ACQUITY UPLC Photodiode Array Detector

Getting Started Guide

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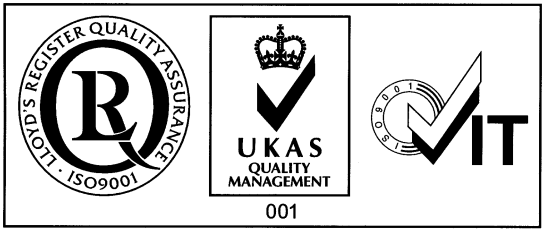
Trademarks



Customer comments

Please contact us if you have questions, suggestions for improvements, or find errors in this document. Your comments will help us improve the quality, accuracy, and organization of our documentation.

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EU General *in vitro* Diagnostic Device Directive 98/79/EC

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Operating this device

When operating this device, adhere to standard quality control procedures and the following equipment guidelines.

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 Funktion-stüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: eventuali modifiche o alterazioni apportate a questa unità e non espressamente approvate da un ente responsabile per la conformità annulleranno l'autorità dell'utente ad operare l'apparecchiatura.

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

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

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

주의 : 기기 검교정 담당자의 승인 없이 무단으로 기기를 변경 또는 수정하는 경우에는, 그 기기 운영에 대한 허가가 취소될 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、本装置のユーザとしての承認が無効になる可能性があります。

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

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

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

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

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

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

Attenzione: prestare attenzione durante l'utilizzo dei tubi di polimero pressurizzati:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Estinguere ogni fonte di ignizione circostante.
- Non utilizzare tubi soggetti che hanno subito sollecitazioni eccessive o son stati incurvati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrato.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi non metallici, riducendo notevolmente la resistenza alla 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：当在有压力的情况下使用管线时，小心注意以下几点：

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

경고: 폴리머재질의 튜빙을 압력하에서 사용할 때는 다음 사항에 유의하십시오.

- 압력을 받은 폴리머 튜빙 부근에서는 반드시 보호안경을 착용할 것
- 모든 화기의 접근을 금함
- 늘리거나 뒤틀린 튜빙은 사용하지 말 것
- 비금속 튜빙을 테트라히드로퓨란(THF)이나 염산 및 황산과 함께 사용하지 말 것
- 디글로로메탄(methylene chloride)와 디메틸설폭시드(dimethyl sulfoxide)는 비금속 튜빙을 팽창시켜 쉽게 파열되므로 주의할 것

警告：ポリマーチューブに圧力をかけて取り扱う場合は、次のように注意してください。

- 加圧したポリマーチューブの付近では、常に保護めがねを着用してください。
- 付近の火はすべて消してください。
- 激しい応力やねじれを受けたチューブは使用しないでください。
- テトラヒドロフラン（THF）、濃硝酸、あるいは濃硫酸には、非金属製のチューブを使用しないでください。
- ジクロロメタンやジメチルスルホキシドは非金属製のチューブを膨張させ、チューブの破断圧力を大幅に低下させますので、注意してください。

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

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

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

Attenzione: l'utente deve essere al corrente del fatto che, se l'apparecchiatura viene usta in un modo specificato dal produttore, la protezione fornita dall'apparecchiatura potrà essere invalidata.

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制造廠商指定的方式使用，那么該設備所提供的保護將被消弱。

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

경고 : 제조사가 지정한 것 이외의 방법으로 기기를 사용하는 경우에는, 사용자가 위험으로부터 보호될 수 없는 경우가 발생할 수 있음에 유념하십시오.

警告: ユーザは製造業者が指定していない方法で装置を使用した場合は装置が提供する保護が損なわれることがあるということを承知しているものとします。

Caution: To protect against fire hazard, replace fuses with those of the same type and rating.

Attention: Remplacez toujours les fusibles par d'autres du même type et de la même puissance afin d'éviter tout risque d'incendie.

Vorsicht: Zum Schutz gegen Feuergefahr die Sicherungen nur mit Sicherungen des gleichen Typs und Nennwertes ersetzen.

Attenzione: per una buona protezione contro i rischi di incendio, sostituire i fusibili con altri dello stesso tipo e amperaggio.

Advertencia: sustituya los fusibles por otros del mismo tipo y características para evitar el riesgo de incendio.

警告：為了避免火災的危險，應更換同种類型及規格的保險絲。

警告: 为了避免火灾的危险, 应更换同种类型及规格的保险丝。

경고 : 화재를 방지하기 위해서는 퓨즈 교체 시 같은 종류, 같은 등급의 것을 사용하십시오.

警告: 火災の危険防止のために、ヒューズの交換は同一タイプおよび定格のもので行なってください。

Caution: To avoid possible electrical shock, disconnect the power cord before servicing the instrument.

Attention: Afin d'éviter toute possibilité de commotion électrique, débranchez le cordon d'alimentation de la prise avant d'effectuer la maintenance de l'instrument.

Vorsicht: Zur Vermeidung von Stromschlägen sollte das Gerät vor der Wartung vom Netz getrennt werden.

Attenzione: per evitare il rischio di scossa elettrica, scollegare il cavo di alimentazione prima di svolgere la manutenzione dello strumento.

Precaución: para evitar descargas eléctricas, desenchufe el cable de alimentación del instrumento antes de realizar cualquier reparación.

警告：要避免觸電，請在修理或保養器材前把電源線拔出。

警告：为避免可能引起得触电危险，在修理前请切断电源连接。

경고: 전기 충격의 가능성을 피하기 위해서는, 기기를 수리하기 이전에 전원 코드를 차단하십시오.

警告：感電の危険性を避けるために、装置の保守を行う前には装置の電源コードを引き抜いてください。

Observing safety precautions

Observe all safety precautions while servicing, repairing, installing, and operating the instrument. Failing to do so violates the safety standards and intended use of the instrument. Waters Corporation assumes no liability for failure to comply with precautions. Precautions can be of these two types:

- Warnings that indicate risk of injury or death
- Cautions that indicate risk of damage to the system or equipment

These are the warning symbols you can encounter on instruments and/or in documents:

Warning: Indicates a potential health or safety hazard. Refer to the manual.

Warning: Indicates hazardous voltages can exist.

Warning: Indicates hot surfaces or high temperatures can exist.

Warning: Indicates danger from needle-stick punctures.

Warning: Indicates danger from ultraviolet radiation.

Warning: Indicates danger from corrosive substances.

Warning: Indicates danger from contamination by a biological agent.

Warning: Indicates danger from toxic substances.

Warning: Indicates danger from flammable substances.

Warning: Indicates danger from laser radiation.

Warning: Indicates danger from moving machinery.

Using Waters equipment

In addition to warning symbols, you may encounter the following symbols and labels on packaging, instruments, and/or in documents.

	Direct current
	Alternating current
	Protective conductor terminal
	Frame or chassis terminal
	Fuse
	Electrical power on
	Electrical power off
	Keep upright
	Keep dry
	Fragile, handle contents with care
	Use no hooks
	Waste disposal

Safety and electromagnetic equipment compatibility

United States – FCC rules

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

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.

Rationale: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio TV technician for help.

Shielded cables must be used with this unit to ensure compliance with the Class B FCC limits.

United States – safety requirements

Waters products meet the safety requirements for laboratory instruments set forth by the Occupational Safety and Health Administration (OSHA). All products are evaluated by an OSHA-approved, Nationally Recognized Testing Laboratory (NRTL) to ensure they meet applicable safety standards. NRTLs perform safety testing on instruments to ensure the safety of the operator.

Waters products carry a safety label from an NRTL to show compliance. The particular safety standard with which Waters complies is UL 61010A-1: Electrical equipment for laboratory use; Part 1: General Requirements.

Canada – spectrum management

This Class B digital apparatus complies with Canadian ICES-003.

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

Waters products meet the safety requirements for laboratory instruments set forth by the Standards Council of Canada. All products are evaluated by an approved laboratory to meet Canada's safety requirements. Waters instruments carry a safety label from an approved testing laboratory to show compliance. The particular Canadian safety standard with which Waters complies is CAN/CAS-C22.2 No. 1010.1: Safety requirements for electrical equipment for measurement, control and laboratory use, Part 1: General Requirements.

Europe – safety and electromagnetic compatibility

Waters products have been tested to meet the safety and electromagnetic requirements of the European community. Display of the CE mark indicates compliance to these requirements. The safety requirements are set forth via the standard EN61010: Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements. The EMC requirements are supported in the standard EN61326: Electrical equipment for the measurement, control, and laboratory use – EMC requirements. Compliance to the EN61010 standard ensures the safety of the operator from any hazardous situations that could have been caused by the instrument. Adherence to the EMC standard guarantees that the instrument will not cause interference to adjacent electronic products nor will other electronic units interfere with its operation.

Australia – emissions requirements

Australian authorities require that instruments do not exceed specified radiation limits. These radiation limits are given in the standard AS/NZS 2064: Limits and methods of measurement of electronic disturbance characteristics of industrial, scientific and medical (ISM) radio frequency equipment. Conformance to this standard is shown by displaying the Australian C-tick mark.

ACQUITY UPLC PDA detector information

Intended use

Use the Waters® ACQUITY UPLC™ PDA detector for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds.

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

Biological hazard

When you analyze physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in "CDC Guidelines on Specimen Handling," *CDC – NIH Manual*, 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples, typical specimens, and atypical specimens.

Quality control

It is recommended that you routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that chromatographic system performance is acceptable.

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1 ACQUITY UPLC PDA Detector Optics Principles

To use the detector's operating software (Empower™ or MassLynx™) effectively, you should understand the principles that underlie operation of the detector's optics and electronics.

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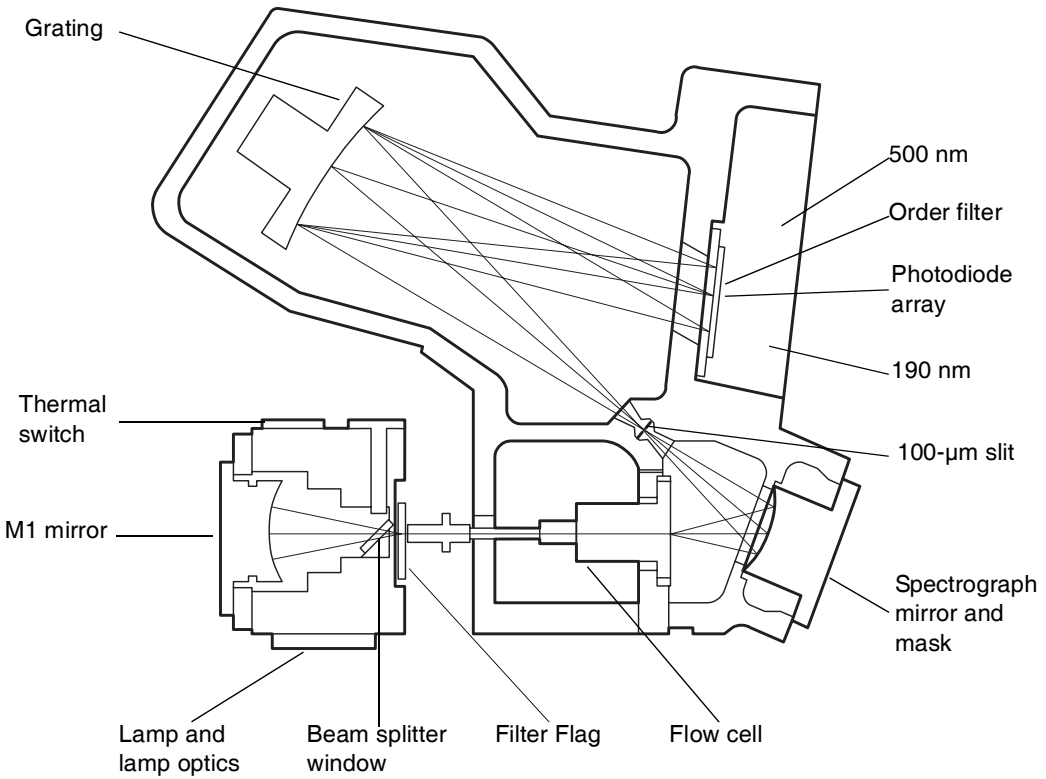
Detector optics

The detector is an ultraviolet/visible light (UV/Vis) spectrophotometer. With a photodiode array of 512 photodiodes and an optical resolution of 1.2 nm, the detector operates within a range of between 190 and 500 nm.

