ACQUITY UPLC TUV Detector

Operator's Overview and Maintenance Guide

715004733 / Revision B

THE SCIENCE OF WHAT'S POSSIBLE."

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Safety considerations

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

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

Considerations specific to the ACQUITY TUV detector

High voltage hazard



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

Safety advisories

Consult the Safety Advisories section on page 62 for a comprehensive list of warning and caution advisories.

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

Applicable symbols

Symbol	Definition
EC REP	Authorized representative of the European Community
CE	Confirms that a manufactured product complies with all applicable European Community directives
ABN 49 065 444 751	Australia C-Tick EMC Compliant
c C Ligger Us	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
C	This product has been tested to the requirements of CAN/CSA-C22.2 No. 61010-1, second edition, including Amendment 1, or a later version of the same standard incorporating the same level of testing requirements
i	Consult instructions for use

Audience and purpose

This guide is intended for personnel who install, operate, and maintain ACQUITY TUV detectors. It gives an overview of the instrument's technology and operation.

Intended use of the ACQUITY TUV detector

The Waters ACQUITY TUV detector is for research use only.

Calibrating

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

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

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. 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.

When analyzing samples from a complex matrix such as soil, tissue, serum/plasma, whole blood, and other sources, note that the matrix components can adversely affect LC/MS results, enhancing or suppressing ionization. To minimize these matrix effects, Waters recommends you adopt the following measures:

- Prior to the instrumental analysis, use appropriate sample pretreatment such as protein precipitation, liquid/liquid extraction (LLE), or solid phase extraction (SPE) to remove matrix interferences.
- Whenever possible, verify method accuracy and precision using matrix-matched calibrators and QC samples.
- Use one or more internal standard compounds, preferably isotopically labeled analytes.

Authorized representative information

Authorized representative



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ISM classification

ISM Classification: ISM Group 1 Class B

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

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Overview

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

The detector offers two basic, primary flow cell options: the analytical flow cell, with a volume of 500 nanoliters and a path length of 10 mm, and the high sensitivity flow cell, with a volume of 2.4 microliters and a path length of 25 mm. The design of both flow cells reflects the Waters' patented, light-guiding, flow cell technology. This guide focuses on the light-guiding, flow cell operating principles and maintenance procedures.

The Waters ACQUITY TUV detector operates at wavelengths ranging from 190 to 700 nm. The detector can sample up to 80 data points per second.

The detector has the following capabilities:

- 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 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.
- Full diagnostic capability Supports built-in diagnostic tools, to optimize functionality and performance.
- One contact closure output The detector has one configurable switch, which can accommodate a maximum of ± 30 VDC, 1.2-A current carrying capacity, and 0.5-A current switching. The switch can trigger fraction collectors and other external devices, as well as activate according to time, absorbance threshold, or ratio criteria.

- Thermal wander management To mitigate thermal instability caused by ambient temperature changes, the detector's insulation ensures air flow across the optics bench, and its variable speed fan runs at higher or lower speeds, as needed. The fan normally changes speeds in response to the thermal changes. This feature can be optimized for two average temperature zones, or disabled for maximum cooling of the optics and flow cell.
- 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.

Principles of operation

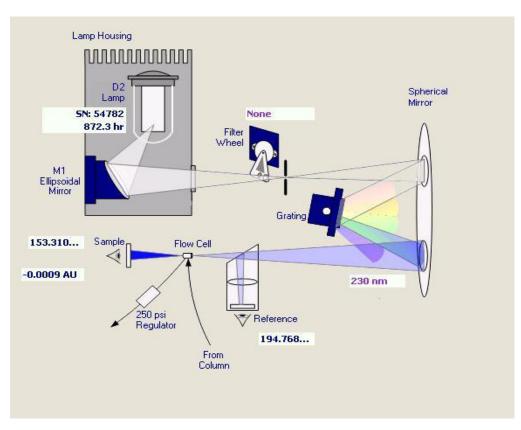
To use the detector effectively, become familiar with its optical and electronic design and the theory and principles of its operation.

This section describes the detector's optics and the wavelength verification process and test.

Detector optics

The Waters ACQUITY TUV detector optics include these components:

- High brightness deuterium (D₂) 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 light-guiding flow cell



Waters ACQUITY TUV detector optics assembly

Optics assembly light path

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

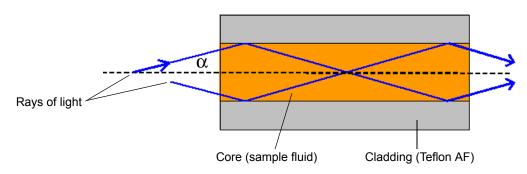
- 1. The ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel onto the entrance slit. The spherical mirror directs light toward the grating. A different portion of the spherical mirror focuses dispersed light of a particular wavelength band, determined by the grating angle, onto the entrance of the flow cell. Light exiting the flow cell passes 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 specify a new wavelength via Empower, MassLynx, or third-party 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, chart recorder, or integrator.

Light-guiding flow cell operating principles

Small-bore, high-capacity columns like those used in UPLC produce small-volume peaks. To avoid bandspreading and maintain concentration, the flow cell volume must be correspondingly small. A rule of thumb is to hold cell volume to $1/10^{\text{th}}$ or less than the peak volume. To achieve the required volume reduction with conventional absorbance detector flow cells, the path length must be reduced to avoid a drastic cut in light throughput. Reduced path length results in less analytical sensitivity as predicted by Beer's law, but high light levels are necessary to preserve a high signal-to-noise ratio.

Fortunately, a small-volume light-guiding flow cell can be designed with optimum path length and high light throughput. Such a flow cell is analogous to an optical fiber, where the core is the fluid sample and the cladding is Teflon[®] AF, a unique, chemically inert, amorphous fluoropolymer made by DuPont. The refractive index of Teflon AF is lower than that of water or other HPLC mobile phases. Light rays entering the liquid core, within the cone half-angle, α , are totally internally reflected when they meet the Teflon AF boundary. These rays are transmitted through the flow cell, theoretically without loss, except for absorption by the sample.



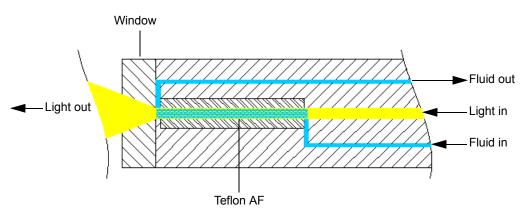
Light transmission through a light-guiding flow cell

This information complements the foregoing illustration:

- The core of the light guide is the fluid sample, with refractive index n_1 .
- The cladding is a Teflon AF tube, with refractive index n_2 . Index $n_2 < n_1$.
- The cross-sectional area of the tube is A and the length d. Cell volume = Ad.

In the figure "Light transmission through a light-guiding flow cell" on page 4, two rays of light are shown reflecting from the core-cladding interface. In a flow cell, the number of "bounces" depends on the length of the Teflon AF tube, its inside diameter (lumen), and the ray angle, " α ". The light beam (which represents the energy transmitted through the cell) is comprised of many such rays, up to a maximum whose angle is theoretically set by the refractive index of the core and cladding. In the ACQUITY UPLC TUV detector, this angle is mechanically controlled by components external to the flow cell so that the variation in refractive index arising from different mobile phases does not materially influence the efficiency of the transmitted energy.

The following schematic diagram of the flow cell shows the light-guiding portion of the cell inside the cell assembly.



Light-guiding portion of flow cell

The sample fluid is introduced and removed from the flow cell via PEEK[™] tubing. Probe radiation from the lamp housing is focused onto the input face of the optical fiber that forms one end of the flow cell. Light travels down this optical fiber until it encounters the fluid channel defined by the internal diameter of the Teflon AF tube. The light then exits the optical fiber and enters the fluid-filled Teflon AF tube. As the light passes through this tube, it interacts with the sample stream. Any absorption by the fluid reduces the

light intensity. The reduction is subsequently converted to absorbance. The light exits the flow cell through a fused silica window, where it projects onto the sample photodiode.

