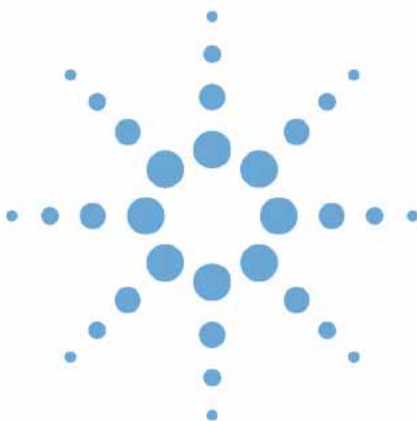




Agilent 1200 Series Diode Array and Multiple Wavelength Detectors

G1315B / G1365B



User Manual



Agilent Technologies

Notices

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Manual Part Number

G1315-90006

Edition

Edition 02/2006

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

Manual Structure

The **User Manual G1315-90006** (English) and its localized versions contain a subset of the Service Manual and is shipped with the detector in printed matter.

Latest versions of the manuals can be obtained from the Agilent web.

The **Service Manual G1315-90106** (English) contains the complete information about the Agilent 1200 Series Diode Array and Multiple Wavelength Detectors. It is available as Adobe Reader file (PDF) only.

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In This Manual...

This manual contains information for using your Diode Array and Multiple Wavelength Detectors.

1 Introduction to the Detector

This chapter gives an introduction to the detector, instrument overview and internal connectors.

2 Site Requirements and Specifications

This chapter gives information on environmental requirements, physical and performance specifications.

3 Installing the Detector

This chapter describes the installation of the detector.

4 Using the Detector

This chapter provides information on how to set up the detector for an analysis and explains the basic settings.

5 How to optimize the Detector

This chapter provides information on how to optimize the detector.

6 Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

7 Maintenance and Repair

This chapter provides general information on maintenance and repair of the detector.

8 Maintenance

This chapter describes the maintenance of the detector and the required tests.

9 Parts and Materials for Maintenance

This chapter provides information on parts for maintenance.

A Appendix

This chapter provides safety and other general information.

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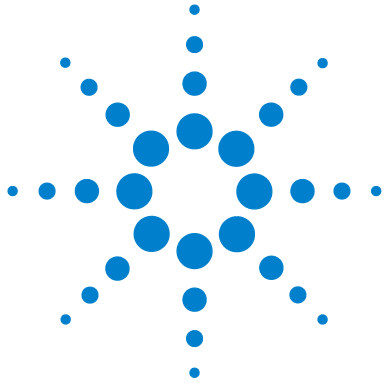
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1

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This chapter gives an introduction to the detector, instrument overview and internal connectors.



Introduction to the Detector

The detector is designed for highest optical performance, GLP compliance and easy maintenance. It includes the following features:

- long-life deuterium and tungsten lamps for highest intensity and lowest detection limit over a wavelength range of 190–950 nm,
- no loss in sensitivity for up to five wavelengths simultaneous,
- programmable slit from 1–16 nm for complete optimization of sensitivity, linearity and spectral resolution,
- optional flow-cell cartridges with I.D. tag (standard 10 mm 13 µl, semi-micro 6 mm 5 µl, micro 3 mm 2 µl, 80 nl/500 nl 10 mm, high pressure 10 mm 1.7 µl and prep-cells) are available and can be used depending on the application needs,
- easy front access to lamps and flow cell for fast replacement, and
- built-in holmium oxide filter for fast wavelength accuracy verification,
- built-in temperature control for improved baseline stability (G1315B DAD and G1365B MWD).

For specifications, see [“Performance Specifications”](#) on page 26.

Optical System Overview

Optical System

The optical system of the detector is shown in [Figure 1](#). Its illumination source is a combination of a deuterium-arc-discharge lamp for the ultraviolet (UV) wavelength range and a tungsten lamp for the visible (VIS) and short-wave near-infrared (SWNIR) wavelength range. The image of the filament of the tungsten lamp is focused on the discharge aperture of the deuterium lamp by means of a special rear-access lamp design which allows both light sources to be optically combined and share a common axis to the source lens. The achromat (source lens) forms a single, focused beam of light through the flow cell. Each cell room and lamp are separated by a quartz window which can be cleaned or replaced. In the spectrograph, light is being dispersed onto the diode array by a holographic grating. This allows simultaneous access to all wavelength information

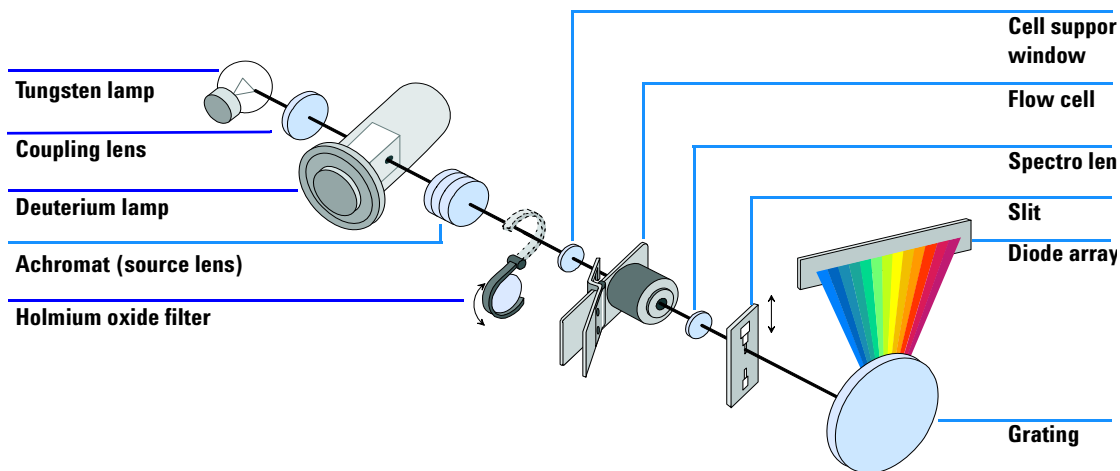


Figure 1 Optical System of the Detector

Lamps The light source for the UV-wavelength range is a deuterium lamp with a shine-through aperture. As a result of plasma discharge in low-pressure deuterium gas, the lamp emits light over the 190-nm to approximately 800-nm

wavelength range. The light source for the visible and SWNIR wavelength range is a low noise tungsten lamp. This lamp emits light over the wavelength range 470 – 950 nm.

Achromat (Source Lens) The achromat receives the light from both lamps and focuses it so that the beam passes through the flow cell.

Holmium Oxide Filter The holmium oxide filter is electromechanically actuated. During the holmium filter test it moves into the light path.

Cell Support Window The cell support window assembly separates the holmium filter area from the flow cell area.

Flow Cell Compartment The optical unit has a flow cell compartment for easy access to flow cells. A variety of optional flow cells can be inserted using the same quick, simple mounting system. The flow cell can be removed to check the optical and electronic performance of the detector without having influences from the flow cell.

Spectrograph The spectrograph material is ceramic to reduce thermal effects to a minimum. The spectrograph consists of the spectrograph lens, the variable entrance slit, the grating and the photodiode array with front-end electronics. The spectrograph lens refocuses the light beam after it has passed through the flow cell. The sampling interval of the diode array is < 1 nm over the wavelength range 190 – 950 nm. Depending on the wavelength this varies from 1.0 to 1.25 diodes per nanometer (for example a diode every 0.8 to 1 nm).

For a small wavelength range, the small non-linearity could be neglected. With the wavelength range from 190 – 950 nm a new approach is required to achieve wavelength accuracy over the full range. Each spectrograph is calibrated individually. The calibration data is stored in the spectrograph on an EEPROM. Based on these data, the built-in processors calculate absorbance data with linear intervals (1.0, 2.0, ...) between data points. This results in an excellent wavelength accuracy and instrument-to-instrument reproducibility.

Variable Entrance Slit System The micro-slit system makes use of the mechanical properties of silicon combined with the precise structuring capabilities of bulk micro-machining. It combines the required optical functions – slit and shutter – in a simple and compact component. The slit width is directly controlled by the micro-processor of the instrument and can be set as method parameter.

Grating The combination of dispersion and spectral imaging is accomplished by using a concave holographic grating. The grating separates the light beam into all its component wavelengths and reflects the light onto the photodiode array.

Diode Array The diode array is a series of 1024 individual photodiodes and control circuits located on a ceramic carrier. With a wavelength range from 190 – 950 nm the sampling interval is < 1 nm.

Early Maintenance Feedback (EMF)

Maintenance requires the exchange of components which are subject to wear or stress. Ideally, the frequency at which components are exchanged should be based on the intensity of usage of the detector and the analytical conditions, and not on a predefined time interval. The early maintenance feedback (EMF) feature monitors the usage of specific components in the instrument, and provides feedback when the user-selectable limits have been exceeded. The visual feedback in the user interface provides an indication that maintenance procedures should be scheduled.

EMF Counters

The detector provides two EMF counters for the lamps. The counters increment increments with lamp use, and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. The counters can be reset to zero after the lamp is exchanged. The detector provides the following EMF counters:

- Deuterium Lamp On-Time
- Tungsten Lamp On-Time

Using the EMF Counters

The user-settable EMF limits for the EMF counters enable the early maintenance feedback to be adapted to specific user requirements. The useful lamp burn time is dependent on the requirements for the analysis (high or low sensitivity analysis, wavelength etc.), therefore, the definition of the maximum limits need to be determined based on the specific operating conditions of the instrument.

Setting the EMF Limits

The setting of the EMF limits must be optimized over one or two maintenance cycles. Initially, no EMF limit should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by lamp counters. Enter these values (or values slightly less than the displayed values) as EMF limits, and then reset the EMF counters to zero. The next time the EMF counters exceed the new EMF limits, the EMF flag will be displayed, providing a reminder that maintenance needs to be scheduled.

