

ACQUITY UPLC Photodiode Array and e λ Photodiode Array Detector

Operator's Overview and Maintenance Guide

Revision A

Waters

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.

Considerations specific to the ACQUITY PDA/eλPDA detector

High voltage hazard



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

Safety advisories

Consult the Safety Advisories section on [page 80](#) for a comprehensive list of warning and caution advisories.

Operating the ACQUITY PDA/eλPDA detector

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

Applicable symbols

Symbol	Definition
 Waters Corporation 34 Maple Street Milford, MA 01757 U.S.A.	Manufacturer
 Waters Corporation Floats Road Wythenshawe Manchester M23 9LZ United Kingdom	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
 ABN 49 095 444 751	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use

Audience and purpose

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

Intended use of the ACQUITY PDA/eλPDA detector

The Waters ACQUITY PDA/eλPDA detector is for research use only and is not intended for use in diagnostic applications.

Calibrating

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

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

Quality-control

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

ISM classification

ISM Classification: ISM Group 1 Class B

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

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Overview

The Waters ACQUITY UPLC[®] photodiode array (PDA) detector and extendable λ photodiode array (e λ PDA) detector are ultraviolet/visible (UV/Vis) spectrophotometers designed for use in the family of ACQUITY UPLC[®] Systems, such as ACQUITY UPLC H-Class or bioACQUITY. The detectors, controlled by Empower[™], MassLynx[™], or third-party software for both LC/MS and LC applications, operate as integral parts of the system.

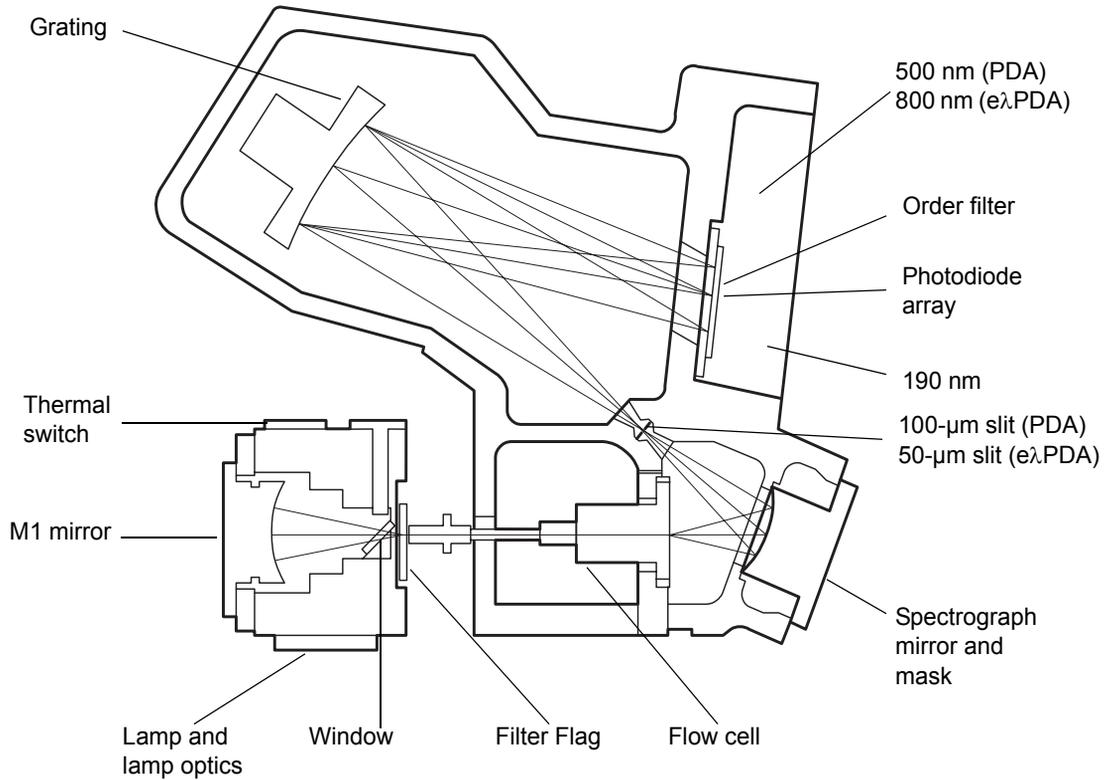
With a photodiode array of 512 photodiodes and an optical resolution of 1.2 nm, the detectors operate within a range of between 190 and 500 nm for the PDA and between 190 and 800 nm for the e λ PDA.

To use the detector's operating software effectively, you must understand the principles that underlie operation of the detector's optics and electronics.

Detector optics

The light path through the optics assembly of the detector is shown in the following figure.

Optics assembly light path



The following table describes the optics assembly components.

Optics assembly components

Component	Function
Thermal switch	Disconnects power to the lamp if the temperature rises to an unacceptable level.
M1 mirror	Off-axis, ellipsoidal mirror that projects light from the lamp into the flow cell.
Lamp and lamp optics	Focuses light from the high-brightness deuterium (D_2) source lamp and, via a mirror, redirects the light through a beam splitter and then to the flow cell.
Window	Used to help minimize air infiltration into the lamp housing.

Optics assembly components (Continued)

Component	Function
Filter flag	<p>Influences the light entering the flow cell. These are the flag settings:</p> <ul style="list-style-type: none"> • Shutter – Prevents light from entering the flow cell. In the shutter position, dark counts are measured at each pixel and subsequently subtracted from observed signal counts to give true signal counts. • Open – Allows light to pass into the flow cell. It is the normal setting when performing runs. • Erbium – Inserts an erbium filter into the light beam that allows the wavelength calibration to be checked or updated. • UV blocking filter – Inserts a UV blocking filter into the light beam that minimizes light with wavelengths shorter than, approximately, 210 nm.
Flow cell	Houses the segment of the flow path (containing eluent and sample) through which the polychromatic light beam passes.
Shunt (not pictured)	Diagnostic tool used in place of the light-guiding flow cell to emulate light transmission without fluid flow.
Spectrograph mirror and mask	The mirror focuses light transmitted through the flow cell onto the slit at the entrance to the spectrographic portion of the optics. The mirror mask defines the size of the beam at the grating.
Slit	Determines wavelength resolution and intensity of light striking the photodiodes. The width of the slit is 100 μm (for the PDA) or 50 μm (for the e λ PDA).
Grating	Blazed, holographic diffraction grating that disperses light into bands of wavelengths and focuses them onto the plane of the photodiode array.
Order filter	Reduces the contribution of second-order diffraction of UV light (less than 340 nm for PDA, or 370 nm for e λ PDA) to the light intensity observed at visible wavelengths (greater than 340 nm for PDA, or 370 nm for e λ PDA).

Optics assembly components (Continued)

Component	Function
Photodiode array	An array of 512-pixel photodiodes arranged linearly. The diode width (50- μm), together with a 100- μm slit (for the PDA) or 50- μm slit (for the e λ PDA), yield single wavelength resolution of 1.2 nm.

Calculating absorbance

The detector computes absorbance by subtracting the dark current (see [“Dark current” on page 12](#)) and reference spectrum from the acquired spectrum. Absorbance is based on the principles of Beer’s law.

Beer’s law

The relationship between the quantity of light of a particular wavelength arriving at the photodiode and the concentration of the sample passing through the flow cell is described by the Beer-Lambert law (commonly called Beer’s law). Beer’s law is expressed as $A = \epsilon lc$ where

A = dimensionless quantity measured in absorbance units

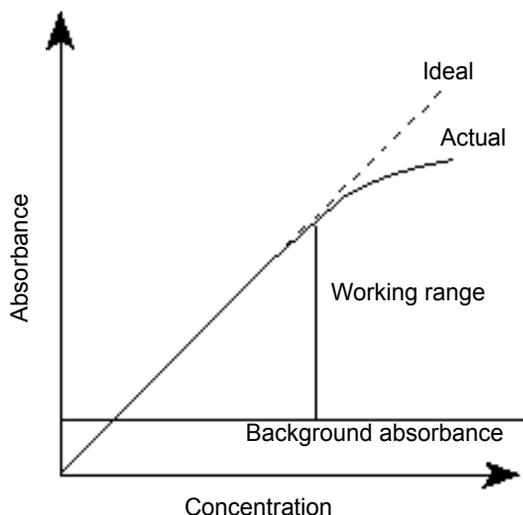
ϵ = constant of proportionality, known as the molar extinction coefficient

l = path length, in centimeters (1.0 cm in the detector’s normal flow cell)

c = concentration, in moles per liter

Beer’s law applies only to well-equilibrated dilute solutions. It assumes that the refractive index of the sample remains constant, that the light is monochromatic, and that no stray light reaches the detector element. As concentration increases, the chemical and instrumental requirements of Beer’s law can be violated, resulting in a deviation from (absorbance versus concentration) linearity. The absorbance of mobile phase can reduce the linear range by the amounts shown in [“Mobile phase absorbance” on page 105](#).

Absorbance as a function of concentration

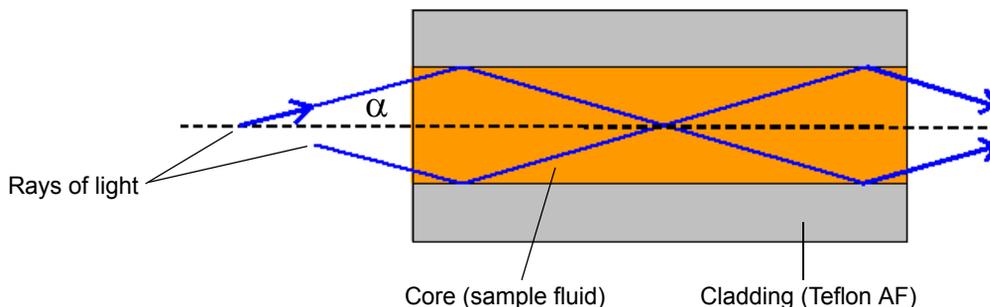


Operating principles of the light-guiding flow cell

Small-bore, high-capacity columns like those used in UPLC produce small-volume peaks. To avoid bandspreading and maintain concentration, the volume of a detector's flow cell must be correspondingly small. A good rule of thumb is to hold the 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 pathlength must be reduced, to avoid a significant decrease in light throughput. Reduced pathlength results in less analytical sensitivity, as predicted by Beer's law, yet high light levels are necessary to preserve a high signal-to-noise ratio.

Using a small-volume, light-guiding flow cell designed with optimum pathlength and high light throughput, resolves the problem. 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 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



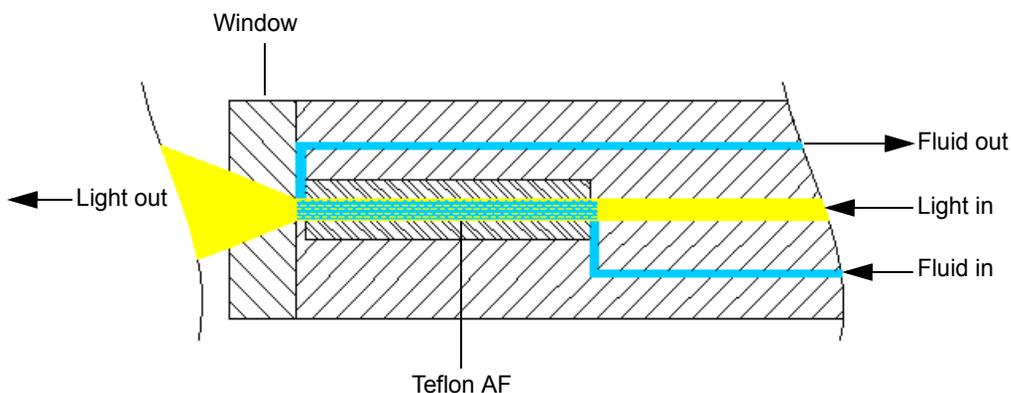
This information complements the foregoing illustration:

- 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 preceding figure, 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 PDA 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 slit of the spectrograph. A concave grating then disperses and projects the light onto the photodiode array.

Tip: Unlike other flow cell designs, in which light beams do not strike the internal walls of the cell, light-guiding relies on internal reflections from the walls of the Teflon AF tubing. Consequently, you must maintain flow cell cleanliness by following the recommended procedures described in [“Maintaining the detector” on page 36](#). With such care, the instrument and flow cell will provide continuous, sensitive detection.