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

Optics assembly light path



The following table describes the optics assembly components.

Optics assembly components

Component	Function
Lamp and lamp optics	Focuses light from the deuterium source lamp, and via a mirror, directs it through a beam splitter to the flow cell.
Beam splitter window	Used to help minimize air infiltration into the lamp housing.
Filter flag	Influences the light entering the flow cell. Flag settings include • 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	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.
Grating	Disperses light into bands of wavelengths and focuses them onto the plane of the photodiode array.

Optics assembly components (Continued)

Component	Function
Order filter	Reduces the contribution of second-order diffraction of UV light (less than 340 nm) to the light intensity observed at visible wavelengths (greater than 340).
Photodiode array	An array of 512 photodiodes arranged linearly. The diode width (50-µm), together with a 100-µm slit, yield single wavelength resolution of 1.2 nm.

Calculating absorbance

The detector computes absorbance by subtracting the dark current (see "Dark current" on page 1-13) and reference spectrum from the acquired spectrum. Absorbance is based on the principles of Beer's law.

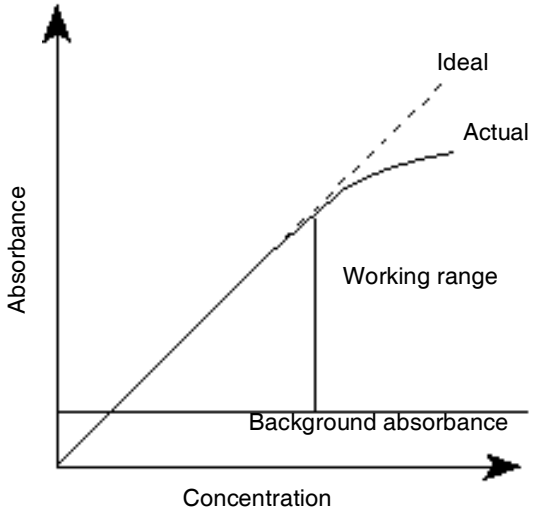
Beer's law

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 $A = \varepsilon lc$ 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 may 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 Appendix C.

Absorbance as a function of concentration

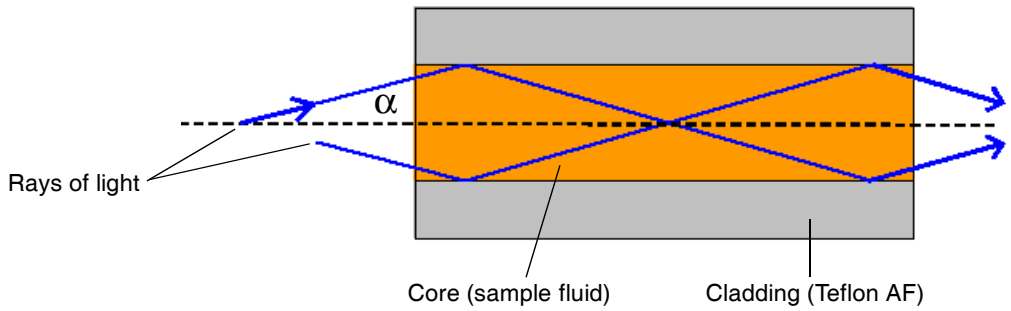


Light-guiding flow cell operating principles

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

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

Light transmission through a light-guiding flow cell



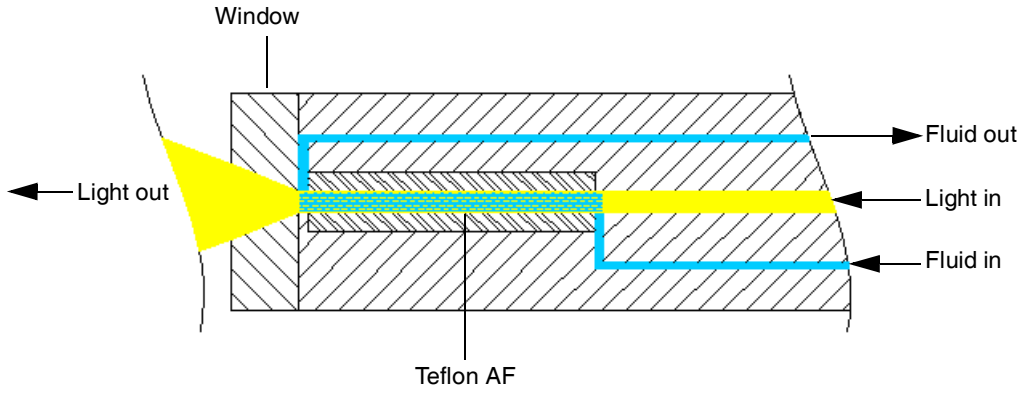
This information complements the foregoing illustration:

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

In the figure on page 1-6, 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, where the light beam is designed to avoid striking the internal walls of the cell, light-guiding relies on internal

reflections from the walls of the Teflon AF tubing. Consequently, it is important to maintain flow cell cleanliness by following the recommended procedures described in Chapter 5. With such care, the instrument and flow cell should provide you continuous sensitive detection.

Caution: To ensure the detector cell is properly aligned and calibrated, the flow cell must be filled with flowing solvent before you power-on the detector. An empty flow cell will cause a calibration error. Refer to the recommended procedures described in Chapter 5 for more information.

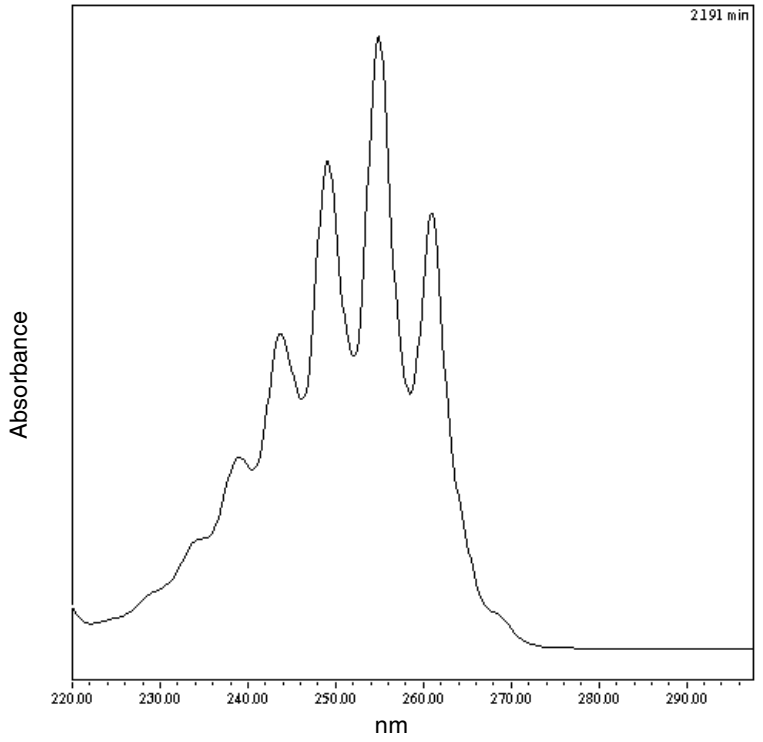
Resolving spectral data

Together with photodiode spacing, the detector's 100-µm wide slit 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



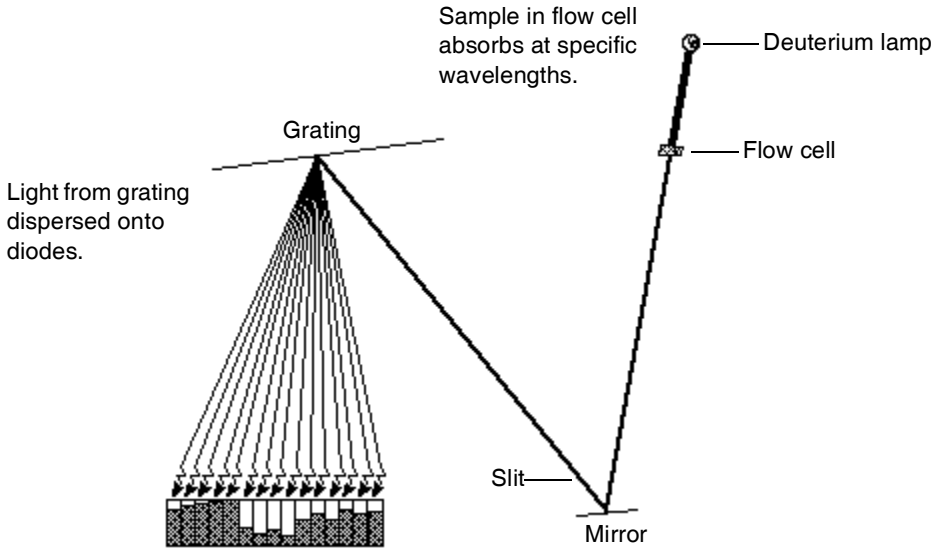
Measuring light at the photodiode array

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 have been recharged and read.

You set the exposure time parameter 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 the Auto Exposure parameter

The Auto Exposure parameter allows the detector's optics 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. 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 proper settings for signal-to-noise and dynamic range for each run

The Auto Exposure time setting may not optimize performance for certain combinations of sampling rates, wavelength ranges, or filter time-constant settings required for your analysis. If this is the case, you can set the exposure time manually, in the instrument editor.

Using the Exposure Time parameter

The Exposure Time parameter lets you 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 topic, "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 Appendix C). The signal-to-noise ratio may also be improved by increasing the Spectral Resolution parameter. 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 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 1-4).
- 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 1-14).
- Also, the detector can apply a filter when calculating absorbance (see “Filtering data” on page 1-15).

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.

Tip: For best results, the reference spectrum should represent the initial mobile phase.

Tip: For extremely long exposure times, the dark current and reference spectrum readings can take several seconds to finish.

Absorbance

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

$$Absorbance_n = \log\left[\frac{(Sn - Dn)}{(Rn - Dn)}\right]$$ 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 reports to the database (Empower or MassLynx) 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 minimal 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 have opposite effects on noise and spectral detail. 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

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. However, 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 runtime. A countdown clock, in the instrument control panel, indicates the length of the delay.