Electrical Connections

- The GPIB connector is used to connect the detector with a computer. The address and control switch module next to the GPIB connector determines the GPIB address of your detector. The switches are preset to a default address which is recognized once after power is switched on.
- The CAN bus is a serial bus with high speed data transfer. The two connectors for the CAN bus are used for internal Agilent 1200 Series module data transfer and synchronization.
- Two independent analog outputs provide signals for integrators or data handling systems.
- The interface board slot is used for external contacts and BCD bottle number output or LAN connections.
- The REMOTE connector may be used in combination with other analytical instruments from Aligent Technologies if you want to use features such as start, stop, common shut down, prepare, and so on.
- With the appropriate software, the RS-232C connector may be used to control the detector from a computer through a RS-232C connection. This connector is activated and can be configured with the configuration switch next to the GPIB connector. See your software documentation for further information.
- The power input socket accepts a line voltage of 100 – 240 V AC $\pm$ 10 % with a line frequency of 50 or 60 Hz. Maximum power consumption is 300 VA/125 W. There is no voltage selector on your detector because the power supply has a wide-ranging capability. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply. The security lever at the power input socket prevents that the detector cover is taken off when line power is still connected.

WARNING

Never use cables other than the ones supplied by Aligent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

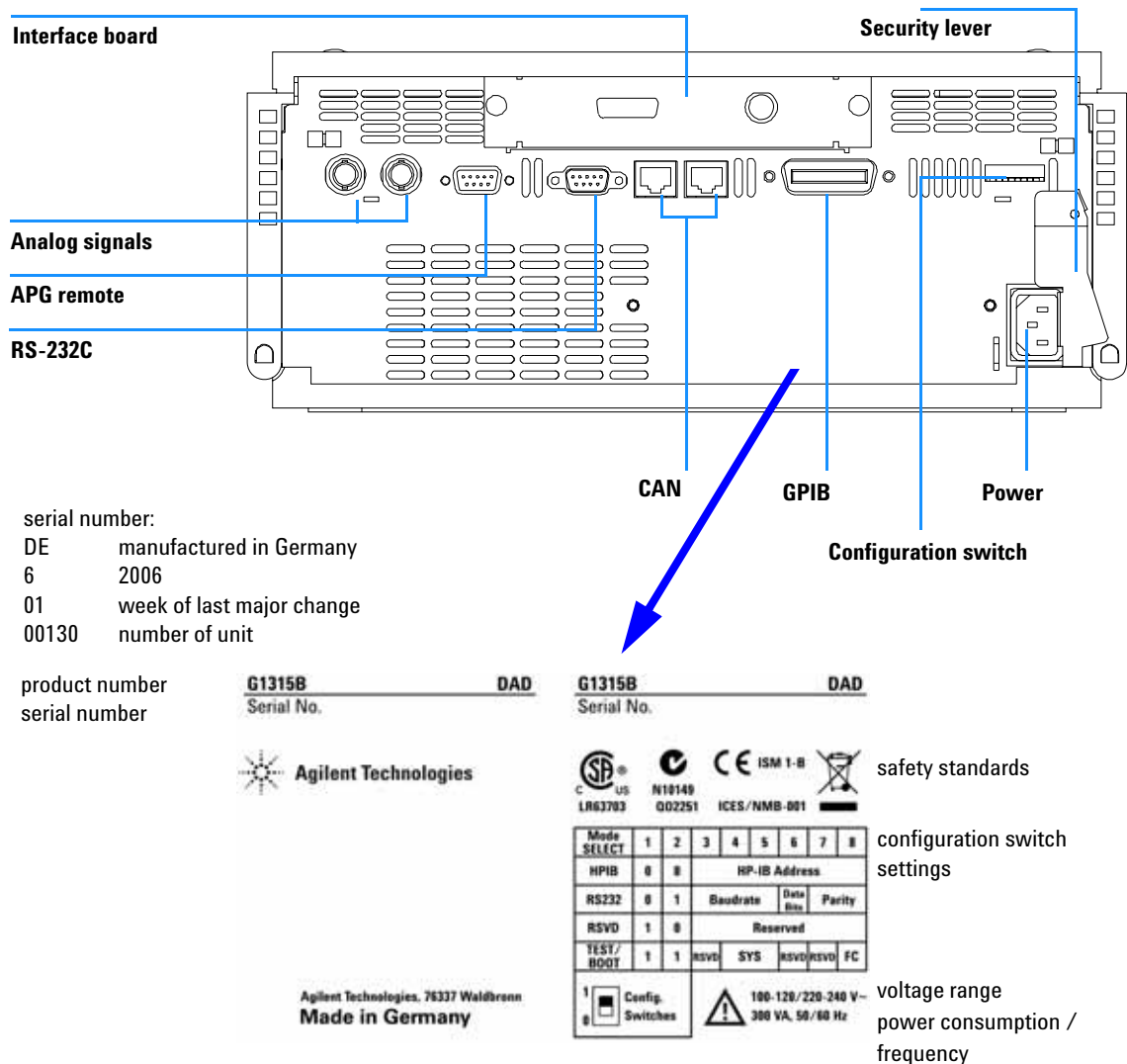


Figure 2 Rear View of Detector - Electrical Connections and Label

Instrument Layout

The industrial design of the detector incorporates several innovative features. It uses Agilent's E-PAC concept for the packaging of electronics and mechanical assemblies. This concept is based upon the use of expanded polypropylene (EPP) layers of foam plastic spacers in which the mechanical and electronic boards components of the detector are placed. This pack is then housed in a metal inner cabinet which is enclosed by a plastic external cabinet. The advantages of this packaging technology are:

- virtual elimination of fixing screws, bolts or ties, reducing the number of components and increasing the speed of assembly/disassembly,
- the plastic layers have air channels molded into them so that cooling air can be guided exactly to the required locations,
- the plastic layers help cushion the electronic and mechanical parts from physical shock, and
- the metal inner cabinet shields the internal electronics from electromagnetic interference and also helps to reduce or eliminate radio frequency emissions from the instrument itself.



2 Site Requirements and Specifications

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This chapter gives information on environmental requirements, physical and performance specifications.



Site Requirements

A suitable environment is important to ensure optimal performance of the detector.

Power Consideration

The detector power supply has wide ranging capabilities and accepts any line voltage in the range mentioned in Table 1. Consequently, there is no voltage selector in the rear of the detector. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

WARNING

To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.

WARNING

Shock hazard or damage of your instrumentation can result, if the devices are connected to a line voltage higher than specified.

CAUTION

Make sure to have easy access to the power cable of the instrument, in order to disconnect the instrument from line.

Power Cords

Different power cords are offered as options with the detector. The female end of all power cords is identical. It plugs into the power-input socket at the rear of the detector. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

WARNING

Never operate your instrumentation from a power outlet that has no ground connection. Never use a power cord other than the Agilent Technologies power cord designed for your region.

WARNING

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Bench Space

The detector dimensions and weight (see Table 1) allows you to place the detector on almost any desk or laboratory bench. It needs an additional 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear for air circulation and electric connections.

If the bench should carry a Agilent 1200 Series system, make sure that the bench is designed to bear the weight of all modules.

The detector should be operated in a horizontal position.

Environment

Your detector will work within the specifications at ambient temperatures and relative humidity described in Table 1.

ASTM drift tests require a temperature change below 2 °C/hour (3.6 °F/hour) over one hour period. Our published drift specification (refer also to “[Performance Specifications](#)” on page 26) is based on these conditions. Larger ambient temperature changes will result in larger drift.

Better drift performance depends on better control of the temperature fluctuations. To realize the highest performance, minimize the frequency and the amplitude of the temperature changes to below 1 °C/hour (1.8 °F/hour). Turbulences around one minute or less can be ignored.

CAUTION

Do not store, ship or use your detector under conditions where temperature fluctuations could cause condensation within the detector. Condensation will damage the system electronics. If your detector was shipped in cold weather, leave it in its box and allow it to warm up slowly to room temperature to avoid condensation.

2 Site Requirements and Specifications

NOTE

The G1315B DAD and G1365B MWD are designed to operate in a typical electromagnetic environment (EN61326-1) where RF transmitters, such as mobile phones, should not be used in close proximity.

Physical Specifications

Table 1 Physical Specifications

Type	Specification	Comments
Weight	11.5 kg (26 lbs)	
Dimensions (width × depth × height)	345 × 435 × 140 mm (13.5 × 17 × 5.5 inches)	
Line voltage	100 – 240 VAC, $\pm 10\%$	Wide-ranging capability
Line frequency	50 or 60 Hz $\pm 5\%$	
Power consumption (G1315B/65B)	300 VA / 125 W / 427 BTU	Maximum
Ambient operating temperature	0 – 55 °C (32 – 131 °F)	.
Ambient non-operating temperature	-40 – 70 °C (-4 – 158 °F)	
Humidity	< 95%, at 25 – 40 °C (77 – 104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6500 ft)	
Non-operating altitude	Up to 4600 m (14950 ft)	For storing the detector
Safety standards: IEC, CSA, UL, EN	Installation category II, pollution degree 2. For indoor use only.	

Performance Specifications

Table 2 Performance Specifications Agilent 1200 Series DAD and MWD

Type	Specification	Comments
Detection type	1024-element photodiode array	
Light source	Deuterium and tungsten lamps	
Wavelength range	190 – 950 nm	
Short term noise (ASTM) Single and Multi-Wavelength	$\pm 1 \times 10^{-5}$ AU at 254 and 750 nm	See on page 27
Drift	2×10^{-3} AU/hr at 254 nm	See on page 27
Linear absorbance range	> 2 AU (upper limit)	See on page 27
Wavelength accuracy	± 1 nm	Self-calibration with deuterium lines, verification with holmium oxide filter
Wavelength bunching	1 – 400 nm	Programmable in steps of 1 nm
Slit width	1, 2, 4, 8, 16 nm	Programmable slit
Diode width	< 1 nm	
Flow cells	Standard: 13 μ l volume, 10 mm cell path length and 120 bar (1760 psi) pressure maximum Semi-Micro: 5 μ l volume, 6 mm cell path length and 120 bar (1760 psi) pressure maximum Micro: 2 μ l volume, 3 mm cell path length and 120 bar (1760 psi) pressure maximum High pressure: 1.7 μ l volume, 6 mm cell path length and 400 bar (5880 psi) pressure maximum 80 nano: 0.08 μ l volume, 10 mm cell path length and 50 bar (725 psi) pressure maximum 500 nano: 0.5 μ l volume, 10 mm cell path length and 50 bar (725 psi) pressure maximum	See “Optimization Overview” on page 69
Control and data evaluation	Agilent ChemStation for LC	
Analog outputs	Recorder/integrator: 100 mV or 1 V, output range 0.001 – 2 AU, two outputs	

Table 2 Performance Specifications Agilent 1200 Series DAD and MWD, continued

Type	Specification	Comments
Communications	Controller-area network (CAN), GPIB, RS-232C, APG Remote: ready, start, stop and shut-down signals, LAN optional	
Safety and maintenance	Extensive diagnostics, error detection and display (through control module and ChemStation), leak detection, safe leak handling, leak output signal for shutdown of pumping system. Low voltages in major maintenance areas.	
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage in terms of lamp burn time with user-settable limits and feedback messages. Electronic records of maintenance and errors. Verification of wavelength accuracy with built-in holmium oxide filter.	
Housing	All materials recyclable.	