Requirement: To ensure the cell’s proper alignment and calibration, fill the flow cell with flowing solvent before you power-on the detector. An empty flow cell causes calibration error. Refer to the recommended procedures described in [“Maintaining the detector” on page 36](#) for more information

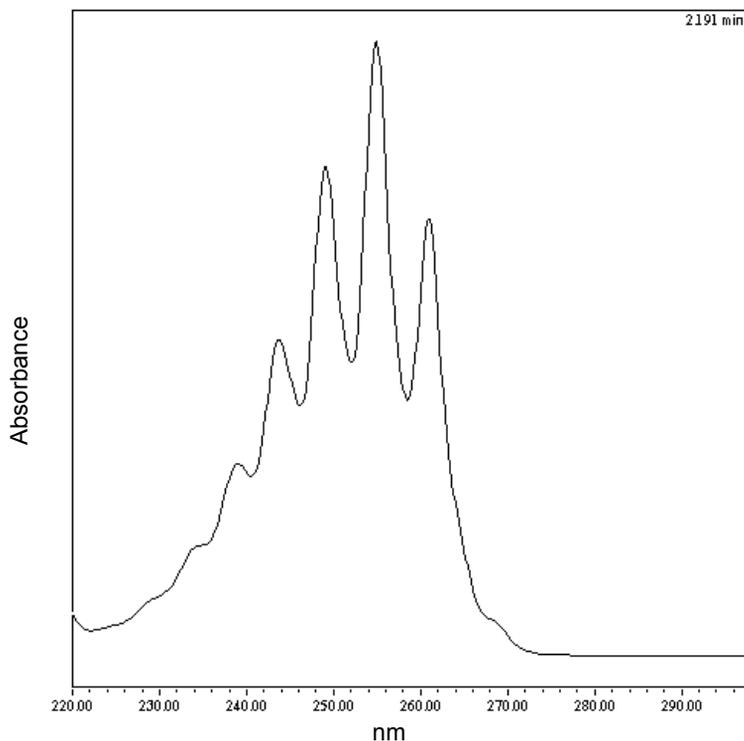
Resolving spectral data

Together with photodiode spacing, the detector's 100- μm -wide slit (50- μm -wide slit for $e\lambda\text{PDA}$) determines the intensity and bandwidth of the light that strikes the photodiode array. Variations in intensity and bandwidth provide the means to distinguish among similar spectra.

The grating images the slit onto the photodiode array. The angle of diffraction from the grating determines the wavelength that strikes a particular photodiode in the array.

The following figure shows an absorbance spectrum of benzene. Note that the wavelength resolution is sufficient to resolve five principal absorption peaks.

Benzene spectrum at 1.2 nm resolution



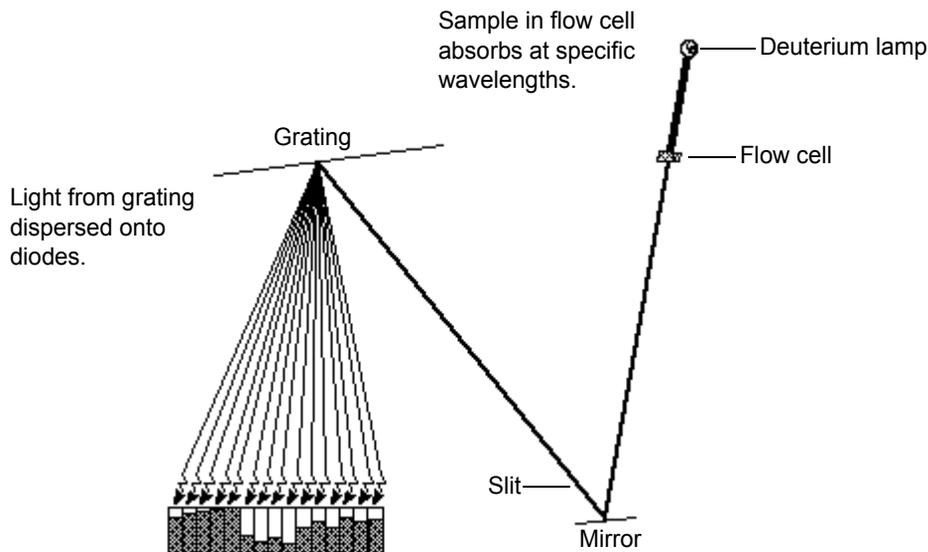
Measuring light at the photodiode array

The photodiode array detector measures the amount of light striking the photodiode array to determine the absorbance of the sample in the flow cell.

The array consists of 512 photodiodes arranged in a row. Each photodiode acts as a capacitor by holding a fixed amount of charge.

Light striking a photodiode discharges the diode. The magnitude of the discharge depends on the amount of light striking the photodiode.

Photodiodes discharged by light



The detector measures the amount of current required to recharge each photodiode. The current is proportional to the amount of light transmitted through the flow cell over the interval specified by the diode exposure time.

Exposure time

The detector recharges each diode and reads the recharging current, one diode at a time. The interval between two readings of an individual diode is the exposure time. The detector requires less than 5 msec to sequentially read all of the diodes in the array and process the data. The minimum exposure time is 5 msec. You can set exposure time from 5 to 500 msec.

For example, if an exposure time is set to 50 milliseconds, the detector performs as follows:

1. Recharges diode 1 and reads the current required to recharge diode 1
2. Recharges diode 2 and reads the current required to recharge diode 2
3. Sequentially recharges and reads the current required to recharge all the remaining 510 photodiodes
4. Waits approximately 45 msec before beginning the recharge-and-reading sequence, with diode 1, after all diodes are recharged and read.

You specify the exposure time in the General tab of the PDA Instrument Method Editor. You can specify either Auto Exposure or Exposure Time. For details, refer to the Empower or MassLynx online Help.

Tip: For best signal-to-noise performance, adjust the wavelength range to optimize autoexposure computations. For details, refer to the Empower or MassLynx online Help.

Using Auto Exposure

Use the Auto Exposure function to calculate the optimum exposure time needed to recharge the diodes, based on lamp energy, lamp spectrum, mobile phase absorbance, and the chosen wavelength range using a single deuterium light source of 190 to 500 nm (for the PDA; 190 to 800 nm for the eλPDA). To minimize detector noise, Auto Exposure adjusts the exposure time to approximately 85% of full scale for the diode generating the highest signal within the selected wavelength range.

With Auto Exposure enabled, the detector performs as follows:

- Produces the highest signals possible, consistent with not saturating due to overexposure
- Calculates exposure time at the start of a sample set based on maximum light intensity within the selected wavelength range
- Limits the exposure so that no diode within the given wavelength range discharges more than approximately 85%
- Provides settings for an optimal signal-to-noise ratio and dynamic range for each run

For certain combinations of sampling rates, wavelength ranges, or filter-time constants, the Auto Exposure time setting does not always optimize performance. If this is the case, you can set the exposure time manually, in the instrument editor.

Using Exposure Time

Use the Exposure Time function to manually set the length of time the photodiodes are exposed to light before they are read. The supported range is 5 to 500 msec.

Tip: Changing exposure times within a set of samples can cause changes in baseline noise.

Note that increasing exposure time can saturate the photodiodes and cause the detector to lose signal at certain wavelengths. To avoid signal loss, select an exposure time-value that provides settings for an optimum signal-to-noise ratio over the wavelength range of your analysis (see the next section, “Optimizing the signal-to-noise ratio”).

Optimizing the signal-to-noise ratio

To optimize signal-to-noise ratios, choose an acquisition wavelength range that includes only the wavelengths of interest. It is also important that the range be one in which the mobile phase absorbs only minimally (see “[Mobile phase absorbance](#)” on page 105). You can also improve the signal-to-noise ratio by increasing the spectral resolution value. For example, you can choose to operate at 3.6 nm instead of at 1.2 nm resolution.

Optimizing filter constants

The filtering constant you select affects the peak intensity. To increase sensitivity, decrease the filtering (filter-time) constant.

Selecting the appropriate sampling rate

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

Tip: The Points Across Peak value appears in the Peaks table, at the bottom of the Review Main window. If the Points Across Peak field is not visible,

right-click anywhere in the table, and then click Table Properties. Click the Columns tab, and then scroll down to find the Points Across Peak field. Clear the check box, and then click OK.

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

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

Computing absorbance data points

The detector calculates absorbance values before transmitting the data to the database (Empower or MassLynx). The detector calculates absorbance as follows:

- It computes the absorbance at each diode using the dark current and reference spectrum (see [“Calculating absorbance” on page 4](#)).
- It averages the absorbances at a particular wavelength, as specified in the spectra-per-second sample rate, and reports the average as a single data point (see [“Resolution” on page 13](#)).
- Also, the detector can apply a filter when calculating absorbance (see [“Filtering data” on page 14](#)).

Dark current

Photodiodes produce thermally excited charge even when they are not exposed to light. The amount of thermally excited charge produced is called dark current.

When a dark current update is necessary, the detector closes the shutter to take a dark current reading for each diode. The shutter closes after the exposure time is calculated and stays closed for the same interval as the exposure time.

The detector subtracts the dark current values from the current values recorded during absorbance measurements for both the sample and the reference spectra.

Reference spectrum

Immediately after the dark current measurement, and before any components elute, the detector records a reference spectrum. The

reference spectrum is a measure of lamp intensity and mobile phase absorbance. With the shutter open, the reference spectrum is determined over the interval specified in the exposure time.

Tips:

- For best results, the reference spectrum should represent the initial mobile phase.
- For extremely long exposure times, the dark current and reference spectrum readings can take several seconds to finish.

Absorbance

The detector calculates the absorbance for each diode at the end of each exposure time using the following equation:

$$Absorbance_n = \log \left[\frac{(S_n - D_n)}{(R_n - D_n)} \right]$$

where

S = obtained during sample analysis

D = obtained during the dark current test

R = obtained from the reference spectrum

n = diode number

Resolution

The data the detector report to the database (Empower, MassLynx, or third-party) can be the average of a number of data points. After calculating absorbance, the detector averages absorbance values based on spectral resolution and sample rate.

Averaging spectral data based on resolution

Spectral resolution (or bandwidth) is the wavelength interval (in nanometers) between data points in an acquired spectrum. The detector's lowest resolution setting is 1.2 nm. For example, in 3D mode, the detector averages six adjacent diodes for each reported wavelength when the spectral resolution is set in the software to 3.6 nm. In 2D mode, absorbance values are computed based on the bandwidth setting.

Averaging chromatographic data based on sample rate

Sample rate is the number of data points acquired per second. The number of times the photodiodes are read during the sample rate

interval depends on the exposure time. For example, if exposure time is 25 msec, and sample rate is 20 Hz, then readings per data point are

$$\frac{1 \text{ sec}}{20 \text{ samples}} \times \frac{1 \text{ exposure}}{25 \text{ msec}} \times \frac{1000 \text{ msec}}{1 \text{ sec}} = 2 \frac{\text{exposures}}{\text{sample}}$$

The readings are averaged and reported as a single data point.

Combining spectral resolution and sample rate

A high value of the spectral resolution parameter and sample rate affect noise and spectral detail in opposite ways. In normal use, high spectral resolution indicates a numerically small spectral resolution parameter.

Tip: The data storage rate is based on wavelength range, spectral resolution, and sample rate. Specify these parameter values in the General tab of the PDA Instrument Method Editor. For details, refer to the Empower or MassLynx online Help.

Filtering data

In the General tab of the PDA Instrument Method Editor (for details, refer to the Empower or MassLynx online Help or the ACQUITY UPLC Console online Help) you can apply an optional noise filter (via the Digital Filtering parameter) to the data acquired. The following table lists the digital filter settings for the allowable data rates.

Digital filter settings for data rates

Data Rate	Slow	Normal	Fast
1	10.000	4.000	1.000
2	5.000	2.000	0.500
5	2.000	0.800	0.200
10	1.000	0.400	0.100
20	0.500	0.200	0.050
40	0.250	0.100	0.025
80	0.125	0.050	0.0125

Filtering noise

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

The behavior of the filter depends on the filtering-time constant, or “filter-time” constant, that you select. You can program a filtering time to be Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you need not enter a value. The filtering constant is determined by the data rate. If you select Other, you can enter a value. Nevertheless, 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 filter-time constant settings produce these effects:

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

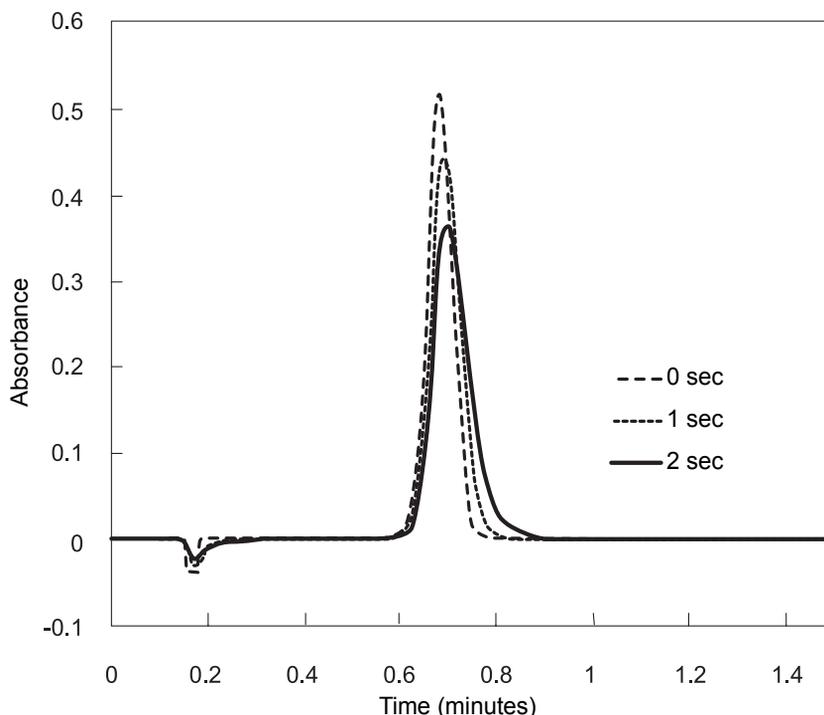
Higher filter-time constant settings produce these effects:

- Greatly decrease baseline noise
- Shorten and broaden peaks

The software includes fast or normal filter-time 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 filter-time constants, the peak area remains the same.