2 Setting Up the Detector

Contents:

Topic	Page
Before you begin	2-2
Installing the detector	2-3
Plumbing the detector	2-5
Making Ethernet connections	2-11
Connecting to the electricity source	2-12

Before you begin

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

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

Before installing the detector, ensure that

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

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

Customers in the USA and Canada should report damage and discrepancies to Waters Technical Service (800 252-4752). Others should phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or they may visit http://www.waters.com, and click Offices.

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

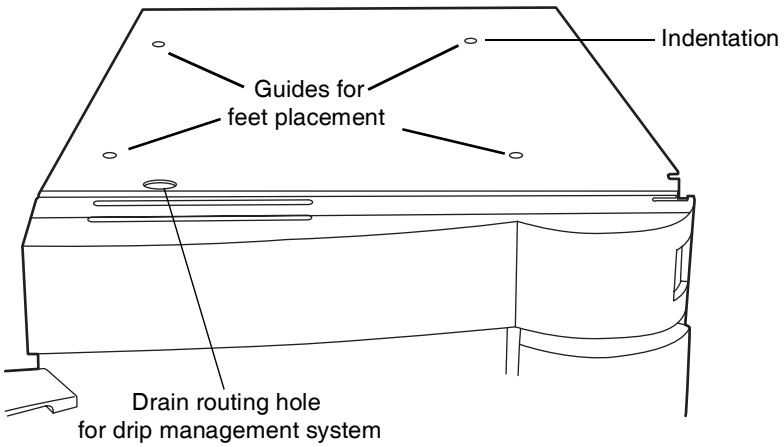
Installing the detector

To install the ACQUITY UPLC PDA detector:

1. Place the detector atop the column manager, ensuring that the feet are properly positioned in the indentations of the column manager. This aligns the detector's drip tray over the drain routing hole, on the top left side of the column manager.

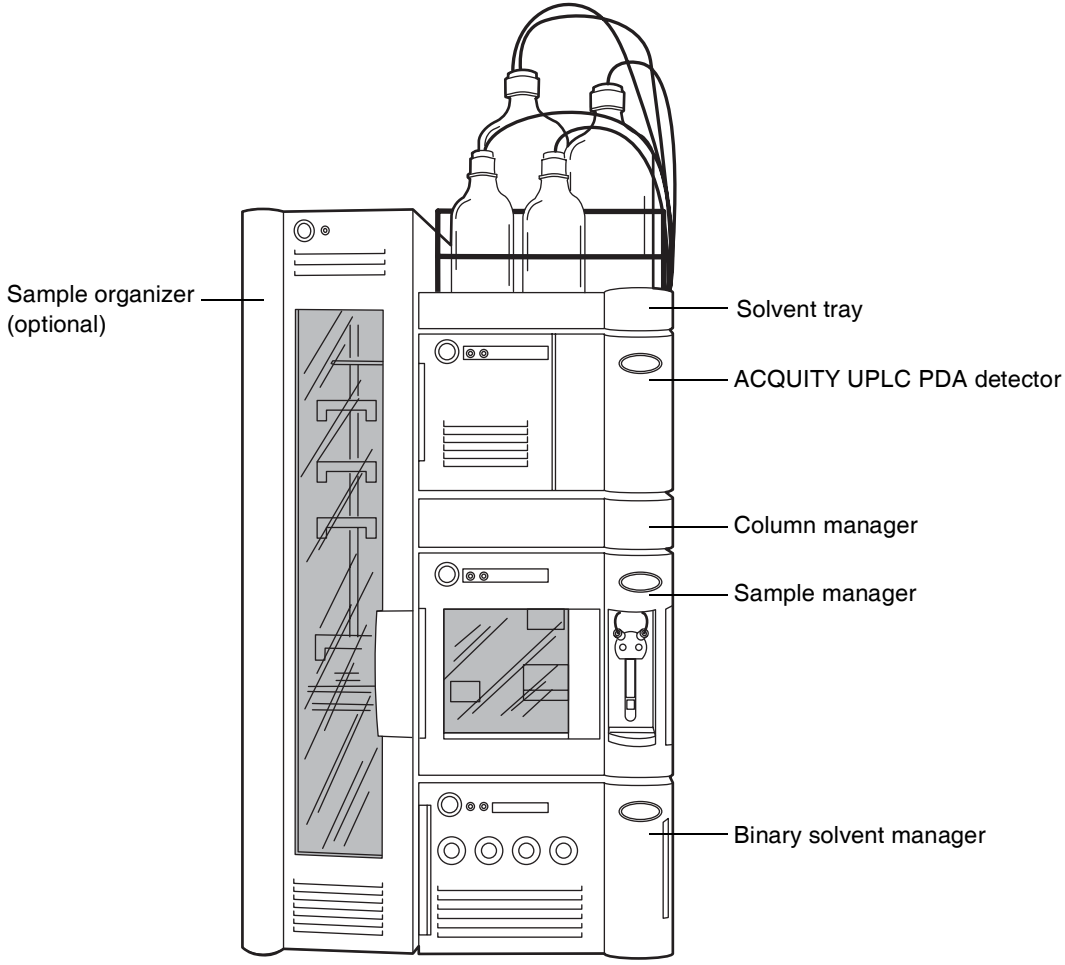
Warning: If only one person is to install the detector he or she should do so using a mechanical lift.

Proper placement for drip management system:



2. Place the solvent tray module atop the detector.

ACQUITY UPLC PDA detector installed in ACQUITY UPLC system:



Plumbing the detector

Warning: Using incompatible solvents may cause severe damage to the instrument and injury to the operator. Refer to Appendix D of the *ACQUITY UPLC System Operator's Guide* for more information.

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

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

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

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

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

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

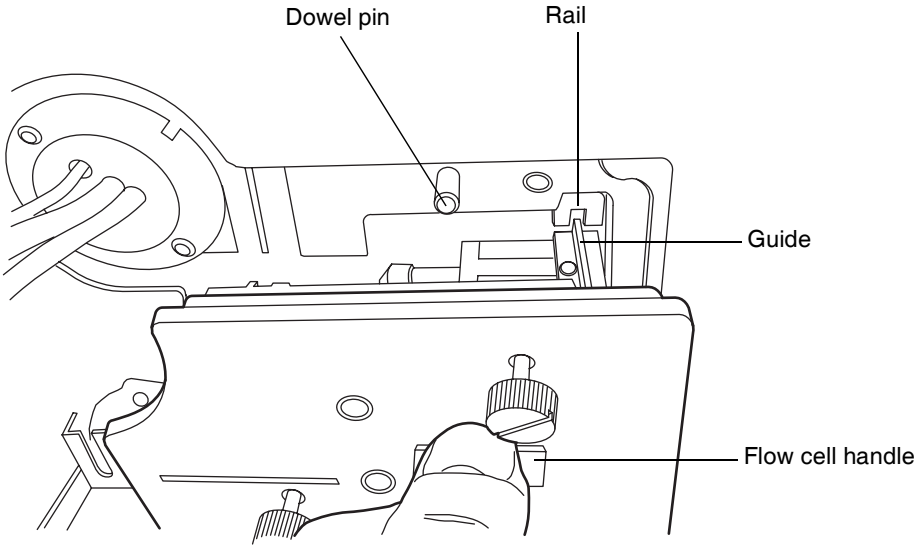
See also: *ACQUITY UPLC System Operator's Guide.*

To plumb the detector:

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

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

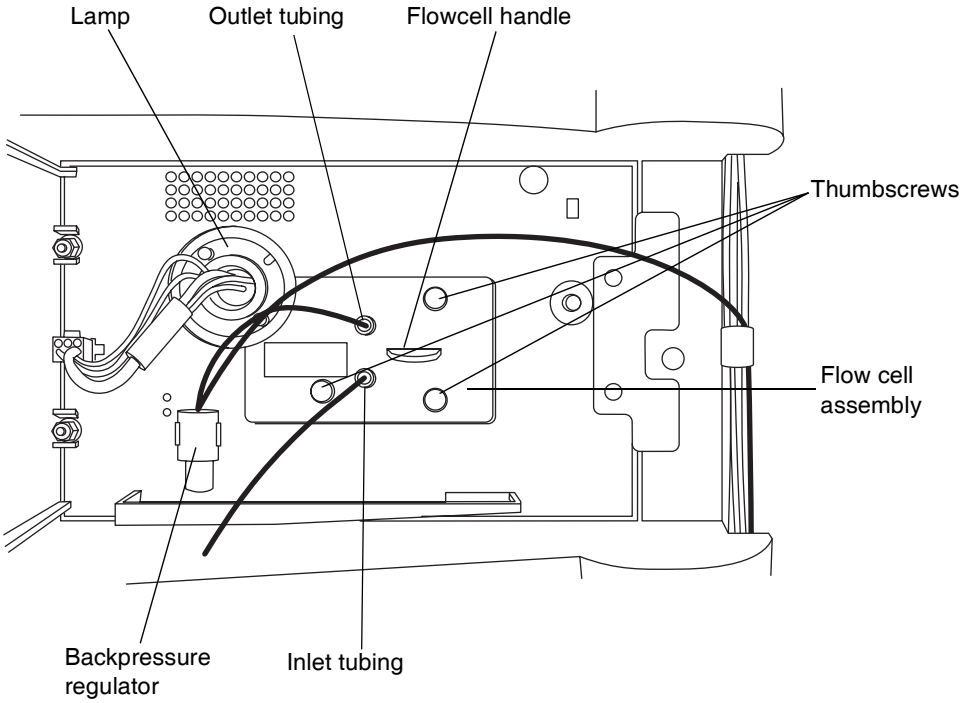
Installing the flow cell assembly:



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.

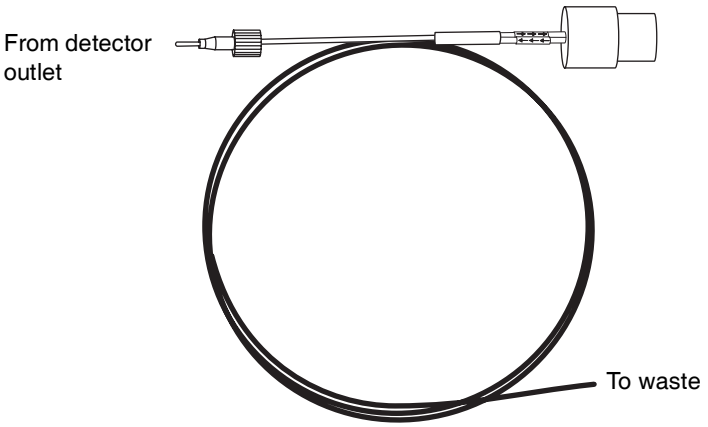
ACQUITY UPLC PDA detector flow cell:



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

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

Backpressure regulator:



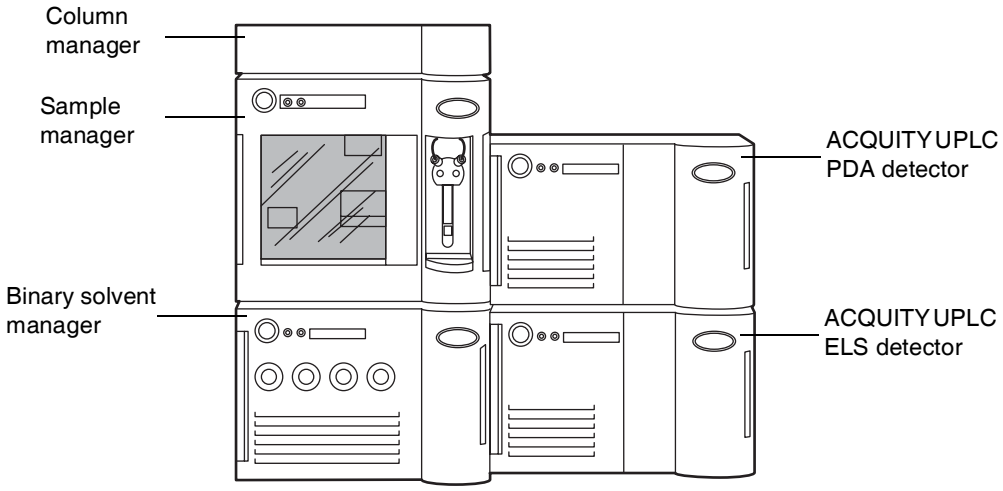
7. Route the long end of the outlet tubing from the backpressure regulator, through the channel clips along the front right side of the system, and into a suitable waste container.