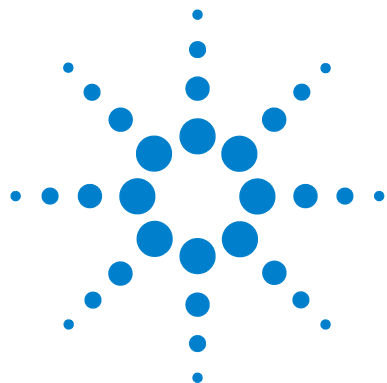
NOTE

ASTM: "Standard Practice for Variable Wavelength Photometric Detectors Used in Liquid Chromatography".

Reference conditions: cell path length 10 mm, response time 2 s, flow 1 ml/min LC-grade Methanol, slit width 4 nm.

Linearity measured with caffeine at 265 nm.

For environmental conditions refer to "[Environment](#)" on page 23.



3 Installing the Detector

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Installing the Detector	35
Flow Connections to the Detector	38

This chapter describes the installation of the detector.



Unpacking the Detector

Damaged Packaging

If the delivery packaging shows signs of external damage, please call your Agilent Technologies sales and service office immediately. Inform your service representative that the detector may have been damaged during shipment.

CAUTION

If there are signs of damage, please do not attempt to install the detector.

Delivery Checklist

Ensure all parts and materials have been delivered with the detector. The delivery checklist is shown below. Please report missing or damaged parts to your local Agilent Technologies sales and service office.

Table 3 Detector Checklist

Description	Quantity
Detector	1
Power cable	1
Flow cell	As ordered
<i>User Manual</i>	1
Accessory kit (see Table 4)	1

Detector Accessory Kit Contents

Table 4 Accessory Kit Contents

Description	Part Number	Quantity
Accessory kit	G1315-68705	
CAN cable 0.5 m	5181-1516	1
Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462	2 m
Corrugated tubing (to waste), re-order 5 m	5062-2463	1.2 m
Fitting male PEEK	0100-1516	2
Capillary column-detector 380 mm lg, 0.17 mm i.d. includes:	G1315-87311	1
Ferrule front SST	0100-0043	2
Ferrule back SST	0100-0044	2
Fitting SST	79814-22406	2
Hex key set 1 – 5 mm	8710-0641	1
Wrench open end 1/4 – 5/16 inch	8710-0510	1
Wrench open end 4 mm	8710-1534	1

Optimizing the Stack Configuration

If your detector is part of a complete Agilent 1200 Series system, you can ensure optimum performance by installing the following configuration. This configuration optimizes the system flow path, ensuring minimum delay volume.

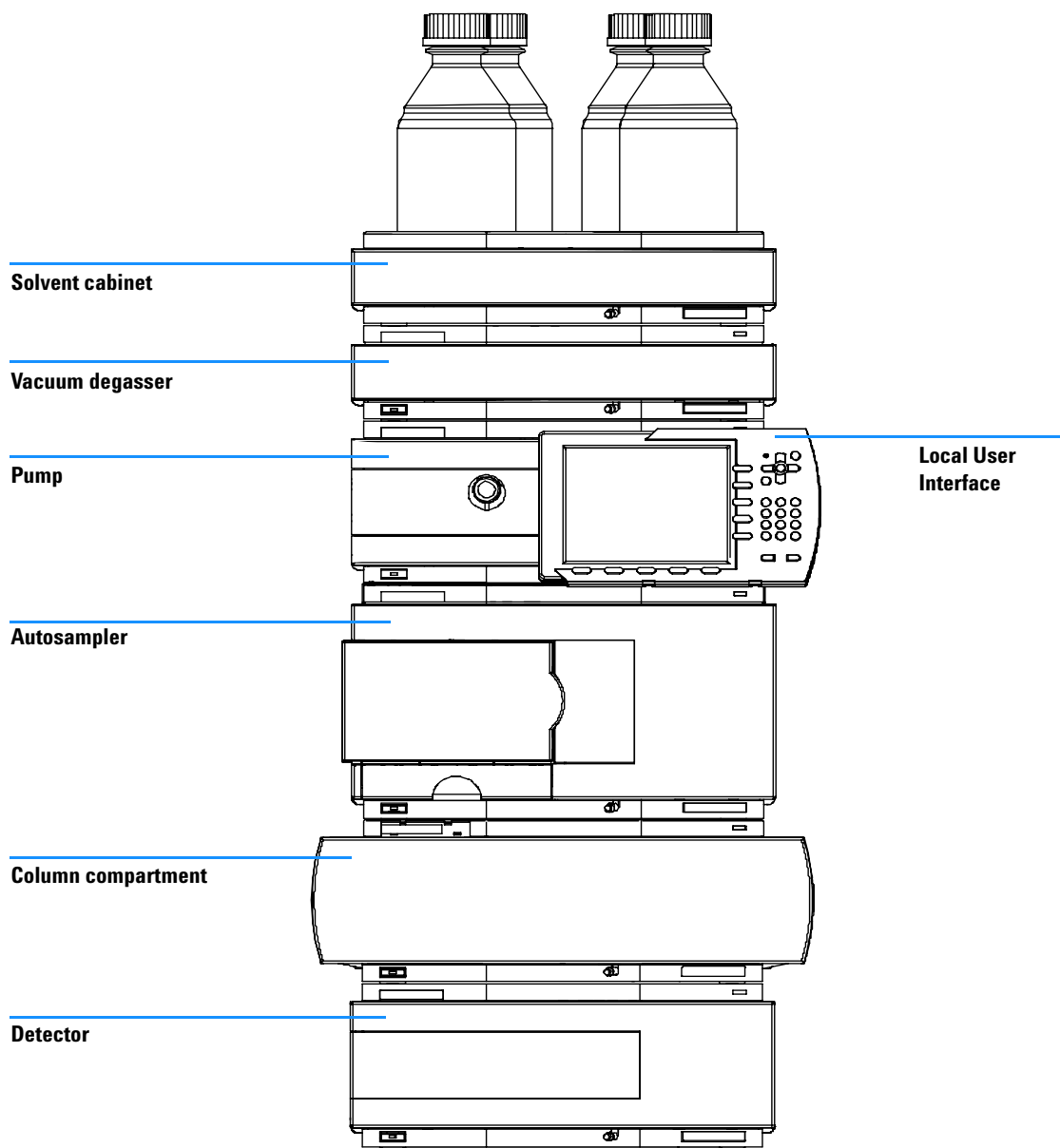


Figure 3 Recommended Stack Configuration (Front View)

