.

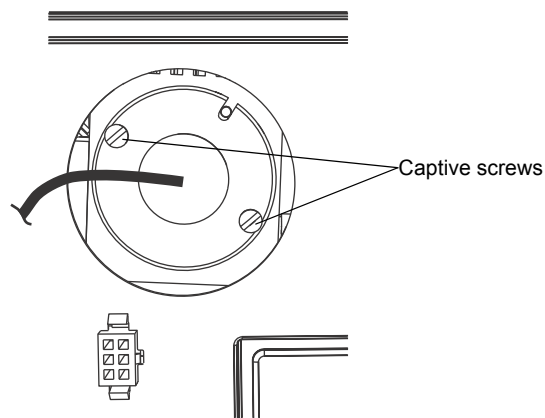
2. Power-off the detector, and disconnect the power cord.
3. After extinguishing the lamp, allow it to cool for at least 30 minutes.
4. Open the front door.
5. Disconnect power to the lamp and disconnect the cord.

Figure 4–5: Lamp assembly and power connector



6. Loosen the two captive screws in the base lamp.

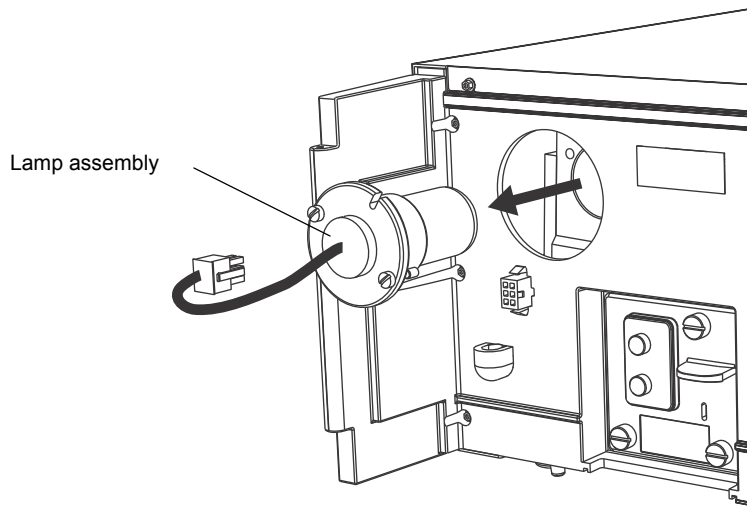
Figure 4–6: Lamp assembly captive screws



7. Withdraw the lamp assembly from the lamp housing.

! **Notice:** To prevent shattering the glass, use care when disposing of the lamp as the lamp gas is under slight negative pressure. Cushion the old lamp by placing it in the packaging material of the new lamp before disposal.

Figure 4–7: Removing the lamp assembly



4.5.4 Installing the new lamp



Warning: To avoid exposing your eyes to harmful ultraviolet radiation, never ignite a lamp while it is outside the instrument or if it is not properly secured in place.

Important: Do not touch the glass bulb of the new lamp. Dirt or fingerprints on the bulb adversely affect detector operation. If the lamp needs cleaning, gently clean the bulb with ethanol and lens tissue. Do not use abrasive tissue or apply excessive pressure.

Before you begin:

1. Unpack the lamp from its packing material.
Note: The new lamp's features can vary slightly from those shown in the figure.
2. If you see any particles or dirt on the new lamp, clean the lamp with a gas duster or lens tissue.
3. Record the serial number, located on a label attached to the lamp connector wire, using the procedure in [“Recording the new lamp serial number” on page 106](#).

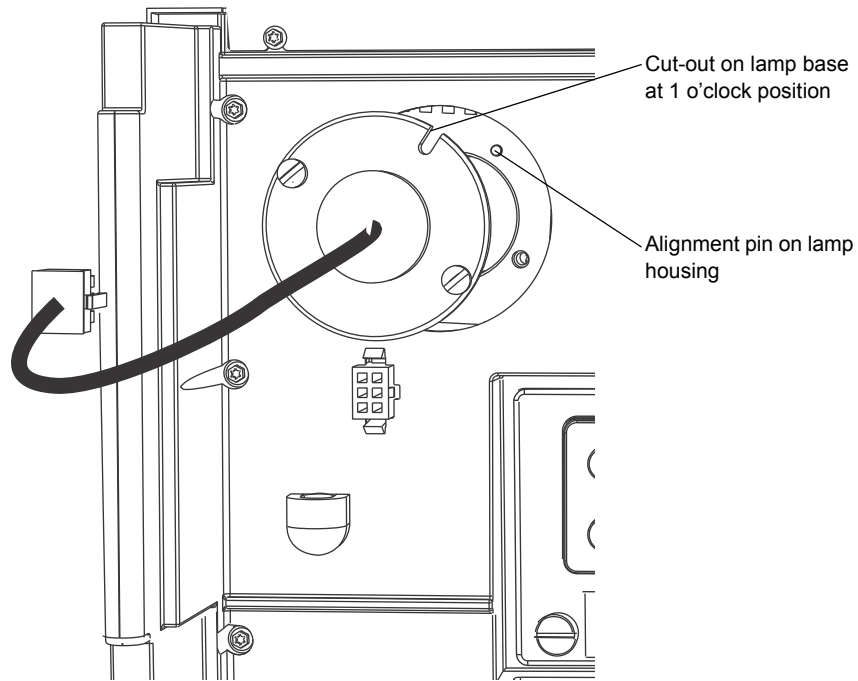
Requirement: Make sure the detector is powered-off and the power cord is disconnected.

Note: When you change the lamp, always power-off the detector. After installing a new lamp, power-on the detector and always wait at least 5 minutes for the new lamp to warm up.