Median baseline filter

The median baseline filter enhances the detector's baseline stability by decreasing the baseline's curvature, facilitating the development of integration methods. The filter's primary purpose is to reduce the effects of mobile phase gradient separations that demonstrate gradual compositional changes. Note that it should not be applied in cases where abrupt gradient changes, such as steps, are evident.

Generally, the filter does not significantly change peak area, peak height, peak width or retention times. Nevertheless, it can create baseline distortions around very wide peaks, and these distortions can affect peak area. Therefore, it is not recommended for situations where peak widths (measured at 5% height) are greater than 5% of run time.

In the ACQUITY UPLC PDA detector, the filter works with 2D channels only. It cannot be applied to 3D or extracted 2D channels. When the MBF data mode is selected for a channel, the presentation of the data in the real-time data display plot is delayed by a percentage (~25%) of the run time. A countdown clock in the instrument control panel indicates the length of the delay.

Flow cell options

The detectors offer 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 a patented, light-guiding, flow cell technology. This guide addresses the light-guiding, flow cell operating principles and maintenance procedures.

The detectors operate at wavelengths ranging from 190 to 500 nm for the PDA and from 190 to 800 nm for the e λ PDA. The detectors can sample up to 80 data points per second.

The detectors have the following capabilities:

- Full three-dimensional spectrum data – Enables collecting the full spectral range throughout the chromatogram.
- Individual 2D channels – Monitor absorbance of one through eight discrete wavelengths.
- Wavelength verification reference filter – Ensures wavelength accuracy.
- Fixed, second-order filter – Filters UV wavelengths above 340 nm (PDA) or 370 nm (e λ PDA).
- 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 and it can activate according to time, absorbance threshold, or ratio criteria.
- Wavelength compensation – Defines a region of the spectrum to be used as a reference, suppressing baseline wander caused by refractive index or other dynamics.

- 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.

Before you begin

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

Tip: Refer to 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 must report damage and discrepancies to Waters Technical Service (800 252-4752). Others must phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or visit the Waters Web site at www.waters.com.

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

Installing the detector

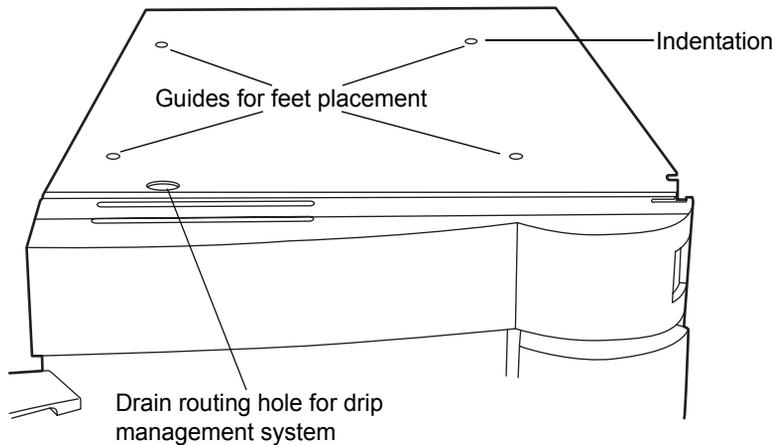
To install the ACQUITY UPLC PDA/eλPDA detector

 **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.

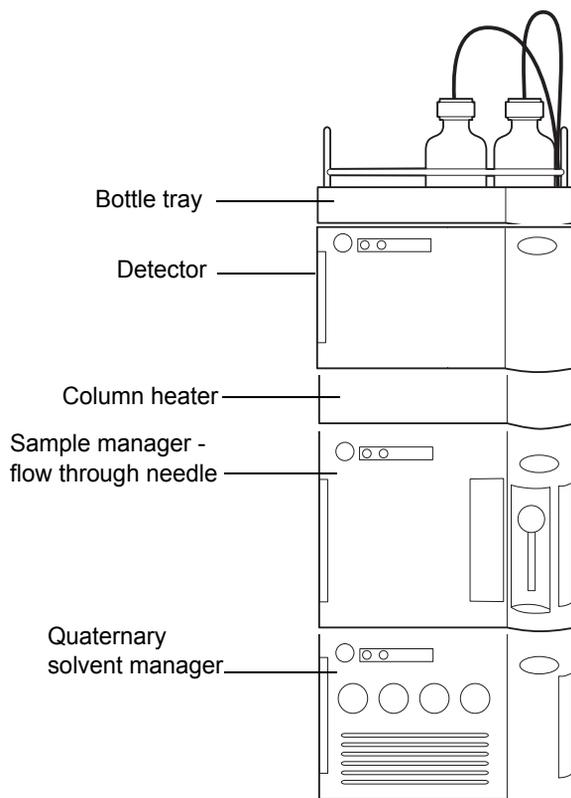
Tip: Doing this aligns the detector's drip tray over the drain routing hole at the top, left side of the column manager.

Proper placement for drip management system



2. Place the solvent tray module atop the detector.

ACQUITY UPLC PDA/eλPDA 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 98 for more information.

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

Although the in-line 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 PDA/eλPDA detector is the last detector in the system, the backpressure regulator is required for optimum performance. Note, however, that if a mass spectrometer or other detector is connected downstream of the detector, you need not install a backpressure regulator. The length of the tubing connecting to the mass spectrometer or other detector helps to maintain the backpressure on the flow cell.

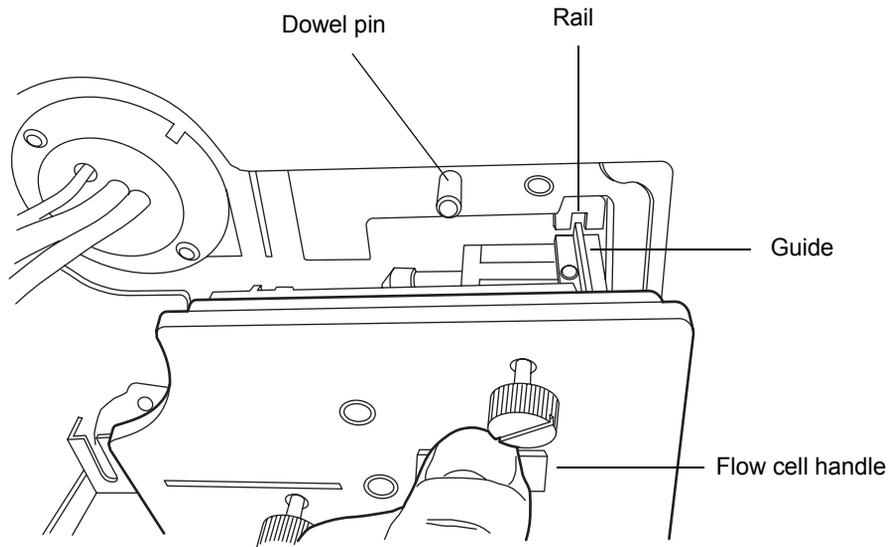
Recommendation: To avoid particulate contamination in the flow cell, flush any columns you are connecting to the detector before connecting them.

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

To plumb the detector

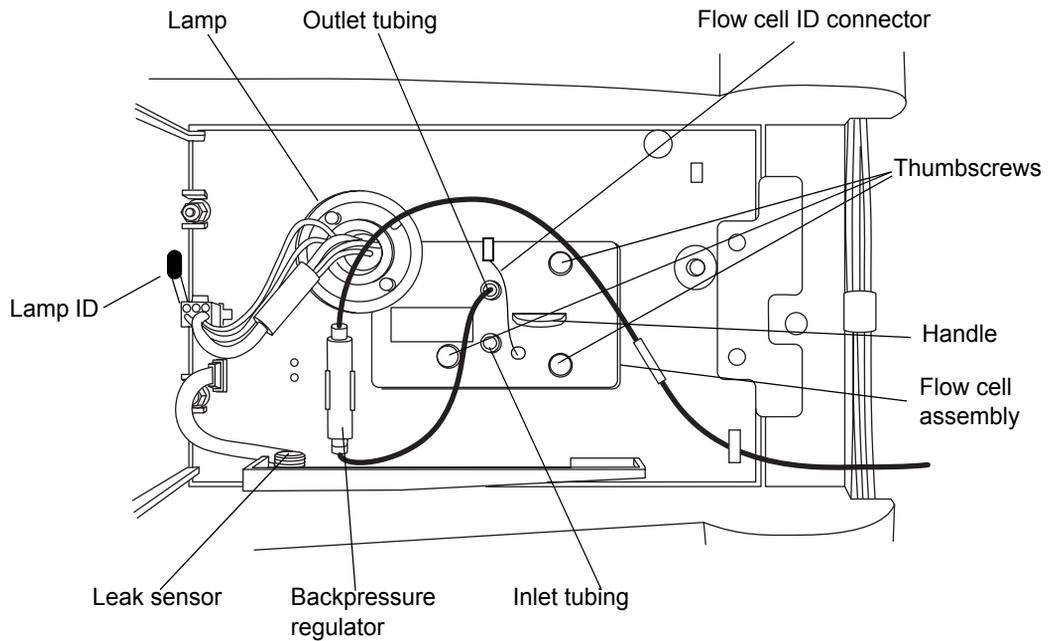
Recommendation: If the detector is already powered on, in the console, select PDA/eλPDA Detector from the system tree and click  (Lamp Off) to extinguish the lamp.

1. Open the detector's front door, and install the flow cell assembly, holding it squarely with respect 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.

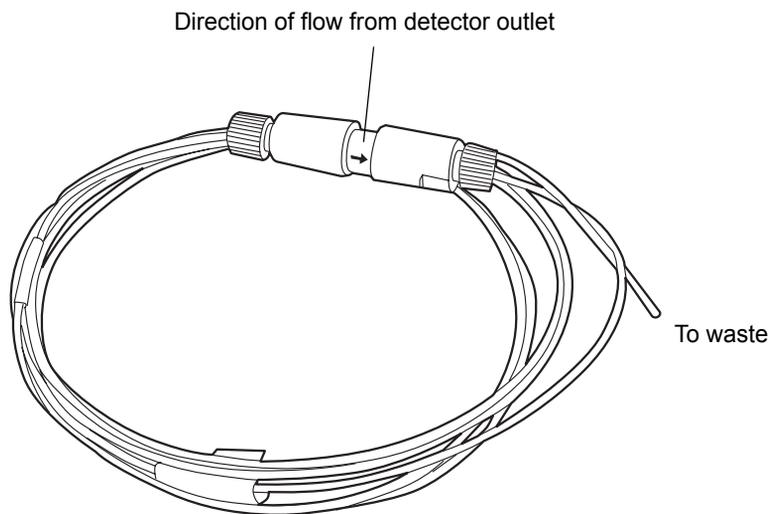
4. Hand tighten the thumbscrews and confirm they are secure using a screw driver.



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



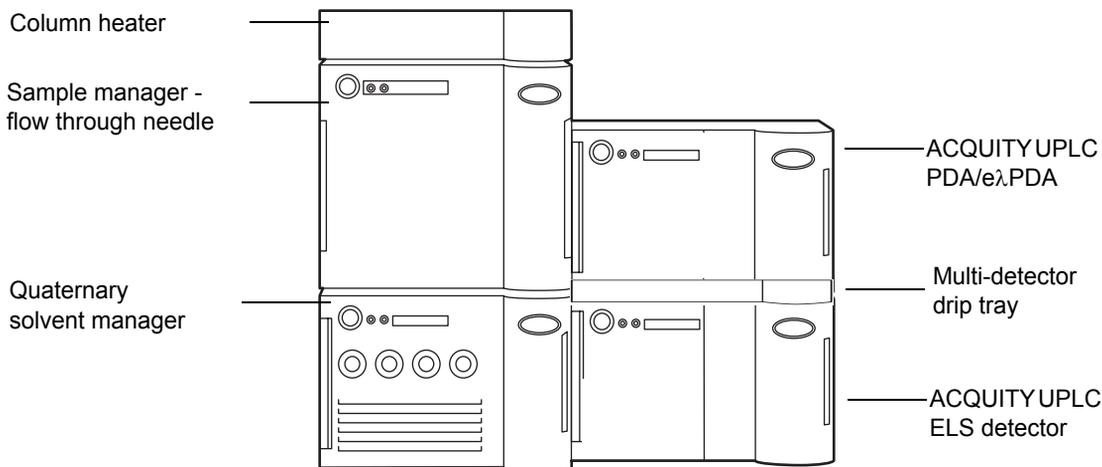
7. Route the long end of the outlet tubing from the backpressure regulator, through the channel clips along the front right side of the system, and into a suitable waste container.