Unlike other flow cell designs, where the light beam is designed to avoid striking the internal walls of the cell, light-guiding relies on internal reflections from the walls of the Teflon AF tubing. Consequently, it is important to maintain flow cell cleanliness by following the recommended procedures described in "Maintaining the Detector" on page 29. With such care, the instrument and flow cell should provide you continuous sensitive detection.

Caution: To ensure the detector cell is properly aligned and calibrated, the flow cell must be filled with flowing solvent before you power-on the detector. An empty flow cell causes a calibration error. Refer to the recommended procedures described in "Maintaining the Detector" on page 29 for more information.

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 data 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 data rate.

The filter time-constant adjusts the filter response time to achieve an optimal signal-to-noise ratio. Selecting Other and entering a value of 0.0 disables all filtering.

Lower time-constant settings produce these effects:

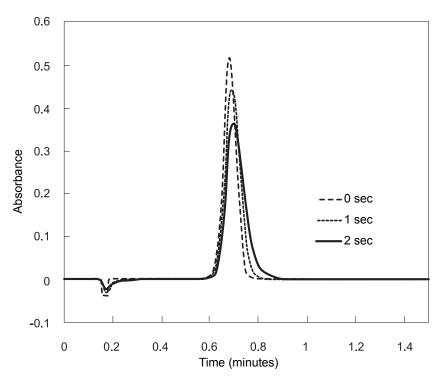
- Narrow peaks with minimal peak distortion and time delay
- Very small peaks become harder to discriminate from baseline noise
- Less baseline noise is removed

Higher time-constant settings produce these effects:

- Greatly decrease baseline noise
- Shorten and broaden peaks

The software includes fast or normal filtering constants at each data 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.



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.

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.

Requirement: Always ensure that the front door is 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.

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 five minutes of lamp warmup time.

Recommendation: If you run the detector continuously, perform wavelength verification weekly by turning off the detector, and then turning it on again.

Operational modes

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

Single-wavelength mode

Single-wavelength is the detector's default mode of operation that supports monitoring a single-wavelength, from 190 nm to 700 nm, settable in 1-nm increments 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.

Primary parameters

The following are the values of major parameters that apply to single-wavelength mode:

- Wavelength, in nanometers Specifies a wavelength for channel A, from 190 nm to 700 nm, settable in 1-nm increments.
- Sensitivity in AUFS Specifies the scaling factor for the analog output channel and corresponds to the absorbance unit (AU) value where the analog outputs saturate at full-scale values. Absorbance units full-scale (AUFS) vary from 0.0001 to 4.000 AU.

Tip: Changing the sensitivity (AUFS) setting affects the 2-V output.

- Chart polarity (+ or –) Reverses the polarity of the charted chromatogram. Select + for a normal chromatogram, or for an inverted chromatogram. This function changes the direction of the plot on the 2-V output, similar to reversing the leads to an external chart recorder.
- Filter time-constant Programs a filter time, in seconds. Options are Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you need not enter a value. The filter constant is determined by the data rate. If you select Other, you can enter a value, but the value you enter is

rounded up or down to a value based on the data rate. Selecting Other and entering a value of 0.0 disables all filtering.

• Analog rate – Specifies a value as high as 80 Hz.

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 use of this mode to more standard chromatography, where peaks 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 ratio plotting (RatioPlot) or maximum-absorbance-volume plotting (MaxPlot) mode.

You 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).

Chart-out selection modes

When operating in dual-wavelength mode, the detector offers more choices for analog output than the selections offered in single-wavelength mode. The default selection for dual-wavelength mode is Absorbance.

- Absorbance (A and B) The standard LC mode where the current absorption is scaled and sent directly out the analog output. The scaling depends on the AUFS 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 AUFS of 2.0000 in single-wavelength mode or in dual-wavelength mode.
- MaxPlot This mode results in the output of the larger of the two absorbance values scaled to the selected AUFS setting. Use this mode

when observing, with one data channel, multiple compounds that exhibit absorbancies at two separate wavelengths. (Dual mode only)

- RatioPlot (A/B) This mode 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 AUFS, 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. (Dual mode only)
- Difference Plot (A-B) This mode plots the arithmetic difference in absorbance for the two monitored wavelengths. (Dual mode only)

Spectrum scanning

When the detector is operating under the control of the Empower, MassLynx, or third-party software, the scanning function is disabled.

You can use the detector as a spectrophotometer, to acquire spectra from the flow cell. The major difference between the detector and a double-beam spectrophotometer is that the detector uses only one flow cell, rather than a sample and a reference pair.

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

- 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 analog output, or acquired and stored in memory for later playback.

To generate a spectrum

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

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

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 chart recorder or data system over the output channel. 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.

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.

Before you begin

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

Tip: Use this guide in conjunction with the ACQUITY UPLC system documentation and online Help.

Before installing the detector, ensure that

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

If you discover any damage or discrepancy when you inspect the contents of the cartons, immediately contact the shipping agent and your local Waters representative. Customers in the USA and Canada should report damage and discrepancies to Waters Technical Service (800 252-4752). Others should 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*.

Installing the detector

To install the ACQUITY UPLC TUV detector

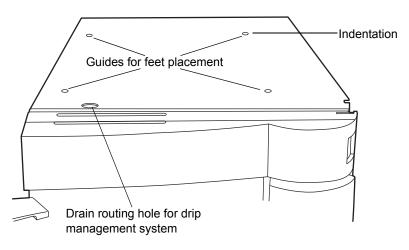


Warning: When installing the detector unassisted by another person, use a mechanical lift to avoid lifting injuries.

1. Place the detector atop the column manager, ensuring that the feet are properly positioned in the indentations of the column manager.

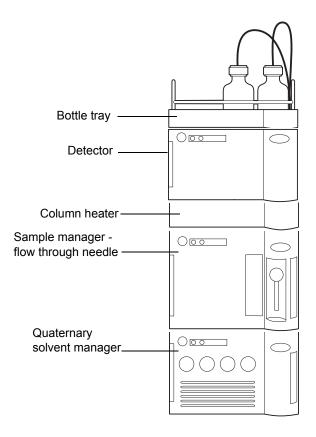
Tip: Doing this aligns the detector's drip tray over the drain routing hole at the top, left side of the column manager.

Proper placement for drip management system



2. Place the solvent tray module atop the detector.

ACQUITY UPLC TUV detector in an ACQUITY UPLC H-Class system



Plumbing the detector



Warning: Using incompatible solvents can injure you and severely damage the instrument. See "Solvent Considerations" on page 77 for more information.

Plumbing the detector involves connecting the flow cell and installing a backpressure regulator, if necessary.

Although the inline degasser removes most of the gas (air) from solvents, some gas is reintroduced during partial loop injections. Under pressure, this gas remains in solution. However, because the post-column pressure is normally much lower than the pre-column pressure, any dissolved gas in the post-column affluent comes out of solution and produces an unstable baseline characterized by large, unexpected spikes.

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

Requirement: If the ACQUITY TUV detector is the last detector in the system, the backpressure regulator is required for optimum performance.

Tip: If a mass spectrometer or other detector is connected downstream of the detector, a backpressure regulator should not be installed. The length of the tubing connecting to the mass spectrometer or other detector helps to maintain the backpressure on the flow cell.

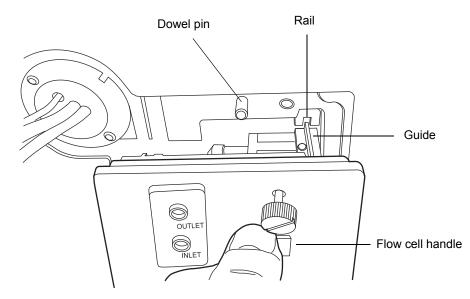
Recommendation: To avoid particulate contamination in the flow cell, flush any columns you are connecting to the detector before connecting them.

See also: ACQUITY UPLC System Documentation CD or ACQUITY UPLC H-Class System Documentation CD.