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

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 detector installed in a split ACQUITY UPLC system:



Required materials

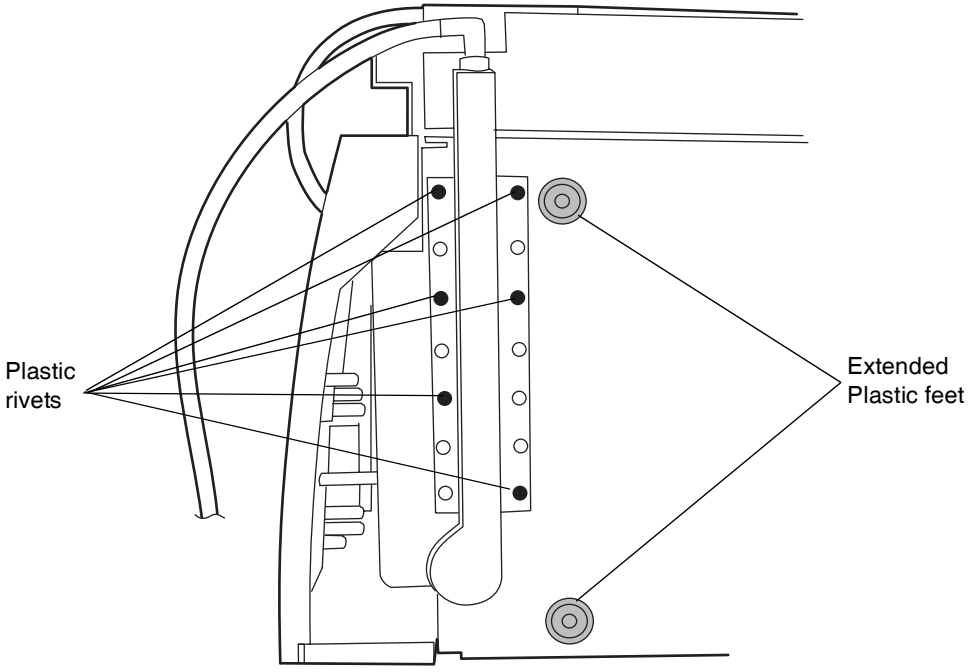
Multi-detector drip tray kit

To install the drip tray:

1. Turn the ACQUITY PDA detector so that it is resting on its left side.
2. Snap the extended plastic feet on to the bottom of the detector, and then snap the anti-skid pads on to the extended plastic feet.

3. Secure the drip tray to the bottom of the detector with the six plastic rivets provided in the multi-detector drip tray kit.

Installing the multi-detector drip tray:



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

Making Ethernet connections

To make Ethernet connections:

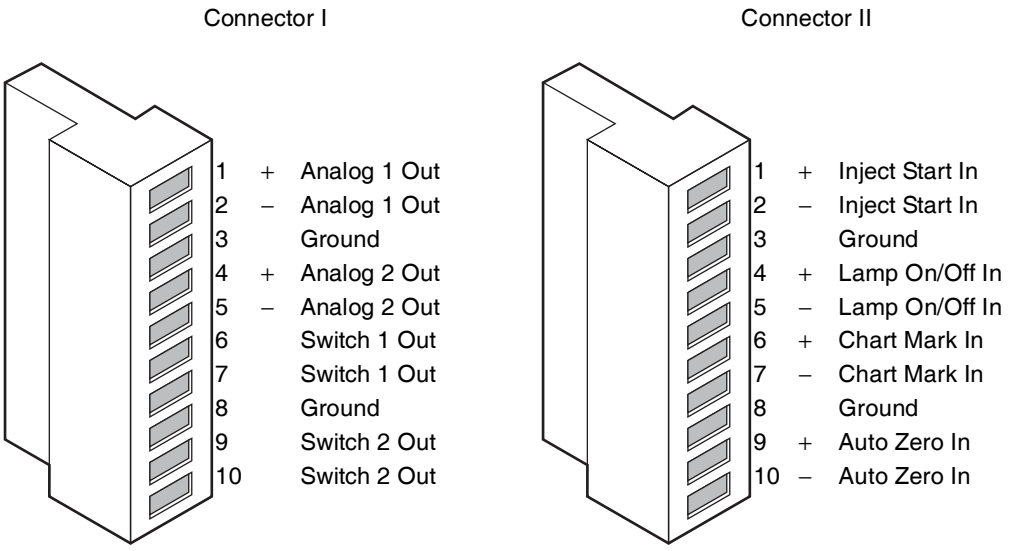
1. Unpack and install the preconfigured ACQUITY workstation.
2. Connect one end of one Ethernet cable to the network switch, and then connect the other end to the Ethernet card, on the workstation.

   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 connectors

The detector's rear panel includes two removable connectors that hold the screw terminals for I/O signals. These connectors are keyed so that they can receive a signal cable inserted only one way.

I/O signal connectors



ACQUITY UPLC PDA detector analog-out/event-in connections:

Signal connections	Description
Analog 1 (Out)	Used for analog chart output functionality.
Analog 2 (Out)	Used for analog chart output functionality.
Switch 1 (Out)	Controlled by threshold and timed events.
Switch 2 (Out)	Controlled by threshold and timed events.
Inject Start (In)	Should not be used.
Lamp On/Off (In)	When triggered, it ignites or extinguishes the lamp.
Chart Mark (In)	Marks all data with a 0.1 AU tick mark.
Auto Zero (In)	Calculates an offset value that, when added to the sample signal, makes the resulting baseline signal zero for all wavelengths.

Connecting to the electricity source

The ACQUITY UPLC PDA 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, use power cord SVT type in the United States and HAR type in Europe or better. For other countries, contact your local Waters distributor.

To connect to the electricity source:

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

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

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.

3 Preparing the Detector for Operation

Contents:

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Starting the detector	3-2
Shutting down the detector	3-6

Starting the detector

Warning: Using incompatible solvents may cause severe damage to the instrument and injury to the operator. Refer to Appendix D of the *ACQUITY UPLC System Operator's Guide* 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 or MassLynx).

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.

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 or MassLynx) by specifying a Lamp On event in the Events table. You may 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 Detector from the system tree and click  (Lamp Off).

See also: *ACQUITY UPLC System Operator's Guide.*

To start the detector:

1. Power-on the workstation.
2. Press the power switch on the top, left side of the binary solvent manager door and sample manager door. 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 binary solvent manager's flow LED and the sample manager's run LED remain unlit.

3. Start Empower or MassLynx. 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 binary solvent manager to deliver the appropriate flow 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.

   Tip: The detector may not initialize correctly if the cell contains air.
8. Press the power switch on the front panel to power-on the detector. 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 or MassLynx, and download an instrument or inlet method. You may monitor the ACQUITY console for messages and visual signals. For best results, wait one hour for the detector to stabilize before acquiring data.

Monitoring detector LEDs

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

Power LED

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

Lamp LED

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

Lamp LED indications

LED mode and color	Description
Unlit	Indicates the detector lamp is extinguished.
Constant green	Indicates the detector lamp is ignited.
Flashing Green	Indicates the detector is initializing or calibrating.
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 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	This image mimics the actual lamp on/off LED mode unless communications with the detector are lost. Clicking it opens the lamp control window.
Status	Displays the status of the current operation.
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	Resets the detector, when present, after an error condition.
Help	Displays the console Help.

Shutting down the detector

Caution: Buffers left in the system can precipitate and damage instrument components, including the flow cell.

Recommendation: You might want to shut down the detector

- between analyses
- overnight
- for a weekend
- for 72 hours or more

Tip: If Empower software controls the system, set system shutdown parameters in the Instrument Method Editor. Consult the Empower online Help or the ACQUITY UPLC Console online Help for more information.

If MassLynx software controls the system, set system shutdown parameters in the Shutdown Editor. Consult the MassLynx Online Help for more information.

Between analyses

To shut down the detector between analyses:

1. Between analyses, continue to pump the initial mobile phase mixture through the column. This maintains the column equilibrium necessary for good retention time reproducibility.
2. 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.

   Tip: Ensure that the shutdown method is deactivated.
3. Keep the detector operating and the column manager at operating temperature during this period.

Shutting down for fewer than 72 hours

To shut down the detector for fewer than 72 hours:

1. Flush the column with 90% HPLC-quality water:10% methanol. This keeps the column bed in an active, wetted state.

Requirement: If you are using buffers, you must first flush the column with a high-water-content mobile phase (90% water). Then stop the pump flow.

2. If possible, extinguish the detector lamp to lengthen lamp life.
3. The column manager can operate overnight but should be shut down over a weekend.

Shutting down for more than 72 hours

To shut down the detector for more than 72 hours:

1. Follow the steps for shutting down the detector for fewer than 72 hours, above.
2. After flushing the column and letting it cool to ambient temperature, disconnect the inlet and outlet tubes, and join them with a union. Install end-plugs in the column inlet and outlet fittings, and then return the column, carefully, to its box for storage.
3. Pump water through the system for 10 to 20 minutes at 0.5 mL/min. Follow with isopropyl alcohol for another 10 to 20 minutes. Then turn the pump off, leaving the alcohol in the fluid lines.

   Caution: If any system instruments are to be used for another type of analysis, ensure that the liquids pumped initially through the system are miscible with methanol, water, methanol/acetonitrile, or isopropyl alcohol. Likewise, before restarting the system, ensure that any residual material not miscible with the initial methanol/water mobile phase has been flushed thoroughly from the system with an appropriate intermediate solvent.

   Caution: 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 lab gases such as helium, nitrogen, or air for 5 to 10 minutes.

4 Verifying Detector Operation

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Topic	Page
Preparing the detector	4-2
Creating the test methods	4-4
Performing the gradient performance test	4-7

This chapter explains how to run a gradient performance test to verify that your detector is operating properly. The sample you use to verify the detector is included in the system startup kit.