Tip: If a mass spectrometer or other detector is connected downstream of the detector, you need not install a backpressure regulator. 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 PDA/eλPDA detector in a split ACQUITY UPLC H-Class system



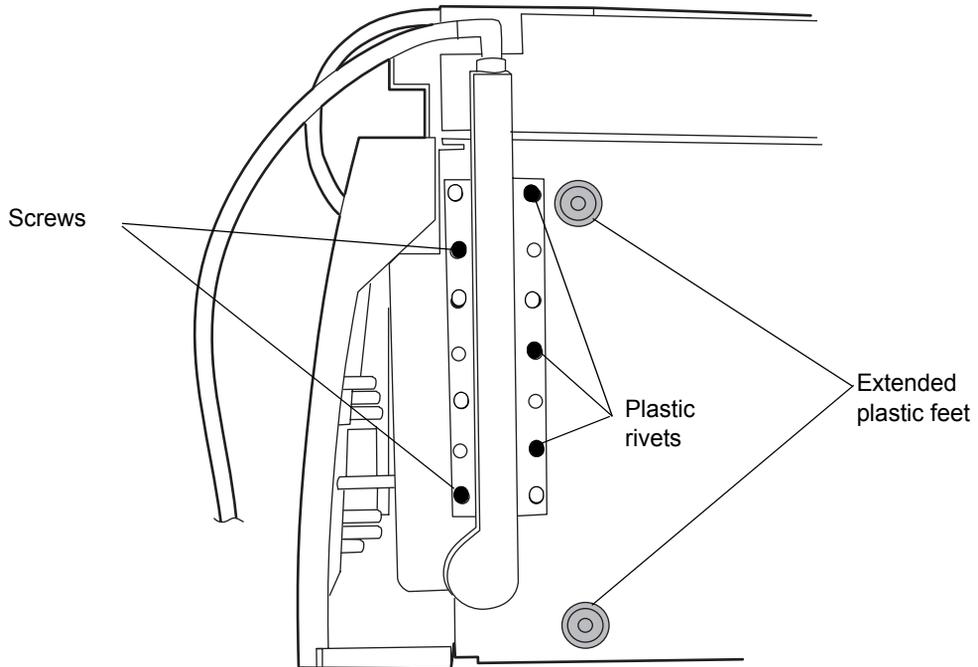
Required materials

Multi-detector drip tray kit

To install the drip tray

1. Turn the ACQUITY PDA/eλPDA detector, laying it on its left-hand side.
2. Snap the extended plastic feet onto the bottom of the detector, and then snap the anti-skid pads onto 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 PDA/e λ PDA 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.

PDA/eλPDA I/O signal connector

	1	+	Inject Start
	2	-	
	3	+	Event Out
	4	-	
	5	+	Analog Out
	6	-	

ACQUITY UPLC PDA/eλPDA 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 PDA/eλPDA 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 98 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 Off 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 PDA/eλPDA 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-hand side 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.

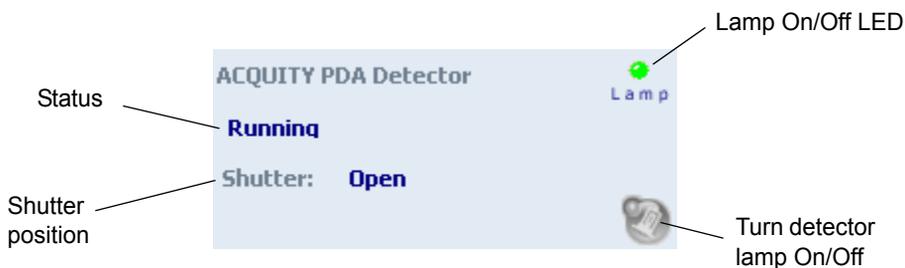
Lamp LED indications

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.
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.

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) and shutter position. 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 communication with the detector is lost.
Status	Displays the status of the current operation. (Appears only if the detector is running.)
Shutter	Displays the shutter position (Open, Closed, Erbium, or UV blocking).
 (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.

Additional functions in the detector control panel

Control panel function	Description
Autozero	Resets the detector offsets.
Reset PDA or Reset eλPDA	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 prevents accumulation of contaminants in the flow cell and 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.



Warning: Risk of electric shock. The power switch on each system instrument controls the basic operational state of that instrument. Nevertheless, some instrument circuits remain live after the instrument is switched off. To completely interrupt power to a system instrument, set the power switch to Off, and then unplug the instrument's power cord from the AC outlet.

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 °C (104 °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.

Maintaining the detector

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). If you are located elsewhere, 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:

- A record of error messages, if any
- 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 from 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 29](#).

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:

- For optimal baseline stability, keep the detector door closed at all times.
- 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 these adverse conditions:
 - Plugging of solvent lines and the flow cell
 - Damaging of 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 after establishing communications.
- 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

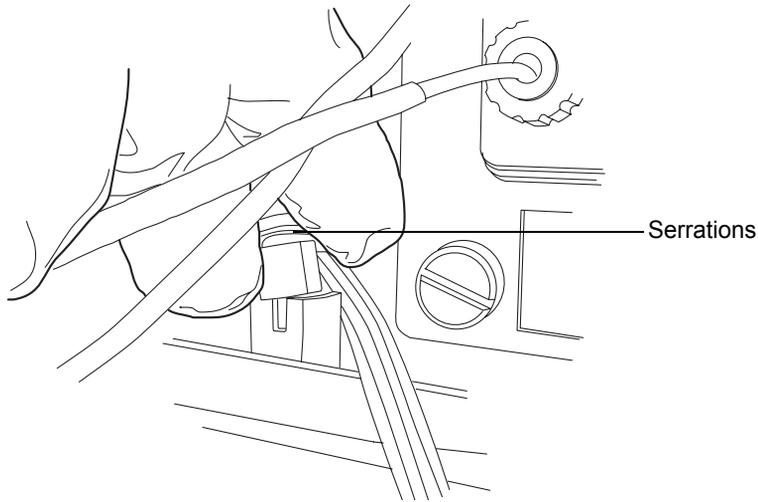
1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to verify that the leak sensor detected a leak.

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.

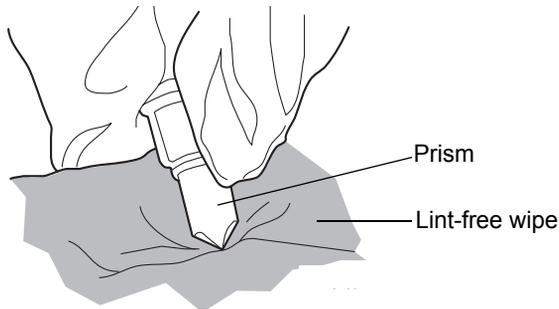
! **Caution:** To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

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

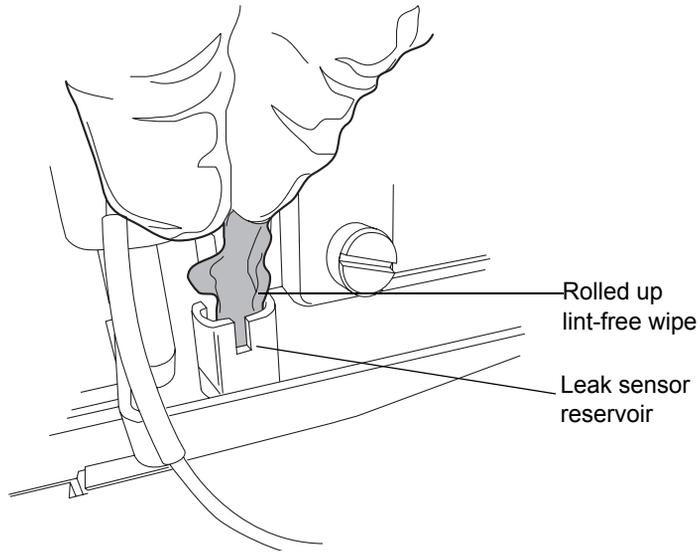


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the leak sensor connector from the front of the instrument (see [“Replacing the detector’s leak sensor”](#) on page 43).

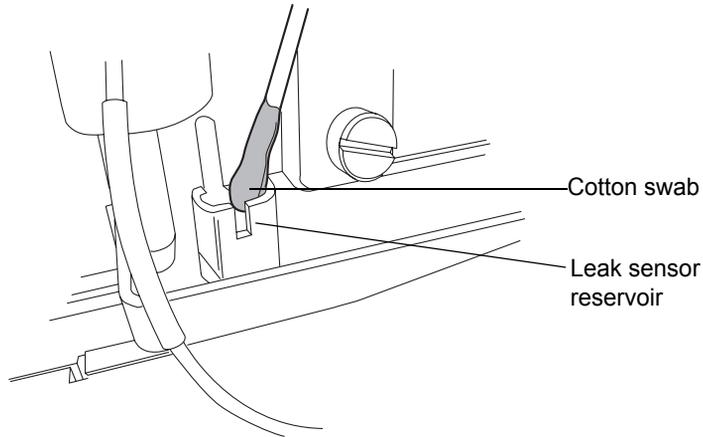
5. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



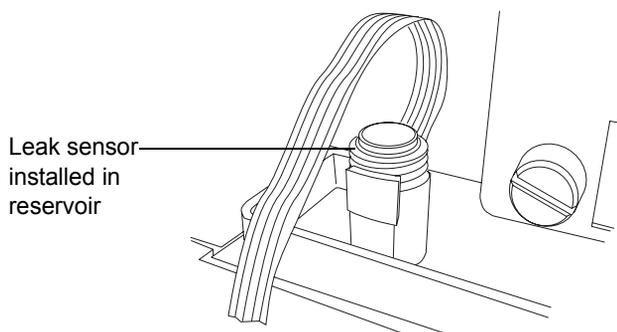
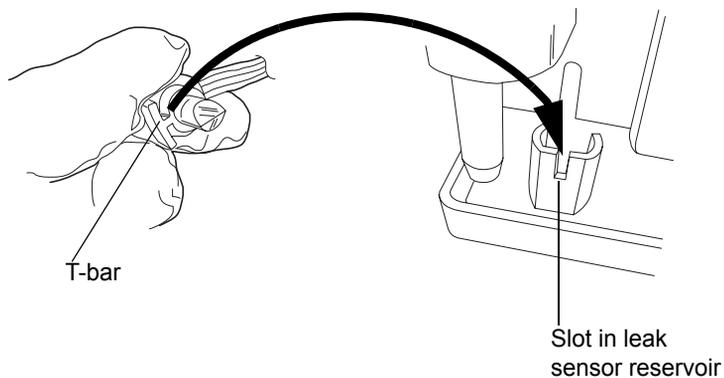
6. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



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



- 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.



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

Replacing the detector's leak sensor



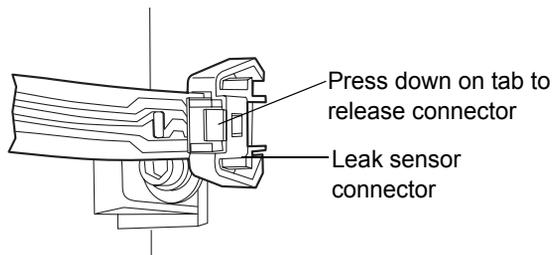
Warning: The leak sensor and its reservoir can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

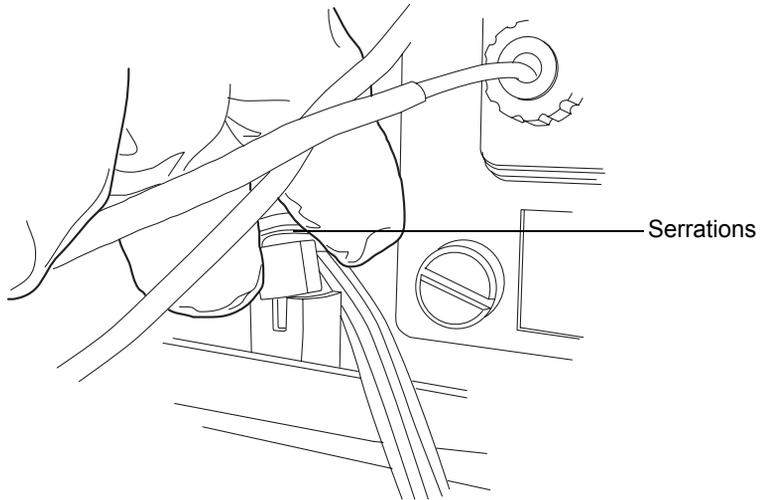
- Clean, chemical-resistant, powder-free gloves
- Leak sensor

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.

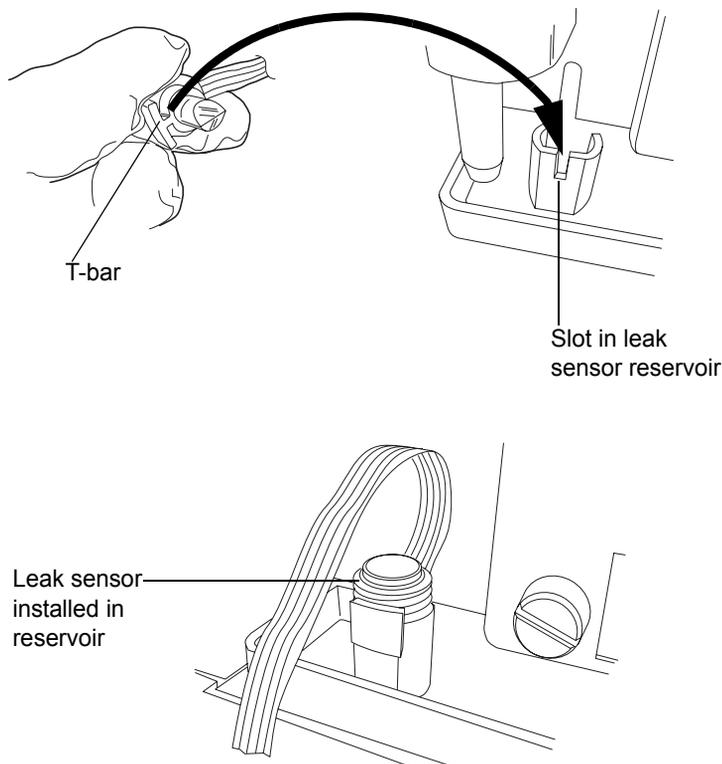


3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



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.