To plumb the detector

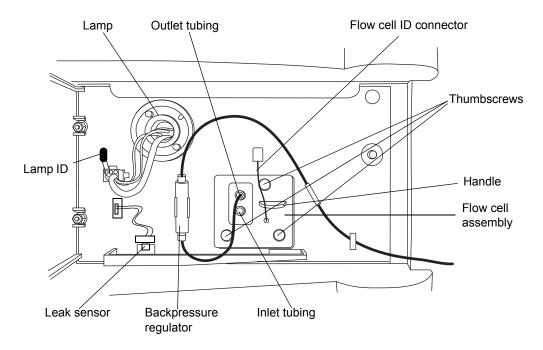
Recommendation: If the detector is already powered on, in the console, select TUV Detector from the system tree and click **(Lamp Off)** to extinguish the lamp.

1. Open the detector's front door, and install the flow cell assembly, holding it squarely to the opening and then inserting it slowly so that the guides on the front part of the flow cell flange engage the rails in the sample cell compartment.



- 2. After the flange and rails are engaged, continue inserting the flow cell until the dowel pins on the detector engage the corresponding holes on the cell holder.
- 3. Continue to insert the flow cell until the three thumbscrews align with their holes in the bulkhead.

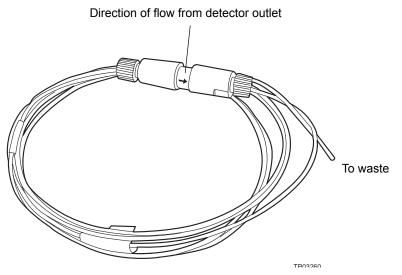
4. Hand tighten the thumbscrews. Confirm the screws are secure using a screw driver.



5. Remove the protective cover from the PEEK cell inlet tubing, and connect the tubing to the flow cell inlet, confirming that the label on the tubing matches the type of detector and flow cell in your system.

6. Attach the short length of outlet tubing from the backpressure regulator to the outlet of the flow cell.

Backpressure regulator



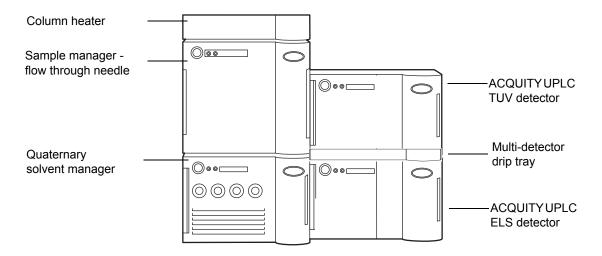
7. Route the long end of the outlet tubing from the backpressure regulator, through the channel clips along the front right side of the system, and into a suitable waste container.

Tip: If a mass spectrometer or other detector is connected downstream of the detector, a backpressure regulator should not be installed. The length of the tubing connecting to the mass spectrometer or other detector helps to maintain the backpressure on the flow cell.

Installing the multi-detector drip tray

If your ACQUITY UPLC system has more than one detector, you must install the multi-detector drip tray.

ACQUITY UPLC TUV detector in a split ACQUITY UPLC H-Class system



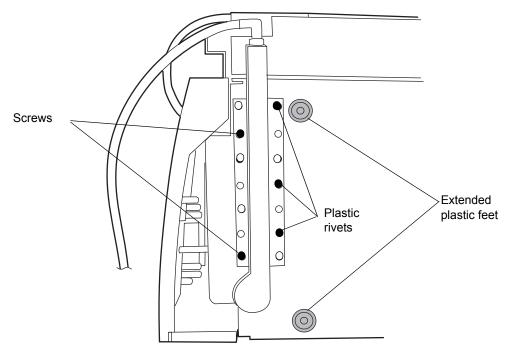
Required materials

Multi-detector drip tray kit

To install the drip tray

- 1. Turn the ACQUITY TUV detector so that it is resting on its left side.
- 2. Snap the extended plastic feet on to the bottom of the detector, and then snap the anti-skid pads on to the extended plastic feet.
- 3. Secure the drip tray to the bottom of the detector, using the screws and plastic rivets provided in the multi-detector drip tray kit.
 - a. Remove the two screws, move the drip tray into place, and then reinstall the screws to secure the tray.
 - b. Install three plastic rivets to further secure the tray.

Installing the multi-detector drip tray (bottom view)



4. Return the ACQUITY TUV detector to its original position atop the other detector.

Making Ethernet connections

To make Ethernet connections

- 1. Unpack and install the preconfigured ACQUITY workstation.
- 2. Connect one end of one Ethernet cable to the network switch, and then connect the other end to the Ethernet card, on the workstation.

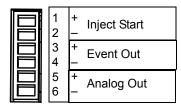
Tip: On preconfigured systems, the Ethernet card is identified as the Instrument LAN card.

3. Connect one end of one Ethernet cable to the back of the detector, and then connect the other end to the network switch.

I/O signal connector

The detector's rear panel includes a removable connector that holds the screw terminals for I/O signals. This connector is keyed so that it can receive a signal cable inserted only one way.

TUV I/O signal connector



ACQUITY UPLC TUV detector analog/event connections

Signal connections	Description
Inject Start	Start injection
Event Out	Output switch to trigger external devices
Analog Out	Analog chart output

Connecting to the electricity source

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



Warning: To avoid electrical shock, observe these precautions:

- 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 all components of the ACQUITY UPLC system to a common ground.

To connect to the electricity source

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

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

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

Starting the detector



Warning: Using incompatible solvents can injure you and severely damage the instrument. See "Solvent Considerations" on page 77 for more information.

Starting the detector entails powering-on the detector and each system instrument individually, as well as the ACQUITY workstation. It also entails starting the operating software (Empower, MassLynx, or third party).

Caution:

- To ensure a long life for the light-guiding flow cell and proper detector initialization, use well-degassed eluents, making sure they are flowing before you power-on the detector.
- To minimize contaminants that can leave deposits on the flow cell's walls, flush new columns for 15 minutes before connecting the flow cell.

If you must power-on the detector before the eluent is flowing, extinguish the lamp. You can do this in the Instrument Method Editor (Empower, MassLynx, or third-party) by specifying a Lamp On event in the Events table. You can also extinguish the lamp in one of these ways:

• If Empower software controls the system, click 🔯 (Lamp Off) in the control panel at the bottom of the Run Samples window.

- If MassLynx software controls the system, click 💢 (Lamp Off) in the control panel at the bottom of the Inlet Editor window.
- In the console, select TUV Detector from the system tree and click 🗽 (Lamp Off).

See also: ACQUITY UPLC System Documentation CD or ACQUITY UPLC H-Class System Documentation CD.

To start the detector

- 1. Power-on the workstation.
- 2. Press the power switch on the top, left side of the solvent manager (QSM/BSM) door and sample manager door.

Result: Each system instrument "beeps" and runs a series of startup tests.

The power and lamp LEDs change as follows:

- Each system instrument's power LED shows green.
- During initialization, each system instrument's status LED flashes green.
- After the instruments are successfully powered-on, all LEDs show steady green. The solvent manager's flow LED and the sample manager's run LED remain unlit.
- 3. Start Empower, MassLynx, or a third-party software. You can monitor the ACQUITY console for messages and LED indications.
- 4. Flush the system with filtered, degassed, and sparged HPLC-grade methanol or acetonitrile.
- 5. In the console, set the solvent manager to deliver a flow rate appropriate for the flow cell in your system.

Tip: Use only thoroughly degassed HPLC-grade solvents. Gas in the mobile phase can form bubbles in the flow cell and cause the detector to fail the Reference Energy diagnostic test.

6. Pump mobile phase for at least 15 minutes.

7. Ensure the detector cell is filled with solvent and free of bubbles.

Caution: The detector can fail to initialize correctly if the cell contains air. To avoid damaging the light-guiding flow cell, do not ignite the detector lamp when no solvent is flowing through the cell or when it is dry.

8. Press the power switch on the front panel 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.

9. When the lamp LED is steady green, start Empower, MassLynx, or a third-party software, and download an instrument or inlet method.

Result: The ACQUITY Console displays messages and visual signals.

10. For best results, wait one hour for the detector to stabilize before acquiring data.

Monitoring detector LEDs

Light emitting diodes on the detector indicate its state of functioning.