3 Installing the Detector

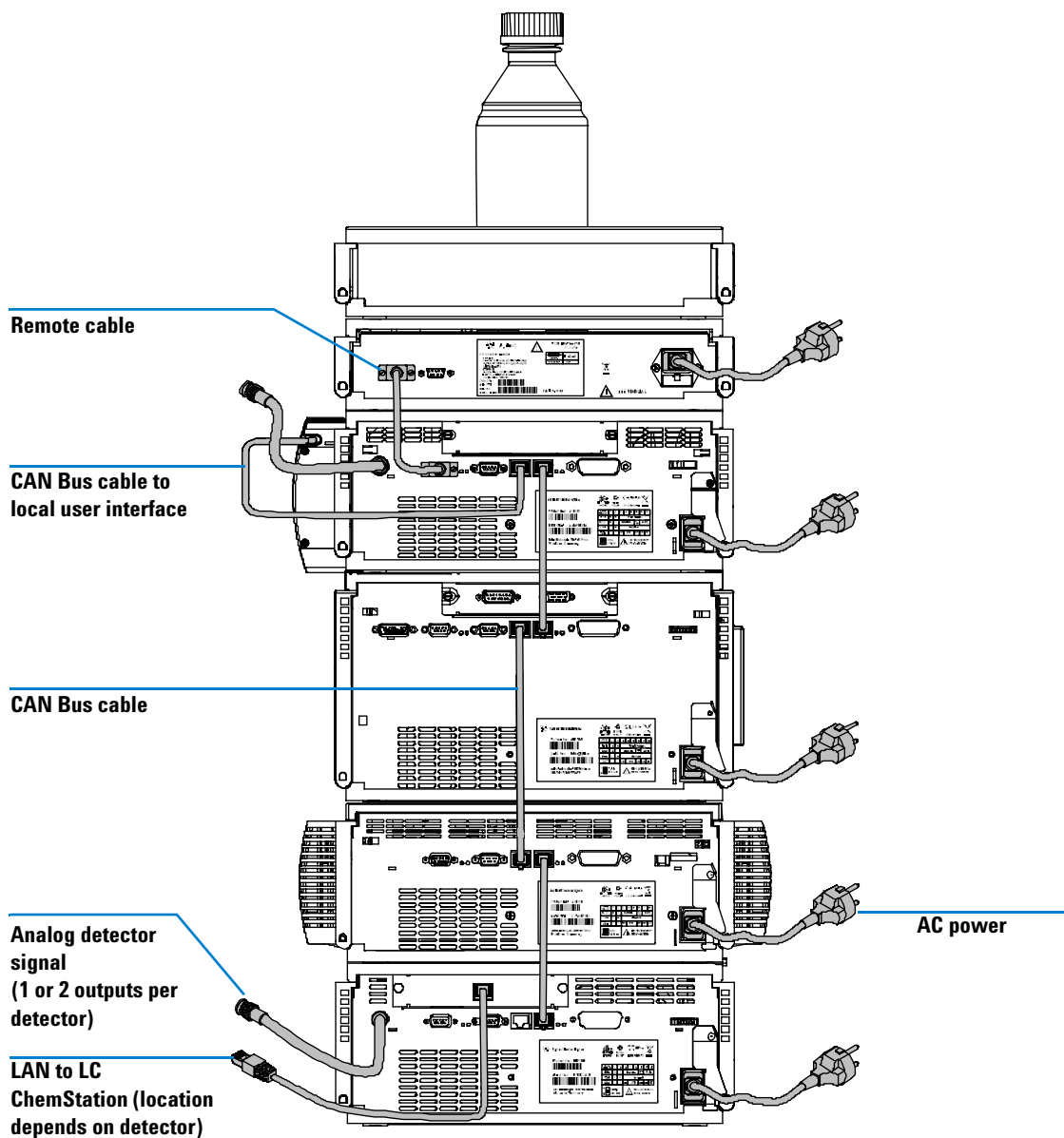


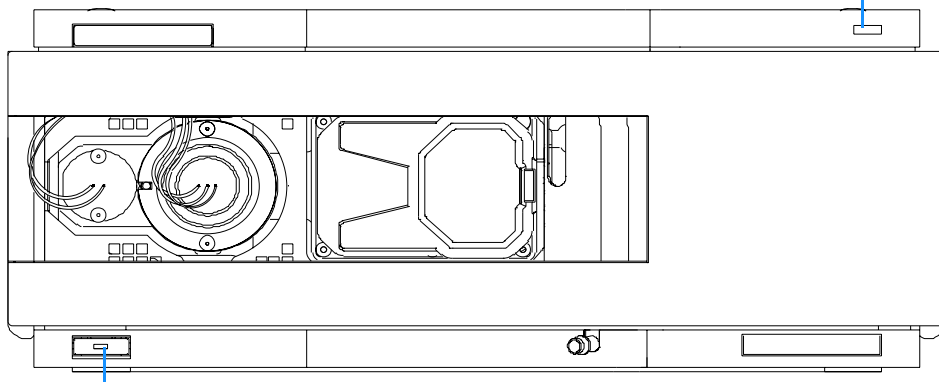
Figure 4 Recommended Stack Configuration (Rear View)

Installing the Detector

Preparations	Locate bench space
	Provide power connections
	Unpack the detector
Parts required	Detector
	Power cord, for other cables see below
	ChemStation and/or Control Module G1323B or Instant Pilot G4208A

- 1 Install the LAN interface board in the detector (if required), see [“Replacing the Interface Board”](#) on page 133.
- 2 Place the detector in the stack or on the bench in a horizontal position.
- 3 Ensure the line power switch at the front of the detector is OFF.

Status indicator
green/yellow/red



Line power switch
with green light

Figure 5 Front View of Detector

3 Installing the Detector

- 4 Connect the power cable to the power connector at the rear of the detector.
- 5 Connect the CAN cable to other Agilent 1200 Series modules.
- 6 If a Agilent ChemStation is the controller, connect the LAN connection to the LAN interface board in the detector.

NOTE

If a Agilent 1200 DAD/MWD/FLD is in the system, the LAN should be connected to the DAD/MWD/FLD (due to higher data load).

- 7 Connect the analog cable(s) (optional).
- 8 Connect the APG remote cable (optional) for non-Agilent 1200 Series instruments.
- 9 Turn ON power by pushing the button at the lower left hand side of the detector. The status LED should be green.

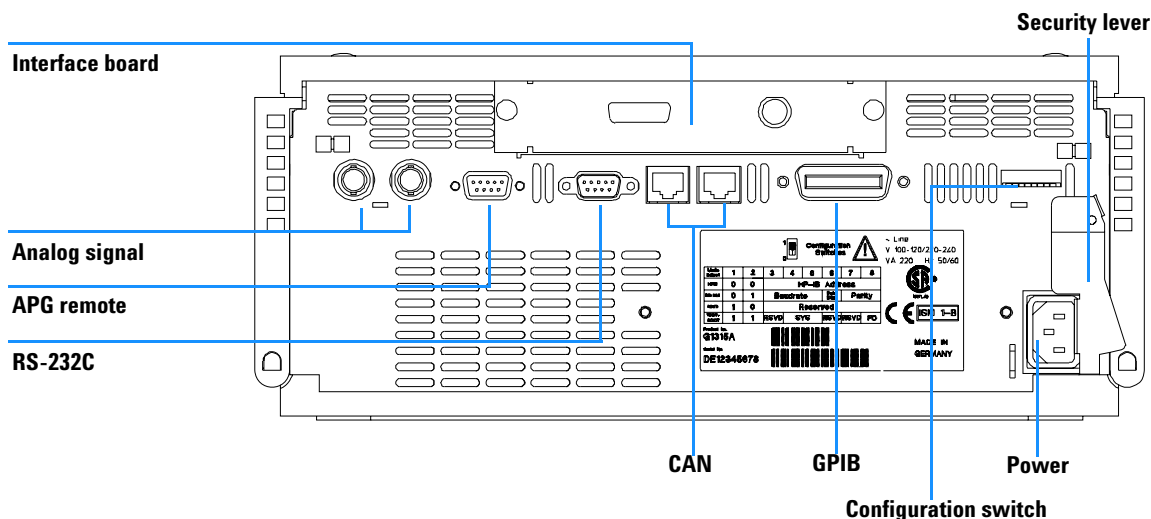


Figure 6 Rear View of Detector

NOTE

The detector is turned on when the line power switch is pressed and the green indicator lamp is illuminated. The detector is turned off when the line power switch is protruding and the green light is OFF.

WARNING

To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch at the front panel is turned OFF.

NOTE

The detector was shipped with default configuration settings.

Flow Connections to the Detector

Preparations	Detector is installed in the LC system.
Parts required	Other modules Parts from accessory kit, see " Detector Accessory Kit Contents " on page 31. Two wrenches 1/4 – 5/16 inch for capillary connections

WARNING

When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

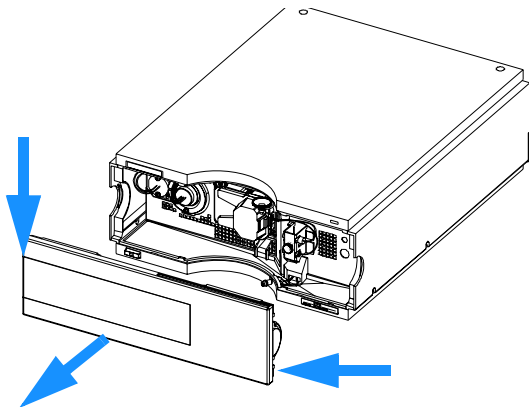
NOTE

The flow cell is shipped with a filling of isopropanol (also recommended when the instrument and/or flow cell is shipped to another location). This is to avoid breakage due to subambient conditions.

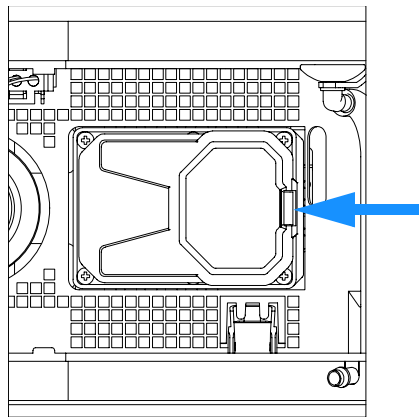
NOTE

The heat exchanger/capillary and the cell body can be fixed mirror symmetrically to have both capillaries routed to the bottom or to the top (depending on the routing of the capillaries to the column). For details see "[Replacing Capillaries on a Standard Flow Cell](#)" on page 114.

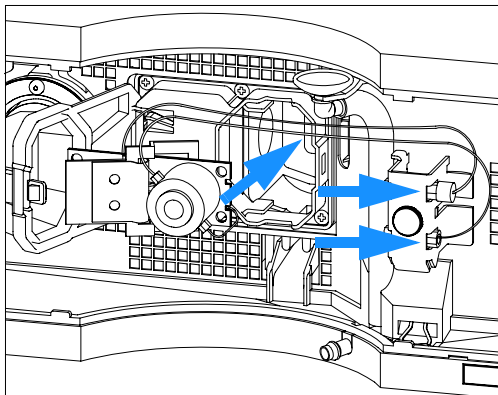
- 1** Press the release buttons and remove the front cover to gain access to the flow cell area.



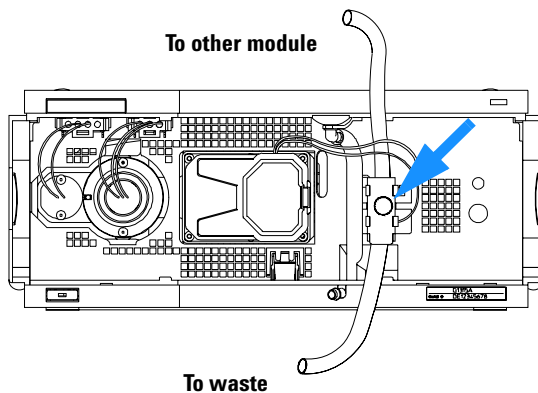
- 2** Press the release button and open the flow cell door.



- 3** Insert the flow cell and install the capillaries to the capillary holder (top is inlet, bottom is outlet).

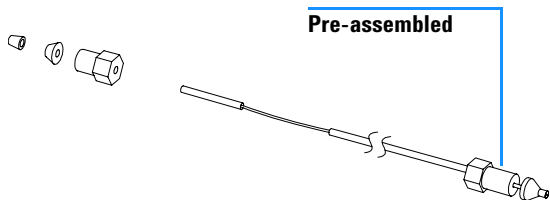


- 4** If another Agilent 1200 Series module is positioned on top of the detector, route the tubing assembly waste from the accessory kit behind the capillary holder and connect the top end to the other module's waste outlet.

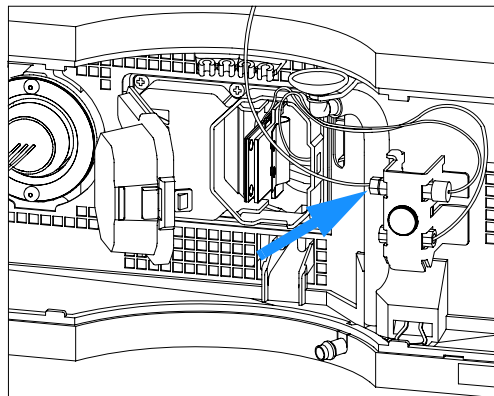


3 Installing the Detector

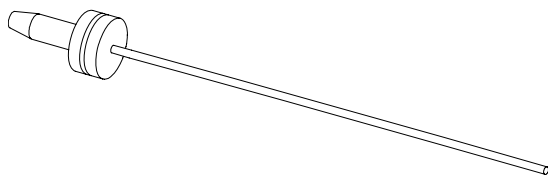
- 5** Assemble the column-detector capillary from the accessory kit. One side is already factory-assembled



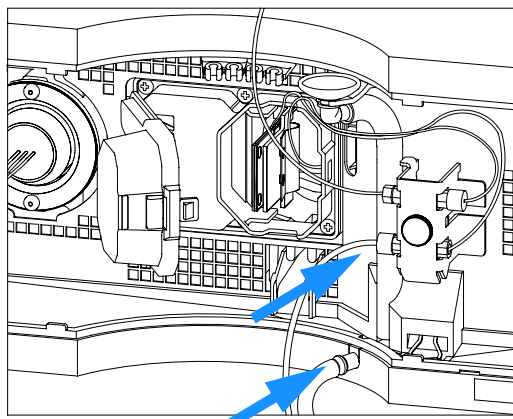
- 6** Connect the newly assembled fitting of the capillary to the top fitting holder and the other end to the column.