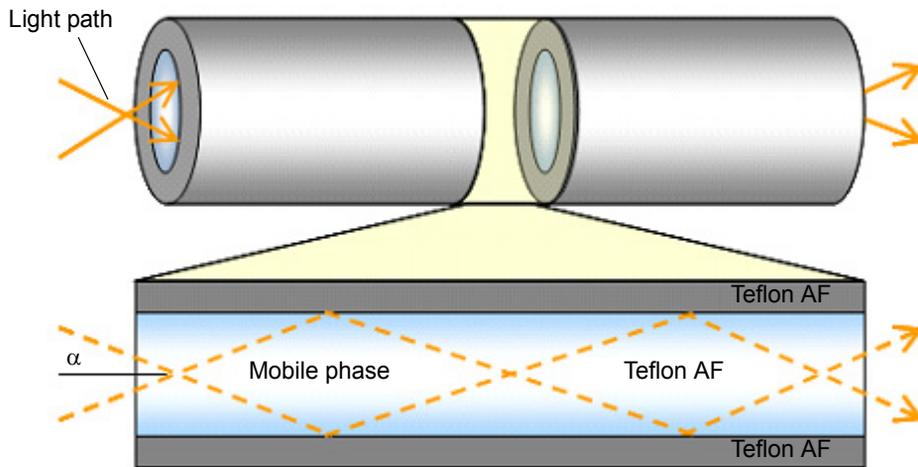


6. Plug the leak sensor connector into the front of the instrument.
7. In the ACQUITY UPLC Console, select your detector from the system tree.
8. In the detector information window, click Control > Reset to reset the detector.

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 transmits 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.

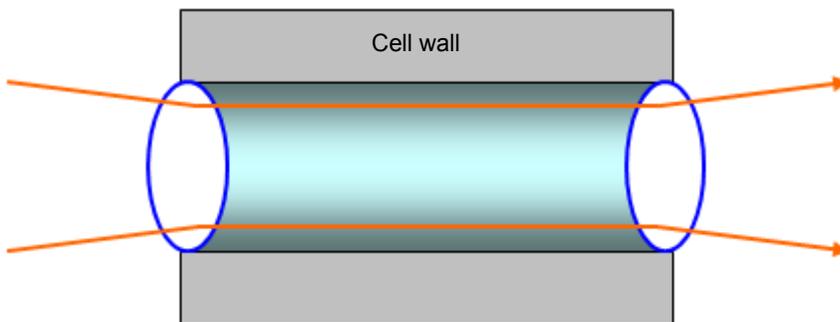
Light transmission through a light guiding flow cell



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.

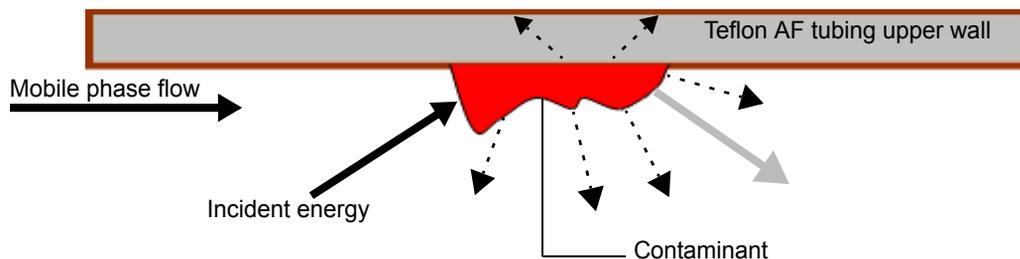
Light transmission through a conventional flow cell



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 50). 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

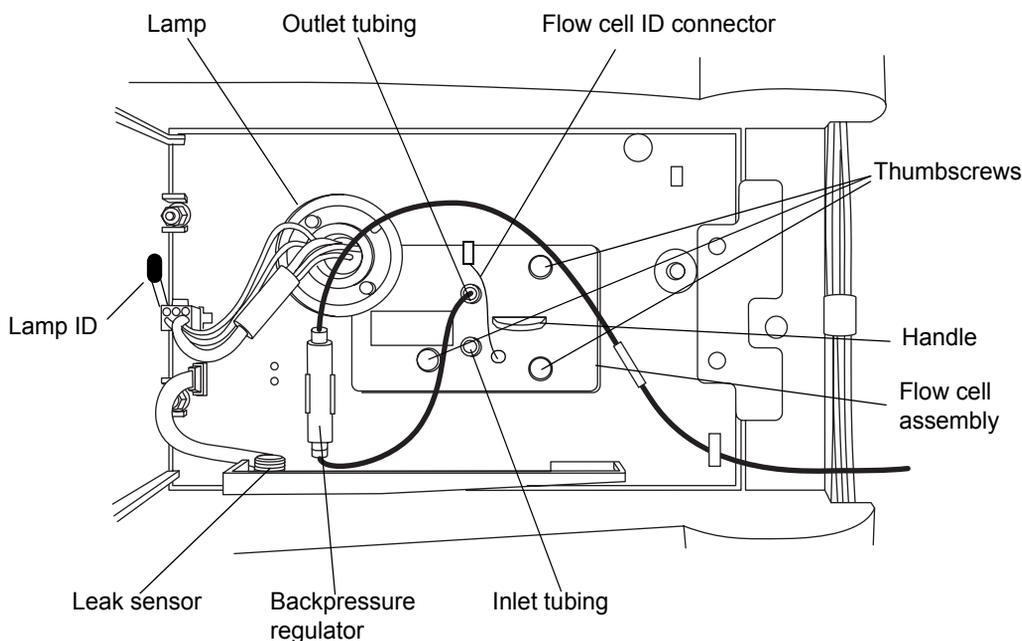
- 1% formic acid
- Clean, chemical-resistant, powder-free gloves
- Water (for flushing buffers)
- Intermediate solvent that is miscible in both the mobile phase and water
- Stainless steel unions (to replace the column during flushing)
- Wrench suitable for removing and replacing the column

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.

! **Caution:** Do not flush while connected to a mass spectrometer.



5. Flush the detector with HPLC-grade water.
Requirement: 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.
7. 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 or instruments from the system.
9. 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.
4. 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 adopting the following parameter settings:
 - 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: Expect this step to 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.

Requirement: 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

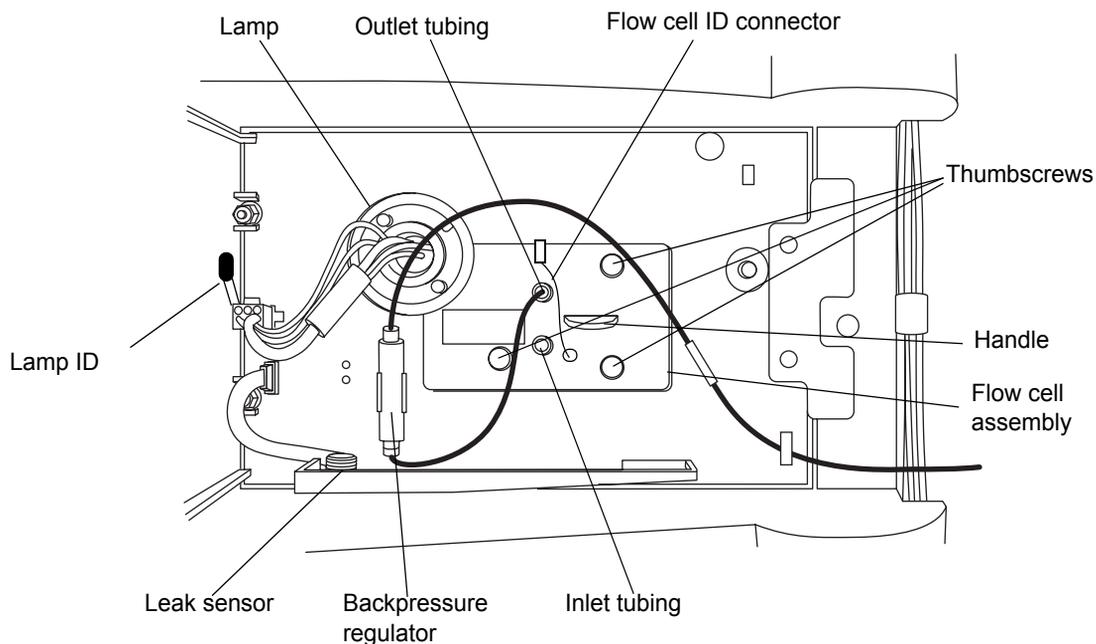
To replace the flow cell



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.

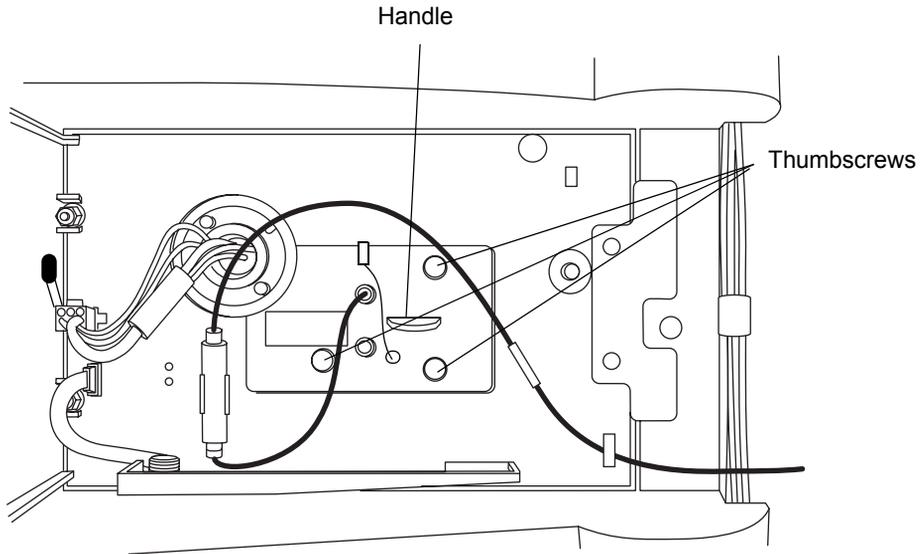
1. Power-off the detector.
2. Stop the solvent flow.
3. Open the detector door, gently pulling its right-hand edge toward you.

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.

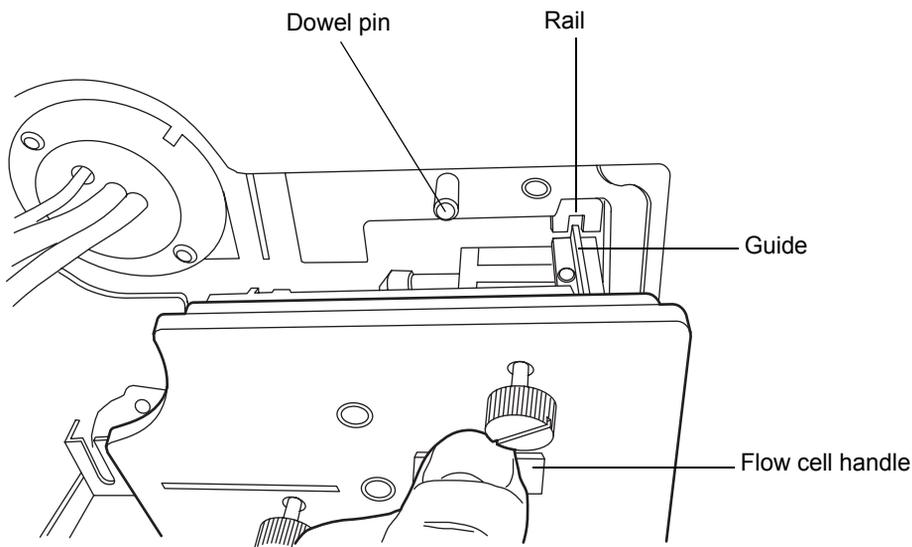


! **Caution:** To avoid damaging the capillary tubing, do not touch it.

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 21).

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.

Clearing bubbles from the flow cell

To clear bubbles from the flow cell

1. Ensure the pressure regulator at the outlet of the flow cell is in place.
Requirement: If the pressure regulator is bypassed, ensure that a device that develops a minimum of 1724 kPa (17 bar, 250 psi), but not greater than 6895 kPa (69 bar, 1000 psi), for the given flow rate and mobile phase is placed downstream from the detector.
2. Establish liquid flow through the detector flow cell using degassed acetonitrile or methanol at a flow rate that you expect to run for the subsequent analysis.

Replacing the lamp

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

Tip: The software automatically senses the ACQUITY PDA/eλPDA lamp when you install it, and records its serial number and installation date in 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 PDA/eλPDA Detector in the left-hand pane of the console, and then click . 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 can be hot. Wait 30 minutes (or 15 minutes with the fan running) for these components to cool before touching them.