Power LED

The power LED, to the left of the detector's front panel, indicates when the detector is powered-on or powered-off.

Lamp LED

The lamp LED, to the right of the power LED, indicates the lamp status.

Lamp LED indications

LED mode and color	Description
Unlit	Indicates the detector lamp is extinguished.
Constant green	Indicates the detector lamp is ignited.
Flashing Green	Indicates the detector is initializing or calibrating.

LED mode and color	Description
Flashing red	Indicates an error stopped the detector. Information regarding the error that caused the failure can be found in the console.
Constant red	Indicates a detector failure that prevents further operation. Power-off the detector, and then power-on. If the LED is still steady red, contact your Waters service representative.

Lamp LED indications (Continued)

About the detector control panel

If Empower software controls the system, the detector's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the detector's control panel appears at the bottom of the Inlet Editor window.

Detector control panel



The detector control panel displays the acquisition status (if the detector is running). You cannot edit detector parameters while the system is processing samples.

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

Modifiable detector control panel items

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

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

Control panel function	Description
Autozero	Resets the detector offsets.
Reset TUV	Resets the detector, when present, after an error condition.
Help	Displays the console Help.

Shutting down the detector

Shutting down for less than 24 hours

Requirement: For short-term idle times (less than 24 hours), maintain the solvent flow to preserve flow cell cleanliness.

If a few hours will pass before the next injection, slow the flow rate in the interim to a few tenths of a mL/min to conserve solvent. Keep the detector operating and the column heater at operating temperature during this period.

To shut down the detector for less than 24 hours

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

🔀 (Lamp Off) in the detector control panel.

Requirement: When operating the detector under MassLynx control, ensure the shutdown method's Auto-Shutdown function is deactivated.

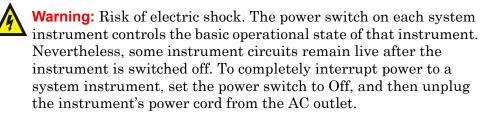
Shutting down for more than 24 hours

Requirement: For extended idle times (greater than 24 hours), plug the flow cell ports to preserve flow cell cleanliness.

To shut down the detector for more than 24 hours

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

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



4. Power-off the system.

Alternative: If you prefer to leave the system powered-on, turn off the column heater or reduce the column heater temperature to 40 $^{\circ}$ C (104 $^{\circ}$ F).



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

5. Cap the flow cell inlet and outlet ports.

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, and click Waters Division.

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

- Any error messages
- Nature of the symptom
- Instrument serial numbers
- Flow rate
- Operating pressure
- Solvent(s)
- Detector settings (sensitivity and wavelength)
- Type and serial number of column(s)
- Sample type
- Empower, MassLynx, or third-party software version and serial number
- ACQUITY workstation model and operating system version

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

Maintenance considerations

Safety and handling

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



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



Warning: To avoid electric shock, do not remove the detector's top cover. No user-serviceable parts are inside.



Caution:

- To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the detector. To completely interrupt power to the detector, set the power switch to Off, and then unplug the power cord from the AC outlet. After power is removed, wait 10 seconds before you disconnect an assembly.
- To prevent circuit damage due to static charges, do not touch integrated circuit chips or other system instruments that do not require manual adjustment.

Proper operating procedures

To ensure your system runs efficiently, follow the operating procedures and guidelines in "Starting the detector" on page 22.

Spare parts

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

Recommendations:

- To prevent dirt from getting into the optics assembly, always keep the detector door closed whenever a flow cell is not installed in the detector.
- Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.

- To conserve lamp life, extinguish the lamp while leaving the detector running but idle. Note, however, that you should do so only when the lamp will remain extinguished more than 4 hours.
- If you use buffered mobile phase, flush it from the detector before powering-off to prevent
 - plugging solvent lines and the flow cell
 - damaging instrument components
 - microbial growth

Caution:

- To ensure optimum performance of the light-guiding flow cell, ensure that eluent is flowing prior to powering-on the detector. If, however, you must power-on the detector before the eluent is flowing, extinguish the lamp first.
- If the light-guiding flow cell will not be used for a period of time, flush it with clean mobile phase, such as a water/acetonitrile or water/methanol mix, and either cap the flow ports or dry the flow cell with pure nitrogen or pure helium for 5 to 10 minutes.
- To avoid damaging the detector or column, remove the column and disconnect the detector before you flush the system.

Flushing the detector

To flush the detector



Caution: To avoid damaging the detector, do not exceed the 6895 kPa (69 bar, 1000 psi) pressure limitation of the flow cell.

- 1. Remove the column from the system.
- 2. Flush the system to waste with 100% HPLC-quality water at a rate of 1.0 ml/minute for 10 minutes.
- 3. Flush the system with a solution of 90:10 methanol/water for 10 minutes.

Maintaining the leak sensor

A leak sensor in the drip tray continuously monitors the detector for leaks. The sensor stops system flow when it detects accumulated, leaked liquid in its surrounding reservoir, and an error message describing the problem appears in the ACQUITY UPLC Console.

Resolving detector leak sensor errors

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



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



Caution: To avoid scratching or damaging the leak sensor

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

Required materials

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

To resolve a detector leak sensor error

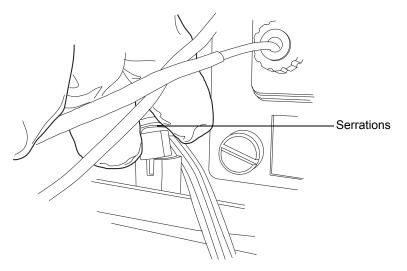
1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to verify that the leak sensor detected a leak.

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

- 2. Open the detector door, gently pulling its right-hand edge toward you.
- 3. Locate the source of the leak, and make the repairs necessary to stop the leak.

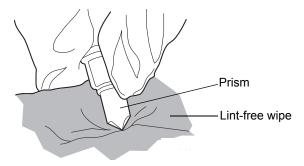
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

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

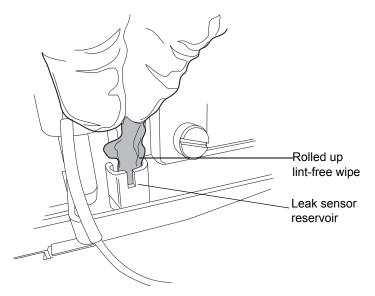


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the leak sensor connector from the front of the instrument (see the figure on page 36).

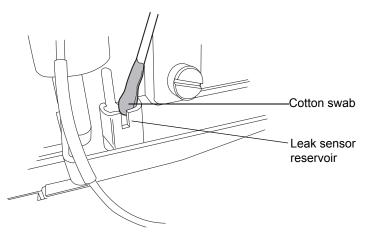
5. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



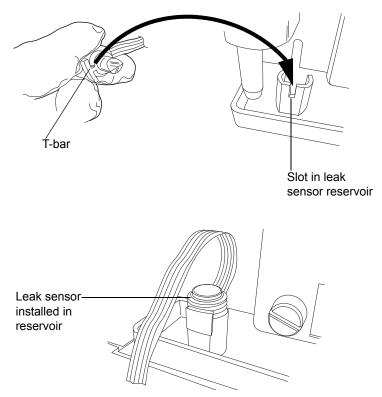
6. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



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



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



- 9. If you detached the leak sensor connector from the front of the instrument, reattach it.
- 10. In the ACQUITY UPLC Console, select your detector from the system tree.
- 11. In the detector information window, click Control > Reset to reset the detector.