To install the new lamp:

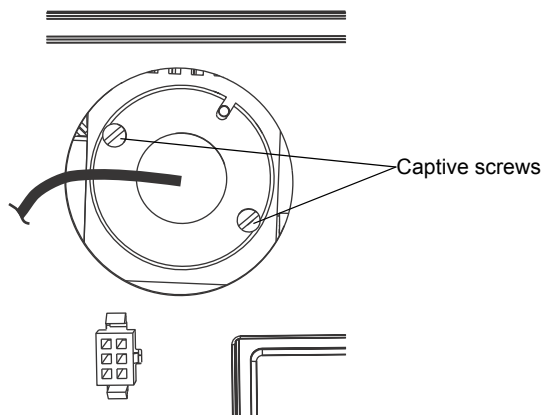
1. Position the lamp so the cut-out located on the lamp base is at the 1 o'clock position, in line with the alignment pin on the lamp housing.
Tip: There is no additional alignment required.

Figure 4–8: Aligning the lamp



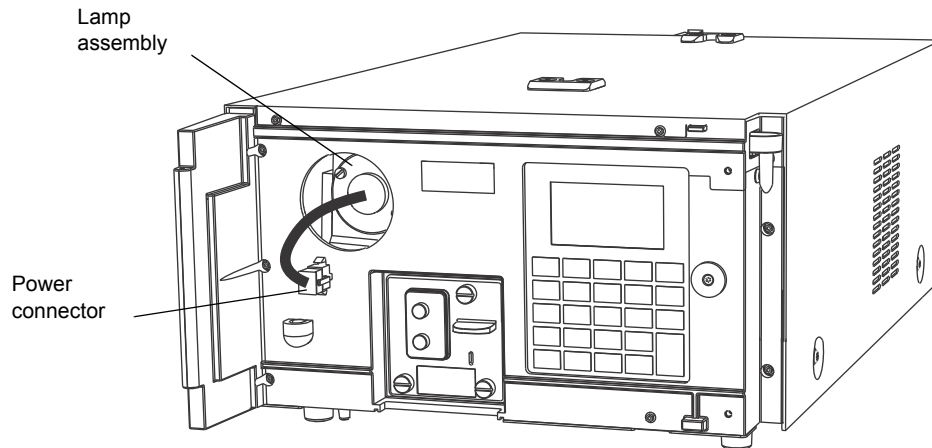
2. Gently push the lamp forward until it bottoms into position.
3. Tighten the two captive screws.

Figure 4–9: Lamp assembly captive screws



4. Reconnect the lamp's power connector.

Figure 4–10: Lamp assembly and power connector



5. When ready to resume operation of the detector, reconnect the power cord and power-on the unit.

Tip: The instrument firmware automatically delays operation for 5 minutes to allow the lamp to warm after it has been reignited.

4.5.5 Recording the new lamp serial number

Notes:

- Always run the Change Lamp diagnostic test after you install a new lamp (see [“Using the lamp, display, and keyboard diagnostic tests” on page 116](#)).
- If you do not record the new lamp serial number following the procedure in this section, the lamp warranty is voided.

The detector software records and stores the serial number and date of installation of a new lamp so that you can periodically check its age and its number of ignitions.

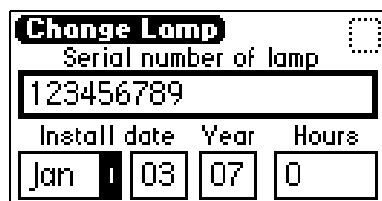
To record the new lamp serial number:

1. When the detector warms up, press the DIAG key.
2. Press 4, Lamp, display & keypad.
3. Press 1, Change lamp.

Tip: Be sure to enter the 9-digit lamp serial number and not the lamp part number when performing this procedure.

4. Enter the 9-digit serial number of the new lamp in the active field.

Figure 4–11: Change lamp screen



5. Press Enter to store the serial number and to move to the “Install date” field.
6. Select the month from the choice list, and press Enter twice to update the month and to select the next field, which specifies the day.
7. Specify the day of the month that the lamp is installed, and then press Enter to enter it and move to the next field, which specifies the year.
8. Specify the year (last two digits only), and press Enter to update the year and to select the Hours field.
Tip: The Hours field is optional. If you are using a lamp for which hours of use are already recorded, enter those hours of use. If this is a new lamp, enter 0 hours are 0.
9. Press the HOME key.
10. At the “OK to store” message, press Enter to store the serial number and date of installation, or press Cancel to cancel the entry.
11. When the confirmation message appears, press Enter.
12. Perform a manual wavelength calibration (see [page 68](#)).

Requirement: To run the verification procedures with the new lamp installed, recalibrate the detector, or cycle power to the detector after replacing the lamp.

4.5.6 Setting the lamp threshold

You can set an alarm threshold for the lamp. When the number of hours meets or surpasses the threshold, an alarm message appears. The default alarm threshold is 2000 hours.

The alarm message also appears when you first power-on the instrument. The lamp threshold screen displays the total hours of lamp use since the lamp’s installation.

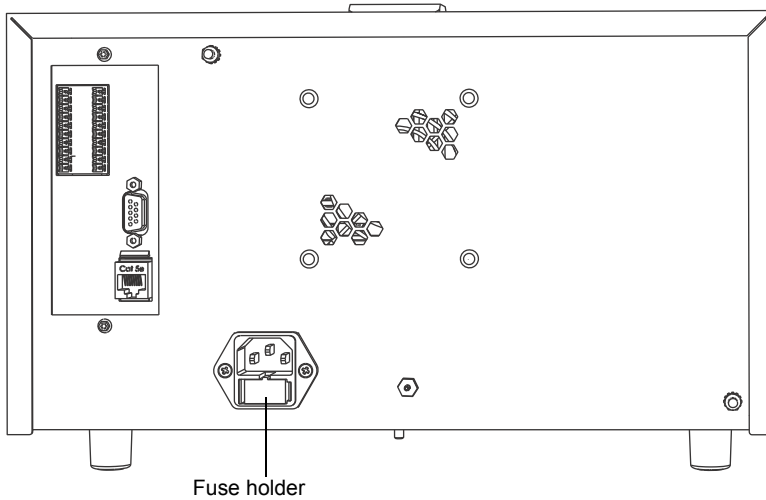
Figure 4–12: Lamp alarm threshold screen



4.6 Replacing fuses

The fuse holder is located on the rear panel of the detector (see figure below). Two fuses are shipped in place with the detector, rated as indicated in [“Electrical specifications” on page 132](#).