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

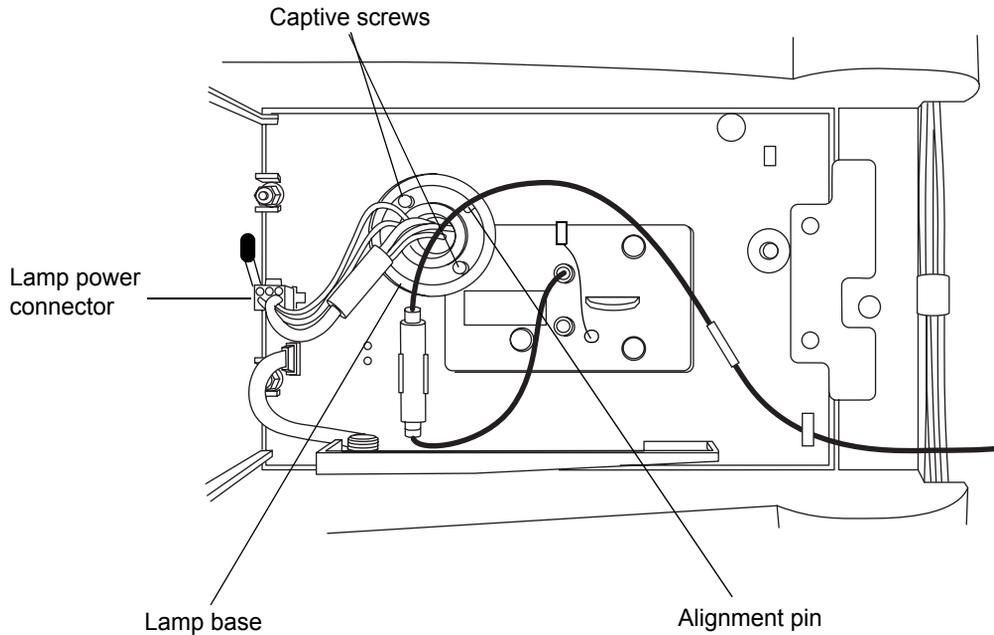


Warning: To avoid electric shock, power-off and unplug the detector before detaching the lamp power connector from the detector.



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

4. Detach the lamp power connector from the detector.



 **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 against 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.

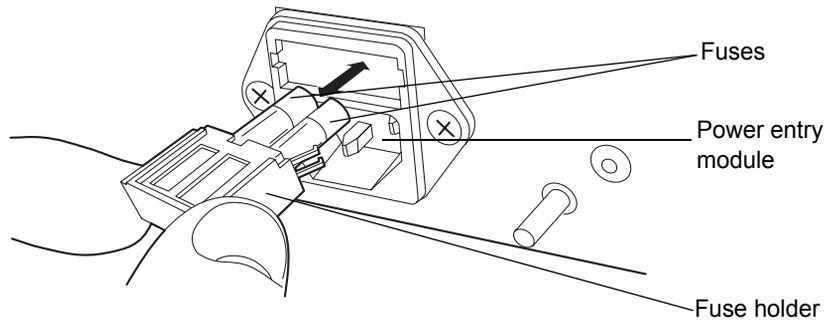
Suspect a fuse is open or otherwise defective when

- 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.

Spectral contrast theory

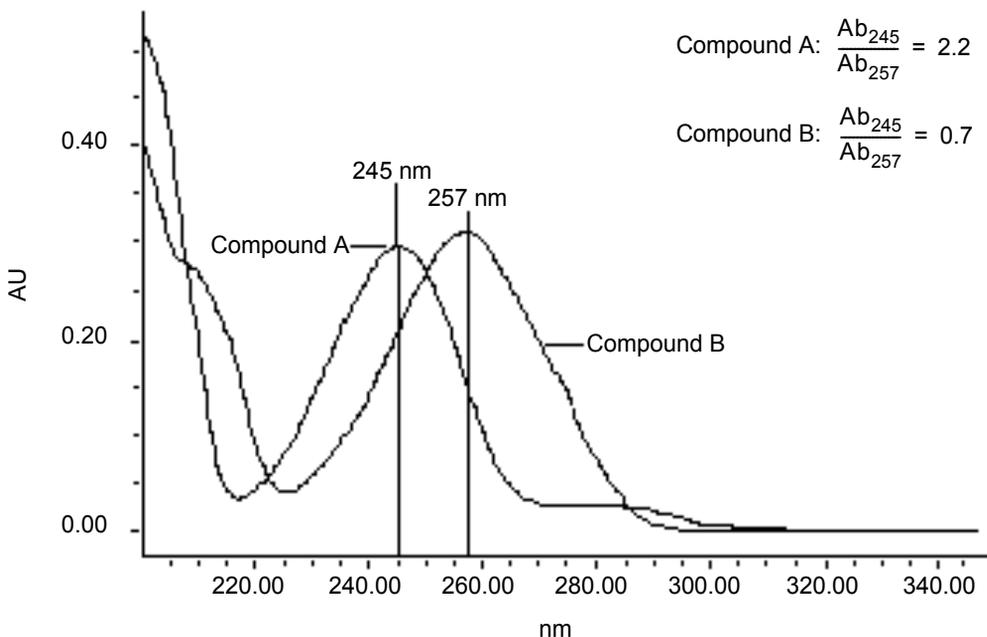
The spectral contrast algorithm compares the UV/Vis absorbance spectra of samples the detector collects. This chapter describes the theory on which the algorithm is based, explaining how it exploits differences in the shapes of the absorbance spectra. It also explains how spectral contrast represents those spectra as vectors, determining whether differences among them arise from the presence of multiple compounds in the same peaks (coelution) or from nonideal conditions such as noise, photometric error, or solvent effects.

Comparing absorbance spectra

When measured at specific solvent and pH conditions, the shape of a compound's absorbance spectrum characterizes the compound. The varying extent of UV/Vis absorbance occurring at different wavelengths produces a unique spectral shape.

The following figure shows the absorbance spectra for two compounds, A and B. The ratio of the absorbance at 245 nm to that at 257 nm is about 2.2 for compound A and 0.7 for compound B. Note that this comparison of a single wavelength pair's absorbance ratios yields little information about a compound. For more information, you must compare the ratios of multiple wavelength pairs.

Comparing spectra of two compounds



Representing spectra as vectors

The spectral contrast algorithm uses vectors to quantify differences in the shapes of spectra, converting baseline-corrected spectra to vectors and then comparing the vectors. Spectral vectors have two properties:

- Length – Proportional to analyte concentration.
- Direction – Determined by the relative absorbance of the analyte at all wavelengths (its absorbance spectrum). Direction is independent of concentration for peaks that are less than 1.0 AU across the collected wavelength range.

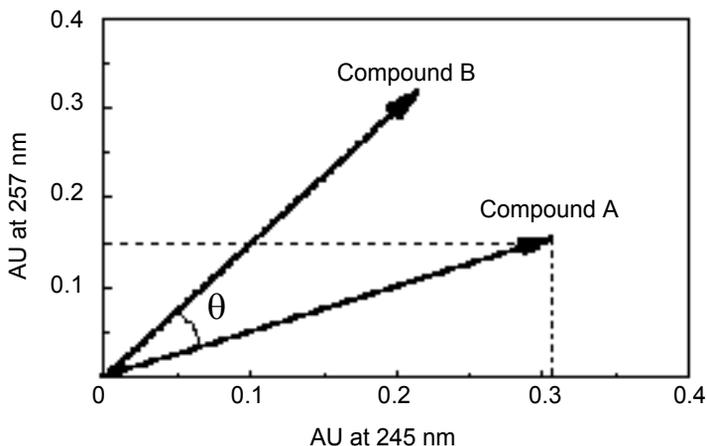
Vector direction contributes to the identification of a compound, since the direction is a function of the absorbance compound's spectrum. The ability of spectral vectors to differentiate compounds depends on the resolution of spectral features. As both wavelength range and spectral resolution increase, the precision of a spectral vector for the resultant spectrum increases. A detector-derived vector can include absorbances in the range of 190 to 500 nm. To enhance spectral sensitivity, set the bench resolution to 1.2 nm.

Tip: To prevent detector noise, don't include wavelengths where there is little or no analyte absorption.

Vectors derived from two wavelengths

The spectral contrast algorithm uses vectors to characterize spectra. To understand the vector principle, consider two vectors, in the figure below, which are based on the spectra depicted in the previous figure.

Plotting vectors for two spectra



In this figure, the axes reflect the absorbance units of the two wavelengths used to calculate the absorbance ratio of the previous figure. The head of the vector for Compound A lies at the intersection of the absorbance values (for Compound A), at the two wavelengths represented by each axis. The remaining vector is similarly derived from the spectrum of Compound B.

Compound B's vector points in a different direction from Compound A's. Expressed by the spectral contrast angle (θ), this difference reflects the difference between the two compounds' absorbance ratios at wavelengths 245 nm and 257 nm. A spectral contrast angle greater than zero indicates a shape difference between spectra (see [“Spectral contrast angles” on page 64](#)).

Finally, note that the length of the vectors is proportional to concentration.

Vectors derived from multiple wavelengths

When absorbance ratios are limited to two wavelengths, the chance that two different spectra share the same absorbance ratio is greater than if comparison is made using absorbance ratios at many wavelengths. Therefore, the spectral contrast algorithm uses absorbances from multiple wavelengths to form a vector in an n -dimensional vector space, where n is the number of wavelengths from the spectrum.

To compare two spectra, the spectral contrast algorithm forms a vector for each spectrum in an n -dimensional space. The two spectral vectors are compared mathematically to compute the spectral contrast angle.

As with the two-wavelength comparison, a spectral contrast angle of zero in n -dimensional space means that all ratios of absorbances at corresponding wavelengths match. Conversely, if any comparison of ratios does not match, the corresponding vectors point in different directions.

Spectral contrast angles

Spectra of identical shape have vectors that point in the same direction. Spectra of varying shapes have vectors that point in different directions. The angle between the two vectors of any two spectra, the spectral contrast angle, quantifies the magnitude of the shape difference between the spectra. The *spectral contrast angle* expresses the difference in direction between the spectral vectors of two spectra.

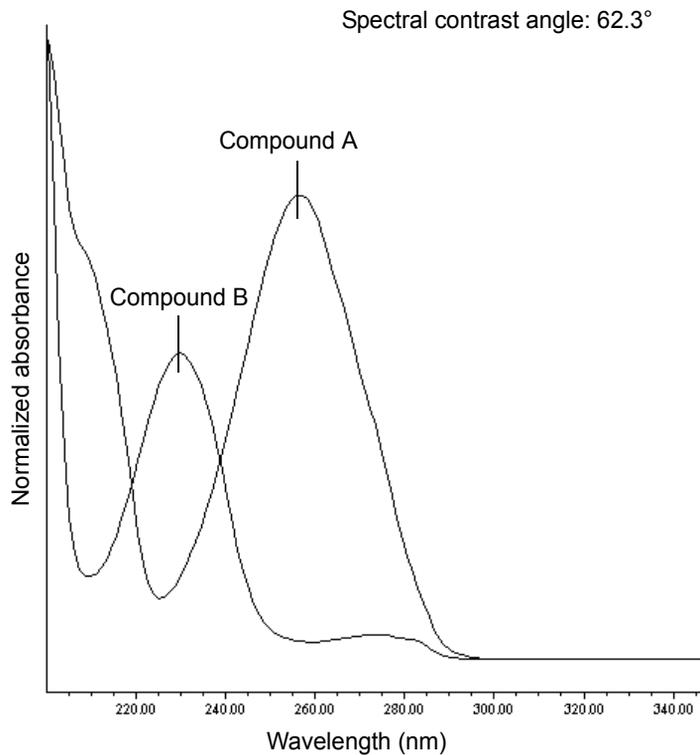
A spectral contrast angle can vary from 0° to 90° . A spectral contrast angle approaching 0° indicates little shape difference between the compared spectra. Matching a spectrum to itself produces a spectral contrast angle of exactly 0° . The maximum spectral contrast angle, 90° , indicates that the two spectra do not overlap at any wavelength.

To illustrate the relationship between the spectral contrast angle and spectral shape differences, consider the pairs of spectra shown in the next three figures.

Spectra with different shapes

In the following figure, the absorbance spectra of two compounds, A and B, are distinctly different. They therefore produce a large spectral contrast angle (62.3°).

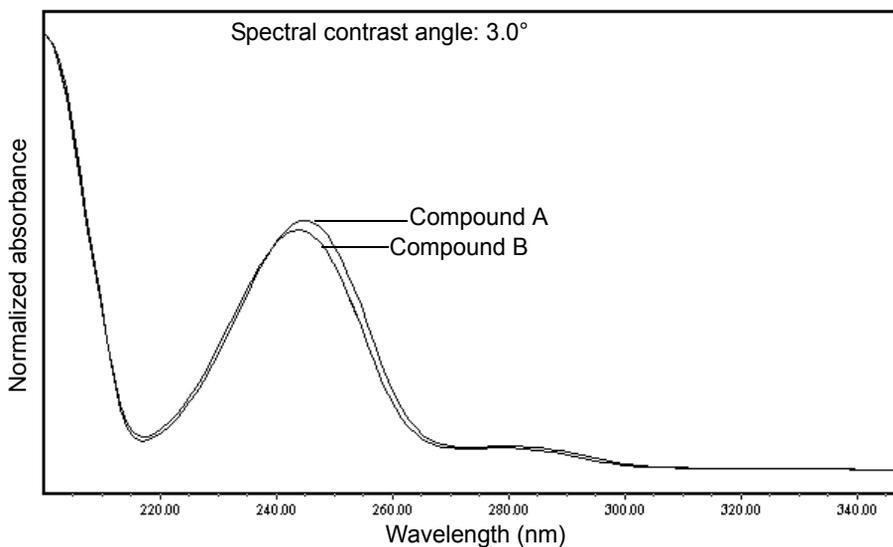
Spectra that produce a large spectral contrast angle



Spectra with similar shapes

In the following figure, the absorbance spectra of two compounds, A and B, are similar. They therefore produce a small spectral contrast angle (3.0°).