Replacing the detector's leak sensor



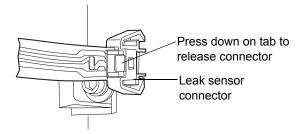
Warning: The leak sensor and its reservoir can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

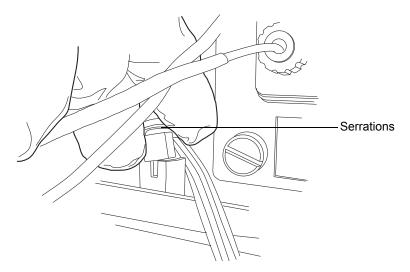
- Clean, chemical-resistant, powder-free gloves
- Leak sensor

To replace the detector leak sensor

- 1. Open the detector door, gently pulling its right-hand edge toward you.
- 2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

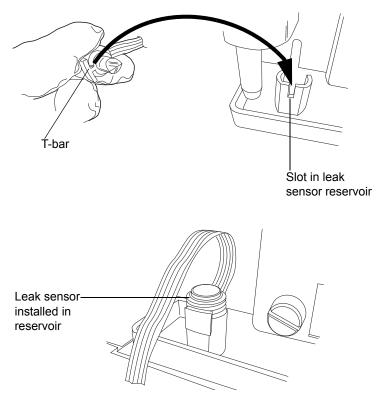


3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



4. Unpack the new leak sensor.

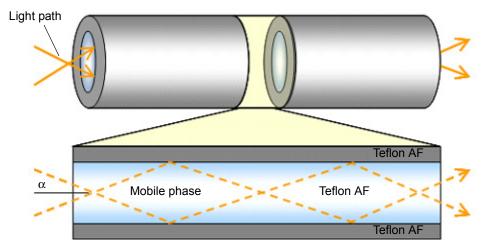
5. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



- 6. Plug the leak sensor connector into the front of the instrument.
- 7. In the ACQUITY UPLC Console, select your detector from the system tree.
- 8. In the detector information window, click Control > Reset to reset the detector.

Maintaining the flow cell

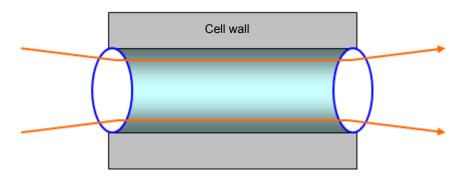
Waters light-guiding flow cells transport light and sample via Teflon AF tubing. The tubing transmits energy through low-volume flow cells, resulting in heightened analytical sensitivity. Light efficiently transits tubing by means of a mechanism known as total internal reflection (TIR) in which the light remains within the fluid stream because the refractive index of the fluid exceeds that of the Teflon tubing material.



Light transmission through a light guiding flow cell

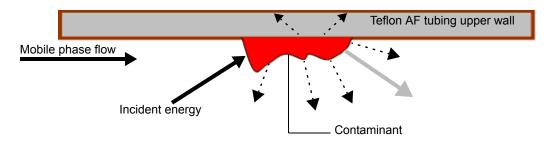
In the figure above, the light path through the cell is depicted by a pair of rays (dashed lines) that bounce off the cell wall. The energy carried by each ray is conserved after each bounce. One hundred percent of the light is reflected, hence the term "total internal reflection". The Teflon AF tubing is an active component in the flow cell light path.

In contrast to the light-guiding flow cell, a conventional cell is typically an all-metal body with lenses at each end.



Light transmission through a conventional flow cell

The light path through a conventional flow cell is designed to avoid contact with the cell walls, primarily to prevent rays that encounter the walls from contributing to the measured signal. The energy associated with errant rays is highly variable depending on the mobile phase composition, quality of wall finish (which is never 100% reflective), or the slow build-up of contaminants. The Teflon AF surface, however, is mirror-like, and the relatively slight RI-dependence associated with a well-maintained cell is negligible. Surface contamination, depicted by the red, irregularly shaped object in the next figure can lead to undesirable beam effects like scattering (dashed arrows) or absorption (gray, thick arrow), both of which decrease energy relative to the incident ray (black, thick arrow).



Unwanted beam effects from a light guiding flow cell

The operational differences between the light-guiding method, where light is transmitted through interactions with the cell wall, and the conventional method, which avoids such interactions, underscores these practical measures for maintaining the liquid core flow cell:

- Periodically determine flow cell transmission under conditions similar to those used to characterize a new cell. (Doing this typically means determining cell transmission with clean mobile phase.)
- Avoid fouling the flow cell by changing or disturbing upstream system components, as in the case when a new column is brought online.

Cleaning the flow cell

Clean the flow cell when it becomes contaminated with the residues of previous runs and also after each detector shutdown. A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems.

Always flush the flow cell with mobile phase as your initial attempt to correct these problems. Afterward, if you observe no improvement, flush the flow cell with pure organic solution, like 100% acetonitrile. If the problems persist, flush the flow cell with 1% formic acid for 30 minutes, and then flush with water until the formic acid is removed or until the pH is neutral. If flushing with the 1% formic acid solution also fails, perform a system acid cleansing flush (see "Performing a system, acid-cleansing flush" on page 43). If you still observe no improvement, call Waters Technical Service.

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

The pressure through the flow cell must not exceed 6895 kPa (69 bar, 1000 psi). Increasing the flow rate usually increases the pressure. High-viscosity fluids generally increase the pressure through the flow cell and therefore require a lower flow rate. Allowable flow rates are based on the limit of pressure that each flow cell can withstand.



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

Rule: Always use clean, well-degassed eluents.

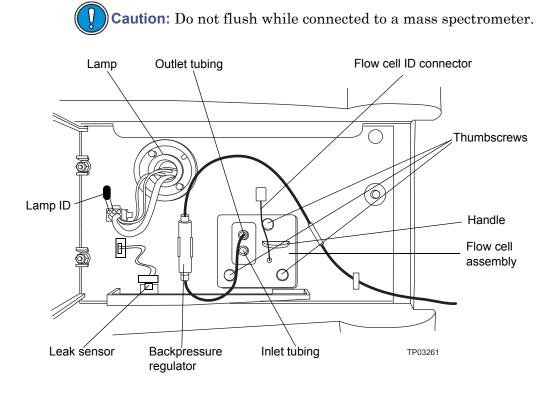
Required materials

- 1% formic acid
- Clean, chemical-resistant, powder-free gloves
- Water (for flushing buffers)
- Intermediate solvent that is miscible in both the mobile phase and water
- Stainless steel unions (to replace the column during flushing)
- Wrench suitable for removing and replacing the column

To clean the flow cell

- 1. In the detector control panel, click 🔀 (Lamp Off).
- 2. Stop the solvent flow and remove the column.
- 3. Replace the column with a union or piece of tubing.

4. If another instrument is downstream of the flow cell outlet, break the connection at the other instrument, and route the outlet tubing to waste while flushing.



5. Flush the detector with HPLC-grade water.

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

- 6. Pump an acid wash composition of 1.0% formic acid in water or 90% water/10% organic mixture.
- Flush the flow cell for at least 4 hours at 0.05 to 0.1 ml/min.
 Requirement: Do not exceed 6895 kPa (69 bar, 1000 psi).
- 8. Remove any other active detectors of instruments from the system.
- Flush the detector with HPLC-grade water until the pH is neutral.
 Tip: If the mobile phase is not compatible with water, flush with an intermediate solvent first.

- 10. Reattach the column.
- 11. Resume pumping mobile phase.

Tip: If the mobile phase is not miscible in water, first flush with an intermediary solvent.

Performing a system, acid-cleansing flush



Caution: If you are running a mass spectrometer, do not perform the system, acid-cleansing flush. Instead, call Waters Technical Service.

General system contamination can spread to the flow cell. If the system becomes contaminated, perform a system, acid-cleansing flush, which cleans the solvent manager, sample manager, and flow cell.

To prepare the solvent

- 1. Prepare a mixture of 50:50 (v/v) methanol/water:
 - a. Measure 500 mL of water in a graduated cylinder.
 - b. In a separate graduated cylinder, measure 500 mL of methanol.
 - c. Add methanol to water, and mix for 5 minutes.
- 2. Prepare a mixture of 30:70 (v/v) phosphoric acid/water:
 - a. Measure 700 mL of water in a graduated cylinder.
 - b. In a separate graduated cylinder, measure 300 mL of phosphoric acid.
 - c. Add phosphoric acid to water, and mix for 5 minutes.
- 3. Fill a 1-L mobile phase reservoir with 100% water.
- Fill a 1-L mobile phase reservoir with 100% isopropanol.
 The cleaning procedure takes approximately 6 hours, once the solvents are prepared.

To perform the system, acid-cleansing flush



Caution: Failure to remove the bottle filters contaminates the flow path.