Figure 4–13: 2489 UV/Vis detector rear panel fuse holder



Warning: To avoid electric shock, power-off and unplug the instrument before inspecting the fuses.

The detector requires two 100 to 240 VAC, 50 to 60 Hz, F 3.15 A, 250 V fast blo, 5 × 20 mm (IEC) fuses.

Suspect a fuse is open or otherwise defective when

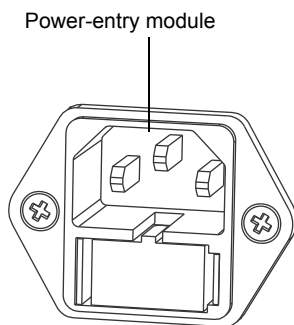
- the detector fails to power-on;
- the fans do not operate.

To replace the fuses:

Requirement: Replace both fuses, even when only one is open or otherwise defective.

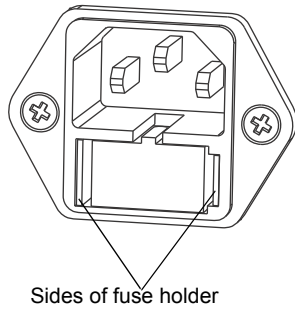
1. Power-off the detector, and disconnect the power cord from the power-entry module.

Figure 4–14: Power-entry module



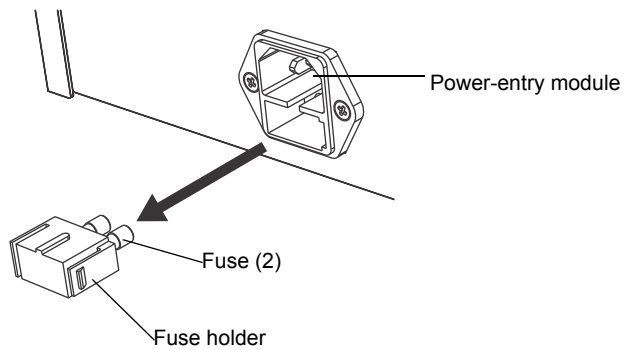
2. Pinch the sides of the spring-loaded fuse holder, which is below the power entry module on the rear panel of the detector.

Figure 4–15: Sides of spring-loaded fuse holder



3. With minimum pressure, withdraw the spring-loaded fuse holder.

Figure 4–16: Removing fuse holder



4. Remove and discard both fuses.
5. Make sure that the new fuses are properly rated, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
6. Reconnect the power cord to the power entry module.

5

Error Messages, Diagnostic Tests, and Troubleshooting

The detector provides both user and service diagnostic tests to troubleshoot system problems.

5.1 Error messages

5.1.1 Startup error messages

Startup diagnostic tests run automatically when you power-on the detector. They verify the proper operation of the detector electronics. If one or more of the tests fail, the detector beeps and displays an error message. For serious errors, it displays the word “Error” in brackets (<Error>) in place of the run-time absorbance on the absorbance screen.

Tip: To reduce the likelihood of errors, be sure the cuvette holder is empty, the flow cell contains degassed transparent solvent (methanol or water), and the front door closed securely.

See also: Online Help, for alarm messages and recommended actions you can take to correct the problem.

5.2 User-selected diagnostic tests

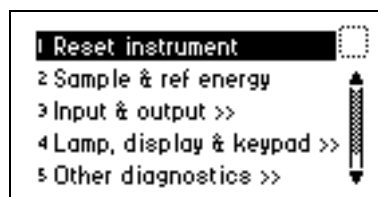
5.2.1 Overview

You can run several diagnostic tests for troubleshooting the detector and verifying that its electronics and optics perform correctly.

To perform user-selectable diagnostic tests:

1. Press the DIAG key from the detector’s front panel.

Figure 5–1: Diagnostic tests choice list



- To access a specific diagnostic test, press the up or down arrow key to select a test, and then press Enter.

Alternative: Press a number on the keypad corresponding to the test number. Choices that display other choices are indicated by >>.

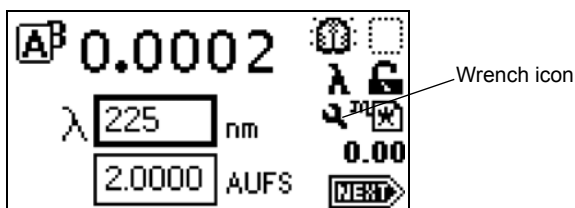
Sticky diagnostic tests remain in effect until you disable them. When a test is active, the detector absorbance screen displays a wrench icon (see the figure below).

You can disable a specific sticky diagnostic test by resetting it to the default settings.

You can disable all active sticky diagnostic tests by pressing DIAG and then selecting “1, Reset instrument.”

If no sticky diagnostic tests are active, the wrench icon does not appear on the absorbance screen. When you power-off the detector, sticky diagnostic tests become inactive.

Figure 5–2: Absorbance screen with sticky diagnostic tests active



The user-selectable sticky diagnostic tests are:

- Fix (Set) Voltage Output
- Fix (Set) Absorbance Input
- Generate Test Peaks
- Optical Filter Override

Note: Application of sticky diagnostic tests affects results. To clear changes to the voltage output or absorbance input or to make a manual optical filter change, select 1, “Reset instrument” from the Diagnostics choice list, or cycle power to the detector.

The table below lists diagnostic tests by their number on the choice list (see [page 113](#) for more information).

Table 5–1: Detector diagnostic tests

Diagnostic test	Description
1. Reset instrument	Resets all diagnostic tests to defaults. Renders sticky diagnostic tests inactive and removes the wrench icon.
2. Sample & ref energy	Makes it possible to view sample and reference energy (displayed in nanoamps) on channel A or B.