Before you begin this procedure, your detector must be set up and configured as described in the *Waters ACQUITY UPLC System Operator's Guide* in Chapter 2, and Chapter 3.

Preparing the detector

Preparation is the same whether the detector is controlled by the Empower or MassLynx data system.

To prepare to verify detector operation:

Warning: Always observe safe laboratory practices when you use this equipment and when you work with solvents and test solutions. Know the chemical and physical properties of the solvents and test solutions you use. See the Material Safety Data Sheet for each solvent and test solution in use.

1. Prepare a 10:90 acetonitrile/water mobile phase:
   a. Measure 100 mL of filtered acetonitrile into a 100-mL graduated cylinder.
   b. Carefully transfer the acetonitrile to a 1-L reservoir bottle.
   c. Measure 900 mL of filtered HPLC-grade water into a 1000-mL graduated cylinder.
   d. Carefully transfer the water to the same 1-L reservoir bottle.
   e. Cap the reservoir bottle and mix well.
   f. Label the reservoir bottle as 10:90 acetonitrile/water.
   g. Submerge lines A1, B2, Seal Wash, Weak Wash, and Strong Wash in the reservoir bottle containing the 10:90 acetonitrile/water mixture.
   h. Place the reservoir bottle in the solvent tray.
2. Prepare a mobile phase of 100% acetonitrile:
   a. Pour approximately 1L of filtered acetonitrile into a 1-L reservoir bottle.
   b. Label the reservoir bottle as acetonitrile.
   c. Submerge lines A2 and B1 in the acetonitrile reservoir bottle.
   d. Place the reservoir bottle in the solvent tray.

3. Install the ACQUITY UPLC hybrid column in the column manager. Close the column tray, and replace the column manager's front cover. If you need more information about installing the column, see the *ACQUITY UPLC System Operator's Guide*.

   Caution: Never change directly between immiscible eluents or between buffered solutions and organic eluents. Immiscible eluents form emulsions in the flow path. Buffered solutions and organic eluents in combination can result in salt precipitation in the gradient proportioning valves, pump heads, check valves, or other parts of the system. Confirm that all fluids in the system are miscible with acetonitrile. If you need additional information about priming your system, see the *ACQUITY UPLC System Operator's Guide*.

4. Before you connect the column to the detector flow cell, flush solvent through the column and out to waste to ensure there are no column particulates that could damage the flow cell.

5. Access the console, and perform these tasks:

   a. Wet prime pump lines A1 and B2 for 5 minutes.

   b. Wet prime pump lines A2 and B1 for 5 minutes.

   c. Prime the seal wash pump.

   d. Prime the sample manager 20 times.

   e. Calibrate the system volume.

6. Prepare the sample as listed on the sample instructions, using 10/90 acetonitrile:water.

7. Place the sample in the vial plate, noting the vial position, and put the plate in position 2 of the Sample Manager.

Creating the test methods

The gradient performance test method parameters are the same whether Empower or MassLynx controls the system. Follow the steps below to create the methods, setting the parameter values to match those pictured in the screen representations.

Tip: Click ? on the tab pages to display online Help.

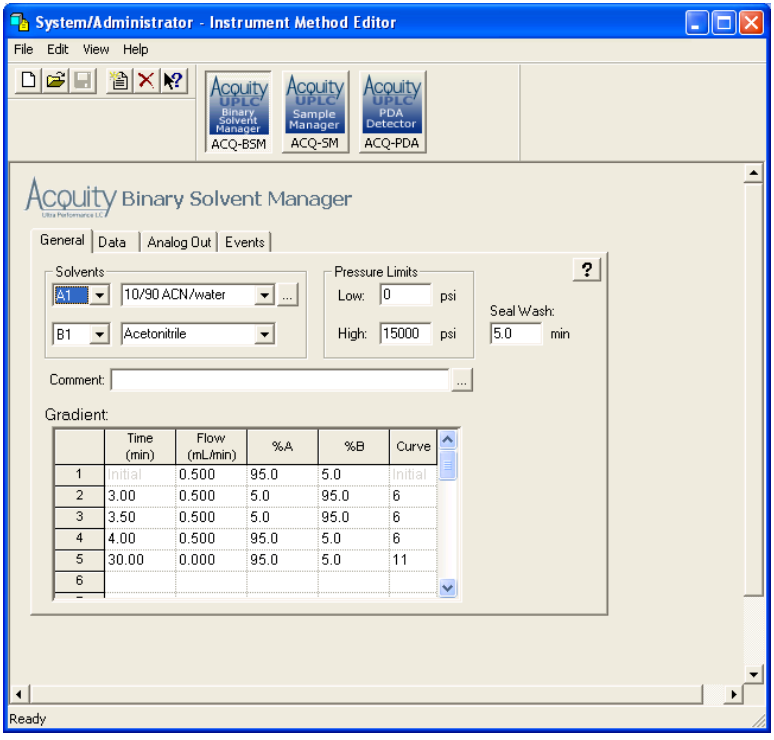
Creating the instrument method

To create the instrument method:

1. Create an instrument method with the binary solvent manager parameters shown in the following screen representation.

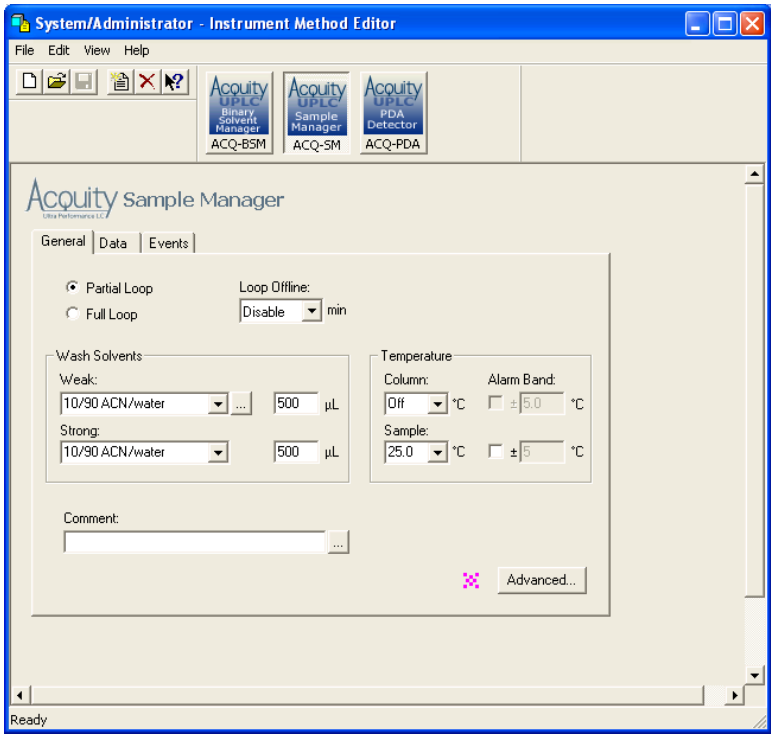
   Tip: The binary solvent manager parameters are identical for Empower and MassLynx.

Binary solvent manager instrument parameters:



2. Set instrument method parameters for the sample manager as shown in the following screen representation.

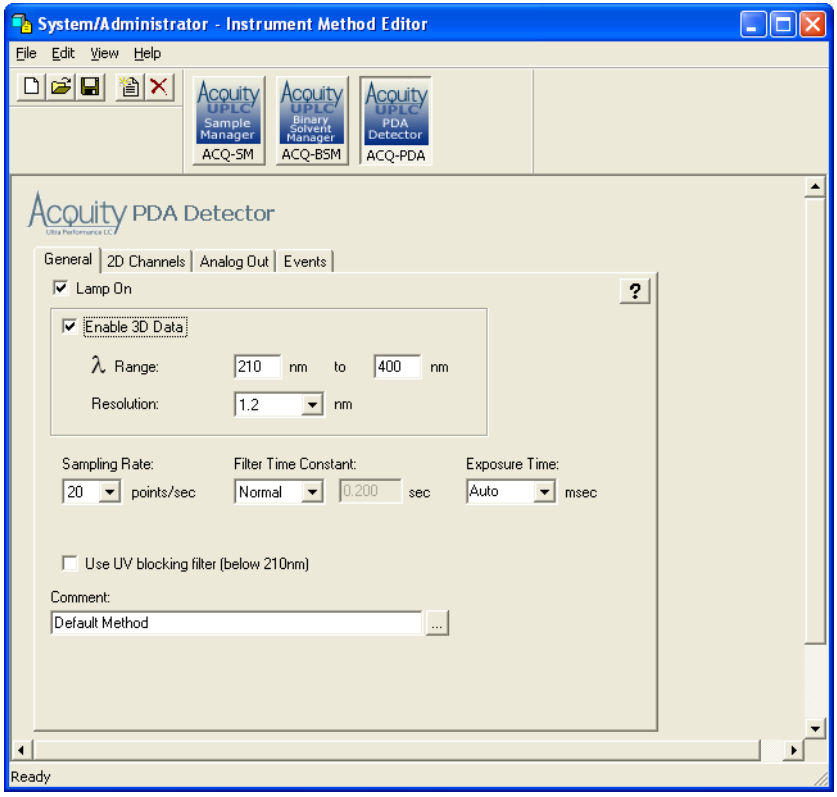
Sample manager instrument parameters:



3. Click Advanced, on the General tab, and set these parameters as follows:
   - Draw rate to 100 µL/min.
   - Pre-aspirate and Post-aspirate air gaps to 4 µL.

4. Set instrument method parameters for the PDA detector as shown in the following screen representation.

PDA detector instrument parameters:



5. Save the instrument method.

Performing the gradient performance test

When the system is prepared and the test methods are created, you are ready to run the gradient performance test. The steps for running the test vary slightly, depending on whether Empower or MassLynx controls your system, but the desired results are the same.

To perform the test:

1. Start the run:
   - If the system is Empower-operated, open the project in Run Samples, select the gradient performance test sample set, and then select Run and Report.
   - If the system is MassLynx-operated, access the MassLynx main page, and select Start from the Run menu.
2. When the sample set is complete, enter the appropriate results in the table, below.