- 1. Remove the sample and solvent manager bottle filters.
- 2. Place all lines A1, A2, B1, B2 seal wash, weak needle wash and strong needles in 50:50 methanol:water.
- 3. Prime the solvent lines for 5 minutes each.
- 4. Prime the seal wash.
- 5. Prime the wash syringes and sample syringe for 4 cycles.
- 6. Connect a pressure restrictor in the fluid path, after the injector, to create 13,800 kPa (138 bar, 2000 psi) backpressure in the system.
- 7. Transfer 1 mL of mobile phase to an autosampler vial, and place the vial in position 1:A,1.
- 8. Create an instrument method with the following parameter values:
 - Flow rate = 0.5 mL/min
 - Gradient composition 50% A1:50% B1
 - Full loop injection
- 9. Make 30, full-loop injections from the vial containing the mobile phase.
- 10. Set the run time to 0.5 minutes.

Tip: This step should take approximately 30 minutes.

11. Repeat steps 1 through 8 using 100% isopropanol as the solvent.

Requirement: Do not pass effluent through optical detector for this wash step. Route the restrictor to waste.

12. Repeat steps 1 through 8 using 100% water as the solvent.

Note: Remove the Seal wash line from the mobile phase bottle prior to performing the phosphoric acid wash.

- 13. Repeat steps 1 through 8 using 30:70 (v/v) phosphoric acid/water as the solvent.
- 14. Continue pumping the phosphoric acid mixture for an additional 3 hours.

- 15. Repeat steps 1 through 8 using 100% water as the solvent.
- 16. Repeat steps 1 through 8 using 50:50 (v/v) methanol/water as the solvent.
- 17. Replace the sample and solvent manager bottle filters.

Replacing the flow cell

Caution:

- To avoid contaminating the flow cell, wear clean, chemical-resistant, powder-free gloves when handling, removing, or replacing it.
- To avoid damaging the flow cell, handle it with care. Do not disassemble the flow cell.

See also: Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems (part number 715001307), available on the Waters web site (www.waters.com).

Required materials

- 1/4-inch, flat-blade screwdriver
- Clean, chemical-resistant, powder-free gloves
- Flow cell

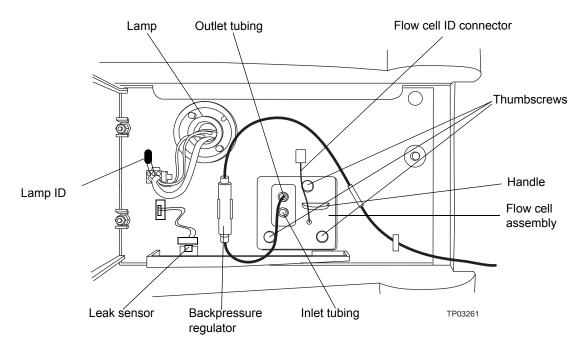
To replace the flow cell



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

- 1. Power-off the detector.
- 2. Stop the solvent flow.
- 3. Open the detector door, gently pulling its right-hand edge toward you.

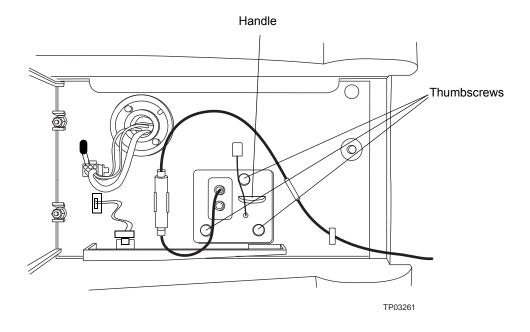
4. Disconnect the detector's inlet and outlet tubing from the main column connection.



- 5. Disconnect the flow cell ID connector (if present).
- 6. Remove the flow cell:
 - Using a 1/4-inch, flat-blade screwdriver, loosen the 3 thumbscrews on the flow cell assembly's front plate.

Grasp the handle and gently pull the assembly toward you.

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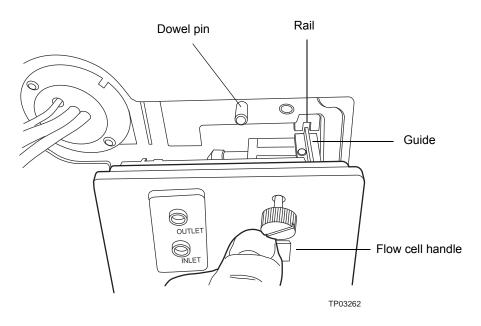


Caution: To avoid damaging the capillary tubing, do not touch it.

7. Unpack and inspect the new flow cell, ensuring the flow-cell type is correct for your application.

Tip: When replacing the flow cell, replace the flow cell inlet tubing with the tubing included with the new flow cell (see "Plumbing the detector" on page 15).

8. Square the flow cell assembly in front of the opening, and then insert it slowly so that the guides on the front part of the cell flange engage the rails in the sample cell compartment.



- 9. After the flange and rails are engaged, continue inserting the flow cell until the dowel pins on the instrument engage the corresponding holes on the cell holder.
- 10. Continue to insert the flow cell until the three thumbscrews align with their holes in the bulkhead.
- 11. Hand tighten the thumbscrews. Confirm the screws are secure using a screw driver.
- 12. Connect the inlet tubing to the main column connection and flow cell inlet, and connect the outlet tubing to the flow cell outlet.
- 13. Reconnect the flow cell ID connector (if present).
- 14. Close the detector door.
- 15. Before you power-on the detector, ensure the flow cell is filled with degassed, transparent solvent (acetonitrile or water) and free of air bubbles.

Replacing the lamp

Change the lamp when it repeatedly fails to ignite or when the detector fails to calibrate.

Tip: The ACQUITY TUV lamp is automatically sensed upon installation, and its serial number and installation date are automatically entered into the Lamp Change Record table.

Waters warrants 2000 hours of lamp life, or one year since date of purchase, whichever comes first.



Warning: To prevent burn injuries, allow the lamp to cool for 30 minutes before removing it. The lamp housing gets extremely hot during operation.



Warning: To avoid eye injury from ultraviolet radiation exposure

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

To remove the lamp

- 1. Power-off the lamp:
 - To power-off the lamp manually, click TUV Detector in the left pane of the console, and then click **X**. The green LED on the console darkens as does the Lamp LED on the door.
 - To power-off the lamp using a timed event, see the instructions in the Empower, MassLynx, or third-party online Help.
- 2. Power-off the detector and disconnect the power cable from the rear panel

Alternative: To save time, leave the detector powered on for 15 minutes after you power-off the lamp. Doing so will allow the fan to blow cool air on the lamp, cooling it faster. Be sure to power-off the detector and disconnect the power cable from the rear panel after 15 minutes has elapsed.



Warning: The lamp and lamp housing may be hot. Wait 30 minutes (or 15 minutes with the fan running) for these components to cool before touching them.

Allow the lamp to cool for 30 minutes (or 15 minutes with the fan 3. running), and then open the door, gently pulling its right edge toward you.

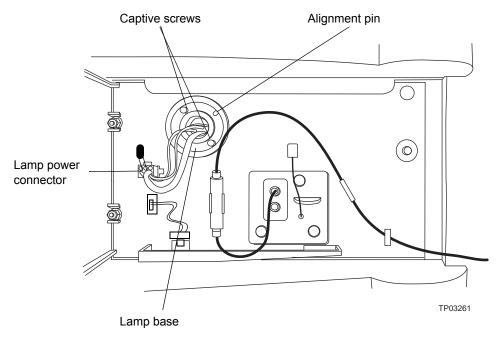


Warning: To avoid electric shock, power-off and unplug the detector before detaching the lamp power connector from the detector.



Caution: To avoid damaging the detector's electronics, power-off and unplug the detector before detaching the lamp power connector from the detector.

Detach the lamp power connector from the detector. 4.



Warning: Lamp gas is under slight negative pressure. To prevent shattering the glass, use care when disposing of the lamp.



