# ACQUITY UPLC Evaporative Light Scattering Detector Getting Started Guide

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Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

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#### Waters contact information

# Safety considerations

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

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

# Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

# **Operating this instrument**

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

# **Applicable symbols**

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

#### Audience and purpose

This guide is intended for personnel who install, operate, and maintain ACQUITY UPLC<sup>®</sup> Evaporative Light Scattering (ELS) detectors.

# Intended use of the ACQUITY UPLC ELS detector

Waters designed the ACQUITY UPLC ELS detector to analyze and monitor many compounds.

#### Calibrating

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

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

## **Quality-control**

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

# **ISM classification**

#### ISM Classification: ISM Group 1 Class B

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

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# **1** ACQUITY UPLC ELS Detector Optics Principles

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

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#### **Overview**

Evaporative light scattering (ELS) detection works by nebulizing the solvent flow from a liquid chromatography (LC) system and entraining the resultant droplets in a gas stream. Mobile phase is then evaporated from the droplets. When an analyte is less volatile than the mobile phase, it remains in the gas stream as a "dry" solute particle and flows to the ELS detector. Once there, the particles scatter the light beam. The amount of scattered light is measured and bears a relationship to the concentration of material eluting.

## Capabilities

Though designed for ultra performance liquid chromatography (UPLC<sup>®</sup>), the ACQUITY UPLC<sup>®</sup> ELS detector is nevertheless compatible with virtually all modes of chromatography including flow injection analysis. The detector responds to all compounds that are, relative to their mobile phase, sufficiently nonvolatile at the conditions of analysis. Applications for ELS detection include combinatorial libraries of small molecules, natural product extracts and libraries, food products, and related materials. For detecting compounds that exhibit little to no UV/Vis response and do not ionize well for mass spectrometry, the ELS detector complements UPLC for analyzing sugars, antibiotics, antivirals, biomolecules, and natural products. You can use ELS detector, applying it as a qualitative tool to demonstrate the purity or complexity of a sample. Quantitation can be achieved by carrying out a calibration plot, as explained later in this guide. Note, however, that the curve will not be linear because ELS detectors give a non-linear response.

ELS detection performs well in isocratic and gradient elution with a wide variety of mobile phases and additives. Waters recommends using mass spectrometry-compatible mobile-phase modifiers. ELS detectors are less sensitive to changes in mobile phase composition and temperature fluctuations than refractive index detectors, but they share response capabilities with them.

# **ELS detection process**

The three separate regions of an ELS detector are nebulization, desolvation, and detection. In all ELS detectors, these three regions are positioned so that the chromatographic effluent is nebulized and mobile phase is evaporated so that dry solute particles, consisting only of analytes, reach the light source for scattering.

#### Low temperature nebulization

In the detector's nebulization region, the chromatographic effluent is transformed into a fine aerosol. A concentric tube, or flow-type nebulizer, mixes chromatographic effluent with a carrier gas (usually nitrogen) developing a series of droplets that forms the aerosol that enters a narrow-orifice drift tube.

#### Nebulization region and drift tube



The concentric flow nebulizer allows you to control the carrier gas flow versus the chromatographic effluent flow rate. High gas flow produces small droplets, requiring less heat to evaporate the solvent. Conversely, low gas flow produces large droplets, requiring more heat to evaporate the solvent.

#### **Desolvation**

In the vaporization region, the mobile phase evaporates and condenses, leaving dried solute particles in the drift tube.

As the aerosol drops exiting the nebulizer pass through the drift tube, they become smaller. The carrier gas sweeps the dried, aerosolized solute particles along to the instrument's detection region.

Evaporation occurs as a function of time, temperature, and pressure of the carrier gas. It is therefore important to use UPLC mobile phases that easily

and quickly evaporate and desolvate. Solvents of fairly low boiling point and low viscosity are best. They include the more commonly used UPLC mobile phases: water, acetonitrile, methanol, and ethanol. Viscous and high-boiling solvents might fail to fully separate from the analyte molecules or species before the detection step. This adds to the background noise and decreases the analyte signal response, which causes low sensitivity (slope of the calibration plot) and high limits of detection (LOD). The evaporated UPLC solvents are condensed and captured in the recommended solvent trap and exhaust routing. Almost all vapors are trapped. Nevertheless, small amounts of residual can persist, and these should be exhausted into a fume hood to prevent their escape into the laboratory.

## **Detection**

The analyte particles enter the detection region where a light source impinges on the particles. The light is thus scattered and focused onto a photomultiplier tube (PMT) where its intensity is measured.

The size (diameter) of the analyte particles determines how the light is scattered. The detector measures the intensity of the scattered light at 60° relative to the excitation beam to minimize polarization effects and stray light. Particles of different sizes exhibit different angular distributions of the scattered light, and particles whose sizes and shapes vary have different light-scattering cross sections. In general, larger particles scatter more light, yielding more intense signals and peak responses.

A photomultiplier tube (PMT) converts the scattered light signal to a voltage that can be recorded and analyzed. The stronger the scattering, the more intense the final signal on the ELS detection chromatogram. The scattered light is a rough measure of the mass of material represented by a chromatographic peak. To some degree, this "mass" response can be compound-independent. However, many factors can also affect the mass response, particularly the density of the analyte in a small dried particle. For example, a popped kernel of corn has a lower density than the unpopped kernel from which it originated. Yet, because it is larger, in most cases it would scatter more light. You should also remember that the output of an ELS detector has no direct relation to the molecular weight of an analyte.

# Types of light scattering

The three possible regimes of light scattering are

- Rayleigh
- Mie
- refraction-reflection

#### Light scattering direction



For a nebulizer that produces an average droplet diameter of  $\mathrm{D}_{0},$  the diameter of an average resulting dry analyte particle is

 $D = D_0 (c/p)^{1/3}$  where

 $D_0$  = Average liquid droplet diameter

c =Concentration of the analyte

p = Density of the dry analyte

For any given analyte peak, the response of an ELS detector can be that of all three light scattering regimes. The light-scattering type depends on the size of the particles going through the light beam. The ratio of particle diameter, D, to the incident wavelength,  $\lambda$ , or  $\frac{D}{\lambda}$ , defines the type of scattering that results.

- Rayleigh scattering occurs for the smallest particles where  $\frac{D}{\lambda}$  <0.1. The scattered light from a particle is proportional to D<sup>6</sup>, and consequently the scattered signal is proportional to c<sup>2</sup>.
- Mie scattering occurs for particles where  $\frac{D}{\lambda} > 0.1$ , but <1.0. The scattered light is proportional to D<sup>4</sup>, and the scattered signal is proportional to c<sup>4/3</sup>.

- Refraction-reflection scattering occurs for particles where  $\frac{D}{\lambda} > 1.0$ . The scattered light is proportional to D<sup>2</sup>, and the scattered signal is proportional to c<sup>2/3</sup>.
- As a chromatographic peak elutes from a column, the concentration of the analyte it represents changes. Concentration goes from near-zero at the baseline to a maximum that corresponds to column efficiency, injection volume, retention time, and concentration of the sample when injected. From the maximum level, the concentration then returns to near-zero. If the concentration is high enough, the diameter of a dry analyte particle can vary through all three scattering regimes— Rayleigh, Mie, and refraction-reflection scattering. It is this variance that prevents linearity in ELS detection calibration plots over more than one order of magnitude.

# **ELS detection limitations**

Consider these limitations when implementing global ELS detection separation methods:

- ELS detection lacks linearity over wide concentration ranges. When you use the detector for assays, you may need to experiment with a variety of "best fits" using linear, quadratic, and log-log responses for the compounds of interest. You might also need to establish groupings for expected concentration ranges.
- ELS detection is a destructive technique; the analyte is sacrificed to generate the scattering particles. Ideally, therefore, the ELS detector should be the final detector in a series. Alternatively, you can place the ELS detector upstream of others, provided you split the column effluent so that the ELS detector receives its own stream from the LC.
- Any particle can interfere with the sample signal, including particulates in poor-grade chromatographic solvents because the detector responds equally to all particulates. This lack of selectivity can cause problematic background noise.
- The detector's sensitivity to the particulates increases noise and, consequently, signal-to-noise variation for a given method arising from differences in the quality of mobile phases. Moreover, stationary phase components can leach from the column and contribute particulates to the sample flow.