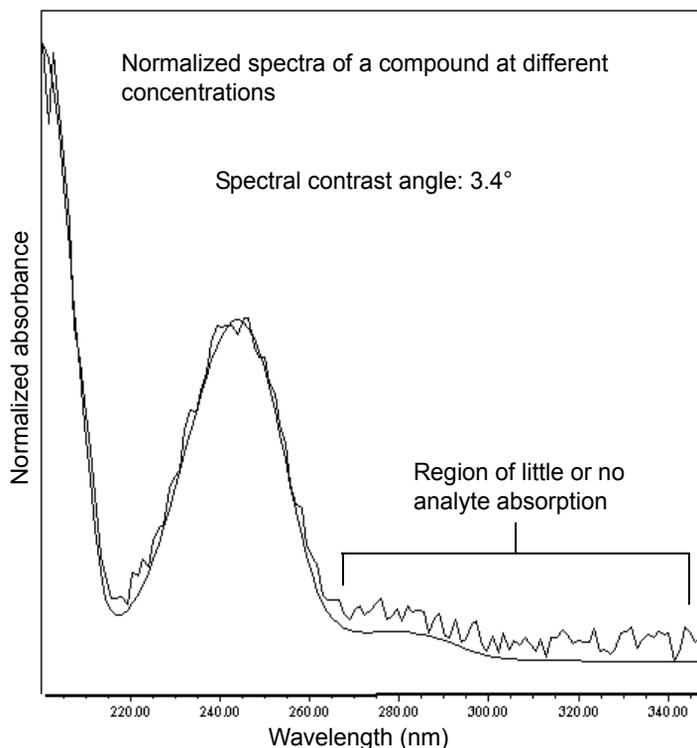
Spectra with a small spectral contrast angle



Differences between spectra of the same compound

Small but significant differences between absorbance spectra can occur because of factors other than those due to the absorbance properties of different compounds. For example, multiple spectra of the *same* compound may exhibit slight differences because of detector noise, photometric error, high sample concentration, or variations in solvent conditions. The spectra in the next figure, for example, show how instrument noise can affect the shape of an absorbance spectrum of one compound. This effect is most likely to occur at low concentrations, where the signal-to-noise ratio is low. Note that the spectral contrast angle between these absorbance spectra of the same compound is 3.4° .

Absorbance spectra of a compound at two concentrations



Undesirable effects

Shape differences between absorbance spectra can be caused by one or more of the following undesirable effects:

- Detector noise
- Photometric error caused by high sample concentration
- Variation in solvent composition

These sources of spectral variation can cause chemically pure, baseline-resolved peaks to exhibit a small level of spectral inhomogeneity. You can assess the significance of spectral inhomogeneity by comparing a spectral contrast angle to a threshold angle (see [“Threshold angle” on page 68](#)).

Detector noise

Statistical and thermal variations add electronic noise to the detector's absorbance measurements. The noise, which manifests itself as fluctuations in the baseline, is known as baseline noise. The magnitude of any absorbance differences caused by statistical and thermal variations can be predicted from the instrument noise in the baseline region of a chromatogram.

Photometric error

At high absorbances (generally those greater than 1 AU), a combination of effects can produce slight departures (about 1%) from Beer's law due to photometric error. Although photometric errors at this level can negligibly affect quantitation, they can nevertheless be a significant source of spectral inhomogeneity. To minimize the effects of photometric error for all spectral contrast operations, the maximum spectral absorbance of a compound should be less than 1 AU. Keep in mind that the absorbance of the mobile phase reduces the working linear dynamic range by the amount of mobile phase absorbance at each wavelength. For examples of mobile phase absorbance, see [“Mobile phase absorbance” on page 105](#).

See also: For more information about the effects of the photometric error curve, refer to *Principles of Instrumental Analysis*, third edition, by Douglas A. Skoog, Saunders College Publishing, 1985, pp. 168–172.

Solvent changes

As long as solvent concentration and composition do not change (isocratic operation), background absorbance, if any, by the solvent remains constant. However, change in solvent pH or composition, such as that which occurs in gradient operation, can affect the intrinsic spectral shape of a compound. (See the figure on [page 69](#)).

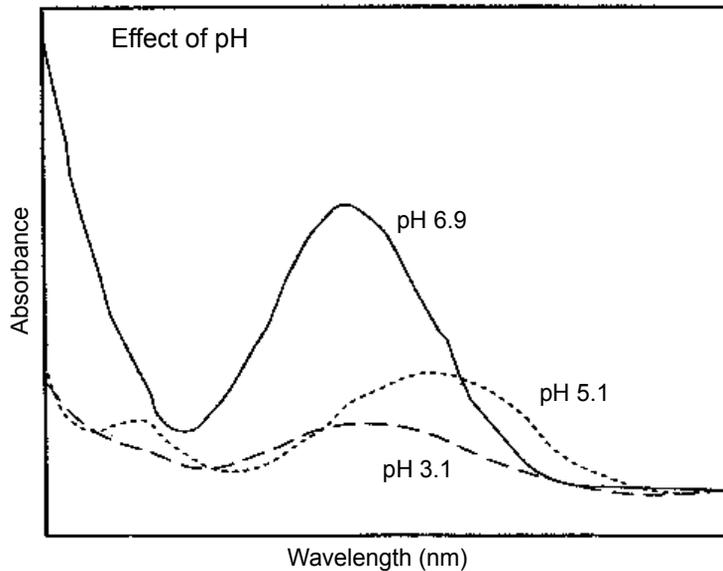
Threshold angle

In addition to computing spectral contrast angles, the spectral contrast algorithm also computes a threshold angle. The threshold angle is the maximum spectral contrast angle between spectra that can be attributed to nonideal phenomena.

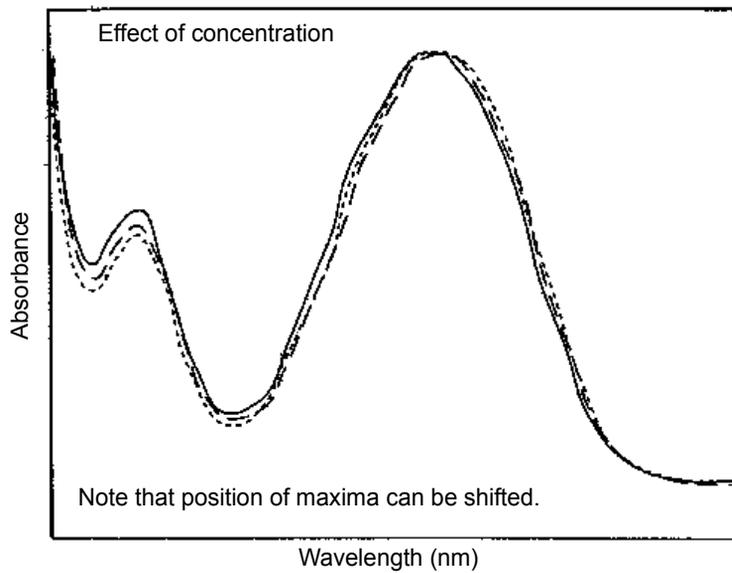
Comparison of a spectral contrast angle to its threshold angle can help determine whether the shape difference between spectra is genuine. In general, a spectral contrast angle less than its threshold angle indicates that

shape differences are attributable to nonideal phenomena alone and that no evidence exists for genuine differences between the spectra. A spectral contrast angle greater than its threshold angle indicates that the shape differences arise from genuine differences between the spectra. When automating the spectral contrast comparison, the maximum absorbance of the spectra must not exceed 1 AU.

Effects of pH on the absorbance spectrum of *p*-aminobenzoic acid



Effects of solvent concentration on the absorbance spectrum of *p*-aminobenzoic acid



Error messages and troubleshooting

The detector provides error messages to help troubleshoot system problems.

The tables in this section are organized as follows:

- Messages requiring you to perform corrective action including messages encountered at startup and during 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 36](#)). Most of these errors arise on startup.
- General troubleshooting problems, containing a description of the symptom, possible causes, and corrective actions.

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

Startup and operating error messages

Error Message	Description	Corrective Action
15-Volt fuse failed	Fuse failure was detected	1. Cycle power to the detector. 2. Contact Waters Technical Service.
24-Volt fuse failed	Fuse failure was detected	1. Cycle power to the detector. 2. Contact Waters Technical Service.

Startup and operating error messages (Continued)

Error Message	Description	Corrective Action
Alarm Dsp transfer failed	Electronic communication with the digital signal processor failed.	<ol style="list-style-type: none"> 1. Cycle power to the detector. 2. If problem persists, contact Waters Technical Service to replace the personality card.
Command received while initializing. Unable to process.	Data system (Empower, MassLynx, 3rd party) is attempting to communicate with the instrument while the instrument is initializing.	<ol style="list-style-type: none"> 1. Close the data system and wait until both front panel LEDs are green (steady, not flashing). 2. Reconnect or restart the data system.
Communication error. Possible data point loss.	Possible problem with the data system PC or personality card.	<ol style="list-style-type: none"> 1. Cycle power to both the detector and the data system PC. 2. If problem persists, contact Waters Technical Service.
Configuration not found, defaults set	The instrument's CPU battery ceased working.	Replace the instrument's CPU battery.
Delta between previous and current timestamp is incorrect	Personality card electronic error.	<ol style="list-style-type: none"> 1. Cycle power to the detector. 2. If error persists, contact Waters Technical Service to replace the personality card.
Ethernet cable disconnected.	The Ethernet cable has been disconnected.	<ol style="list-style-type: none"> 1. Connect (or reconnect) the Ethernet cable. 2. Reset communications from the control panel or console.

Startup and operating error messages (Continued)

Error Message	Description	Corrective Action
Flow cell memory device not detected	Unable to electronically communicate to flow cell	Connect flow cell identification cable.
Lamp data not found, defaults set	Stored lamp data are invalid.	Cycle power to the detector. This action removes the error.
Lamp energy low	Energy level low.	<ol style="list-style-type: none"> 1. Clear the flow cell and establish flow. 2. If step 1 does not work, replace the lamp. 3. If problem persists, contact Waters Technical Service.
Lamp hours counter exceeded	Lamp hour usage threshold exceeded.	Replace lamp.
Lamp memory device not detected	Unable to electronically communicate to lamp memory device.	Replace lamp with one that has the memory device attached.
Leak detector Enabled	Leak detector has been enabled.	Informational message. Enable or disable the leak detector if needed.
Leak detector Disabled	Leak detector has been disabled.	Informational message. Enable or disable the leak detector if needed.
Method not found, defaults set	Stored method data are invalid.	Cycle power to the detector. This action removes the error.
Serial number was modified	Instrument serial number was modified by the user.	Informational message. No action needed.

Startup and operating error messages (Continued)

Error Message	Description	Corrective Action
Vcc fuse failed	Fuse failure was detected.	<ol style="list-style-type: none">1. Cycle power to the detector.2. Contact Waters Technical Service.

Error messages preventing operation

During initialization 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.

Instrument error messages

Error Message	Description	Corrective Action
Both lamp and flow cell memory devices are blank.	Neither the lamp nor the flow cell memory device has been programmed.	<ol style="list-style-type: none">1. Cycle power to the detector.2. If problem persists, contact Waters Technical Service.
Communications with the Dsp failed. Dsp firmware is not running.	Personality card electronic problem.	<ol style="list-style-type: none">1. Cycle power to the detector.2. If problem persists, contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Energy level too low.	Energy level is too low.	<ol style="list-style-type: none"> 1. Clear the flow cell and establish flow. 2. If step 1 does not work, replace the lamp. 3. If problem persists, contact Waters Technical Service.
Flow cell memory device not detected.	Unable to electronically communicate to flow cell	Connect flow cell identification cable.
Flow cell memory device not programmed.	Flow cell memory device has not been programmed.	<ol style="list-style-type: none"> 1. Cycle power to the detector. 2. If problem persists, contact Waters Technical Service.
Lamp failure	Lamp indicates Off when it should be On.	<ol style="list-style-type: none"> 1. Check lamp icon. 2. Cycle power to the detector. 3. Replace lamp.
Lamp lighting failure	The lamp failed to ignite.	<ol style="list-style-type: none"> 1. Cycle power to the detector. 2. Check lamp power connection. 3. Replace lamp.
Lamp memory device not detected.	Unable to electronically communicate to lamp	Change lamp.
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 39 .
Leak Detector not present	Leak detector is not present or is not connected.	<ol style="list-style-type: none"> 1. Connect the leak detector. 2. Contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Shutter failed to home	Shutter could not home.	<ol style="list-style-type: none"> 1. Flush the flow cell. 2. Establish flow. 3. Reset communications from the control panel or console. 4. Contact Waters Technical Service
Temperature controller A/D failed	Personality card electronic error.	Contact Waters Technical Service to replace the personality card.
Temperature sensor hardware fault	Sensor error.	<ol style="list-style-type: none"> 1. Replace the temperature sensor. 2. If the problem persists, contact Waters Technical Service to replace the personality card.
Thermal controller disabled	Thermal controller was disabled.	<ol style="list-style-type: none"> 1. Cycle power to the detector. 2. Contact Waters Technical Service.
Wavelength outside of allowed range	Instrument method usage error.	<ol style="list-style-type: none"> 1. Modify the instrument method. Ensure wavelength end is lower than 500 nm.
Wavelength verification failure	Instrument failed power-up initialization wavelength verification step.	<ol style="list-style-type: none"> 1. Establish flow and reset the instrument. 2. If step 1 fails, perform erbium calculation.