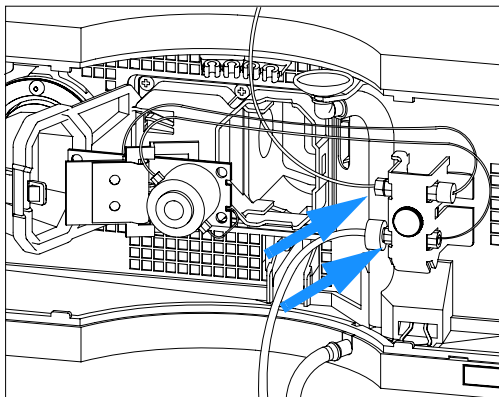
- 7** Assemble the waste tubing from the accessory kit.



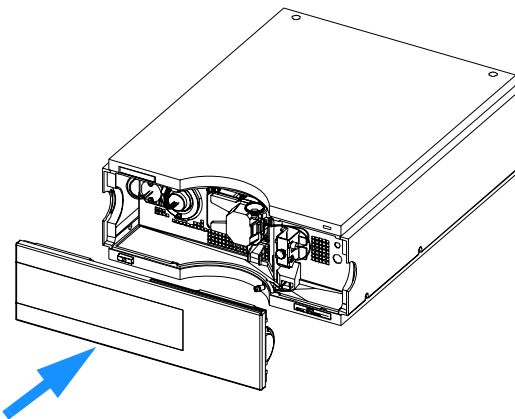
- 8** Connect the waste tubing to the bottom fitting holder and a waste tubing to the leak outlet.



- 9** Remove the flow cell and establish a flow and observe for leaks.



- 10** Insert the flow cell, close the cover and replace the front cover.

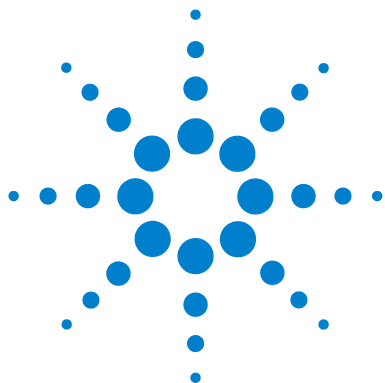


The installation of the detector is now complete.

NOTE

The detector should be operated with the front cover in place to protect the flow cell area against strong drafts from the outside and to cover the deuterium lamp.

Some types of the Agilent deuterium lamps show a light ring during operation. This is not harmful, refer to ["UV-Radiation"](#) on page 173.



4 Using the Detector

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This chapter provides information on how to set up the detector for an analysis and explains the basic settings.



Setting up an Analysis

This chapter can be used for

- preparing the system,
- to learn the set up of an HPLC analysis and
- to use it as an instrument check to demonstrate that all modules of the system are correctly installed and connected. It is not a test of the instrument performance.
- to learn about special settings.

Before Using the System

Solvent Information

Observe recommendations on the use of solvents in chapter “Solvents” in the pump’s reference manual.

Priming and Purging the System

When the solvents have been exchanged or the pumping system has been turned off for a certain time (for example, overnight) oxygen will re-diffuse into the solvent channel between the solvent reservoir, vacuum degasser (when available in the system) and the pump. Solvents containing volatile ingredients will slightly lose these. Therefore priming of the pumping system is required before starting an application.

Table 5 **Choice of Priming Solvents for Different Purposes**

Activity	Solvent	Comments
After an installation	Isopropanol	Best solvent to flush air out of the system
When switching between reverse phase and normal phase (both times)	Isopropanol	Best solvent to flush air out of the system
After an installation	Ethanol or Methanol	Alternative to Isopropanol (second choice) if no Isopropanol is available
To clean the system when using buffers	Bidistilled water	Best solvent to re-dissolve buffer crystals
After a solvent change	Bidistilled water	Best solvent to re-dissolve buffer crystals
After the installation of normal phase seals (P/N 0905-1420)	Hexane + 5% Isopropanol	Good wetting properties

NOTE

The pump should never be used for priming empty tubings (never let the pump run dry). Use a syringe to draw enough solvent for completely filling the tubings to the pump inlet before continuing to prime with the pump.

- 1** Open the purge valve of your pump (by turning it counterclockwise) and set flow rate to 3-5 ml/min.
- 2** Flush all tubes with at least 30 ml of solvent.
- 3** Set flow to required value of your application and close the purge valve.

Pump for approximately 10 minutes before starting your application.

Requirements and Conditions

What You Will Need

Table 6 lists the items you need to have for the set up of the analysis. Some of these are optional (not required for the basic system).

Table 6 What you will need

1200 system	Pump (plus degassing)
	Autosampler
	Detector, standard flow cell installed
	Agilent ChemStation (B.02.01 and above) or Instant Pilot G4208 (A.01.01 and above) (optional for basic operation) or Control Module G1323B (B.04.02 and above) (optional for basic operation)
	System should be correctly set up for LAN communication with the Agilent ChemStation
Column:	Zorbax Eclipse XDB-C18, 4.6 x 150 mm, 5 μ m Part No. 993967-902 or Part No. 5063-6600
Standard:	Part No. 01080-68704 0.15 wt.% dimethylphthalate, 0.15 wt.% diethylphthalate, 0.01 wt.% biphenyl, 0.03 wt.% o-terphenyl in methanol

Conditions

A single injection of the isocratic test standard is made under the conditions given in Table 7:

Table 7 Conditions

Flow	1.5 ml/minute
Stoptime	8 minutes
Solvent	100% (30% water/70% Acetonitrile)
Temperature	Ambient
Wavelength	sample 254 nm (4 nm bandwidth) reference 360 nm (100 nm bandwidth)
Injection Volume	1 µl

Typical Chromatogram

A typical chromatogram for this analysis is shown in [Figure 7](#). The exact profile of the chromatogram will depend on the chromatographic conditions. Variations in solvent quality, column packing, standard concentration and column temperature will all have a potential effect on peak retention and response.

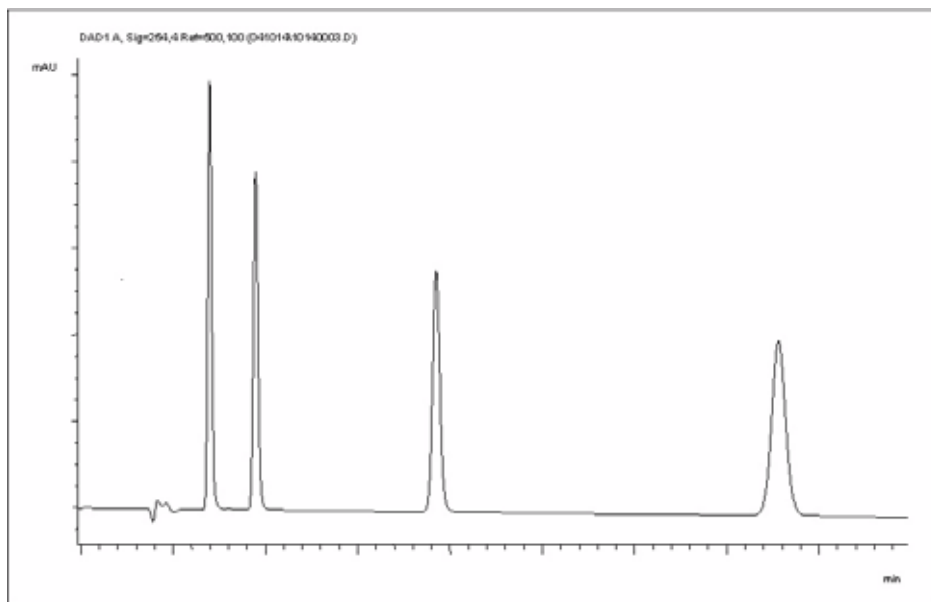


Figure 7 Typical Chromatogram with UV-detector

Optimization of the System

The settings used for this analysis are specific for this purpose. For other applications the system can be optimized in various ways. Please refer to the section [“Optimizing the Detector”](#) on page 66 or *“Optimizing”* in the your module’s *Reference Manual*.

Preparing the HPLC System

- 1 Turn on the Agilent ChemStation PC and the monitor.
- 2 Turn on the 1200 series HPLC modules.
- 3 Start the Agilent ChemStation software (B.02.01). If the pump, autosampler, thermostatted column compartment and detector are found, the ChemStation screen should look like shown in [Figure 8](#).

The System status is red (Not Ready).