Table 5–1: Detector diagnostic tests (continued)

Diagnostic test	Description
3. Input & output >>	List of diagnostic tests to control four contact closure inputs and two switch outputs: <ul style="list-style-type: none"> • Auto-zero offset • Fix absorbance • Fix voltage • Contact closures & events • Previous choices <<
4. Lamp, display, & keypad >>	List of diagnostic tests used to test lamp, display, and keypad functions: <ul style="list-style-type: none"> • Change lamp • Test keypad • Test display • Previous choices <<
5. Service	Diagnostic tests used by Waters Service personnel.
6. Other diagnostics >>	Diagnostic tests that generate test peaks, with which you determine wavelength accuracy or override the default filter setting: <ul style="list-style-type: none"> • Generate test peaks • Optical filter override • Previous choices <<

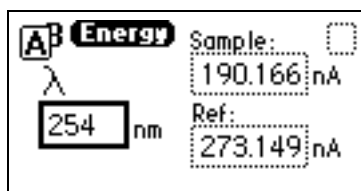
5.2.2 Using the diagnostic tests

The detector employs both user-selectable and service diagnostic tests. You access user diagnostic tests by pressing the DIAG key. Only qualified Waters service personnel can access service diagnostic tests.

5.2.2.1 Using the sample and reference energy diagnostic tests

You use the sample and reference energy diagnostic tests to plot the output of the analog channels to examine noise fluctuations and to compare with the AU time trace. The current sample and reference energy readings appear in nanoamps (nA).

Figure 5–3: Sample and reference energy diagnostic test



To use the sample and reference energy diagnostic test:

1. Press DIAG, and then press 2.
2. Change the wavelength, if necessary.

3. Press Enter.
Result: When the new wavelength shifts to the left, the corresponding sample and reference energies appear.
4. If you are operating the detector in dual-wavelength mode, press the A/B key to view sample and reference energy on the other wavelength.

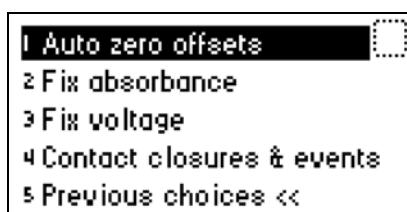
5.2.2.2 Using the input and output diagnostic tests

You use the input and output diagnostic tests to

- display and reset the autozero offsets;
- fix (set) absorbance;
- fix (set) the voltage on the 2-V output;
- monitor contact closures and toggle event switches;
- generate test peaks;
- override the optical filter.

To perform any of the input and output diagnostic tests, press DIAG, and then press 3, for Input & output. A choice list appears.

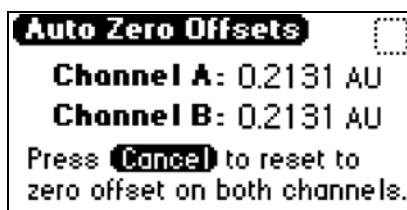
Figure 5-4: Input & output diagnostic test choice list



5.2.2.3 Displaying auto-zero offsets

From the Input & Output choice list, press 1 (Auto-zero offsets). This diagnostic test also allows you to display and reset the offset to zero on both channels by pressing Cancel (Shift 0).

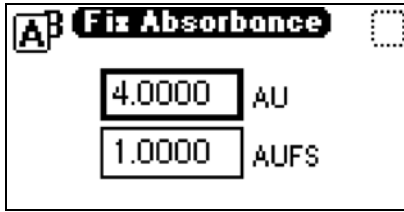
Figure 5-5: Auto-zero offsets diagnostic display



5.2.2.4 Setting a fixed absorbance value

From the Input & Output choice list, press 2 (Fix absorbance) to set a fixed absorbance value for channel A or channel B. The allowable range is from -4.0000 AU to $+4.0000$ AU. For convenience, you can also specify sensitivity (AU) using this diagnostic test. The allowable AU range is from $+0.0001$ to $+4.0000$ AU.

Figure 5–6: Fix absorbance diagnostic display

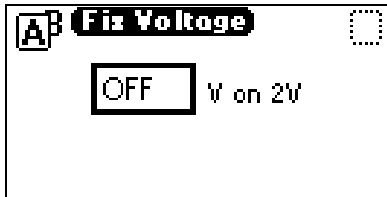


This test sets the voltages on the analog output channels based on the current AU setting. This is a sticky diagnostic test.

5.2.2.5 Setting a fixed voltage output

From the Input & Output choice list, press 3 (Fix voltage) to select a voltage for the analog output. You can select a voltage for both output channels in the -0.10 V to $+2.10$ V range.

Figure 5–7: Fix Voltage diagnostic display

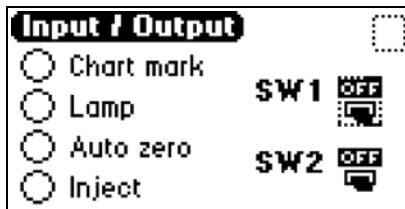


The voltage is driven on the selected analog channel (A or B). This is a sticky diagnostic.

To monitor contact closures and setting switches:

1. From the Input & Output choice list, press 4 (Contact closures & events) to monitor the four contact closure inputs and to control the two switch outputs.

Figure 5–8: Contact closures & events diagnostic display



The Input & Output diagnostic test allows real-time monitoring of the state of the contact closure inputs. A solid (filled in) circle indicates the contact closure is closed (ON = High). An open (empty) circle indicates the contact closure is open (OFF = Low).

2. For the outputs (SW1 and SW2):
 - a. Press Enter to display the active switch (surrounded by a dotted-line border).
 - b. Press any numerical key to change the status of the switch (ON to OFF or vice versa).
 - c. Press Enter to select the second switch.

5.2.2.6 Using the lamp, display, and keyboard diagnostic tests

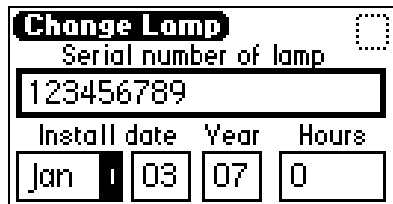
To access the lamp, display, and keypad diagnostic tests, press DIAG and then press 4.