- You can reduce the load of unwanted particulates by filtering LC effluent and the instrument's carrier gas.
- ELS detection cannot detect compounds whose volatility resembles that of the mobile phase. When the analyte and mobile phase have similar volatility, it is impossible to evaporate the mobile phase from droplets without also evaporating the analyte.
- In many cases the detector is minimally sensitive to baseline drift caused by gradient changes in an LC separation. However, its performance is not completely independent of the effects of changing solvent composition, which affects the nebulizer's ability to form droplets and influence their size.

# **Detector description**

The detection of a sample peak occurs as follows:

1. Eluent from the column flows into the nebulizer where a steady supply of gas converts it into a fine aerosol. Carefully controlled gas flow and flow rates determine the size of eluent droplets found in the aerosol.



**Warning:** Fire and explosion hazard. Do not use air as the carrier gas when the mobile phase contains flammable components.

- 2. Droplets are vaporized in the evaporation drift tube, leaving a rising column of particles, suspended in gas and vaporized solvent, to pass into the center of the light scattering chamber.
- 3. Two condensing lenses, L1 and L2, focus light from the lamp through a slit.



#### ACQUITY ELS detection process (representative)

- 4. Lens L3 relays the light from the slit to the center of the scattering chamber. A baffle between the slit and relay lens minimizes stray light reaching the scattering chamber.
- 5. Only light scattered at a 60° angle relative to the incident light is channeled through the snout and collector lens, L4. The positioning and design of the snout, together with the aid of two light traps, minimize stray light that can be detected. The first light trap houses a photodiode to intercept a portion of the stray incident light by monitoring lamp intensity variations. The second light trap minimizes stray light opposite the collection optics.
- 6. The collector lens focuses light onto the M1 mirror to change the direction of light before reflecting it onto the photomultiplier tube.
- 7. The PMT converts the light to an electrical signal.
- 8. Remaining gaseous effluent is vented.

## Signal processing and noise calculations

Power source fluctuations can introduce noise in the detector output and be a major source of noise at high signal levels. To offset their effect, a reference signal tracks lamp fluctuations and corrects the sample (PMT) signal accordingly.

# Calibrating the photomultiplier tube (PMT)

The full scale sensitivity of the instrument is controlled by the gain setting, which increases the voltage to the PMT to amplify response. The instrument gain is achieved by controlling the high voltage supply to the PMT. However, the PMT response is not linear, so each unit must be individually calibrated to determine the required voltage settings for each gain value. PMT calibration is performed by Waters after the assembly and alignment of the detector and whenever the PMT or any PC boards are replaced.

## **Filtering noise**

In the General tab of the ELS Instrument Method Editor (for details, refer to the Empower or MassLynx online Help), you can apply an optional noise filter (the Time Constant parameter) to the data acquired.

# **Electronics and data acquisition**

The detector's electronics control the following components:

- Preamplifier board Collects and processes the analog input signals from the PMT and photodiode to the microprocessor for further signal conditioning. Sample and reference signals are integrated, and A/D conversion is performed simultaneously. This ensures the best rejection of common mode noise in the two beams, leading to a very quiet baseline.
- Control board Receives inputs from the preamplifier board, keyboard, and external events.
- CPU board Contains the digital signal processor, communication ports, nonvolatile (battery backed-up) RAM, and Flashable RAM space (in which the firmware resides).
- Ethernet communications interface Allows the detector to communicate with the Waters chromatography workstation.
- Lamp power supply Control board provides stable tungsten halogen lamp operation.
- DC power supply Provides voltage for the analog and digital circuitry. It is the DC power source for the detector.

## **Nebulizer**

The nebulizer has been optimized for UPLC performance.

# **Optics bench**

The detector's optics bench consists of four major systems:

- Illumination
- Desolvation
- Light scattering chamber
- Collections

#### **Illumination system**

The illumination system uses these components to direct broadband light from the lamp into the light scattering chamber:

- Tungsten halogen lamp
- Entrance mask
- Two convex lenses, L1 and L2, acting as a condenser
- Slit
- Baffle
- Convex relay lens L3

#### Light scattering chamber

The light scattering chamber is the equivalent of a flow cell in other detectors. It provides an environment where the sample in the gas stream and the incident light beam can interact. The chamber contains these components:

- Two light traps
- Reference photodiode

To prevent the solvent and analyte from condensing on the chamber walls or optical surfaces, the chamber is heated to 50 °C (122 °F) and cannot be varied. A thermistor for temperature regulation and an over-temperature switch are included in its heating circuit.

#### **Collections system**

The collections system collects scattered light from the scattering chamber and directs it to the PMT for conversion to an electrical signal. It consists of these components:

- Snout
- Biconvex collector lens, L4
- Mirror M1
- PMT

#### **Temperature control**

To vaporize and evaporate the solvent, the nebulizer and drift tube are heated by two variable-control heaters.

#### Nebulizer

The nebulizer heater, represented as a power function, can heat the sample solution to improve mass flow into the drift tube. The power function indicates the power available to the nebulizer heater circuit. In certain cases, the nebulization process of the mobile phase can be endothermic, as with 100% organic solvents such as methanol and acetonitrile. These require more power than other eluents. Besides the ability to heat the nebulizer, Waters added the ability to cool the nebulizer when faster equilibration times are required.

#### **Drift Tube**

You can set the drift tube heater up to 100 °C (212 °F) to evaporate any residual solvent. RTD (resistance temperature detector) sensors provide temperature feedback to the heater control to ensure the desired temperature is maintained. The RTD is placed at the end of the drift tube, where the temperature is hottest, so it can give accurate feedback of the most extreme temperature the particles will be exposed to. This is particularly important for semivolatile substances.

## **Startup diagnostics**

On starting the detector, the presence of many electronic devices and components is verified. Some can self calibrate, a process that takes place at this time. The startup diagnostics include these tests:

- Central processing unit (CPU) test
- Serial communication interface (SCI) test
- Electrically erasable programmable read-only memory (EEProm) test
- RAM test
- Application program checksum verification (firmware)
- Lamp test
- Photodiode test
- PMT test

The signal of the lamp is measured, and the normalization constant is adjusted accordingly to compensate for lamp intensity variations. This minimizes the influence of lamp intensity changes on detected signal levels. All settings are restored to the values present when the unit was shut down, except for the heater setpoints and gas flow, which must be specified. **Recommendation:** Power-off and on weekly to compensate for lamp aging.

## Lamp energy and performance

In conventional designs of ELS detectors, the signal-to-noise performance of the instrument is directly proportional to the lamp energy input to the instrument. These factors can affect lamp energy input to the detector:

- Age and efficiency of the lamp
- Improperly maintained optics and/or flow cell
- Normal degradation of optical components (including the PMT)

Optical components degrade slowly. In conventional ELS detectors, response increases by incrementally increasing the PMT gain. However, a sample's response varies with energy throughput. If the lamp energy is degraded, peak response degrades accordingly. If lamp intensity diminishes, peak response decreases and noise increases. During normal operation, lamps are usually replaced when the reference energy falls below a user-set threshold. The useful lamp life depends on the method's specific requirements for noise performance. Eventually, the detector's performance becomes unacceptable and the lamp replacement is necessary.

**Recommendation:** Inspect the detector's general condition when replacing a lamp.

Predicting when the detector's performance degrades to an unacceptable level based solely on reference energy is unsatisfactory. Each user's analyses require different levels of sensitivity. Determining reference energy alone to evaluate performance assumes that lamps exhibit the same longevity and degradation patterns. Waters therefore designed the detector to operate as independently of lamp output as possible. Ultimately, the detector's performance is a function of unique application requirements.

Signal-to-noise measurements are the best way to evaluate performance and set boundaries for acceptable operational sensitivity limits. Waters guarantees 2000 hours of lamp life, or one year since date of purchase, whichever comes first.

# **ACQUITY UPLC console**

The ACQUITY UPLC system console is a software application that provides a convenient way to configure settings, monitor performance, run diagnostic tests, and maintain the system and its modules. It replaces the keypads and

small screen displays traditionally found on the fronts of system instruments. The console functions independently of Empower and MassLynx and does not recognize or control the data systems.