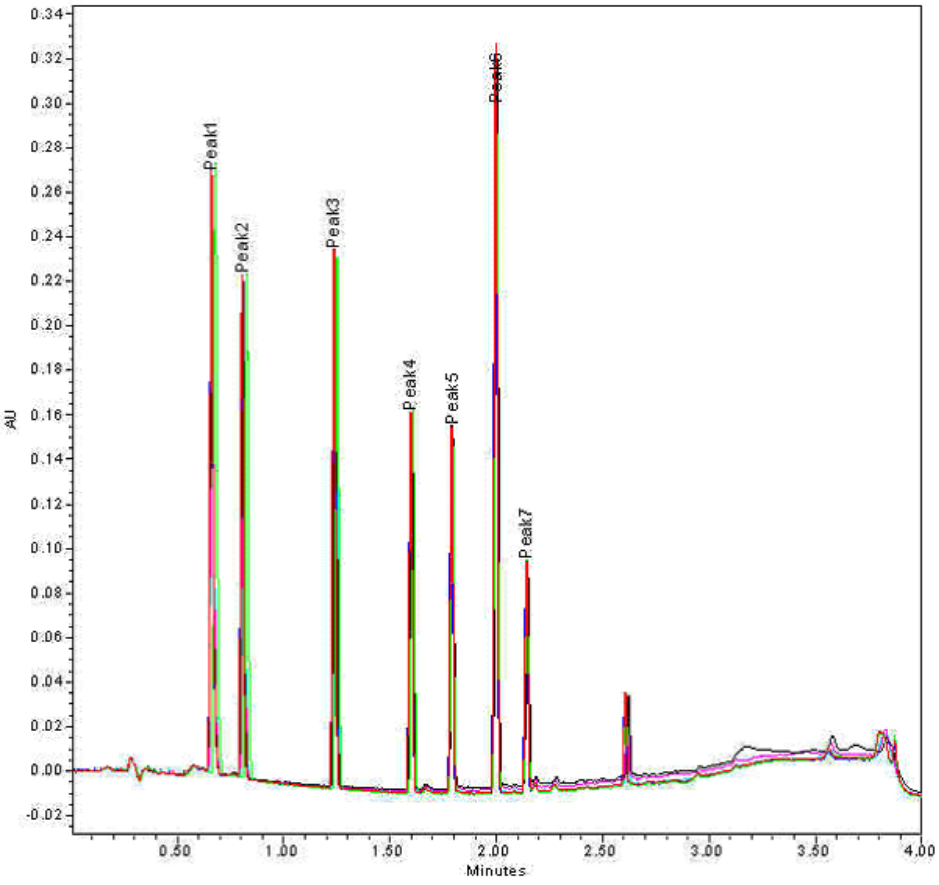
Retention Time Reproducibility (Three Replicates)

Peak	Component	Peak Retention Time Mean Value	%RSD	Acceptable %RSD
1	Acetylfuran			
2	Acetanilide			
3	Acetophenone			
4	Propiophenone			
5	Butylparaben			
6	Benzophenone			
7	Valerophenone			

3. Review the gradient performance report. The gradient performance test result is “passing” when these conditions are realized:
   - The peaks are symmetrical, integrated, and identified correctly. (Compare the chromatogram on the report to the sample chromatogram, below, to determine this.)

- The peak retention times show a standard deviation of less than or equal to 2.0 seconds. (Consult the table you completed to determine this.)

Sample gradient performance test chromatogram:



Note that this is a representative chromatogram. The results from your system may vary slightly.

5 Maintaining the Detector

Contents:

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Maintenance considerations	5-3
Maintaining the flow cell	5-6
Replacing the lamp	5-11
Testing the backpressure regulator	5-15
Replacing the fuses	5-15
Cleaning the instrument's exterior	5-16

Contacting Waters technical service

Customers in the USA and Canada should report maintenance problems they cannot resolve to Waters Technical Service (800 252-4752). Others should phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or visit http://www.waters.com, and click Offices.

When you phone Waters Technical Service, be prepared to provide this information:

- Error message (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 or MassLynx software version and serial number
- ACQUITY workstation model and operating system version

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

Maintenance considerations

Safety and handling

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

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

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

Caution:

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

Proper operating procedures

To ensure your system runs efficiently, follow the operating procedures and guidelines in Chapter 3.

Spare parts

See Appendix B, for spare parts information. You should not attempt to replace any parts not listed in Appendix B.

Recommendations:

- To prevent dirt from getting into the optics assembly, always keep the detector door closed whenever a flow cell is not installed in the detector.
- Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- To conserve lamp life, extinguish the lamp while leaving the detector running but idle. Note, however, that you should do so only when the lamp will remain extinguished more than 4 hours.
- If you use buffered mobile phase, flush it from the detector before powering-off to prevent
  - plugging solvent lines and the flow cell
  - damaging instrument components
  - microbial growth

Caution:

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

Flushing the detector

To flush the detector:

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.

   Caution: To avoid damaging the detector, do not exceed the 69 bar (1000 psi) pressure limitation of the flow cell.
3. Flush the system with a solution of 90:10 methanol/water for 10 minutes.

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

Maintaining the flow cell

Flush the flow cell if 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 and purge the flow cell as your initial attempt to correct these problems. If the problems persist, reverse flush the flow cell. If reverse flushing also fails, replace the flow cell.

Precautions

Observe these precautions when handling, removing, or replacing a flow cell:

- To prevent contamination, use powder-free finger cots or gloves.
- Take care to avoid scratching the flow cell.

Caution: To avoid damaging the flow cell:

- Handle it with care. Do not disassemble the flow cell.
- Pre-flush columns with at least 10 column volumes of clean mobile phase before connecting them to the flow cell. For example, flush a 2.1 × 50 column for 10 minutes at a rate of .5 ml/minute.

Required tools and supplies

- Wrench, suitable for removing and replacing the fittings
- Stainless steel union and tubing
- A solvent like methanol, which is miscible in both the mobile phase and water
- Powder-free finger cots or gloves
- Strong cleaning solvent suitable for your system
- HPLC-quality water
- Separate container for acid waste

Flushing the flow cell

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

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

Caution:

- 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, then cap the flow ports or dry the flow cell with pure nitrogen or pure helium.
- To prevent flow cell failure, do not connect any tubing or device that can create backpressure exceeding the flow cell's maximum rating of 69 bar (1000 psi).

Tip: Always use well-degassed eluents.

To flush the flow cell:

1. Extinguish the detector lamp.
2. Stop the solvent flow, and remove the column.
3. Replace the column with a union or piece of tubing.
4. If another instrument is downstream of the flow cell outlet, break the connection at the other instrument, and route the outlet tubing to waste while flushing.
5. Flush the detector with HPLC-quality water. If the mobile phase is not compatible with water, flush with an intermediate solvent first.
6. Pump 100% methanol through the flow cell to clean it internally. Do not exceed 69 bar (1000 psi).
7. Pump a strong cleaning solvent, such as isopropanol, through the flow cell (optional). Do not exceed 69 bar (1000 psi).

Tip: If the flow cell is dirty, remove any other active detectors from the system, and then pump 1% weak concentration acid solution (such as formic acid solution) through the flow cell at 0.5 mL/min.

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

8. Flush with HPLC-quality water at 0.5 mL/min until the pH of the effluent is neutral.
9. Reattach the column.
10. Resume pumping mobile phase. If the mobile phase is not miscible in water, first use an intermediary solvent.

Reverse flushing the flow cell

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

To reverse flush the flow cell:

1. Reverse the inlet and outlet tubing connections to the flow cell.
2. Flush the flow cell for approximately 15 minutes. Decreasing system pressure indicates the flow cell is clean.
3. If the flow cell remains dirty or blocked, remove and replace it. Return the blocked flow cell to Waters (see “Contacting Waters technical service” on page 5-2).

Replacing the flow cell

Required materials

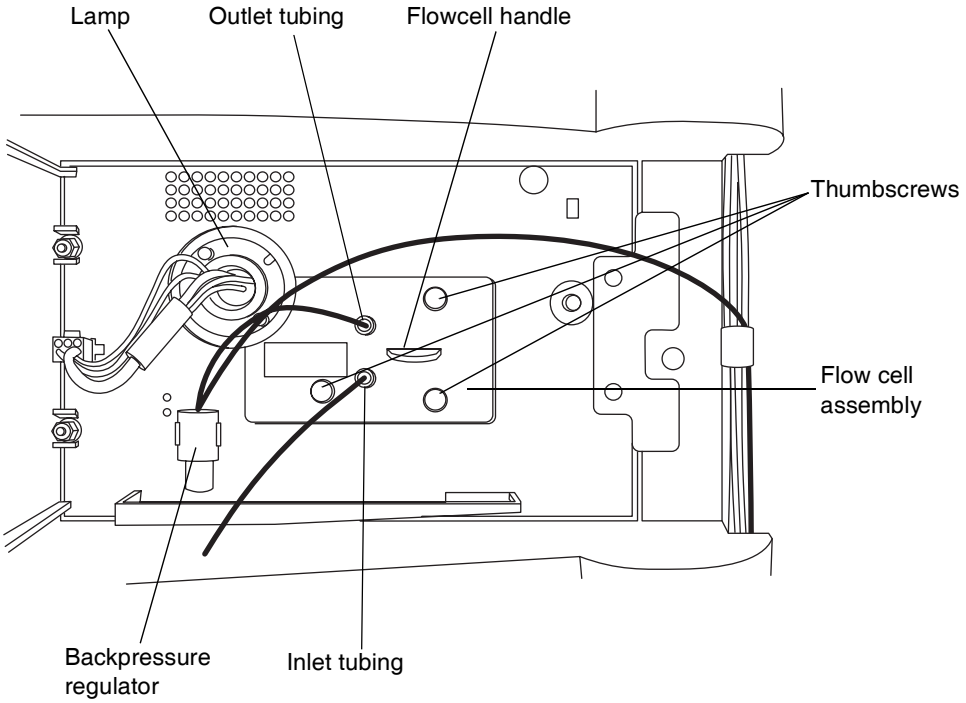
- 1/4-inch flat-blade screwdriver

To replace the flow cell:

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

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

ACQUITY UPLC PDA detector flow cell:

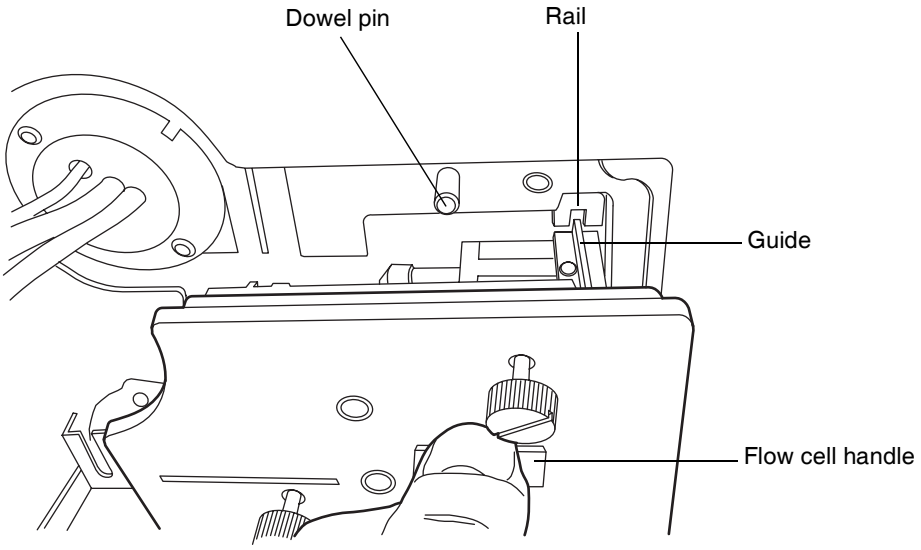


5. Disconnect the backpressure regulator, if present.
6. Remove the flow cell:
   - Loosen the three thumbscrews on the flow cell assembly's front plate.
   - Grasp the handle and gently pull it toward you.
7. Unpack and inspect the new flow cell. Ensure the flow-cell type is correct for your application. If you are replacing the flow cell with a different type, you must change the flow cell inlet tubing (see "Plumbing the detector" on page 2-5).

   Caution: To avoid damaging the tubing, do not touch it. During normal handling, the shield protects the tubing.

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 flow cell flange engage the rails in the sample cell compartment.