Requirement: Make sure the detector is powered-off and the power cord is disconnected before you replace the lamp.

To change the lamp:

1. From the Lamp, display & keypad choice list, press 1 (Change lamp) to access the Change Lamp diagnostic display.

Figure 5–9: Change Lamp diagnostic display



The screen displays the following information:

- Change Lamp** (title)
- Serial number of lamp: 123456789
- Install date: Jan 03 07 0

2. Enter the 9-digit serial number and date of installation for the new lamp. Press Enter after each entry.

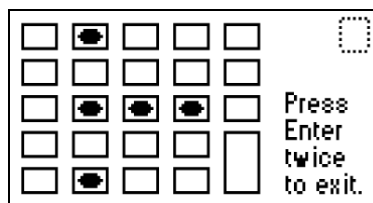
Tip: Use this function whenever you change the lamp to enter a new serial number and installation date (see [page 104](#) and [page 106](#) for a complete explanation of the lamp replacement procedure).

Important: Neglecting to record the new lamp serial number following the prescribed procedures referenced above voids the lamp warranty. The date of the previous lamp installation remains in the detector memory.

To use the Test keypad diagnostic test:

1. From the Lamp, display & keypad choice list, press 2 (Test keypad) to run the keypad test.

Figure 5–10: Keypad diagnostic test display



The screen displays a 5x5 grid of keys. The top-left key is filled with a black circle. The text "Press Enter twice to exit." is displayed to the right of the grid.

2. On the keypad diagnostic test display, press any key to begin the test. Then press each key until you pressed all of them. If the keypad is operating properly, each key location is filled in and then cleared with another press of the key. If any key does not respond when pressed, contact your Waters service representative.

Rule: You must press Enter twice to exit the keypad diagnostic display.

To run the test display diagnostic test:

1. From the Lamp, display & keypad choice list, press 3 (Test display) to run the test.

Result: The display fills from top to bottom and from right to left, then returns to the Lamp, display & keypad choice list. If the display does not completely fill, either horizontally or vertically, contact your Waters service representative.

2. From the Lamp, display & keypad choice list, press 4.

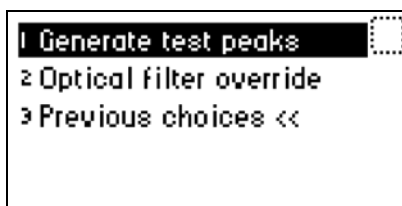
5.2.2.7 Using the other detector diagnostic tests

The user diagnostic test display provides two additional tests:

- Generate test peaks – Specifies the generation of test peaks to calibrate your data system.
- Manually override the optical filter – Selects a filter different from that specified as part of the detector's normal operating mode.

To run either test, press DIAG and then press 5 (Other diagnostics).

Figure 5–11: Other diagnostics choice list



To generate test peaks:

1. From the Other diagnostics choice list, press 1 (Generate test peaks) to generate test peaks every 100 seconds on the chart, trace, or other output.

Restriction: The Generate test peaks diagnostic test works only in single-wavelength mode.

Every 100 seconds, the detector generates an approximate 1-AU peak with a standard deviation of 10 seconds on the trace, chart, or data system display until you disable the Generate test peaks diagnostic test. The amplitude of the test peaks is affected by your choice of filter time constant.

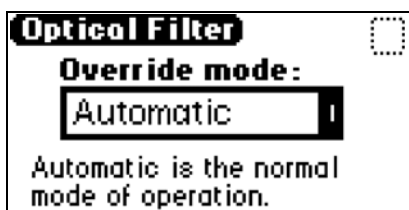
This is a sticky diagnostic test. When this routine is selected, the choice list changes to read “Disable test peaks.”

2. Press 1 (Disable test peaks) from the Other diagnostics choice list to turn off this diagnostic test.

To override the optical filter:

1. From the Other diagnostics choice list, press 2 (Optical filter override) to manually override the detector's automatic filter choice.

Figure 5–12: Optical Filter Override diagnostic test display



2. In the Optical Filter Override diagnostic test display, press Enter to display the following filter choice list.

Automatic	1
Second Order	2
None	3
Erbium	4
Shutter	5

Tip: The detector normally operates with the filter in the Automatic position. This is a sticky diagnostic test.

3. Press the number corresponding to your filter choice, or leave the default filter choice (Automatic) on.
4. To deactivate this diagnostic test, press DIAG, and then press 1, or select Automatic from the choice list.

5.2.3 Service diagnostic tests

Only qualified Waters service personnel can access the detector service diagnostic tests.

5.3 Troubleshooting

This section provides some causes of errors and recommended troubleshooting actions. Keep in mind that the source of apparent detector problems may lie within the chromatography or your other instruments, as well as the detector.

Most detector problems are relatively easy to correct. If you are unable to correct a problem or a failed condition, contact Waters Technical Service (see [page 91](#)).

5.3.1 Diagnostic tests

The detector performs some user-selected diagnostic tests to help you troubleshoot basic system problems. (See Online Help, for diagnostic descriptions and instructions on how to use them.)

Error messages that can appear as you start up or operate the detector and suggested corrective actions are described in Online Help.

5.3.2 Power surges

Power surges, line spikes, and transient energy sources can adversely affect detector operations. Be sure that the electrical supply used for the detector is properly grounded and free from any of these conditions.

5.3.3 Hardware troubleshooting

This section contains general hardware troubleshooting for the detector.