Detector troubleshooting

Detector troubleshooting

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	<ol style="list-style-type: none"> 1. Inspect line cord connections. 2. Test electrical outlet for power.
	Open (spent) or defective fuse	Replace fuse (see page 59).
Change in reference spectrum	Mobile phase contains gas or is contaminated	Prepare fresh mobile phase and degas thoroughly.
	Air bubbles trapped in flow cell	<ul style="list-style-type: none"> • Reseat and check alignment of flow cell (see page 45). • Flush the flow cell (see page 47), or apply slight backpressure on the detector waste outlet. • Ensure the backpressure regulator is connected to the detector waste outlet.
Communication problems	Configuration problem	Check Ethernet or MassLynx configuration.
	Improper or defective Ethernet cable	Replace the cable with a shielded Ethernet cable.

Detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Detector not responding to ACQUITY UPLC Console	Bad or disconnected cable	Inspect cable connections, tighten connectors, or replace cable.
	Configuration problem	Check Ethernet or MassLynx configuration. For details, see Empower or MassLynx online Help.
Leak detected	Leak in detector	Follow the procedure to resolve a detector leak sensor error on page 39 .
Shutter failure message	Shutter failed	<ol style="list-style-type: none"> 1. Clear bubbles from the flow cell (see page 56). 2. Power-off and then power-on the PDA detector.
Solvent in drain line	Leak from flow cell gasket	Replace the flow cell (see page 52).
	Leak from flow cell inlet and outlet fittings	Check fittings for overtightening or undertightening, and replace fittings if necessary.
Status light blinks and lamp light is off	The detector is running confidence tests	No corrective action required. Wait until tests finish.

Detector troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Status light blinks and lamp light is on	Failed startup diagnostic tests	<ul style="list-style-type: none"> • Reseat and check alignment of flow cell (see page 45). • Flush the flow cell (see page 47).
	Dirty flow cell causing shutter diagnostic test to fail	Flush the flow cell (see page 47).
	Insufficient energy reaching photodiode array because of air bubble	To prevent air bubbles from forming, check that there is a 30- to 60-cm (1- to 2-foot) length of 0.009-inch (0.23-mm) ID tubing connected to the detector waste outlet.
	Weak lamp	Replace the lamp (see page 56).

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.)

Warning: (Risk of injury caused by moving machinery.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



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



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



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

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

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

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



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

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

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

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

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

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



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

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- 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砜会导致非金属管线膨胀, 大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

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

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

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



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

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

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

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

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

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

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

版絆: 力炼诀眉啊 疙矫窍瘤 白篮 规饶栏肺 厘厚甫 荤侏且 版快 厘厚啊 力傍窍绰 焊鹵 荐窠捞 力措肺 累悼窍瘤 白阑 荐 乐促绰 痢阑 荤侏磊俊霸 馆靛矫 牢饶矫难具 钦聪促 .

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 boîte à fusible de l'instrument.



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



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



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



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



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



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

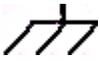
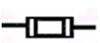


警告：火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols

Electrical symbols

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

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

Handling symbols

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

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

Specifications

This section lists individual specifications for the ACQUITY UPLC PDA Detector and the ACQUITY UPLC eλPDA Detector.

ACQUITY UPLC PDA detector specifications

Physical specifications

Attribute	Specification
Height	20.6 cm (8.1 inches)
Depth	61.0 cm (24.0 inches)
Width	34.3 cm (13.5 inches)
Weight	13.6 kg (30.0 pounds)

Environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<90%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing
Acoustic noise (instrument generated)	<58 dBA

Electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPX0)

Electrical specifications (Continued)

Attribute	Specification
 Line voltages, nominal	Grounded AC
Voltage range	100 to 240 VAC nominal
Frequency	50 to 60 Hz
Fuse	100 to 240 VAC, 50 to 60 Hz, F 3.15-A, 250-V FAST BLO, 5 × 20 mm (IEC)
Power consumption	185 VA nominal

- a. Protection Class I – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. Overvoltage Category II – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. Pollution Degree 2 – A measure of pollution on electrical circuits, which may produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.
- d. Moisture Protection – Normal (IPX0) – IPX0 means that *no* Ingress Protection against any type of dripping or sprayed water exists. The X is a placeholder that identifies protection against dust, if applicable.

Performance specifications

Item	Specification
Data rate	1, 2, 5, 10, 20, 40, and 80 Hz
Digital filter	Variable with data rate
Digital resolution	1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, 12.0 nm
Drift ^a (medium shunt cell)	1000 μ AU/hour, 1 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 120 minute warm-up time. Environmental stability: ± 2 °C/hour. Analytical flow cell conditions 0.4 mL/min, 10/90 Acetonitrile/water.
Linearity	Deviation at 2.0 AU $\leq 5\%$, propylparaben series at 257 nm, 10 mm analytical flow cell

Performance specifications (Continued)

Item	Specification
Nitrogen purge	Purge fitting present on optics bench and rear panel
Noise ^b (3D channel; 10 mm analytical flow cell)	9 μ AU, peak to peak, 1 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 0.4 mL/min, 10/90 Acetonitrile/water, 120 minute warm-up time
Noise ^b (2D channel; 10 mm analytical flow cell)	6 μ AU, peak to peak, 2 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 0.4 mL/min, 10/90 Acetonitrile/water, 120 minute warm-up time, wavelength compensation 310 nm to 410 nm
Optical resolution	1.2 nm (nominal), full width, half maximum
Order filter	Fixed, 340 nm to 500 nm
Photodetector	512 element selective access photodiode array
Wavelength accuracy	± 1.0 nm
Wavelength calibration filter	Erbium filter, used at startup or on-demand
Wavelength range	190 to 500 nm
Wavelength repeatability	± 0.1 nm

- a. ASTM drift tests require a temperature change of less than 2 °C/hour (3.6 °F/hour) over a one hour period. Larger ambient changes will result in larger drift. Better drift performance depends on better control of temperature fluctuations. To realize the highest performance, minimize the frequency and the amplitude of temperature changes to below 1 °C/hour (1.8 °F/hour). Turbulences around one minute or less can be ignored.
- b. Perform wet testing with water/acetonitrile, 90/10, to minimize the effects of oxygen at 230 nm. Water/methanol, 90/10, can be substituted with appropriate solvent conditioning.

ACQUITY UPLC eλPDA detector specifications

Physical specifications

Attribute	Specification
Height	20.6 cm (8.1 inches)
Depth	61.0 cm (24.0 inches)
Width	34.3 cm (13.5 inches)
Weight	13.6 kg (30.0 pounds)

Environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	<90%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	<90%, noncondensing
Acoustic noise (instrument generated)	<58 dBA

Electrical specifications

Attribute	Specification
Protection class ^a	Class I
Overvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPX0)
 Line voltages, nominal	Grounded AC
Voltage range	100 to 240 VAC nominal
Frequency	50 to 60 Hz

Electrical specifications (Continued)

Attribute	Specification
Fuse	100 to 240 VAC, 50 to 60 Hz, F 3.15-A, 250-V FAST BLO, 5 × 20 mm (IEC)
Power consumption	185 VA nominal

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

Performance specifications

Attribute	Specification
Data rate	1, 2, 5, 10, 20, 40, and 80 Hz
Digital filter	Variable with data rate
Digital resolution	1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, 12.0 nm
Drift ^a (medium shunt cell)	1000 μAU/hour, 1 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 120 minute warm-up time. Environmental stability: ±2 °C/hour. Analytical flow cell conditions 0.4 mL/min, 10/90 Acetonitrile/water.
Linearity	Deviation at 2.0 AU ≤5%, propylparaben series at 257 nm, 10 mm analytical flow cell
Nitrogen purge	Purge fitting present on optics bench and rear panel

Performance specifications (Continued)

Attribute	Specification
Noise ^b (3D channel; 10 mm analytical flow cell)	9 μ AU, peak to peak, 1 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 0.4 mL/min, 10/90 Acetonitrile/water, 120 minute warm-up time
Noise ^b (2D channel; 10 mm analytical flow cell)	6 μ AU, peak to peak, 2 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 0.4 mL/min, 10/90 Acetonitrile/water, 120 minute warm-up time, wavelength compensation 310 nm to 410 nm
Optical resolution	1.2 nm (nominal), full width, half maximum
Order filter	Fixed, 370 to 800 nm
Photodetector	512 element selective access photodiode array
Wavelength accuracy	± 1.0 nm
Wavelength calibration filter	Erbium filter, used at startup or on-demand
Wavelength range	190 to 800 nm
Wavelength repeatability	± 0.1 nm

- a. ASTM drift tests require a temperature change of less than 2 °C/hour (3.6 °F/hour) over a one hour period. Larger ambient changes will result in larger drift. Better drift performance depends on better control of temperature fluctuations. To realize the highest performance, minimize the frequency and the amplitude of temperature changes to below 1 °C/hour (1.8 °F/hour). Turbulences around one minute or less can be ignored.
- b. Perform wet testing with water/acetonitrile, 90/10, to minimize the effects of oxygen at 230 nm. Water/methanol, 90/10, can be substituted with appropriate solvent conditioning.

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- μm 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.

Solvent miscibility

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
-0.3	N-decane	0.92	174.1	29	—
-0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	—
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	—
1.8	Triethylamine	0.38	89.5	26	—
2.2	Isopropyl ether	0.33	68.3	—	220
2.3	Toluene	0.59	100.6	23	285
2.4	P-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3	—	—
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	—
3.9	Butyl alcohol	3.00	117.7	—	—

Solvent miscibility (Continued)

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
3.9	Butanol	3.01	177.7	15	—
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—
4.8	Dioxane	1.54	101.3	17	220
5.2	Ethanol	1.20	78.3	14	210
5.3	Pyridine	0.94	115.3	16	305
5.3	Nitroethane	0.68	114.0	—	—
5.4	Acetone	0.32	56.3	15, 17	330
5.5	Benzyl alcohol	5.80	205.5	13	—
5.7	Methoxyethanol	1.72	124.6	13	—
6.2	Acetonitrile	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	—
6.4	Dimethylformamide	0.90	153.0	12	—
6.5	Dimethylsulfoxide	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
7.3	Formamide	3.76	210.5	3	—
9.0	Water	1.00	100.0	—	—

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 100.)

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.

UV cutoff wavelengths for common chromatographic solvents

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

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.

Wavelength cutoffs for different mobile phases

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoroacetic acid, 0.1%	190
EDTA, disodium, 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
HEPES, 10 mM, pH 7.6	225	Triton-X™ 100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC® Reagent A, 1 vial/liter	200
MES, 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225
Potassium phosphate, monobasic, 10 mM dibasic, 10 mM	190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

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. Because of the diversity possible within a given sample, Waters recommends scanning over a range of wavelengths to determine the optimal wavelength for a particular analysis.

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*.

Mobile phase absorbance

This section lists the absorbances, at several wavelengths, for commonly used mobile phases. Choose a mobile phase carefully, to reduce baseline noise.

The best mobile phase for an application is transparent at the chosen detection wavelengths. Such a mobile phase ensures that any absorbance is attributable only to the sample. Absorbance by the mobile phase also reduces the linear dynamic range of the detector by the amount of absorbance the autozero function cancels, or “autozeroes,” out. Wavelength, pH, and concentration of the mobile phase affect its absorbance. Examples of several mobile phases are provided in the table below.

Mobile phase absorbance measured against air or water

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Solvents										
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<0.01	—	—	—	—
Methanol (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	—
Methanol (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	—	—
Isopropanol	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Unstabilized tetrahydrofuran (THF, fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstabilized tetrahydrofuran (THF, old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45
Acids and bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	—
Hydrochloric acid, 0.1%	0.11	0.02	<0.01	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—
Trifluoroacetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Buffers and Salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	—	—	—
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	—	—	—	—	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
EDTA, disodium, 1 mM	0.11	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
HEPES, 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	—	—
MES, 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	—	—	—
Potassium phosphate, monobasic (KH ₂ PO ₄), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K ₂ HPO ₄), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	—	—	—
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	—	—	—	—	—
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	—	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Sodium phosphate, 100 mM, pH 6.8	1.99	0.75	0.19	0.06	0.02	0.01	0.01	0.01	0.01	<0.01
Tris HCl, 20 mM, pH 7.0	1.40	0.77	0.28	0.10	0.04	<0.01	—	—	—	—
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	—	—	—	—
Waters® PIC® reagents										
PIC A, 1 vial/L	0.67	0.29	0.13	0.05	0.03	0.02	0.02	0.02	0.02	<0.01
PIC B6, 1 vial/L	2.46	2.50	2.42	2.25	1.83	0.63	0.07	<0.01	—	—
PIC B6, low UV, 1 vial/L	0.01	<0.01	—	—	—	—	—	—	—	—
PIC D4, 1 vial/L	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01
Detergents										
BRIJ 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	—	—	—
CHAPS, 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
SDS, 0.1%	0.02	0.01	<0.01	—	—	—	—	—	—	—
Triton® X-100, 0.1%	2.48	2.50	2.43	2.42	2.37	2.37	0.50	0.25	0.67	1.42
Tween™ 20, 0.1%	0.21	0.14	0.11	0.10	0.09	0.06	0.05	0.04	0.04	0.03

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