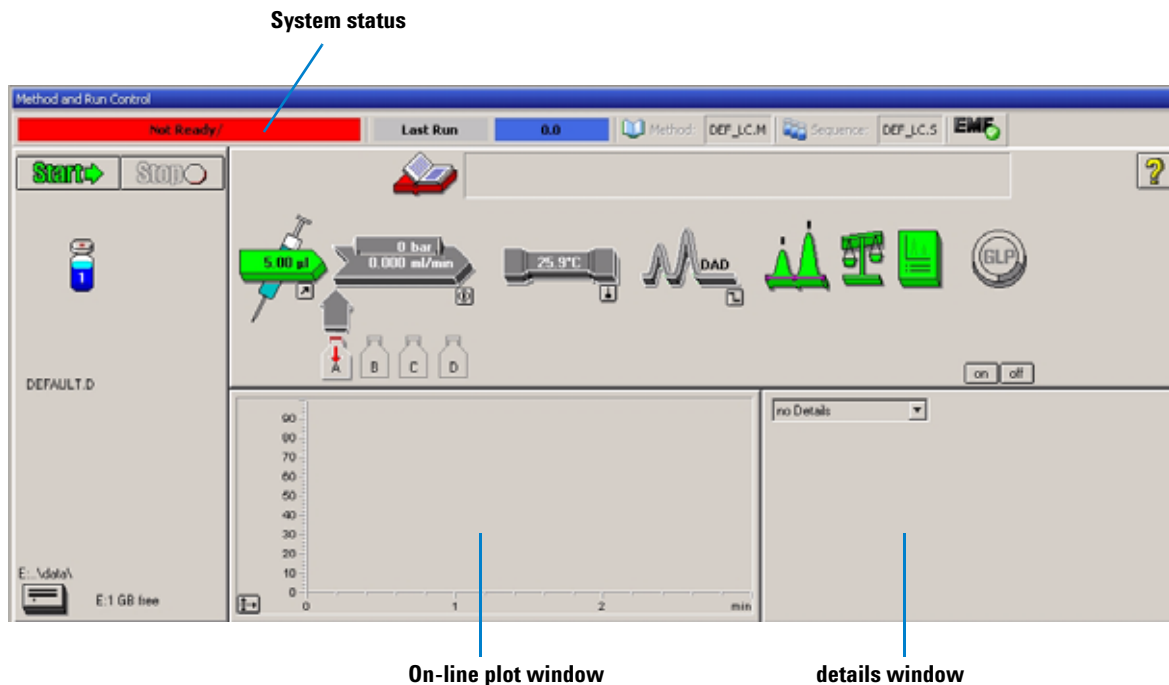


Figure 8 Initial ChemStation screen (Method and Run Control)

- 4 Turn on the detector lamp, pump and autosampler by clicking the *System On* button or the buttons below the module icons on the graphical user interface (GUI). After some time, the pump, thermostatted column compartment and detector module will turn to green.

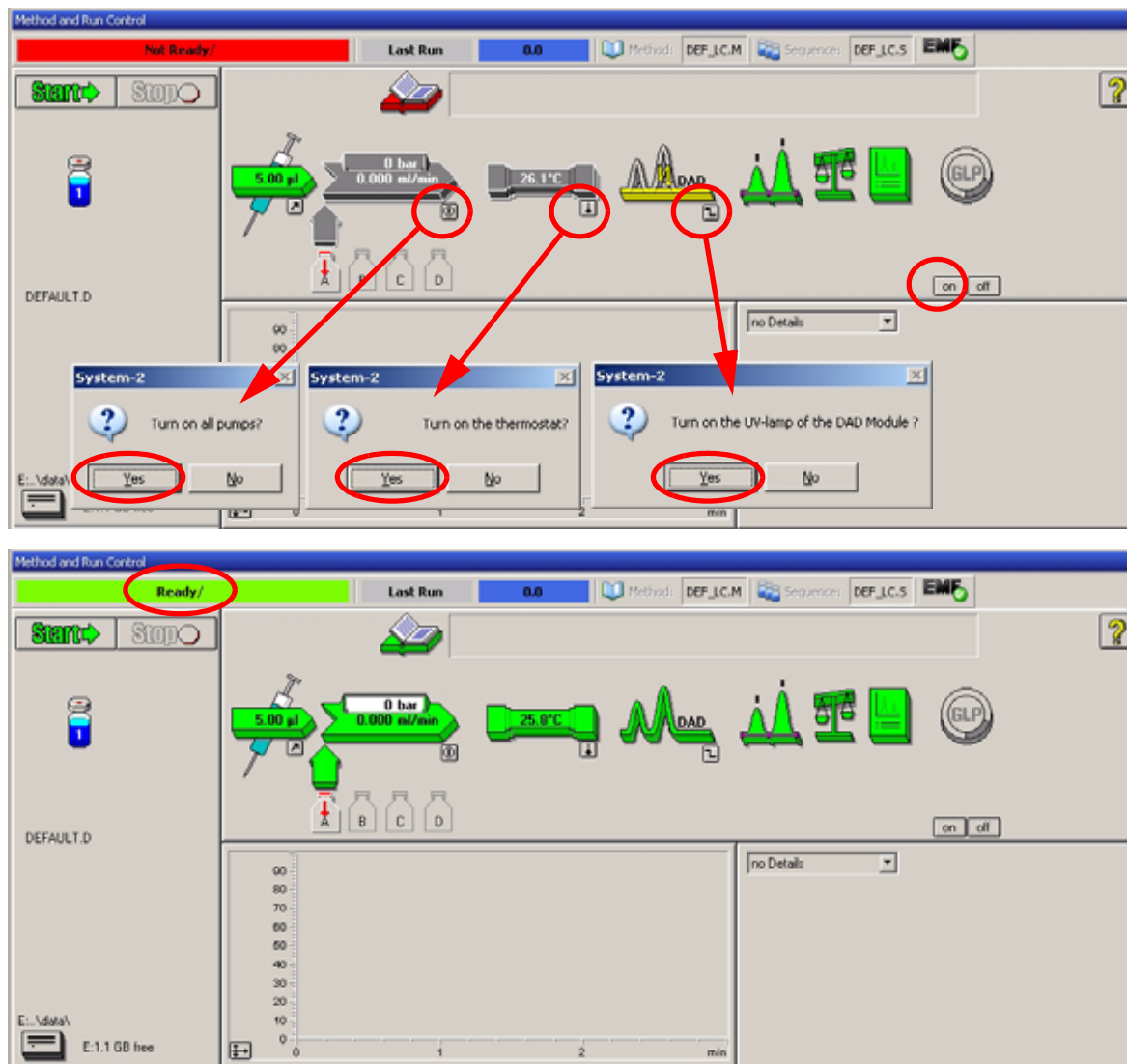


Figure 9 Turning on the HPLC Module

- 5 Purge the pump. For more information see “[Priming and Purging the System](#)” on page 44.
- 6 Allow the detector to warm up of at least 60 minutes to provide a stable baseline (see example in [Figure 10](#) and [Table 8](#)).

NOTE

For reproducible chromatography, the detector and lamp should be on for at least one hour. Otherwise the detector baseline may still drift (depending on the environment).

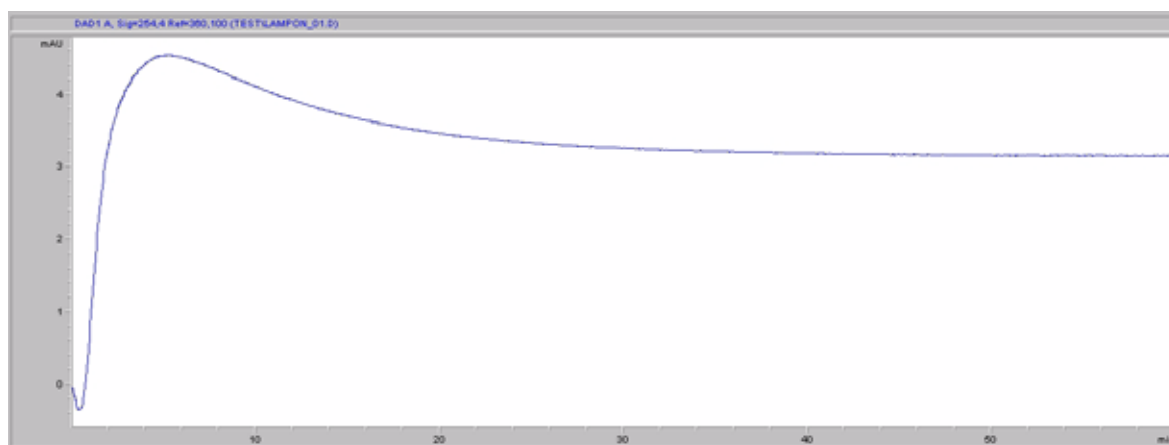


Figure 10 Stabilization of Baseline (both lamps turned on at the same time)

Table 8 Baseline drift after lamp turn on (example from [Figure 10](#))

Time [minutes]	Drift [mAU/hr]
17 - 20	2.6
27 - 30	0.8
37 - 40	0.4
47 - 50	0.2
57 - 60	< 0.2

4 Using the Detector

- 7 For the isocratic pump, fill the solvent bottle with the mixture of HPLC-grade bi-distilled water (30 %) and acetonitrile (70 %). For binary- and quaternary pumps you can use separate bottles.
- 8 Click on the *Load Method* button and select DEF_LC.M and press *OK*.
Alternative double-click on the method in the method window. The default LC method parameters are transferred into the 1200 modules.