From the console's interface, you can quickly navigate to visual representations of each instrument and its components. You can also navigate to interactive diagrams, which show interconnections and provide diagnostic tools for troubleshooting problems. Consult the console Help for more information.



#### ACQUITY UPLC console window

#### **Rear panel**

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



#### ACQUITY UPLC ELS detector rear panel

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# **2** Setting Up the Detector

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# Before you begin

**Requirement:** To install the detector, you must know how, in general, to set up and operate laboratory instruments and computer-controlled devices and also 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.

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

#### To install the ACQUITY UPLC ELS detector



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

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.

#### Proper placement for drip management system



2. Place the solvent tray module atop the detector.

# Example of an ACQUITY UPLC ELS detector installed in an ACQUITY UPLC system



# Making the gas supply connection

**Requirement:** A constant supply (450 to 700 kPa [4.5 to 6.9 bar, or 65 to 100 psi] at regulator) of dry, oil-free, filtered nitrogen (or zero-grade, oil-free, filtered air) is required to operate the detector.

Warning: Do not use gases that would allow the combustion of combustible solvents. Always use inert gases.

**Caution:** A pressure relief valve vents gas to protect the detector when the input gas pressure is too high. If you can hear gas leaking from the relief valve, lower the input pressure to avoid wasting gas.

**Tip:** Gas cylinders are not recommended for extended operation of the detector due to the rapid consumption of gas. For example, a standard tank of nitrogen (300 cubic feet) running a standard flow nebulizer at 170 kPa (1.7 bar, 25 psi) would last approximately 40 hours.

The detector is connected to the gas supply via 6-mm plastic tubing (supplied in startup kit) using the fitting on the back of the unit.

#### To make the gas supply connection

- 1. Cut the 6-mm tube squarely.
- 2. Insert the tube into the fitting until it bottoms.

#### Inserting the gas supply tube



3. Pull the tube to check engagement of the grab ring.

# Installing the nebulizer assembly

**Tip:** The Waters 2420 ELS Detector low-flow and high-flow nebulizers are not compatible with the ACQUITY UPLC ELS Detector.

The following table lists the ACQUITY UPLC ELS detector nebulizer's approximate pressure to flow rates.

Pressure	Flow (L/min) <sup>a</sup>
140 kPa (1.4 bar, 20 psi)	1.5
210 kPa (2.1 bar, 30 psi)	2.0
280 kPa (2.8 bar, 40 psi)	2.5
350 kPa (3.5 bar, 50 psi)	3.0
410 kPa (4.1 bar, 60 psi)	3.5

#### Approximate gas pressure to gas flow rates

a. Standard liters per minute of nitrogen, according to nebulizer manufacturer settings.

#### To install the nebulizer assembly

- 1. Remove the nebulizer assembly from the shipping container.
- 2. Slide the packing ring onto the nebulizer assembly.
- 3. Remove the protective cap from the nebulizer assembly.
- 4. Remove the protective cap from the end of the nebulizer tube.

Nebulizer assembly with packing ring installed



5. Align the two pins inside the nebulization chamber with the grooves in the nebulizer.

#### Pins inside the nebulization chamber



The quick-disconnect tubing fitting will be at the twelve o'clock position.

#### Installing the nebulizer



6. Push the nebulizer into the nebulization chamber, and turn it clockwise until it snaps into place.

# Connecting to the waste system

ELS detector process waste is expelled from the vapor exhaust hose and the siphon drain tube.

# Venting the exhaust hose to a fume hood



**Caution:** Gas from the fume hood could enter the detector, carrying foreign material that can contaminate it.

Vent the exhaust hose, at the back of the unit, to a fume hood. Ensure that the fume hood withdraws gas from the detector. A positive pressure should exist between the detector and the hood.

To properly vent the exhaust vapor to waste, a vapor trap exhaust bottle is provided (startup kit). The vessel traps condensates that form from vented vapor exiting the detector.
#### Vapor trap bottle





- Failure to use the vapor trap could result in too strong a vacuum, which could adversely affect the vapor flow through the drift tube. This could cause loss of sensitivity and excessive high-frequency noise in the baseline.
- To avoid condensate flowing backward into the detector and thus damaging it, run at least 61 cm (24 inches) of the instrument's exhaust hose vertically toward the bench top.

#### **Exhaust venting requirements**

The following exhaust venting requirements apply to ACQUITY UPLC systems that have one detector.

**Requirement:** Ensure the instrument's exhaust hose runs straight down, toward the bench top, a minimum of 61 cm (24 inches).

- Attach the vapor trap bottle to the end of the exhaust hose.
- Place the vapor trap bottle's exit hose close to an evacuation source, but do not apply a vacuum.

- Direct the exhaust from the detector into a fume hood or exhaust vent.
- Ensure both hoses are free of restrictions.

Warning: Inhalation risk. Do not allow detector exhaust to enter the laboratory atmosphere.

#### To connect the vapor trap

1. Connect one end of the exhaust hose exiting from the rear of the detector directly onto one of the barbed fittings on the vapor trap bottle.

#### Vapor trap bottle and exhaust hose





- To avoid excessive electronic noise, do not kink the exhaust hose, which creates an unintended trap. The hose must slope downward, without sharply bending, when exiting the detector.
- To avoid operational problems, do not cut the exhaust hose.
- 2. Using the 1.5 meter (5-foot) hose, attach one end of the tubing to the remaining fitting on the bottle cap.

3. Position the other end of the tube at a perpendicular angle to a laboratory exhaust system that applies gentle vacuum. There should be a minimum negative pressure of -0.21 kPa (-0.00 bar, -0.03 psi) between the detector and the laboratory exhaust system.

#### Proper position of exhaust hose



**Caution:** To avoid excessive detector noise or loss of sample, do not create a strong vacuum on the gas exiting the detector. Loosely attach the end of the exhaust hose to the exhaust vent.

# Connecting the siphon drain in a closed drain system

In a closed drain system, the siphon drain tube is routed to the waste container from either the primary or secondary instrument stack.

**Recommendation:** At initial setup, fill the siphon with water or mobile phase. Failure to do so will delay the detector's satisfactory performance.

#### To route the siphon drain to the waste container

1. If the detector is located in the primary UPLC instrument stack, route the siphon drain tube to the right of the stack.

# Siphon drain tube routing for a configuration with the detector located in the primary stack



- 2. Insert the siphon drain tube into the clip.
- 3. An additional support for the siphon drain tube is in the detector door. Ensure the door is closed before operating the detector.
- 4. Insert the end of the convoluted tube onto the heat-formed siphon drain tube.

5. Route the convoluted tube to the waste container.



Waste container siphon tube connection

**Caution:** To prevent excessive detector noise and drift tube flooding, ensure that the siphon tube is not immersed in the solvent waste.

## 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 ELS detector installed in a split ACQUITY UPLC system



### **Required materials**

- Multi-detector drip tray kit
- T20 TORX<sup>®</sup> driver

#### To install the drip tray

- 1. Turn the ACQUITY UPLC ELS detector so that it is resting on its left side.
- 2. Using the T20 TORX driver, remove the screws that secure the four short, rubber feet to the bottom of the detector.



3. Using the T20 TORX driver, fasten the short and long rubber feet on to the bottom of the detector using 30-mm screws.



4. Secure the drip tray to the bottom of the detector by inserting the snap rivets in the unobstructed holes.

**Tip:** Not all rivets are required to secure the drip tray. The number of rivets you use depends on the type of detector you are securing it to.



- 5. Turn the ELS detector to its normal position.
- 6. Return the ACQUITY PDA or TUV detector to its original position atop the ELS detector.
- 7. Slide a waste line over the barbed drain fitting located on the right side of the drip tray, and route it to a suitable waste container.

# Connecting the nebulization gas to the nebulizer

**Caution:** To avoid contaminating the detector, connect the ACQUITY UPLC system during operation. Gas flow in the nebulizer creates a slight vacuum that can attract solvent or dust from the detector's inlet port.

Insert the Teflon gas inlet tubing into the quick-disconnect tubing fitting on the right side of the nebulizer.