Table 5–2: General system troubleshooting

Symptom	Possible cause	Corrective action
Analog output incorrect	AU setting changed	Reset the AU setting.
Calibration or energy error on startup	Cuvette is installed, or a UV absorber in flow cell	1. Remove the cuvette. 2. Flush the flow cell. 3. Perform manual calibration.
Detector inoperative	Fuse blown	Confirm the front panel display is operational; if not, replace the ac rear panel fuse.
	No power at ac outlet	Test the outlet by connecting another electrical unit known to be in working order and determine whether it operates.
Deuterium lamp does not light	Faulty lamp	Replace the lamp.
	Lamp not plugged in	Plug in the lamp connector.
	Nonperforming or malfunctioning lamp power supply board	Contact Waters Technical Service.
	Lamp switch in the “off” position	Inspect the rear panel connections or timed event within a method.
Ethernet problems	Nonperforming or malfunctioning Ethernet cable	Replace the Ethernet cable.
Front panel display fails to illuminate	Broken electrical connection	Inspect electrical connections.
	Fuse blown	Inspect and, if necessary, replace fuse(s).
	Nonperforming or malfunctioning LCD or control board	Contact Waters Technical Service.
Front panel displays odd characters	Faulty EPROMs Nonperforming or malfunctioning LCD control board	Contact Waters Technical Service.
In dual-wavelength mode, time scale is incorrect	Sampling rate on controller is > 1 pt/s	In dual-wavelength mode, you must select a data sampling rate of 1 point per second.

Table 5–2: General system troubleshooting (continued)

Symptom	Possible cause	Corrective action
Keypad not functioning	Keypad defective	1. Cycle power to the detector and run the keypad diagnostic test. 2. Contact Waters Technical Service.
No sample and reference energy	Lamp burned out	Attempt to reignite using the lamp key. Replace the lamp.
	Lamp extinguished	Check the lamp icon. Run the Sample & ref energy diagnostic test.
Peak out of range error at start up	Cuvette in cuvette holder; mobile phase absorbance too high; air bubbles	1. Remove cuvette from cuvette holder. 2. Be sure the flow cell mobile phase does not absorb above 250 nm. 3. Ensure that no air bubbles are in the flow cell. 4. Recalibrate the detector. If problems persist, contact Waters Technical Service.

5.3.4 Lamp troubleshooting

An apparent lamp-related problem could be caused by air bubbles in the flow cell or incorrect installation of the lamp. Before replacing the lamp, ensure the detector cell is filled with solvent and free of air bubbles, and the current lamp is installed correctly.

Flushing the flow cell can sometimes correct the problem of apparently low lamp intensity, which can occur when you have not operated the detector for 2000 hours or more.

Mobile phase absorbance affects apparent lamp intensity. For example, water or acetonitrile is more transparent than methanol at wavelengths below 220 nm.


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 Bedienungsbefugnis des Systems führen.



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



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



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



注意: 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。



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



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



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

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



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

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Éteignez toute flamme se trouvant à proximité de l'instrument.
- Évitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Évitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylsulfoxyde 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.
- Spegner tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.



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

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.

Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.



警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。

要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。



警告: 当有压力的情况下使用管线时, 小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。

要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀, 大大降低管线的耐压能力。



경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.

염화 메틸렌 (Methylene chloride) 및 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：** 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。







 **경고：** 화재의 위험을 막으려면 유지관리 절차 단원의 "퓨즈 교체" 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.



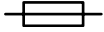



 **警告：** 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

A.7 Electrical and handling symbols

A.7.1 Electrical symbols

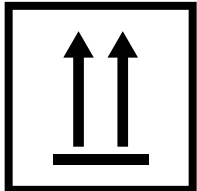


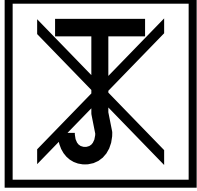
The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.

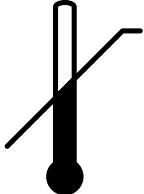

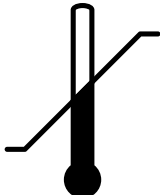
Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (3 phase)

Symbol	Description
	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!

Symbol	Description
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation

B Specifications

This appendix lists individual operating specifications for the 2489 UV/Vis detector.

Note: Allow the detector to warm for a minimum of two hours, and reach an equilibrium state (stable baseline), before conducting the performance test.

B.1 Physical specifications

The following table lists the physical specifications for the 2489 UV/Vis detector.

Table B-1: Physical specifications

Attribute	Specification
Height	20.8 cm (8.2 inches)
Width	34.3 cm (13.5 inches)
Depth	61.0 cm (24.0 inches)
Weight	13.8 kg (30.5 pounds)

B.2 Environmental specifications

The following table lists the environmental specifications for the 2489 UV/Vis detector.


Table B-2: Environmental specifications

Attribute	Specification
Operating temperature range	4 to 40 °C (39.2 to 104 °F)
Operating relative humidity	20 to 80%, non-condensing
Shipping and storage temperature range	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity range	20 to 85%, non-condensing
Audible noise (instrument generated)	≤58 dBA

B.3 Electrical specifications

The following table lists the electrical specifications for the 2489 UV/Vis detector.

Table B-3: Electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overtoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC
Power requirements	100 to 240 Vac nominal
Frequency	50 to 60 Hz
Fuse	Two fuses: 100 to 240- Vac, 50 to 60 Hz F 3.15-A 250-V fast blo, 5 × 20 mm (IEC)
Power consumption	195 VA (nominal)
Attenuated analog output channel: 2 VFS	Attenuation range: 0.0001 to 4.000 AU 2-V output range: -0.1 to +2.1 V
Two event outputs	Type: Contact closure Voltage: +30 V Current: 1 A
Four event inputs	Input voltage: +30 V maximum 100-ms minimum period

- a. **Protection Class I** – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. **Overtoltage Category II** – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. **Pollution Degree 2** – A measure of pollution on electrical circuits, which may produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.
- d. **Moisture Protection** – Normal (IPXO) – IPXO means that *no* Ingress Protection against any type of dripping or sprayed water exists. The X is a placeholder that identifies protection against dust, if applicable.

B.4 Performance specifications

The following table lists the performance specifications for the 2489 UV/Vis detector.