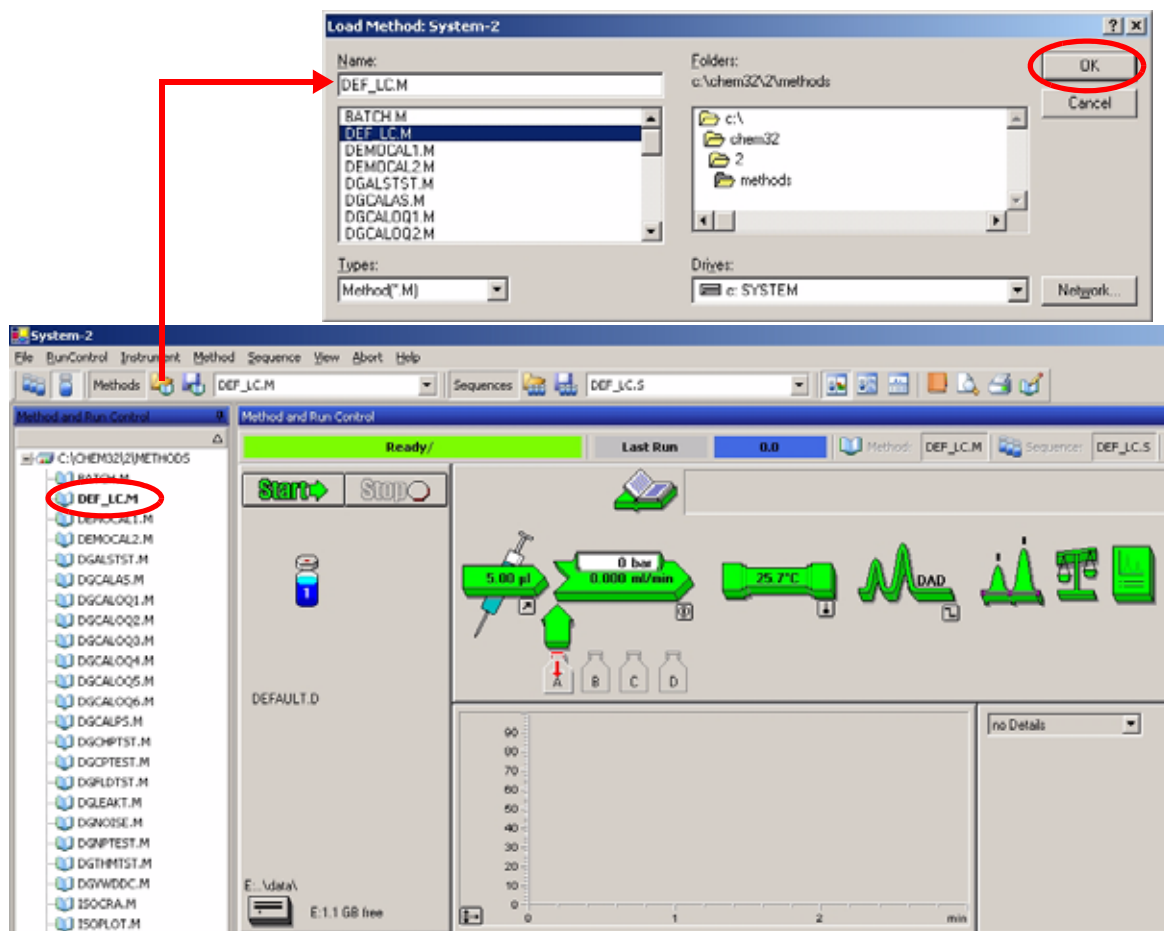


Figure 11 Loading Default LC Method

- 9 Click on the module icons (Figure 12) and open the *Setup* of these modules. Figure 13 on page 54 shows the detector settings (do not change the detector parameters at this time).

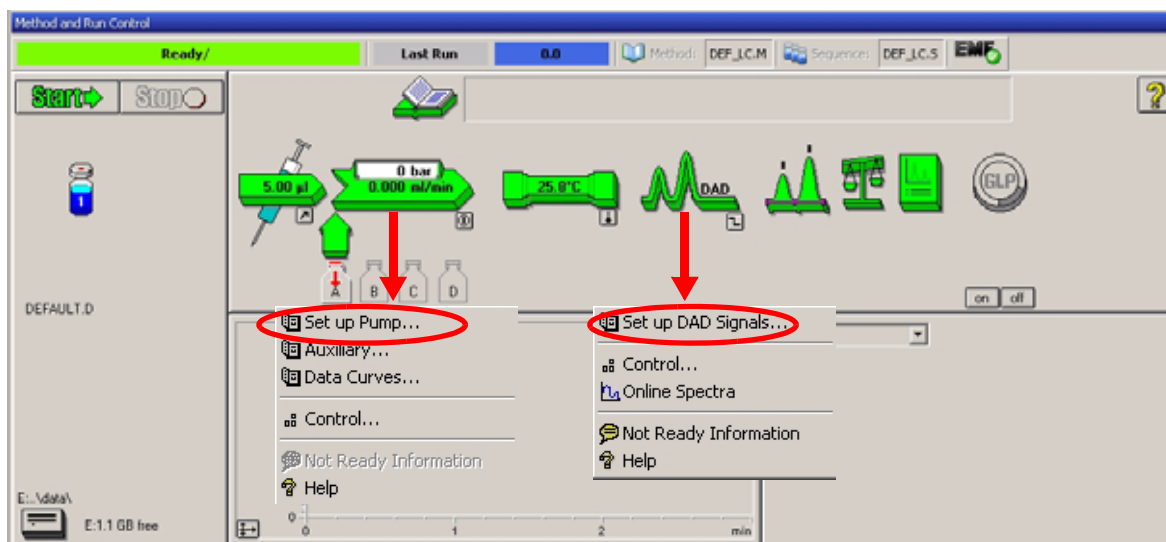
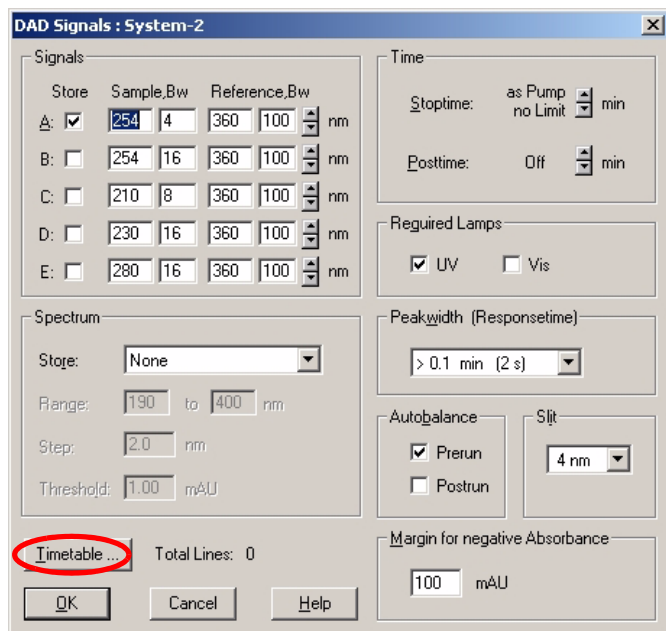


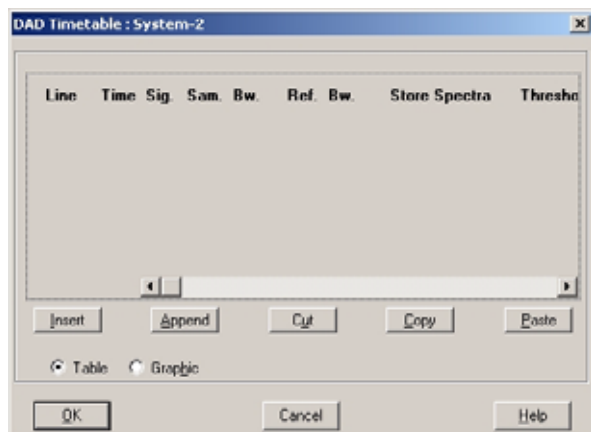
Figure 12 Open the module menu

- 10 Enter the pump parameters mentioned under “Conditions” on page 47.

4 Using the Detector



- up to 5 signals (A to E) with individual wavelength settings can be selected.
- spectrum settings, see [page 62](#).
- stop and post time can be set (if required)
- depending on the application, the lamps can be selected (one or both).
- peak width depends on the peaks in the chromatogram, see [page 64](#).
- autobalance to zero absorbance (on the analog output plus offset) at begin and/or end of run.
- mechanical slit width can be changed for further optimization, see [page 65](#).
- margin for negative absorbance, see [page 66](#).



- time table for programmable actions during the run.

Figure 13 Detector parameter (default)

- 11 Pump the water/acetonitrile (30/70 %) mobile phase through the column for 10 minutes for equilibration.
- 12 Click the button  and select *Change...* to open the Signal Plot information. Select the *Pump: Pressure* and the *DAD A: Signal 254,4* as signals. Change the Y-range for the DAD to 1 mAU and the offset to 20% and the pressure offset to 50%. The X-axis range should be 15 minutes. Press *OK* to exit this screen.

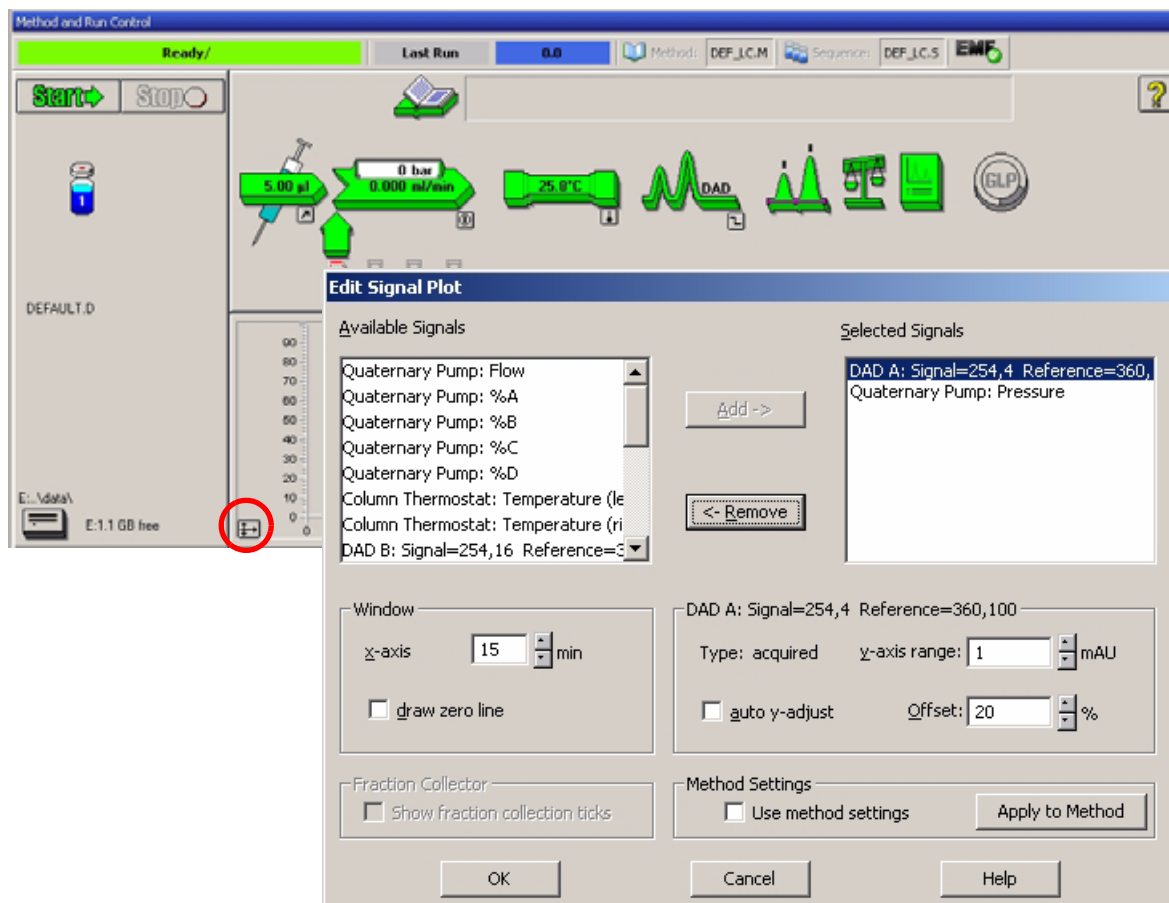


Figure 14 Edit Signal Plot Window

4 Using the Detector

The Online Plot (Figure 15 on page 56) shows both, the pump pressure and the detector absorbance signals. Pressing *Adjust* the signals can be reset to the offset value and *Balance* would do a balance on the detector.