Installing the flow cell assembly:



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.
12. Connect the inlet tubing to the column outlet connection and flow cell inlet, and connect the outlet tubing to the back pressure regulator.
13. Close the detector door.
14. Before you power-on the detector, prime the system to fill the flow cell with solvent and remove any air.

    Caution: To ensure the detector cell is properly aligned and calibrated, the flow cell must be filled with solvent before you power-on the detector. An empty flow cell will cause a calibration error.

Replacing the lamp

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

Tip: If you do not record a new lamp's serial number in the ACQUITY console, the date of the previous lamp installation remains in the detector's memory, voiding the new lamp's warranty.

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.

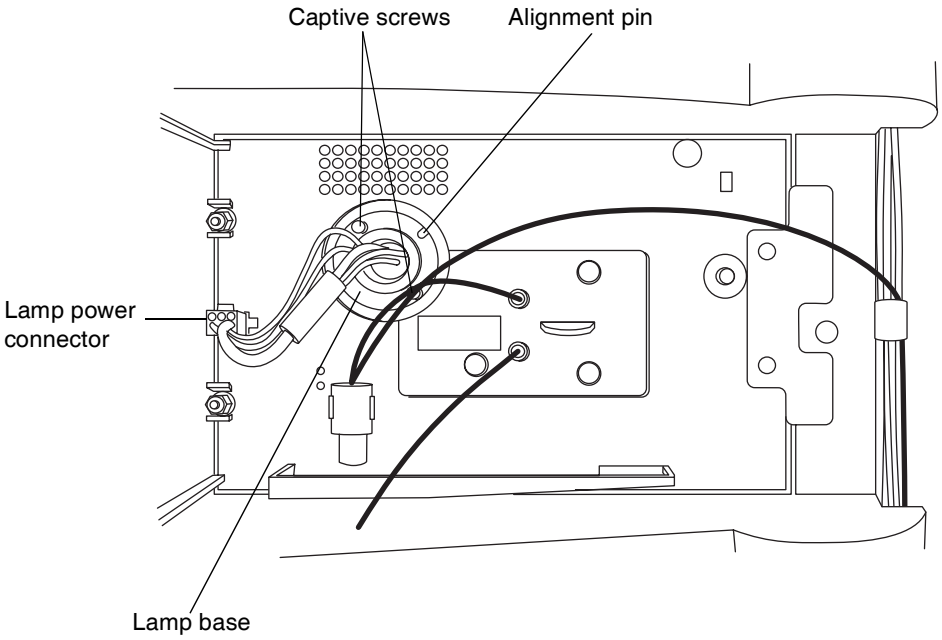
To remove the lamp:

1. Power-off the lamp:
   - To power-off the lamp manually, click PDA Detector in the left 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 or MassLynx online Help.
2. Power-off the detector and disconnect the power cable from the rear panel.
3. Allow the lamp to cool for 30 minutes, and then open the door, gently pulling its right edge toward you.

Warning: The lamp and lamp housing may be hot. Wait 30 minutes after powering off the detector for these components to cool before touching them.

4. Detach the lamp power connector from the detector.

Removing the lamp:



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

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.

To install the lamp:

1. Unpack the new lamp from its packing material without touching the bulb.
2. Inspect the new lamp and lamp housing.
3. Position the lamp so that the cut-out on the lamp base plate is at the 1 o'clock position, in line with the alignment pin on the lamp housing, and

then gently push the lamp forward until it bottoms into position. Ensure that it is flush to the optics bench.

4. Tighten the two captive screws, and then reconnect the lamp power connector.

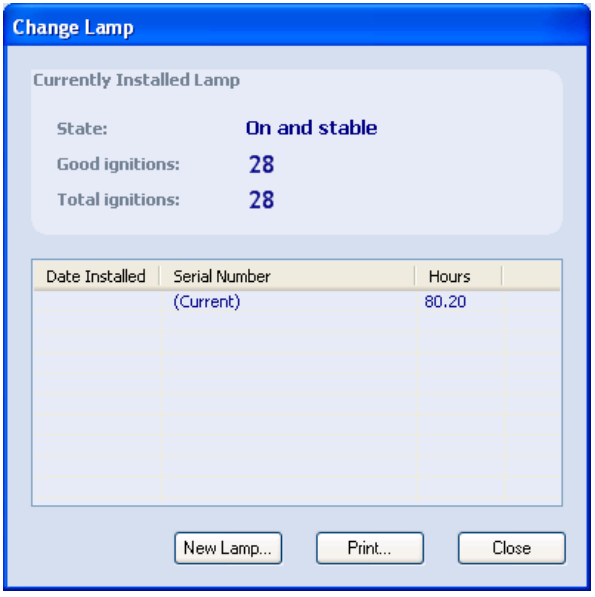
   Caution: To prevent the lamp from binding and ensure that it is properly seated in the lamp housing, alternate between tightening the captive screws and pushing the lamp forward.

5. Power-on the detector, and then wait about 30 minutes 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.

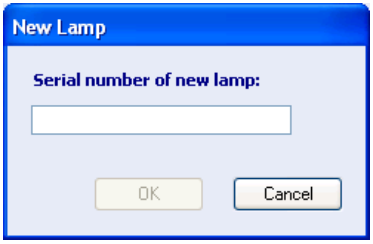
6. In the console, select Maintain > Change Lamp.

Change Lamp dialog box:



7. Click New Lamp.

New Lamp dialog box:



8. Type the serial number for the new lamp (see the label attached to the lamp connector wire), and then click OK.

Testing the backpressure regulator

The back pressure regulator connects from the outlet of the detector flow cell out to the waste container and maintains a minimum post-column pressure of 17 bar (250 psi), even when there is no flow, eliminating post-column outgassing and ensuring a smooth baseline.

Restriction: If a mass spectrometer is connected to the ACQUITY PDA detector, the backpressure regulator should not be installed.

To test the back pressure regulator:

1. In the console, select Sample Manager from the system tree.
2. Click Maintain > Test backpressure.
3. In the Backpressure Test dialog box, click Start.
4. When the test is complete, the Results pane appears.

Replacing the fuses

Warning: To avoid electric shock, power-off and unplug the ACQUITY UPLC PDA 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.

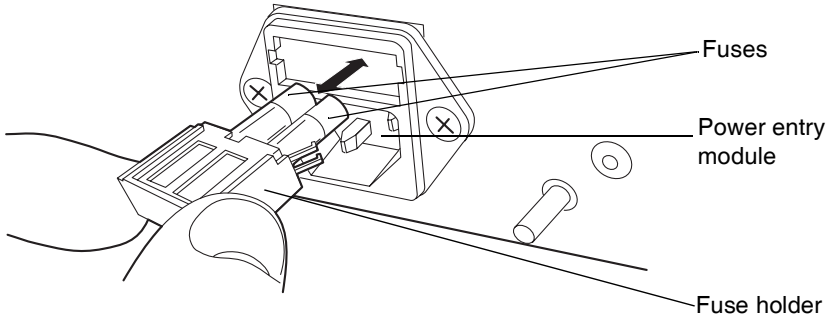
To replace the fuses:

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.

Removing the fuseholder:



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.

6 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 like noise, photometric error, or solvent effects.

Contents:

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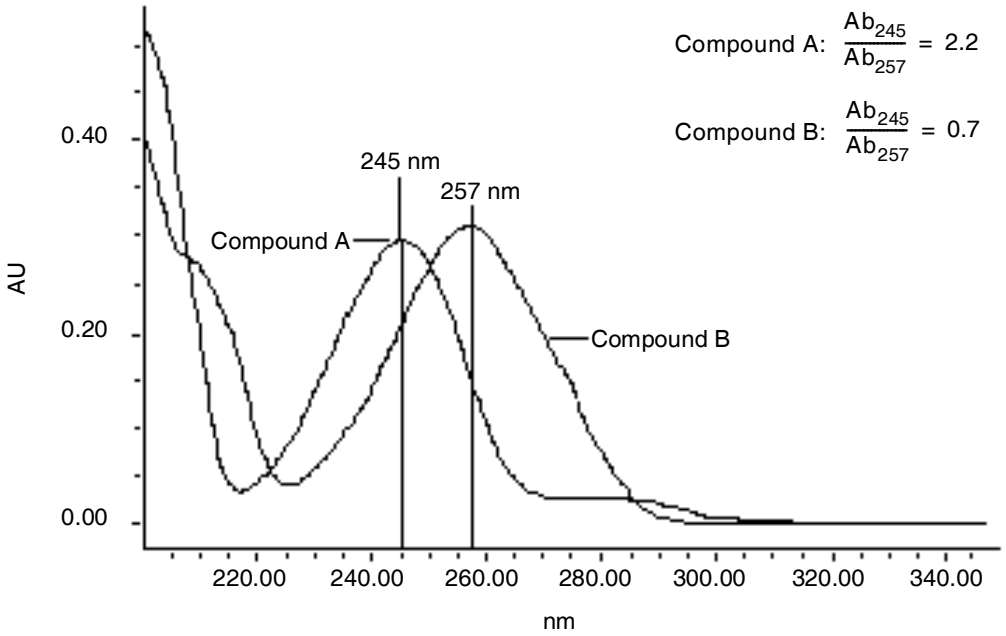
Representing Spectra as Vectors	5-3
Spectral Contrast Angles	5-5
Undesirable Effects	5-9

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



344.5063 nm, 0.4595 AU

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.

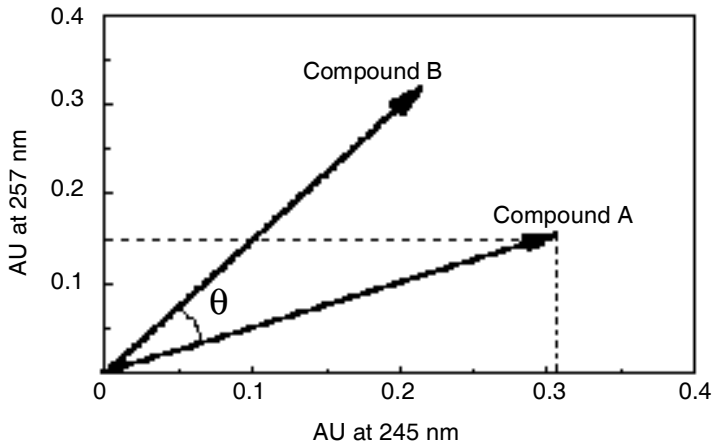
Vector direction contributes to the identification of a compound, since the direction is a function of the absorbance compound's spectrum. The ability of spectral vectors to differentiate compounds depends on the resolution of spectral features. As both wavelength range and spectral resolution increase, 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 6-5).

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.