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

5. Loosen the two captive screws in the lamp base. Gently withdraw the lamp from the lamp housing.

To install the lamp

- 1. Unpack the new lamp from its packing material without touching the bulb.
- 2. Inspect the new lamp and lamp housing.
- 3. Position the lamp so that the cut-out on the lamp base plate is at the 1 o'clock position, in line with the alignment pin on the lamp housing, and then gently push the lamp forward until it bottoms into position. Ensure that it is flush to the optics bench.



Caution: To prevent the lamp from binding and ensure that it is properly seated in the lamp housing, alternate between tightening the captive screws and pushing the lamp forward.

- 4. Tighten the two captive screws, and then reconnect the lamp power connector.
- 5. Power-on the detector, and then wait about 1 hour for the lamp to warm before resuming operations.

Tip: Cycling power to the detector (that is, powering-off and then powering-on the instrument) initiates the verification procedures.

Result: The lamp's serial number and installation date are automatically entered into the Lamp Change Record table.

Replacing the fuses



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

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.

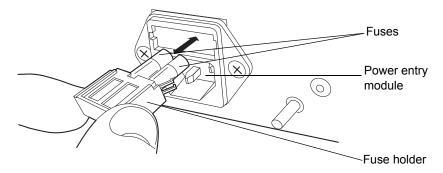
Suspect a fuse is open or otherwise defective when

- the detector fails to power-on.
- the fan does 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.
- 2. Pinch the sides of the spring-loaded fuse holder, which is above the power entry module on the rear panel of the detector. With minimum pressure, withdraw the spring-loaded fuse holder.



3. Remove and discard the fuses.

- 4. Make sure that the new fuses are properly rated for your requirements, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
- 5. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the detector.

Error Messages

The detector provides error messages to help troubleshoot system problems.

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 the control panel and in the console.

Tip: To reduce the likelihood of errors, be sure the flow cell contains degassed transparent solvent (methanol or water), and the front cover is attached securely.

The tables in this section are organized as follows:

- Messages requiring you to perform corrective action including messages encountered at startup and during calibration or operation.
- Messages requiring you to cycle power, and then contact Waters Technical Service personnel if an error persists (see "Contacting Waters technical service" on page 29). Most of these errors arise on startup.

The table below provides startup, calibration, and operating error messages, descriptions, and recommended actions you can take to correct the problem. These messages appear in the console log.

Error Message	Description	Corrective Action
Calibration differs: <i>n</i> nm	At startup, the unit performs a complete verification, which includes remeasurement of all calibration points. New calibration points are compared to stored information from the most recent manual calibration. If any one of the points differs by more than 1.0 nm, the detector displays this message.	 Cycle power to the detector. Perform manual calibration. Contact Waters Technical Service.
Calibration not found	Stored calibration data not valid.	Perform manual calibration procedure.
Calibration unsuccessful: Peak out of range <i>n.nn</i> nm	Results of a calibration operation differ by more than 1.0 nm. Unit uses previously stored calibration points.	 Be sure the front door is closed. Flush flow cell.
Flow cell memory device not detected	Unable to electronically communicate to flow cell	Connect flow cell identification cable.
Lamp failure	Lamp indicates Off when it should be On.	 Check lamp icon. Cycle power to the detector. Replace lamp.

Startup, calibration, and operating error messages

Error Message	Description	Corrective Action
Lamp lighting failure	The lamp failed to ignite.	 Cycle power to the detector. Check lamp power connection. Replace lamp.
Lamp memory device not detected	Unable to electronically communicate to lamp	Change lamp.
Peak not found: Erbium <i>n</i> nm	Erbium filter calibration range does not contain a local maximum.	 Be sure the front door is closed. Flush flow cell.
Peak not found: 656 nm deuterium	Unit sensors cannot determine 656-nm peak.	 Be sure the front door is closed. Flush flow cell.

Startup, calibration, and operating error messages (Continued)

Error Message	Description	Corrective Action
Wavelengths span 370 nm: Order filter not in use	In dual-wavelength mode: • If both selected wavelengths > 370 nm, the detector applies the second-order filter to block unwanted UV light. • If both selected wavelengths < 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 wavelengths above 370 nm may contain inaccuracies because of possible UV light interference (second-order effects).	Select wavelengths that are each above or below 370 nm.

Startup, calibration, and operating error messages (Continued)

Error messages preventing operation

During initialization, calibration, and operation, the detector can display "<Error>" in the control panel, signifying a usually terminating malfunction and preventing further operation of the detector.

When you encounter such an error, ensure that

- the flow cell is clean.
- the front door is shut securely.

Cycle power to the detector. If the terminating error persists, contact Waters Technical Service.

Error Message	Description	Corrective Action
24-Volt fuse failed	Fuse failure was detected	1. Cycle power to the detector.
		2. Contact Waters Technical Service.
Communication failure: Reference A/D	A/D communication test failed.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.
Communication failure: Sample A/D	A/D communication test failed.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.
Configuration not found	Stored configuration data are invalid.	Cycle power to the TUV Detector. This action removes the error.
Dark current too high: nnnnnn	The dark energy level is above 1000000.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.
Dark current too low: 0	The dark energy level equals 0.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.

Instrument error messages

Error Message	Description	Corrective Action
Electronic A/D failure	Lamp optimization is adjusted at the minimum level.	Cycle power.
	Data acquisition via A/D converters is interrupt-driven. If interrupt is too long, problem with data acquisition is indicated.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: Erbium position	Unit sensors cannot find erbium filter position.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: No filters found	Unit sensors observe transition to dark before homing the optical filter.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: No reference energy	Unit sensors cannot find any light energy before homing the optical filter.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: No response	Unit sensors cannot identify any dark regions.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: Order filter position	Unit sensors cannot find the order filter position.	 Cycle power to the detector. Contact Waters Technical Service.
Filter initialization failure: Shutter position	Unit sensors cannot find the shutter position.	 Cycle power to the detector. Contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Grating initialization failure: Backlash too high	Backlash is the difference between the forward and reverse peak positions of deuterium at 656 nm. If this difference is greater than 1 step, the unit displays this message.	 Cycle power to the detector. Contact Waters Technical Service.
Grating initialization failure: No home sensor	Search for the home sensor failed.	 Cycle power to the detector. Contact Waters Technical Service.
Lamp data not found	Stored lamp data are invalid.	Cycle power to the detector. This action removes the error.
Method not found	Stored method data are invalid.	Cycle power to the detector. This action removes the error.
Scan not found	Stored scan data are invalid.	Cycle power to the detector. This action removes the error.
System cannot respond	Error occurs while unit is positioning next wavelength or changing modes. Occurs during initialization or calibration.	 Cycle power to the detector. Contact Waters Technical Service.
System not calibrated.	The calibration read from nonvolatile memory is not valid.	 Cycle power to the detector. Perform manual calibration. Contact Waters Technical Service.

Instrument error messages (Continued)

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Thermal controller disabled	Thermal controller was disabled.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.
Vcc fuse failed	Fuse failure was detected.	1. Cycle power to the detector.
		2. Contact Waters Technical Service.

Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This section presents all the safety symbols and statements that apply to the entire line of Waters products.

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)

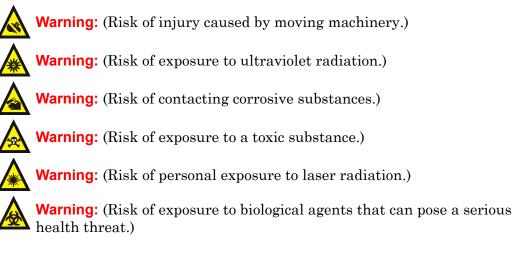


Warning: (Risk of electric shock.)



Warning: (Risk of fire.)

Warning: (Risk of needle puncture.)



Specific warnings

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

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



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

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

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.

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

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

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.

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

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



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

Biohazard warning

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

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

Chemical hazard warning

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



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

Caution symbol

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



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

Warnings that apply to all Waters instruments

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

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



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

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

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

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

警告: 當在有壓力的情況下使用聚合物管線時, 小心注意以下幾點。

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



警告: 当有压力的情况下使用管线时,小心注意以下几点:

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

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

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

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

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



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

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

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

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

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

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

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

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

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



Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers. Attention: pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boite à fusible de l'instrument.