Table B-4: Performance specifications

Attribute	Specification
Wavelength range	190 to 700 nm

Table B-4: Performance specifications (continued)

Attribute	Specification
Bandwidth	≤5 nm
Wavelength accuracy	±1.0 nm (via patented erbium filter ^a)
Wavelength repeatability	±0.1 nm
Baseline noise, single wavelength (dry) ^b	≤5 × 10 ⁻⁶ AU, peak to peak, 230 nm, 10 points/s, 1.0 s filter, 30-s segments, dry analytical flow cell
Baseline noise, single wavelength (wet) ^b	≤8 × 10 ⁻⁶ AU, peak to peak, 230 nm, 10 points/s, 1.0 s filter, 30-s segments, 1 mL/min acetonitrile, analytical flow cell
Baseline noise, dual wavelength (dry) ^b	≤35 × 10 ⁻⁶ AU, peak to peak, 230 nm, 280 nm, 1 point/s, 2.0 s filter, 30-s segments, dry analytical flow cell
Baseline noise, dual wavelength (wet) ^b	≤45 × 10 ⁻⁶ AU, peak to peak, 230 nm, 280 nm, 1 point/s, 2.0 s filter, 30-s segments, 1 mL/min acetonitrile, analytical flow cell
Linearity ^b	≤5% at 2.5 AU, propylparaben at 257 nm
Drift ^b	≤1.0 × 10 ⁻⁴ uAU/hr, 230 nm, 2 points/s, 1.0 s filter, 30-s segments, dry analytical flow cell
Measurement range	0.0001 to 4.0000 AU
Filter time constant range	Single wavelength: 0.0125 to 5.0 seconds Dual wavelength: 1 to 5 seconds
Sampling rate	up to 80 points/s
Analog sampling rate	10, 20, 40, 80 Hz (channel A) 10 Hz only (channel B)

a. US Patent Numbers: 6,423,249 and 6,783,705

b. Other than documented deviations, follow ASTM E685-93

B.5 Optical component specifications

The following table lists the optical component specifications for the 2489 UV/Vis detector.

Table B-5: Optical component specifications

Attribute	Specification
Light source	Deuterium arc lamp, Warranty: 2000 hours or 1 year (whichever comes first)
Photodiodes	2 silicon photodiodes (matched pair)
Second-order filter	Automatic for wavelengths ≥ 370nm
Wavelength calibration filter	Erbium filter, used at startup or on demand
Flow cell design	Patented TaperSlit™ ^a
Nitrogen purge	Purge fitting present on rear of chassis
Path length	10 mm (analytical cell)

Table B-5: Optical component specifications (continued)

Attribute	Specification
Cell volume	16.3 µL (analytical cell)
Pressure limit	6895 kPa (69 bar, 1000 psi) (analytical cell)
Wetted materials	<ul style="list-style-type: none"> Standard: 316 stainless steel, fluoropolymer, fused silica, PEEK™ ACQUITY Arc Bio: Fluoropolymer, fused silica, MP35N, PEEK, titanium

a. US Patent Number: US 5,883,721

B.6 Flow cell specifications

The following table lists the Waters flow cell specifications for the 2489 UV/Vis detector. In the ACQUITY Arc® system, the low dispersion analytical flow cell is standard. In the ACQUITY Arc Bio system, the inert, low-dispersion analytical flow cell is standard.

Important:

- The low-dispersion analytical flow cell is only compatible with the 2489 UV/Vis detector as a component in an ACQUITY Arc system.
- The inert low-dispersion analytical flow cell is only compatible with the 2489 UV/Vis detector as a component in an ACQUITY Arc system or in an ACQUITY Arc Bio system.

Table B-6: Waters TaperSlit flow cell specifications:

Description	Volume (µL)	Pathlength (mm)	Tubing internal diameter (inches)		Pressure rating (bar/psi)
			Inlet	Outlet	
Analytical	16.3	10	0.009	0.009	69/1000
Low-dispersion analytical	16.3	10	0.005	0.005	69/1000
Inert, low-dispersion analytical	16.3	10	0.005	0.005	69/1000
Semi-prep cell	4.4	3	0.040	0.040	69/1000
Microbore cell	4.4	3	0.005	0.005	69/1000
Inert (titanium) cell	16.3	10	0.010	0.010	69/1000
High pressure cell	16.3	10	0.009	0.009	207/3000
Variable path length flow cell (VPF)	0.06 to 1.2	0.15 – 3 (factory preset at 0.5 mm)	0.04	0.04	69/1000
Autopurification cell	6.0	1.0	0.009 0.020 0.040	IN 1 IN 2 OUT	138/2000

C Solvent Considerations



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

C.1 Introduction

C.1.1 Preventing contamination

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

C.1.2 Clean solvents

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

A dirty solvent can cause baseline noise and drift. It can also block the solvent filters with particulate matter.

C.1.3 Solvent quality

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

C.1.4 Preparation checklist

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

- Filter solvents using a 0.45- μ m filter.
- Degas the solvent, sparge it, or perform both operations.
- Stir the solvent.
- Keep solvents in a place free from drafts and shock.

C.1.5 Water

Use water only from a high-quality water purification system. If the water system does not deliver filtered water, filter it through a 0.45- μ m membrane filter before use.

C.1.6 Using buffers

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

C.1.7 Tetrahydrofuran

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



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

C.2 Solvent miscibility

Before you change solvents, refer to the table below to determine the miscibility of the solvents to be used. When you change solvents, be aware that,

- changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as isopropanol);
- 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 C-1: Solvent miscibility:

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
-0.3	N-decane	0.92	174.1	29	—
-0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	—
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	—
1.8	Triethylamine	0.38	89.5	26	—
2.2	Isopropyl ether	0.33	68.3	—	220
2.3	Toluene	0.59	100.6	23	285
2.4	P-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3	—	—

Table C-1: Solvent miscibility: (continued)

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
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	—
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	—	—

C.2.1 How to use miscibility numbers

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see “Solvent miscibility” on page 136).

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 (59 °F).

- A difference of 16 indicates a critical solution temperature from 25 to 75 °C (77 to 167 °F), with 50 °C (122 °F) as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or their critical solution temperature is above 75 °C (167 °F).