**Tip:** The ACQUITY ELS detector requires only one nebulizer for the entire flow rate range of the ACQUITY UPLC system.

#### Connecting the gas inlet tubing



# Connecting the column to the detector

Connect the 15-cm (6-inch) piece of tubing (included in the startup kit) from the nebulizer's solvent inlet port to the column.

# **Connecting a second detector**

Connect the 35.6-cm (14-inch) piece of tubing (included in the startup kit) from the outlet of the optical detector to the inlet of the ELS 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 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



All of the functions in the following table are integrated into the ACQUITY UPLC system.

Signal connections	Description
Signal out	Provides a high resolution output for the sample signal.
	Output voltage range: -0.1 to 2.1 VDC. User may apply an offset to prevent negative-going signals due to drift during a chromatographic run.
Auxiliary out	Provides a high resolution output for monitoring the nebulizer temperature, drift tube temperature, or gas pressure.
	Output voltage range: -0.1 to 2.1 VDC.
Stop flow out	Stops the flow to the chromatographic system when a potential safety problem such as a low input gas flow condition, or temperature control problem occurs.
Switch out	Controls a timed event or threshold level and is a user-programmable auxiliary output.
Inject start in	Activates timed events by triggering the run-time clock to start.
Lamp on in	When triggered, ignites or extinguishes the lamp.
Chart mark in	When triggered, causes both analog output channels and the digital data sent to the data system to increase their value for a period of time.
Auto zero in	Calculates an offset value that, when added to the sample signal, makes the resulting baseline signal zero.

#### ACQUITY UPLC ELS detector analog-out/event-in connections

# Connecting to the electricity source

The ACQUITY UPLC ELS 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 electric shock

- use an SVT-type power cord in the United States and an HAR type (or better) in Europe. For information regarding which cord to use in other countries, contact your local Waters distributor.
- power-off and unplug the detector before performing any maintenance on the instrument.
- connect all components of the HPLC system to a common ground.

### To connect to the electricity source

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

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

### **Contents:**

Торіс	Page
Starting the detector	3-2
Setting up a run	3-5
Conserving lamp life	3-14
Changing chromatographic conditions	3-15
Shutting down the detector	3-15

# Starting the detector

Starting the detector entails powering-on the detector and each system instrument individually, as well as the ACQUITY workstation. It also involves starting the operating software (Empower or MassLynx). The entire startup initialization takes less than one minute. When completed, you should allow the detector to warm up for at least an hour before running an analysis.

See also: ACQUITY UPLC System Operator's Guide.

#### To start the detector

- 1. Turn the gas supply on.
- 2. Power-on the workstation.
- 3. Press the power switch on the top, left side of each instrument's door. Each system instrument beeps and runs a series of startup tests.

The power and status 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, each one's power LED shows steady green. The binary solvent manager's flow LED and the sample manager's run LED remain unlit. The detector's lamp LED shows steady green.
- 4. When the lamp is steady green, start Empower or MassLynx, and download an instrument or inlet method. You can monitor the ACQUITY console for messages and LED indications.
- 5. Set the parameters for the drift tube, nebulizer, and gas pressure in the ACQUITY console. See "Setting up a run" on page 3-5.

## **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.

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.

## ACQUITY UPLC ELS detector control panel



The detector control panel displays light scattering units, PMT gain factor, gas pressure, nebulizer temperature, and drift tube temperature. You can edit

these when the system is idle by clicking on the underlined value. You cannot edit detector parameters while the system is processing samples.

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

Control panel item	Description
Lamp On/Off LED	Imitates the actual lamp on/off LED on the front panel of the detector. This image should mimic the actual lamp on/off LED mode unless communications with the detector are lost.
Status	Displays the status of the current operation.
Light scattering units	Displays the current light scattering units.
Gain	Displays the photomultiplier tube (PMT) gain factor.
Gas pressure	Displays the current gas pressure in psi.
Nebulizer temperature	Displays the current temperature of the nebulizer.
Drift tube temperature	Displays the current temperature of the drift tube.
[ (Lamp On)	Ignites the detector lamp.
🥘 (Lamp Off)	Extinguishes the detector lamp.

## **Detector control panel items**

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 ELSD	Resets the detector, when present, after an error condition.

### Additional functions in the detector control panel (Continued)

Control panel function	Description
Help	Displays the console Help.

# Setting up a run

**Tip:** If you transfer a method from another manufacturer's ELS detector, operating conditions will need to be optimized for maximum performance. These conditions include drift tube, nebulizer power, PMT gain, and gas pressure parameters.



**Caution:** To avoid damaging the nebulizer, preflush columns with at least 10 column volumes of clean mobile phase before connecting them to the nebulizer. For example, flush a 2.1 × 50-mm column for 10 minutes at a rate of 0.5 mL/minute.

Before starting a run, you must set it up by specifying operating parameters and allowing all temperatures to stabilize.

#### To set up a run

- 1. Set the drift tube temperature.
- 2. Set the nebulizer parameter.
- 3. Set the PMT gain and gas pressure.
- 4. Equilibrate the detector for about an hour.

## Important parameters

Gain – Controls the full-scale sensitivity of the detector by defining the PMT gain factor from 1 to 1000. Each gain setting relates linearly with the actual light scattering signal.

Gas pressure – Gas pressure is controlled at the nebulizer by a pneumatic control system.

Filter time constant – Lets you adjust the noise filter (time constant) to achieve the optimum signal-to-noise ratio.

You need to program several other parameters, depending on the functions you may want to perform during a run.

Parameter	Default	Range
Data rate	10 Hz	1, 2, 5, 10, 20, 40, or 80 Hz
PMT gain	500	0 to 1000
Filter time constant	Fast	Slow, Normal, Fast, Off
Sample full-scale	2000	10 to 2000
Sample offset	0 mVDC	±2000 mVDC
Lamp state	On	On, off
Nebulizer gas pressure	280 kPa (2.8 bar, 40 psi)	Off, 140 to 410 kPa (1.4 to 4.1 bar, 20 to 60 psi)
Auto zero on inject	Yes	Yes, No
Auto zero on gain change	Disable	Disable, Zero, Baseline
Nebulizer heater power level	Off	Heating (0 to 100%), Off
Nebulizer cooler power level	Cooling	Cooling, off
Drift tube heater set-temp	50 °C (122 °F)	Off, 5 to 100 °C (41 to 212 °F)
Drift tube heater alarm band	±25 °C (±45 °F)	5 to 25 °C (41 to 77 °F)
Auxiliary output	Nebulizer	Drift Tube, Nebulizer Temperature, Gas Pressure
Voltage offset	0 mVDC	±2000 mVDC
Threshold level	1.0 LSU	±2.0 LSU
Threshold switch mode	Off	On, Off, Pulse, R.Wave, No Change
Pulse switch mode period	0.1 sec	0.1 to 60.0 sec
Rect Wave switch mode period	0.2 sec	0.2 to 60.0 sec

## ACQUITY UPLC ELS detector method parameters

# **Standard UPLC settings**

The following table lists the standard UPLC operating settings for the detector.

#### Standard UPLC settings

Parameter	Setting
Drift tube	50 °C (122 °F)
Nebulizer	Cooling
Gas pressure	280 kPa (2.8 bar, 40 psi)
Gain	500

## Setting the drift tube temperature

**Requirement:** The drift tube temperature must be high enough to remove all solvent; otherwise, detector noise will result.

#### Tips:

- A drift tube temperature setting that is too high could vaporize the sample and cause loss of sensitivity.
- A drift tube temperature setting that is too low will result in detector noise and possibly flood the detector.
- For reversed phase chromatography, start with a drift tube temperature of 50 °C (122 °F).