Vorsicht: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.



Attenzione: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.



Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.



警告:為了避免火災,更換保險絲時,請使用與儀器保險絲蓋旁面板上所印刷之相同類 型與規格的保險絲。



警告:为了避免火灾,应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。



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



警告:火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols

Electrical symbols

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

	Electrical power on
0	Electrical power off
\bigcirc	Standby
	Direct current
\sim	Alternating current
	Protective conductor terminal
m	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

<u> </u>	Keep upright!
×	Keep dry!
Y	Fragile!
\mathbf{X}	Use no hooks!

Specifications

This section lists individual operating specifications for the Waters ACQUITY UPLC TUV Detector, as follows:

- Operational specifications
- Optical specifications

Operational specifications

Condition	Specification
Wavelength range	190 to 700 nm
Bandwidth	<5 nm
Wavelength accuracy	<u>+</u> 1.0 nm
Wavelength repeatability	±0.1 nm
Single λ dry noise	<6 μAU (at 230 nm, 1-second digital filter, 30-second segments, 2-Hz data rate, medium shunt in place)
Dual channel noise (dry)	10.0×10^{-5} AU (measured at 230 nm, running at 230 to 280 nm with 2-second filter at 2-Hz data rate; dry, medium shunt in place)
Linearity	<5% at 2.5 AU, propylparaben at 257 nm
Drift	5.0×10^{-4} AU/hr. max ($\Delta T = \pm 2$ °C/hr.) at 230 nm, after 1 hr. warmup, using medium shunt
Sensitivity setting range	0.0001 to 4.0000 AUFS
Filter setting range	Single-wavelength: 0.1 to 5.0 seconds Dual-wavelength: 1 to 50 seconds
time-constant	0.0- to 5.0-second Hamming filter
Digital data rates	1, 2, 5, 10, 20, 40, 80 Hz (single channel) 1, 2 Hz (dual channel)
Analog output data rates (single λ mode)	10, 20, 40, 80 Hz (channel A) 10 Hz only (channel B)

Operational specifications (Continued)

Condition	Specification		
Optical Component Sp	pecifications		
Lamp source	30-W High Brightness Deuterium lamp,0.5 nm aperture, pre-aligned2000-hour warranty, front accessible		
Photodiodes	2 silicon photodiodes (matched pair)		
Second-order filter	Automatic for wavelengths ≥ 370 nm		
Wavelength calibration filter	Erbium filter, used at startup or on demand		
Nitrogen purge	Purge fitting present on optics bench		
Path length	10 mm (standard analytical)		
Cell volume	0.5 μL (or 500 nL; standard analytical)		
Pressure limit	6895 kPa (69 bar, 1000 psi)		
Materials	316 stainless steel, fused silica, Teflon AF, PEEK		
Environmental Specifi	cations		
Operating temperature	4 to 40 °C (39 to 104 °F)		
Operating humidity	20% to <95%, noncondensing		
Shipping and storage temperature	-30 to +60 °C		
Shipping and storage humidity	0% to <95%, noncondensing		
Electrical Specification	าร		
Line frequency	50 to 60 Hz		
Line voltage	100 to 240 Vac		
Max VA input	200 VA		
Fuse ratings	Two fuses: 100 to 240-Vac, 50 to 60-Hz F 3.15-A 250-V fast blo, 5 × 20 mm (IEC)		
Attenuated analog output channel: 2 VFS	Attenuation range: 0.0001 to 4.000 AU 2-V output range: -0.1 to +2.1 V		

Operational specifications (Continued)

Condition	Specification	
One event output	Type: Contact closure Voltage: +30 V Current: 1 A	
One event input	Input voltage: +30 V maximum 100-ms minimum period	
Dimensions		
Height	19.3 cm (7.6 inches)	
Length	61.0 cm (24.0 inches)	
Width	34.3 cm (13.5 inches)	
Weight	12.0 kg (26.5 pounds)	

Solvent Considerations

Warning: To avoid chemical hazards, always observe Good Laboratory Practices when operating your system, handling solvents, or changing tubing. See the Material Safety Data Sheets for the solvents you use.

Introduction

Preventing contamination

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

Clean solvents

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

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

Solvent quality

Use MS-grade solvents for the best possible results; the minimum requirement is HPLC-grade. Filter solvents through an appropriate membrane filter.

Recommendation: Ensure your solvent choices are consistent with the recommendations of the membrane filter manufacturer or supplier.

Solvent preparation

Proper solvent preparation, primarily filtration, can prevent many pumping problems.

Recommendation: Use brown-tinted glassware to inhibit microbial growth.

Water

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

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

Using buffers

Adjust the pH of aqueous buffers. Filter them to remove insoluble material, and then blend them with appropriate organic modifiers. After you use a buffer, flush it from the pump by running a wet-prime with at least five system volumes of HPLC-grade distilled or deionized water.

For shutdowns of more than a day, flush the pump with a 20% methanol/water solution to prevent microbial growth.



Caution: Some buffers can be incompatible with mass spectrometers. Consult the documentation that accompanies your instrument for compatible buffers.

Tip: To avoid salt precipitation, nonvolatile buffer concentrations must not exceed 100 mM.

Buffered solvents

When using a buffer, choose good quality reagents, filtering them through a 0.2- μ m membrane filter.

Recommendation: To discourage microbial growth, replace 100% mobile aqueous phase daily.

See also: Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems (part number 715001307).

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.

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
-0.3	N-decane	0.92	174.1	29	—
-0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	
1.8	Triethylamine	0.38	89.5	26	
2.2	Isopropyl ether	0.33	68.3		220
2.3	Toluene	0.59	100.6	23	285
2.4	P-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3		
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	
3.9	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

Solvent miscibility

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
4.3	2-propanol	2.35	117.7	15	
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—
4.8	Dioxane	1.54	101.3	17	220
5.2	Ethanol	1.20	78.3	14	210
5.3	Pyridine	0.94	115.3	16	305
5.3	Nitroethane	0.68	114.0		
5.4	Acetone	0.32	56.3	15, 17	330
5.5	Benzyl alcohol	5.80	205.5	13	
5.7	Methoxyethanol	1.72	124.6	13	—
6.2	Acetonitrile	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	
6.4	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	—	—

Solvent miscibility (Continued)

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 78.)

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.

Wavelength selection

This section includes UV cutoff ranges for

- common solvents.
- common mixed mobile phases.
- chromophores.

UV cutoffs for common solvents

The table below shows the UV cutoff (the wavelength at which the absorbance of the solvent 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.

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

UV cutoff wavelengths for common chromatographic solvents

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.

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	Trifluoracetic acid, 0.1%	190
EDTA, disodium, 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
HEPES, 10 mM, pH 7.6	225	Triton-X [™] 100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC [®] Reagent A, 1 vial/liter	200
MES, 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225
Potassium phosphate, monobasic, 10 mM dibasic, 10 mM	190 190	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

Wavelength cutoffs for different mobile phases

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.

Chromophore	Chemical configuration	λ _{max} (nm)	∈ _{max} (L/m/cm)	λ _{max} (nm)	∈ _{max} (L/m/cm)
Ether	-0	185	1000		
Thioether	—S—	194	4600	215	1600
Amine	-NH ₂	195	2800		
Thiol	—SH	195	1400		
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	—СНО	210	strong	280-300	11-18
Carboxyl	—СООН	200-210	50-70		
Sulfoxide	>S→O	210	1500		

Electronic absorption bands of representative chromophores*

Chromophore	Chemical configuration	λ _{max} (nm)	∈ _{max} (L/m/cm)	λ _{max} (nm)	∈ _{max} (L/m/cm)
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 (shoulde r)	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—NO2	229	9,500		

Electronic absorption bands of representative chromophores* (Continued)

*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.

See also: For further details on solvent recommendations, common solvent properties, solvent stabilizers, and solvent viscosity, consult the ACQUITY UPLC System Documentation CD or the ACQUITY UPLC H-Class System Documentation CD.

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