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, 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

C.3 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 fluid paths with HPLC-quality water before shutting the system down and leave distilled water in the system (flush with 90% HPLC-quality water: 10% methanol for shutdowns scheduled to be more than one day). Use a minimum of 15 mL for sparge-equipped units, and a minimum of 45 mL for in-line vacuum degasser-equipped units.

C.4 Head height

Position the solvent reservoirs at a level above the HPLC equipment or on top of the pump or detector (with adequate spill protection).

C.5 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 will affect the analysis is not known, monitor the pressure during the run using the Chart Out terminal.

C.6 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,

- stable baselines and enhanced sensitivity;
- reproducible retention times for eluting peaks;
- reproducible injection volumes for quantitation.
- stable pump operation.

C.6.1 Gas solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount depends on,

- 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 all lead to outgassing.

C.6.1.1 Effects of intermolecular forces

Nonpolar gases (N_2 , O_2 , CO_2 , He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent with intermolecular attractive forces similar to those in the gas (like dissolves like).

C.6.1.2 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 H_2O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

C.6.1.3 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.

C.7 Solvent degassing methods

This section describes the solvent degassing techniques that will help you to attain a stable baseline. Degassing your solvent also improves reproducibility and pump performance.

You can use either of the following methods to degas solvents:

- Sparging with helium
- Vacuum degassing

C.7.1 Sparging

Sparging removes gases from solution by displacing dissolved gases in the solvent with a less soluble gas, usually helium. Well-sparged solvent improves pump performance. Helium sparging brings the solvent to a state of equilibrium, which may be maintained by slow sparging or by keeping a blanket of helium over the solvent. Blanketing inhibits reabsorption of atmospheric gases.

Sparging can change the composition of mixed solvents.

C.7.2 Vacuum degassing

The in-line vacuum degasser operates on the principle of Henry's Law to remove dissolved gases from the solvent. Henry's Law states that the mole fraction of a gas dissolved in 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.

Vacuum degassing can change the composition of mixed solvents.

C.7.3 Solvent degassing considerations

Select the most efficient degassing operation for your application. To remove dissolved gas quickly, consider the following.

C.7.3.1 Sparging

Helium sparging gives stable baselines and better sensitivity than sonication in a detector, and prevents reabsorption of atmospheric gases. Use this method to retard oxidation when you are using tetrahydrofuran or other peroxide-forming solvents.

C.7.3.2 Vacuum degassing

The longer the solvent is exposed to the 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.

The in-line degasser is available as an option or factory-installed in the Waters 2695 Separations Module, XE model.

C.8 Wavelength selection

C.8.1 UV cutoffs for common solvents

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

Table C-2: UV cutoff wavelengths for common chromatographic solvents:

Solvent	UV cutoff (nm)	Solvent	UV cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Iso-octane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	<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
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

C.8.2 Mixed mobile phases

The table below contains approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's Law, because absorbance is proportional to concentration.

Table C-3: Wavelength cutoffs for different mobile phases:

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
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 dibasic, 10 mM	190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

C.8.3 Wavelength selection for chromophore detection

Certain functional groups found in most compounds absorb light selectively. These groups, known as chromophores, and their behavior can be used to categorize the detection of sample molecules.

The table below lists some common chromophores, and their detection wavelengths (λ_{\max}), as well as the molar absorptivity (ϵ_{\max}) of each group. Use this information as a guide to select the optimal operating wavelength for a particular analysis. Because of the diversity possible within a given sample, scanning over a range of wavelengths may be necessary to determine the best wavelength for a particular analysis.

Table C-4: Electronic absorption bands of representative chromophores*:

Chromophore	Chemical configuration	λ_{\max} (nm)	ϵ_{\max} (L/m/cm)	λ_{\max} (nm)	ϵ_{\max} (L/m/cm)
Ether	—O—	185	1000		
Thioether	—S—	194	4600	215	1600
Amine	—NH ₂	195	2800		
Thiol	—SH	195	1400		

Table C-4: Electronic absorption bands of representative chromophores*: (continued)

Chromophore	Chemical configuration	λ_{\max} (nm)	ϵ_{\max} (L/m/cm)	λ_{\max} (nm)	ϵ_{\max} (L/m/cm)
Disulfide	—S—S—	194	5500	255	400
Bromide	—Br	208	300		
Iodide	—I	260	400		
Nitrile	—C≡N	160	—		
Acetylide	—C≡C—	175-180	6000		
Sulfone	—SO ₂ —	180	—		
Oxime	—NOH	190	5000		
Azido	>C=N—	190	5000		
Ethylene	—C=C—	190	8000		
Ketone	>C=O	195	1000	270-285	18-30
Thioketone	>C=S	205	strong		
Esters	—COOR	205	50		
Aldehyde	—CHO	210	strong	280-300	11-18
Carboxyl	—COOH	200-210	50-70		
Sulfoxide	>S→O	210	1500		
Nitro	—NO ₂	210	strong		
Nitrile	—ONO	220-230	1000-2000	300-400	10
Azo	—N=N—	285-400	3-25		
Nitroso	—N=O	302	100		
Nitrate	—ONO ₂	270 (shoulder)	12		
Allene	—(C=C) ₂ — (acyclic)	210-230	21,000		
Allene	—(C=C) ₃ —	260	35,000		
Allene	—(C=C) ₄ —	300	52,000		
Allene	—(C=C) ₅ —	330	118,000		
Allene	—(C=C) ₂ — (alicyclic)	230-260	3000-8000		
Ethylenic/ Acetylenic	C=C—C≡C	219	6,500		
Ethylenic/ Amido	C=C—C=N	220	23,000		
Ethylenic/ Carbonyl	C=C—C=O	210-250	10,000- 20,000		
Ethylenic/ Nitro	C=C—NO ₂	229	9,500		

*Willard, H. H. and others. *Instrumental Methods of Analysis*, 6th ed. Litton Educational Publishing, Inc., 1981. Reprinted by permission of Wadsworth Publishing Co., Belmont, California, 94002.