#### To set the drift tube temperature

1. In the console, select ELS Detector from the system tree. **See also:** "ACQUITY UPLC console" on page 1-12.

#### ACQUITY UPLC Console for System ELSD\_Beta 5 on Node CORE-EMPOWER-29 - [ELS Detector] \_ 8 × ACOUITY LIPLC Susten Control Configure Maintain Troubleshoot Help Power Lamp Binary Solvent Manager Sample Manager ELS Detector conditions temperatures performance Maintenance Counters 100. ° c ELSD Lamp Life Logs 0.2368 150 Drift Tube 100. 778.0 hours 2000.0 0.0 31.4 ° ⊂ Nebulizer Gain 500 Off Auto Zero Auto Zero Home Home Realtime 39.9 Gas psi 40.0 ELSD Signa 0.40 and dimensional 31.40 System Status 30.20 I SD Drift Tube Temperatu 99,94 39.89 39.890 -10.00

## ACQUITY UPLC console window

2. In the temperatures area, click the Drift Tube field, which contains an underlined set point value.

Alternatives: You can specify the drift tube temperature

- in the Empower or MassLynx detector control panel (see page 3-3).
- in the Empower or MassLynx method editor.

**Rule:** When the set point value is not underlined, Empower or MassLynx software controls the system. You cannot control or modify any system functions from the console until the software completes the run and relinquishes control of the system.

#### **ELSD Drift Tube Temperature dialog box**

ELSD Drift Tube Temperature
Set Point:
50.0 °C
Temperature Limit:
± 25 or
- <u> </u>
S 🛞 🔊

3. Enter the drift tube heater temperature set point and limit, and then click OK 🐼.



**Warning:** The flash point of a chemical is the lowest temperature at which a flame will propagate through the vapor of a combustible material to the liquid surface. It is determined by the vapor pressure of the liquid. Only when a sufficiently high concentration is reached, can a vapor support combustion.

## Setting up the nebulizer parameters

The nebulizer has three settings: heating, cooling, and Off.

The nebulizer heater, which is regulated by a heater power level setting, resides in the nebulizer heat exchanger. The nebulizer heater allows more of the nebulized sample to enter the drift tube.

**Tip:** As you increase the amount of material entering the drift tube, you might need to compensate by increasing the temperature of the drift tube.

The nebulizer cooler, which is regulated by an on/off setting, decreases the nebulizer temperature for reverse phase liquid chromatography.

**Requirement:** The nebulizer temperature must be at least 5 °C (9 °F) below the drift tube temperature.

#### To set up the nebulizer parameters

- 1. In the console, select ELS Detector from the system tree.
- 2. In the Temperatures area, click the Nebulizer field, which contains an underlined set point value.

Alternatives: You can specify the set point

- in the Empower or MassLynx detector control panel (see page 3-3).
- in the Empower or MassLynx method editor.

#### **ELSD Nebulizer Setup dialog box**

ELSD Nebulizer Setup
Mode:
Power Level:
۲

3. Select the nebulizer mode from the drop-down list. Heating and cooling are mutually exclusive.

#### Nebulizer modes

Mode	Description
Cooling	Turns the nebulizer cooler on and deactivates the Power Level text box.
Off	Turns the nebulizer heater and cooler off and deactivates the Power Level text box.
Heating	Turns the nebulizer heater on and activates the Power Level text box.

4. If you selected Heating from the nebulizer mode drop-down list, enter the nebulizer power percent level, and then click OK 🐼.



**Warning:** The flash point of a chemical is the lowest temperature at which a flame will propagate through the vapor of a combustible material to the liquid surface. It is determined by the vapor pressure of the liquid. Only when a sufficiently high concentration is reached, can a vapor support combustion.

# Setting the PMT gain factor

The PMT gain factor controls the full-scale sensitivity of the detector by defining a setting from 1 to 1000. Each gain setting has a linear relationship with the actual light scattering signal.

**Tip:** Start with a gain setting of 500 to display all the peaks in the chromatogram on scale. You can then enlarge the chromatogram with the software to see all the minor peaks.

## To set the PMT gain factor

- 1. In the console, select ELS Detector from the system tree.
- 2. In the conditions area, click the Gain field, which contains an underlined gain factor value.

Alternatives: You can specify the gain factor

- in the Empower or MassLynx detector control panel (see page 3-3).
- in the Empower or MassLynx method editor.

**Rule:** When the gain factor is not underlined, the software controls the system. You cannot control or modify any system functions from the console until the software completes the run and relinquishes control of the system.

#### **ELSD Gain dialog box**



3. Enter the PMT gain factor in the Gain field. Once you enter a gain factor, the PMT is activated. Click OK *⊗*.

## Setting the gas pressure

The gas pressure setting monitors the condition of gas flow through the nebulizer. The nebulizer has maximum gas pressure to 410 kPa (4.1 bar, 60 psi).

Recommendation: Gas pressure should be high enough to prevent

- extended exposure time of the sample in the drift tube area. Gas pressure that is too low could cause higher-than-desired dispersion of particles and, consequently, loss of sensitivity.
- the formation of large particles that could cause detector noise.

Tip: The recommended starting pressure is 280 kPa (2.8 bar, 40 psi).

The following table lists the nebulizer's approximate pressure to flow rates.

#### Approximate gas pressure to gas flow rates

Pressure	Flow (L/min) <sup>a</sup>
140 kPa (1.4 bar, 20 psi)	1.5
210 kPa (2.1 bar, 30 psi)	2.0
280 kPa (2.8 bar, 40 psi)	2.5
350 kPa (3.5 bar, 50 psi)	3.0
410 kPa (4.1 bar, 60 psi)	3.5

a. Standard liters per minute of nitrogen, according to nebulizer manufacturer settings.

#### To set the gas pressure

- 1. In the console, select ELS Detector from the system tree.
- 2. In the conditions area, click the Gas field, which contains an underlined gas flow value in psi.

**Alternative:** You can specify the gas pressure in the Empower or MassLynx detector control panel.

**Rule:** When the gas pressure psi value is not underlined, the software controls the system. You cannot control or modify any system functions from the console until the software completes the run and relinquishes control of the system.

#### **ELS Detector Gas Pressure dialog box**

ELSD Gas Pressure	
Gas Pressure:	
Off psi	
8	

3. Specify a gas pressure (psi) value. Once you enter a value, the gas valve is activated.

**Requirement:** An external minimum supply of 450 kPa (4.5 bar, 65 psi) is required for the gas regulator to activate.

4. To shut the gas regulator off, enter 0 or Off in the psi field.



**Caution:** To prevent the detector from flooding, ensure that no liquid is flowing into it when you specify the gas value of 0.

**Recommendation:** When running from an unlimited gas source, such as a nitrogen generator, Waters recommends using a low gas flow when the detector is idle.

5. Click OK 🧭.

## Stop flow output switch

The detector has an internal, dedicated switch output that activates when the drift tube heater, nebulizer heater/cooler, or gas pressure fails. The stop flow output switch communicates a signal to the binary solvent manager through the network to stop liquid flow to the ELS detector. When the cause of the error is corrected, the switch can be reset by reactivating the previously faulty detector function or by right-clicking in the control panel and selecting Reset ELSD.

# **Conserving lamp life**

To conserve the tungsten lamp without shutting down the detector, you can leave the instrument on, and extinguish only the lamp.

Without powering-off the system, you can conserve lamp life as follows:

- Extinguishing the lamp from the console when you are not running samples.
- Extinguishing the lamp from the control panel by clicking Lamp 🥘.
- Programming a timed event to extinguish the lamp when you are not running samples.

**Recommendation:** To save lamp life, extinguish the lamp when you do not plan to run samples for at least four hours.

#### To ignite or extinguish the lamp

- 1. In the console, select ELS Detector from the system tree.
- 2. Click Lamp 🥘.
- 3. In the Lamp dialog box, click Yes.

#### To determine the amount of time the lamp has been ignited

- 1. In the console, select ELS Detector from the system tree.
- 2. In the performance area, the Lamp Life status bar shows the total number of hours the lamp has been ignited since it was installed.

**Tip:** If the Lamp Life status bar is yellow, the lamp has exceeded its lamp life maintenance threshold as set by the user.

# **Changing chromatographic conditions**

When the detector is plumbed to the chromatographic system and the buffer nature or pH of the mobile phase is being changed, you should remove the previous buffered mobile phase from the fluid path.



**Caution:** Failure to remove any buffered mobile phase from the fluid path before changing new conditions could result in precipitation and clogging of the nebulizer.