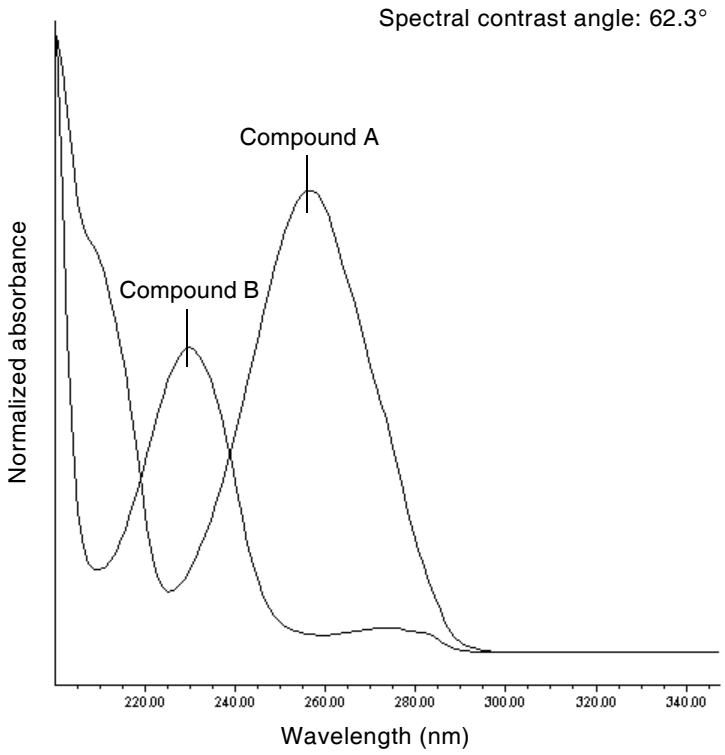
A spectral contrast angle can vary from 0° to 90°. A spectral contrast angle approaching 0° indicates little shape difference between the compared spectra. Matching a spectrum to itself produces a spectral contrast angle of exactly 0°. The maximum spectral contrast angle, 90°, indicates that the two 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°).

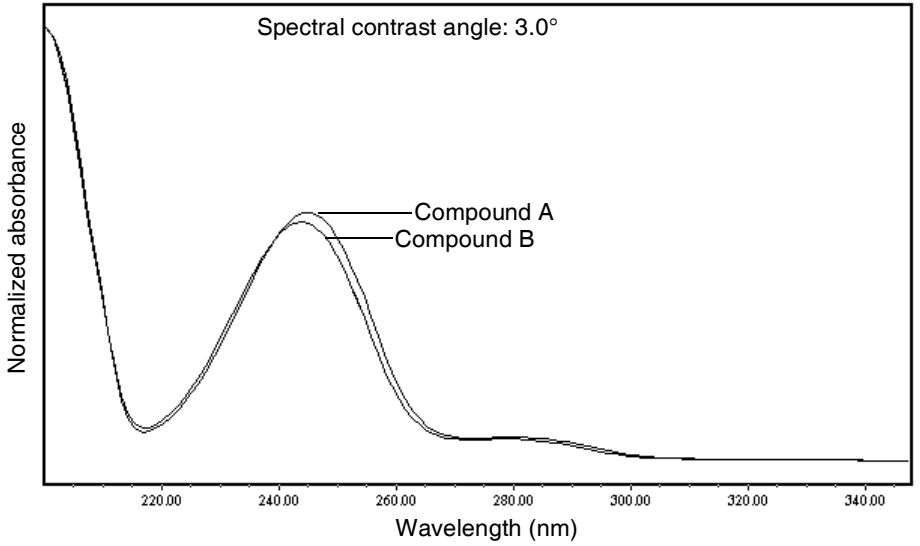
Spectra that produce a large spectral contrast angle



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°).

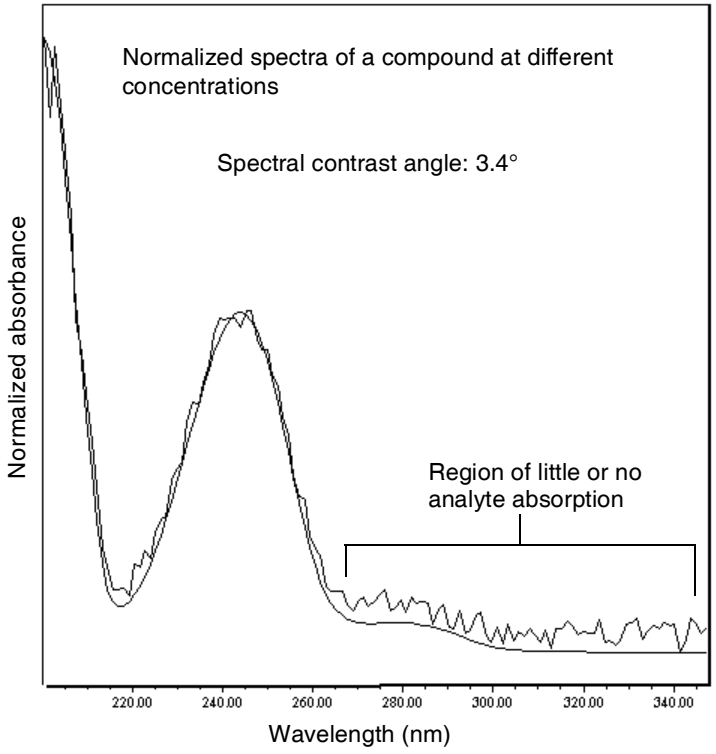
Spectra with a small spectral contrast angle



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 6-10).

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 Appendix C.

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

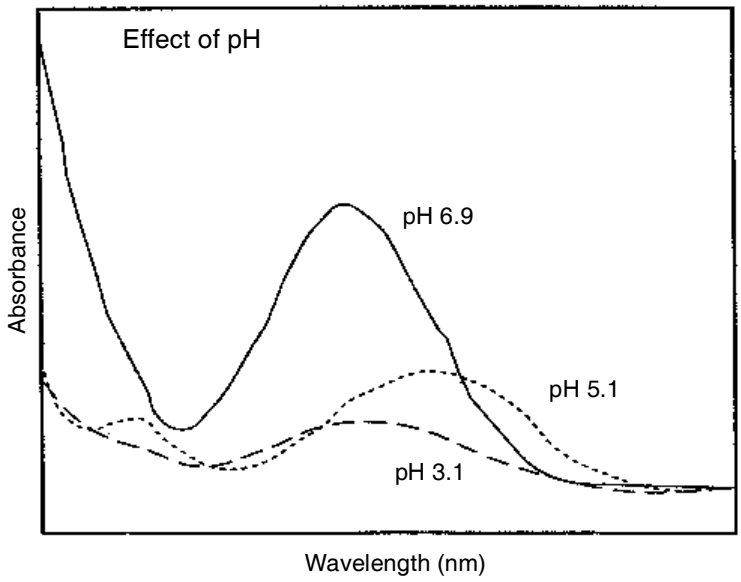
As long as solvent concentration and composition do not change (isocratic operation), background absorbance, if any, by the solvent remains constant. However, change in solvent pH or composition, such as that which occurs in gradient operation, can affect the intrinsic spectral shape of a compound. (See the figure on page 6-11).

Threshold angle

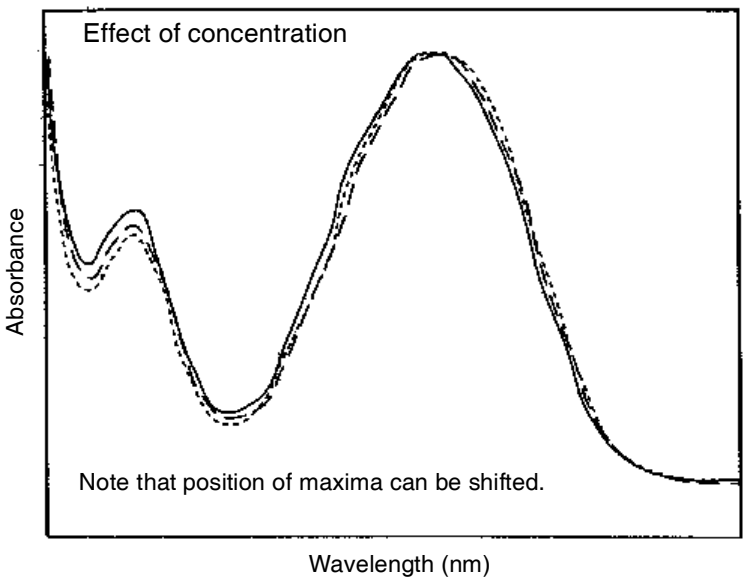
In addition to computing spectral contrast angles, the spectral contrast algorithm also computes a threshold angle. The threshold angle is the maximum spectral contrast angle between spectra that can be attributed to nonideal phenomena.

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



A Specifications

ACQUITY UPLC PDA detector specifications

Physical specifications

Attribute	Specification
Height	20.57 cm (8.1 inches)
Depth	61 cm (24.0 inches)
Width	29.21 cm (11.5 inches)
Weight	15.6 kg (34.4 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)	<65 dBA

Electrical specifications

Attribute	Specification
Protection class[a]	Class I
Overvoltage category[b]	II

Electrical specifications (Continued)

Attribute	Specification
Pollution degree[c]	2
Moisture protection[d]	Normal (IPXO)
⚠ 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	100 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

Item	Specification
Wavelength range	190 to 500 nm
Optical resolution	1.2 nm
Digital resolution	1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, 12.0
Wavelength accuracy	±1.0 nm
Wavelength repeatability	±0.1 nm
Digital filter	Variable with data rate

Performance specifications (Continued)

Item	Specification
Order filter	Fixed 340 nm to 500 nm
Noise (shunt in place of flow cell)	10 µAU, Peak to peak, 2 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, in a 240 micron medium shunt cell, 60 minute warm-up time
Noise (10 mm analytical flow cell)	14 µAU, Peak to peak, 2 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 0.5 mL/min, 10/90 Acetonitrile/water, 60 minute warm-up time
Drift (medium shunt cell and 10 mm analytical flow cell)	1000 µAU/hour, 2 sec time constant, 30 sec interval at 230 nm, 3.6 nm digital resolution, 2 Hz, 60 minute warm-up time. Environmental stability: ±2 °C/hour. Analytical flow cell conditions 0.5 mL/min, 10/90 Acetonitrile/water.
Linearity	< 5% at 2.0 AU, propylparaben series at 257 nm
Data rate	1, 2, 5, 10, 20, 40, and 80

B Spare Parts

This appendix lists recommended spare parts and options for the ACQUITY UPLC™ PDA detector. Unlisted parts are not recommended for customer replacement.

Recommended spare parts for the ACQUITY UPLC PDA detector

Item	Part Number
Backpressure regulator	700002571
Flow cell, analytical, 500nL, 10-mm path length	205015004
Flow cell, high sensitivity, 2400nL, 25-mm path length	205015005
Fuse, 3.15A, 250V, 5 × 20mm, fast acting (5 pack)	WAT055634
I/O signal connectors, 10 position	323000247
Inlet tube, 10-mm, path length	430001226
Inlet tube, 25-mm, path length	430001227
Multi-detector drip tray	205000355
Performance maintenance kit	201000186

C Mobile Phase Absorbance

This appendix 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
Unstablized tetrahydro-furan (THF, fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstablized tetrahydro-furan (THF, old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Acids and bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	—
Hydro-chloric acid, 0.1%	0.11	0.02	<0.01	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—
Trifluoro-acetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Diammo-nium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
Triethy-lamine, 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	—	—	—	—	—	—
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	—	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Potassium phosphate, monobasic (KH_2PO_4), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K_2HPO_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	—	—	—
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	—	—	—	—

Mobile phase absorbance measured against air or water (Continued)

	Absorbance at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
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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