**Recommendation:** Consult the *ACQUITY UPLC System Operator's Guide* before you perform this procedure.

#### To change chromatographic conditions

1. Set the drift tube temperature at the appropriate desolvation temperature setting.

**Tip:** 50 °C (122 °F) is an appropriate desolvation temperature for most solvents.

- 2. To remove buffered mobile phase from the fluid path of the detector, replace the buffered mobile phase with 100% HPLC-grade water and flush the system for 30 minutes at 0.5 mL/min at 280 kPa (2.8 bar, 40 psi).
- 3. Replace the 100% HPLC-grade water with new mobile phase and equilibrate the system for 30 minutes at 0.5 mL/min at 280 kPa (2.8 bar, 40 psi).

# Shutting down the detector

Before you power off the detector, you need to remove any buffered mobile phase present in the fluid path.



**Caution:** To avoid damaging your column, remove all buffers from the column before you shut down the detector.

#### To shut down the detector

1. Remove all buffers from the column and detector by flowing an unbuffered mobile phase through the system.

- 2. Set the pump to Off.
- 3. Allow the nebulization gas to flow through the detector for 10 minutes to drain the evaporation tube and detection chamber.
- 4. Stop the gas flow.
- 5. Power-off the detector.

## Tips:

- It is not harmful to leave the detector powered-on overnight when it is not in use. To increase the life of the lamp, you can extinguish it by clicking Lamp .
- It is recommended to leave the nebulizer gas flowing, at full or reduced rate, when the mobile phase pump has been shut down or the solvent flow set to zero.

Caution: To avoid flooding the detector

- do not allow solvent flow to continue if no gas flow is present.
- do not shut off the drift tube heater if solvent and gas pressure is allowed.



#### **Contents:**

Торіс	Page
Preparing for the run	4-2
Creating the test method	4-2

This chapter illustrates how to perform a run using an evaporative light scattering detector.

Before you begin this procedure, your detector must be set up and configured as described in Chapter 2 and Chapter 3.

# Preparing for the run

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

#### To prepare for a run

- 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 mobile phase of HPLC-grade water with 0.05% TFA.
- 2. Submerge line A1 in the reservoir bottle containing the 0.05% TFA/water mixture. This mobile phase will be referred to as "A".
- 3. Prepare a mobile phase of acetonitrile with 0.05% TFA.
- 4. Submerge line B1 in the reservoir bottle containing the 0.05% TFA/acetonitrile mixture. This mobile phase will be referred to as "B".
- 5. Prime the ACQUITY system.
- 6. After the ACQUITY system has been primed, equilibrate the ACQUITY UPLC 2.1 × 50 mm BEH C18 column with 95/5 A/B.

# Creating the test method

The sample used in this experiment is oxymetazoline. It is also possible to use opthalmic drops or nasal spray solutions containing the decongestant oxymetazoline at concentrations ranging from 0.025 to 0.05%. These products are commonly found in pharmacies throughout the world under both generic and brand names. Brand name products would be Visine L.R.<sup>®</sup> or Afrin<sup>®</sup>.

Set up a gradient run from 5% B to 95% B in 90 seconds with these parameters:

- Flow rate: 0.84 mL/min
- Injection volume: 2 µL
- Nebulizer: cooling

- Gain: 500
- Drift tube temperature: 50 °C (122 °F)
- Gas pressure:

The following figure shows a representative ELS detector chromatogram of one of the solutions detailed above.

#### ELS detector chromatogram



The early eluting peaks correspond to the formulation agents used in the opthalmic solution. The peak eluting after 0.75 min is oxymetazoline.

# **5** Maintaining the Detector

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# **Contacting Waters technical service**

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

However you contact Waters, be prepared to provide your detector's serial number and this information:

- Error message (if any)
- Nature of the symptom
- Instrument serial numbers
- Flow rate
- Operating gas flow
- 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 open the power supply cover. The power supply does not contain user-serviceable parts.

**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.

## **Proper operating procedures**

To ensure your system runs efficiently, follow the operating procedures and guidelines in Chapter 2.

## **Spare parts**

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

**Tip:** Do not remove the detector's top cover. No user-serviceable parts are inside. If you open the door, close it firmly before resuming normal operation.

**Recommendation:** 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.

# Maintaining the leak sensor

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

## **Resolving detector leak sensor errors**

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



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



Caution: To avoid scratching or damaging the leak sensor

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

## **Required materials**

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

#### To resolve a detector leak sensor error

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

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

- 2. Open the detector door, gently pulling its right-hand edge toward you.
- 3. Locate the source of the leak, and make the repairs necessary to stop the leak.
**Caution:** To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

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



**Tip:** If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see page 5-8).

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



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



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



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



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

# Replacing the detector's leak sensor



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

#### **Required materials**

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

#### To replace the detector leak sensor

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



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



4. Unpack the new leak sensor.

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



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

# **Clearing the flow path**

To maintain the best detector performance, Waters suggests removing the mobile phase from the flow path whenever the detector will remain idle for an extended period of time.



**Caution:** To avoid damaging your column, remove the column before you remove the mobile phase from the flow path.

#### To clear the flow path

1. Set the drift tube temperature at the appropriate desolvation temperature setting.

**Tip:** 50 °C (122 °F) is an appropriate desolvation temperature for most solvents.

2. Replace the buffered mobile phase with 100% HPLC-grade water and flush the system for 30 minutes at 0.5 mL/min, at 280 kPa (2.8 bar, 40 psi).

# Replacing the lamp cartridge

**Recommendation:** Because lamp alignment is critical to proper detector operation, it is recommended that only Waters pre-aligned lamp cartridges be used.

# **Required materials**

- #2 Phillips screwdriver
- Lamp cartridge

#### To replace the lamp cartridge

- 1. Power-off the lamp:
  - To power-off the lamp manually, click ELS 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.

**Alternative:** To save time, leave the detector powered on for 15 minutes after you power-off the lamp. Doing so will allow the fan to blow cool air on the lamp, cooling it faster.



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

- 3. Allow the lamp to cool for 30 minutes (or 15 minutes with the fan running), and then open the door, gently pulling its right edge toward you.
- 4. Using a Phillips screwdriver, completely loosen the two captive screws and pull the assembly out slightly to relieve stress on the lamp connector wires.



#### Removing and replacing the lamp cartridge

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



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

5. Disconnect the lamp connector from the front panel.

# Lamp cartridge

**Disconnecting the lamp connector** 

6. Remove the lamp cartridge assembly and replace it with a new one.

7. Reconnect the lamp connector.



- Do not touch the new lamp with your bare fingers. The oil from your fingers can greatly reduce the lifetime of the lamp. If fingerprints do get on the lamp, remove them with a lint-free tissue saturated with ethanol.
- To avoid misaligning the lamp, do not touch the bulb height adjustment lever.
- 8. Push the assembly back in and tighten the two captive screws with a Phillips screwdriver.
- 9. Close the detector door.
- 10. Power-on the detector, and then wait about 30 minutes for the detector 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.

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

#### Change Lamp dialog box

CI	iange Lamp			
	Currently Insta	lled Lamp		
	State:	On		
	Total ignitio	ns: 283		
	Date Installed	Serial Number		Hours
	27-Apr-05	1234567890		478.08
				1185.98
				0.12
				0.07
				147.40
				13.32
	,			
		NewLamp	Print	Close
		How Edinp		

12. Click New Lamp.

#### New Lamp dialog box

New Lamp				
Serial number of new la	amp:			
ОК	Cancel			

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

# **Replacing the nebulizer**

# **Required materials**

- 5/16-inch wrench
- ACQUITY nebulizer
- Nebulizer packing ring

#### To replace the nebulizer

- 1. Stop the liquid flow and remove the solvent inlet line.
- 2. Power off the detector and disconnect the power cable from the rear panel.



Warning: To avoid burn injuries, do not touch the nebulizer until its temperature cools to less than 30 °C, as displayed on the console. If its temperature exceeds 30 °C, let the nebulizer cool in one of two ways before touching it:

- Wait 30 minutes after powering-off the detector.
- Wait 10 minutes after specifying cooling in the ELSD Nebulizer Setup dialog box (see page 3-10).
- 3. Open the detector door, gently pulling its right edge toward you.

4. Use a 5/16-inch wrench to loosen the compression screw in the front of the nebulizer that holds the inlet tubing, and then remove the solvent inlet tubing.

Removing the solvent inlet tubing with wrench



- 5. Stop the gas flow.
- 6. Push in the quick-disconnect tubing fitting on the right-hand side of the nebulizer, and pull out the gas inlet tubing.



7. Push in and turn the nebulizer counterclockwise so that the quick-disconnect tubing fitting is at the twelve o'clock position. Then remove it from the nebulization chamber.



- 8. Remove the packing ring from the old nebulizer and slide it onto the new nebulizer. If the old packing ring is damaged, replace it with a new one.
- 9. Align the two pins inside the desolvation chamber with the grooves in the new nebulizer. The quick-disconnect tubing fitting will be at the twelve o'clock position.



- 10. Push the nebulizer into the nebulization chamber, and turn it clockwise until it snaps into place.
- 11. Insert the gas inlet tubing into the quick-disconnect tubing fitting on the right-hand side of the nebulizer.
- 12. Reconnect the solvent inlet tubing to the front of the nebulizer.
- 13. Power-on the detector.

# Cleaning the nebulizer using sonication

#### To clean the nebulizer using sonication (ultrasonic agitation)

- 1. Stop the liquid flow, and remove the solvent inlet line.
- 2. Power-off the detector and disconnect the power cable from the rear panel.



Warning: To avoid burn injuries, do not touch the nebulizer until its temperature cools to less than 30 °C, as displayed on the console. If its temperature exceeds 30 °C, let the nebulizer cool in one of two ways before touching it:

- Wait 30 minutes after powering-off the detector.
- Wait 10 minutes after specifying cooling in the ELSD Nebulizer Setup dialog box (see page 3-9).
- 3. Open the detector door, gently pulling its right edge toward you.
- 4. Use a 5/16-inch wrench to loosen the compression screw that holds the inlet tubing in place, and then disconnect the solvent inlet tubing.

#### Removing the solvent inlet tubing with wrench



a. Remove the solvent inlet tubing from the nebulizer.

- 5. Stop the gas flow.
- 6. Push in the quick-disconnect tubing fitting on the right side of the nebulizer, and pull out the gas inlet tubing.



7. Push in and turn the nebulizer counterclockwise so that the quick-disconnect tubing fitting is at the twelve o'clock position, and then remove it from the nebulization chamber.



- 8. Remove the red packing ring from the nebulizer.
- 9. Place the nebulizer upright in a beaker so that the solvent inlet fitting stands up.

10. Pour 100% HPLC-grade water or a mixture of organic solvent compatible with your mobile phase into the beaker, but do not submerge the gas inlet fitting or solvent inlet fitting in the water.

#### Nebulizer in the beaker



- 11. Place the beaker in an ultrasonic bath for 10 to 15 minutes.
- 12. Remove the beaker from the bath.
- 13. Remove the nebulizer from the beaker.
- 14. Insert the gas inlet tubing into the quick-disconnect tubing fitting on the right side of the nebulizer, and place the nebulizer in a dry beaker so that it stands up.
- 15. Run gas at 410 kPa (4.1 bar, 60 psi) for 5 to 10 minutes through the nebulizer to blow out any excess liquid.
- 16. Reinstall the nebulizer. See "Installing the nebulizer assembly" on page 2-5.
- 17. Reset the system to operating conditions and evaluate chromatography.

# **Cleaning the drift tube**

#### To clean the drift tube

1. In the console, select ELS Detector from the system tree.

- 2. In the temperatures area, click the Nebulizer field, which contains an underlined set point value. The ELSD Nebulizer Setup dialog box appears.
- 3. Select On from the nebulizer mode drop-down list.
- 4. Enter a nebulizer power percent level of 75%.
- 5. In the temperatures area, click the Drift Tube field, which contains an underlined set point value. The ELSD Drift Tube Temperature dialog box appears.
- 6. Enter a drift tube heater temperature set point of 100 °C (212 °F), and then click OK 🔗.
- 7. Remove the column.
- 8. Flush the system with 100% HPLC-grade water or a mixture of organic solvent compatible with your mobile phase for 60 minutes at 1 mL/min.
- 9. Reset the system to operating conditions.
- 10. Reinstall the column.
- 11. Evaluate chromatography.

# Servicing the vapor trap

#### To service the vapor trap

1. Unscrew the vapor trap bottle from the cover, and empty the contents of the bottle into an appropriate waste container.

#### Removing the vapor trap bottle from the cover



2. Replace the vapor trap cover.

# Testing noise and drift

#### To test the detector noise and drift

- 1. In the console, select ELS Detector from the system tree.
- 2. Click Troubleshoot > Noise and drift.
- In Test Parameters, select Noise Test, and then click Start.
  Tip: Click Results to display intermediate readings.
- 4. When results appear, verify that they are acceptable.

**Tip:** You can determine acceptable values after performing multiple tests over a period of time.

5. In Test Parameters, select Drift Test, and then click Start.

# **Replacing the fuses**



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

The detector requires two 100 to 240 VAC, 50 to 60 Hz, F 5.0-A, 250-V FAST BLO,  $5 \times 20$  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 fits above the power entry module on the rear panel of the detector. With minimum pressure, withdraw the spring-loaded fuse holder.



3. Remove and discard the fuses.

- 4. Make sure that the new fuses are properly rated for your requirements. 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.

# Troubleshooting

The following table contains general hardware troubleshooting for the ELS detector.

Symptom	Possible cause	Corrective action
Both LEDs unlit	No power	Test electrical outlet for power.
	Cable loose or defective	Inspect line cord connections.
	Open (spent) or defective fuse	Replace fuse (see page 5-22).

#### **ELS** detector troubleshooting

# 6 Optimizing Detection and Preparing Solvents

Proper solvent selection and preparation are critical in differential evaporative light scattering detection to prevent baseline changes such as drift, noise, or an erratic baseline. This chapter presents information on:

- Detector performance
- Common solvent problems
- Solvent selection
- Degassing a solvent



**Warning:** To avoid chemical hazards, always observe safe laboratory practices when operating your system. Refer to the Material Safety Data Sheets shipped with solvents for handling information.

#### **Contents:**

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Optimizing detector performance	6-2
Selecting a solvent	6-2
Optimization protocol	6-4

# Optimizing the mobile phase

Particulate matter in the mobile phase increases the background and the noise. In most cases, distilled water and HPLC-grade solvents are sufficient. When comparing solvents, the most critical parameter is the amount of residue after evaporation, which should be less than 1 ppm.

The mobile phase should not contain nonvolatile solvent modifiers, such as phosphoric acids, sulfuric acid, phosphates, and sulfates. MS-compatible, volatile solvent modifiers, such as  $CF_3COOH$  (Trifluoroacetic Acid) and  $CH_3COOH$  (Acetic Acid), can be used with the ELS detector.

# Sample pretreatment

If the sample contains any particulate matter, it is recommended that it be filtered through a 0.2- $\mu$ m or 0.45- $\mu$ m filter before injection.

# **Column treatment**

The chromatographic column contains microparticles to separate the compounds being analyzed. Under certain circumstances, the column packing will undergo chemical and/or mechanical breakdown, which can introduce particulate matter into the detector, followed by an increase in noise.

Column breakdown depends on particle size, the type of column used, column manufacturer, and the nature of the mobile phase. For example, high pH will degrade silica-based columns.



**Caution:** To avoid damaging the nebulizer, preflush columns with at least 10 column volumes of clean mobile phase before connecting them to the nebulizer. For example, flush a  $2.1 \times 50$ -mm column for 10 minutes at a rate of 0.5 mL/minute.

# Selecting a solvent

An ideal solvent for your analysis has good solubility characteristics for your application, and gives satisfactory baseline noise performance.

# Solvent quality

Use spectral-grade or HPLC-grade solvents to ensure reproducible results, and minimal instrument maintenance.

A dirty or impure solvent can cause these problems:

- Baseline noise and drift
- Plugged columns
- Blockages in the fluid path

#### **Preparation checklist**

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

- Filter solvents with a 0.45-µm filter.
- Degas and/or sparge the solvent.
- Protect solvents from shock and drafts.

#### Water

Use water only from a high-quality water purification system. If the water system does not provide filtered water, filter it through a 0.45-µm membrane filter before use. The total organic carbon reading should be as low as possible (<5 ppb).

#### **Buffer compatibility**

The detector cannot be used with nonvolatile solvents such as salt-buffer solutions. Volatile modifiers, such as acetic acid and ammonium formate, may be used successfully.

Mobile phase modifiers that are suitable for mass spectrometry (for example, ammonium acetate, ammonium bicarbonate, ammonium formate) can be used for evaporative light scattering detection in concentrations of less than 0.01 M, or 0.1% (v/v %). Higher concentrations of nonvolatile materials in the mobile phase will cause greater baseline noise, lower sensitivity, and nebulizer and small-bore-tubing blockages. High purity mobile phases with low boiling points are recommended.



**Caution:** Do not use nonvolatile buffers. They cause noise and block detector components.

#### **Organic solvent compatibility**

The ELS detector is fully compatible with standard chromatographic solvents including both reversed phase and normal phase organic solvents. The limitations of detector solvent compatibility are limits imposed by the chromatographic system in use.

#### Tetrahydrofuran (THF)

When you use unstabilized THF, ensure that it is fresh. Previously opened bottles of THF contain peroxide contaminants, which cause baseline drift.



Warning: THF contaminants (peroxides) are potentially explosive if concentrated or evaporated to dryness.



Warning: Fire and explosion hazard. Do not use air as the carrier gas when the mobile phase contains flammable components.

# **Optimization protocol**

You must select the appropriate application operating parameters to obtain the best performance from your detector. Nebulizer gas flow rate, nebulizer temperature, and drift tube temperature must all be optimized for the best results.

# Nebulizer gas pressure

Increased nebulizer gas flow rate causes a decreased signal response because of the resulting formation of smaller droplets that scatter less light. Lower gas flow rates tend to be more favorable because less gas is consumed and a better sensitivity is achieved. However, at some point this benefit is offset by an increase in baseline noise from the inefficient nebulization of the eluent, resulting in large droplets. The particle size of these droplets results in complex scattering mechanisms and poor detector performance. If you reduce the eluent flow, you must also reduce the nebulizer gas flow rate to maintain the optimum nebulized droplet size. Never decrease the nitrogen flow rate below 170 kPa (1.7 bar, 25 psi).

#### Nebulizer temperature

The detector starts more quickly when you decrease the temperature of the nebulizer. Nevertheless, increasing the temperature reduces the viscosity and surface temperature of the droplets. Note, however, that specifying too high a nebulizer temperature can cause the chromatography to deteriorate if the solvent in the nebulizer boils. Evidence of this deterioration is manifested as increased baseline noise, the result of spiking.

**Recommendation:** An increased nebulizer chamber temperature results in an increased amount of material nebulized and introduced into the drift tube that may require an increased drift tube temperature. Therefore, a lower nebulizer temperature is recommended.

Applying heat to the nebulizer chamber increases the amount of analyte vapor in the drift tube. This increases signal levels but also tends to require a higher drift tube temperature, which can cause problems for samples that require drift tube temperature to be minimized.

#### **Drift tube temperature**

The effects of modifying the evaporator temperature are not as significant as those that result from changing the nebulizer gas flow rate. However, the evaporator temperature must be high enough to evaporate the solvent and sufficiently dry the particle plume without adversely affecting the sample. If the evaporator temperature is too low, the solvent can saturate the diffuser, resulting in high noise and spikes. If the evaporator temperature is too high, the sample may be volatilized, resulting in a small response.

#### Selecting the optimum temperature

When setting up a system, set the temperature of the drift tube to 50 °C (122 °F) if you are using reversed-phase chromatography. You can adjust these values during method optimization.

If you think your compound is thermally labile, you can use a lower temperature to improve detector sensitivity to minimize thermal loss. However, for a given solvent and flow rate, there is a point at which the noise in the chromatogram dramatically increases because all of the eluent is not vaporized. At higher flow rates, higher temperatures are required to minimize the noise level.

# A Safety Advisories

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

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Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-12

# Warning symbols

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

# Task-specific hazard warnings

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

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

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



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



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of needle puncture.)



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



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



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

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

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

# **Specific warnings**

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

#### **Burst warning**

This warning applies to Waters instruments fitted with nonmetallic tubing.



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

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

#### Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



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

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

#### Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



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

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



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

#### **Biohazard warning**

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



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

#### Chemical hazard warning

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

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

# **Caution symbol**

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



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

# Warnings that apply to all Waters instruments

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

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



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

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

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

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

**警告**: 當在有壓力的情況下使用聚合物管線時, 小心注意以下幾點。

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



警告: 当有压力的情况下使用管线时,小心注意以下几点:

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

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

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

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

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



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

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

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

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

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

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

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

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

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


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



Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.



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



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



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

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



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



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

# **Electrical symbols**

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

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

# Handling symbols

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

<u> </u>	Keep upright!
×	Keep dry!
¥	Fragile!
Ж	Use no hooks!

A-14 Safety Advisories

# **B** Specifications

# **ACQUITY UPLC ELS detector specifications**

Attribute	Specification
Height	21.6 cm (8.5 inches)
Depth	51.8 cm (20.4 inches)
Width	34.3 cm (13.5 inches)
Weight	14.7 kg (32.5 pounds)

# Physical specifications

#### **Environmental specifications**

Attribute	Specification
Operating temperature	4 to 30 °C (39.2 to 86 °F)
Operating humidity	<90%, noncondensing
Shipping and storage temperature	$-30$ to $60\ensuremath{^\circ C}$ (–22 to 140 $\ensuremath{^\circ F}$ )
Shipping and storage humidity	<90%, noncondensing

# **Electrical specifications**

Attribute	Specification
Protection class <sup>a</sup>	Class I
Overvoltage category <sup>b</sup>	II
Pollution degree <sup>c</sup>	2
Moisture protection <sup>d</sup>	Normal (IPXO)

#### **Electrical specifications (Continued)**

Attribute	Specification
🕂 Line voltages, nominal	Grounded AC
Input voltage range	85 to 264 VAC
Input frequency range	47 to 63 Hz
Fuse	100 to 240 VAC, 50 to 60 Hz, F 5.0-A, 250-V FAST BLO, 5 × 20 mm (IEC)
Max VA input	200 VA

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.

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 identi-fies protection against dust, if applicable.

Item	Specification
Nebulizer	Front mounted, snap-in design
Temperature control nebulizer chamber	Heater 0 to 100%, thermally controlled, cooler on/off
Gas	Nitrogen, to be supplied, at least 490 kPa (4.9 bar, 65 psi)
Temperature range drift tube	0.1 °C increments, feedback accuracy to 0.1 °C
Eluent flow rate maximum	100% water at 2 mL/min

#### **Operating specifications**

# **Operating specifications (Continued)**

Item	Specification
Filter setting	Hamming, 0 to 5.0 seconds in 0.1 second increments
Unattended operation	Leak sensors, full diagnostic data captured through console software

# Optical component specifications

Item	Specification
Optics	Heated optics bench (constant 50 °C [122 °F])
Light source	Tungsten halogen polychromatic, front mounted, pre-aligned, user installable
Lamp calibration	PMT calibration/normalization to compensate for lamp degradation over time
Lamp normalization	On demand and at startup diagnostic corrects for lamp signal output decrease
Detector	Photomultiplier tube
PMT calibration	Based on individual PMT
Voltage range	0 to 1250 VDC

# Data specifications

Item	Specification
Range	0.1 to 2000 light scattering units full scale
Analog outputs	Two, LS units and nebulizer, drift tube
Analog data output signal range	-0.1V to +2.0 VDC fully attenuated
Attenuation settings	10 to 2000 LSU full scale; 10 to 2000 mV
RS232 output	Used for service diagnostics and firmware updates

# Data specifications (Continued)

Item	Specification
Digital data	24 bit digital data, 80 Hz using Ethernet connection
Analog output	Two, fully programmable output either ELS detector signal and temperature, can monitor temperature of either nebulizer, drift tube 0 to 2 volts, with user programmable voltage or data offset

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