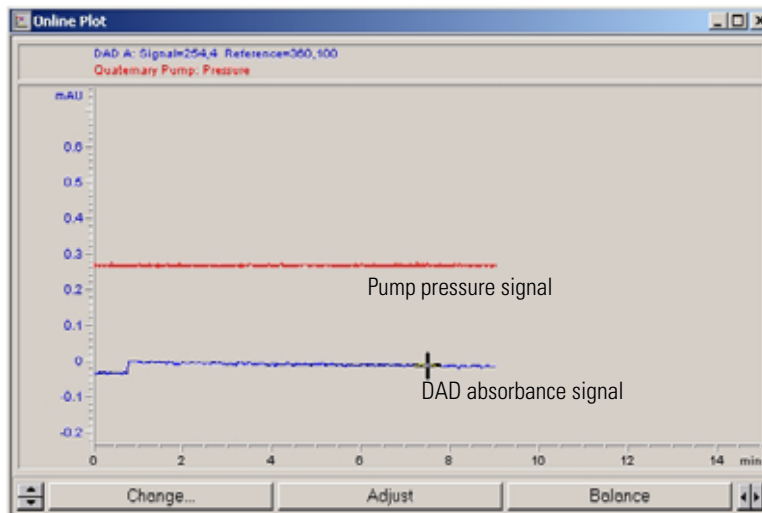


Figure 15 Online Plot Window

13 If both baselines are stable, set the Y-range for the detector signal to 100 mAU.

NOTE

If you start with a new UV-lamp for the first time, the lamp may show initial drift for some time (burn-in effect).

- 14 Select the menu item *RunControl* -> *Sample Info* and enter information about this application (Figure 16 on page 57). Press *OK* to leave this screen.

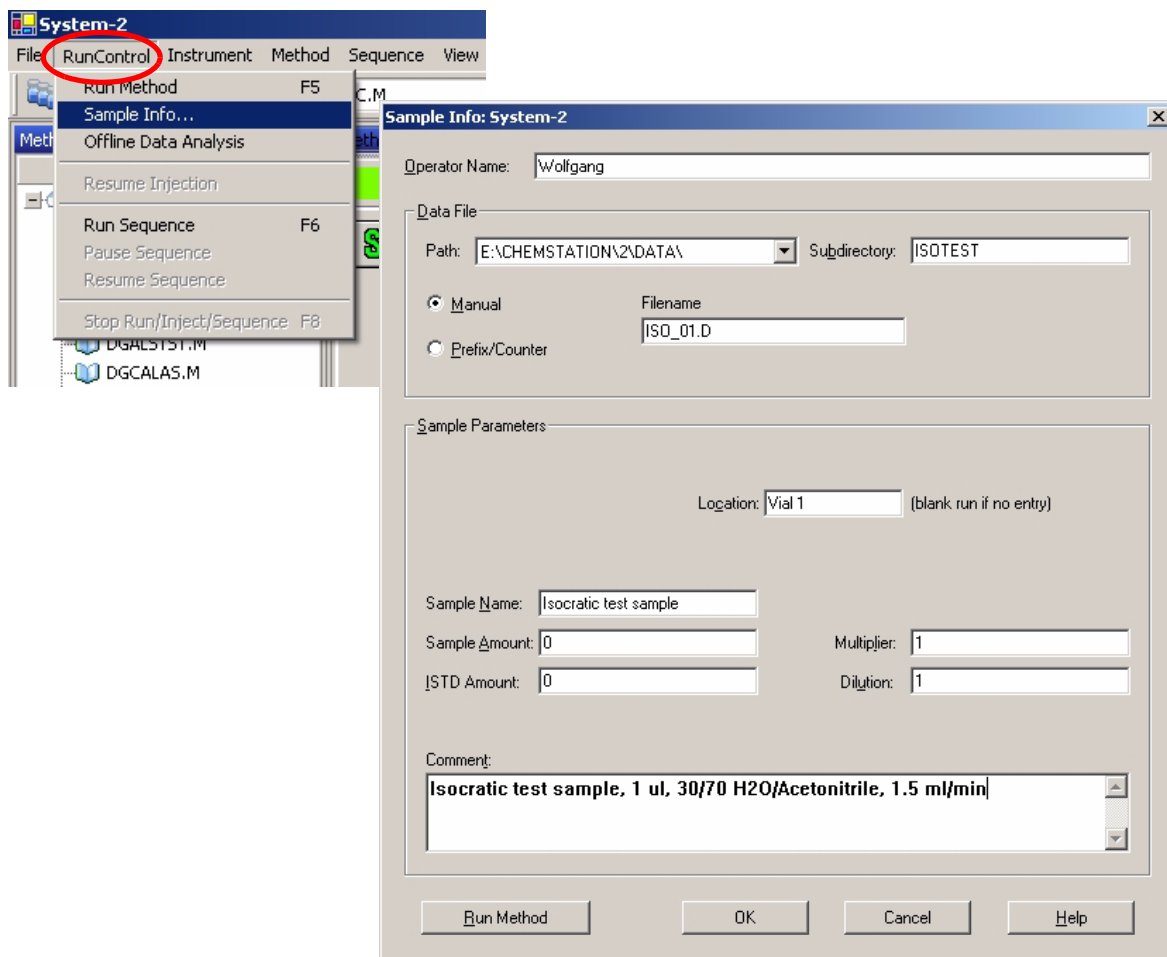


Figure 16 Sample Information

- 15 Fill the content of an isocratic standard sample ampoule into a vial and seal the vial with a cap and place the vial into autosampler tray (position #1).

Running the Sample and Verifying the Results

- 1 To start a run select the menu item *RunControl -> Run Method*.
- 2 This will start the 1200 modules and the online plot on the Agilent ChemStation will show the resulting chromatogram.

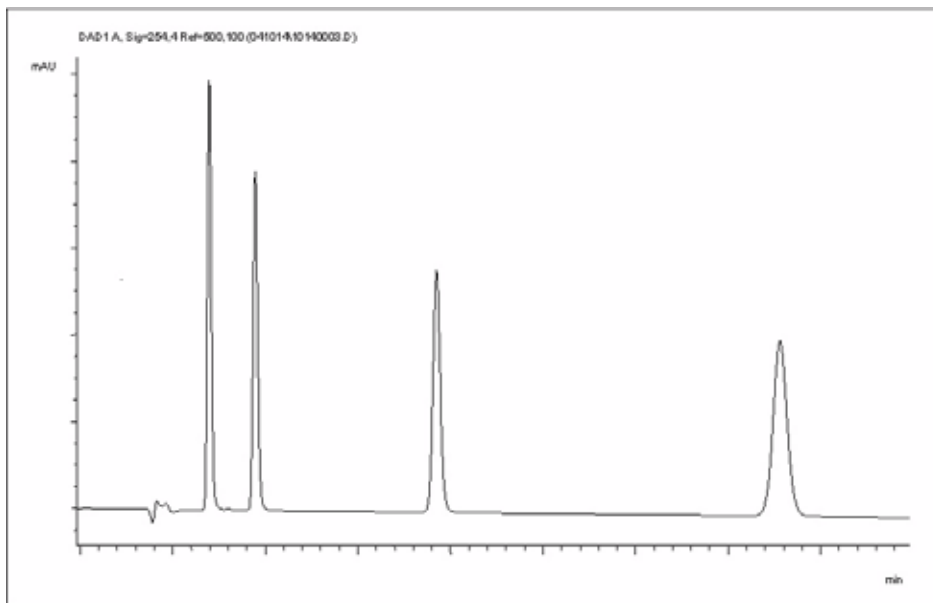


Figure 17 Chromatogram with Isocratic Test Sample

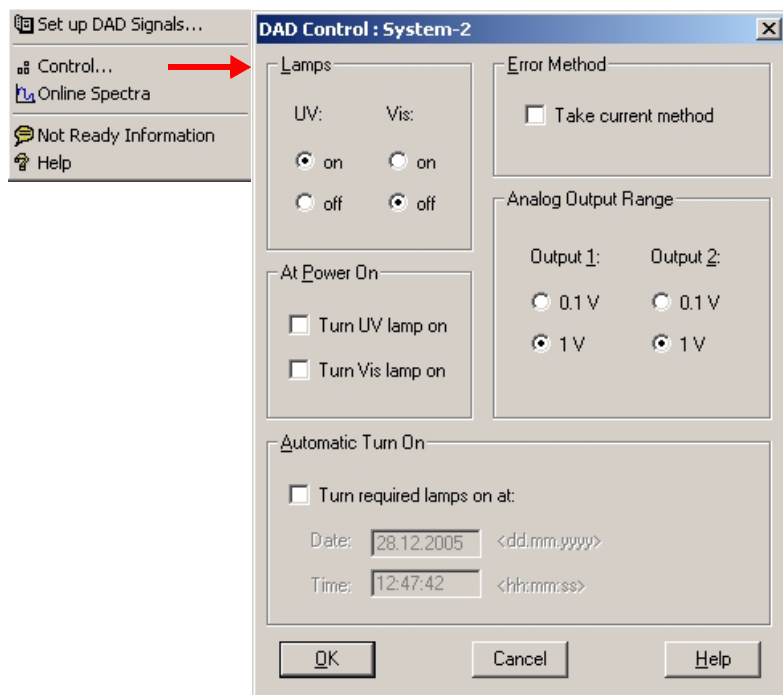
NOTE

Information about using the Data Analysis functions can be obtained from the *Using your ChemStation* manual supplied with your system.

Special Settings of the Detector

In this chapter special settings of the G1315B DAD and G1365B MWD are described (based on the Agilent ChemStation B.02.01).

Control Settings



- **Lamps:** turn on and off of UV- and Vis lamp.
- **At Power On:** automatic lamp-on at power on.
- **Error Method:** take error method or current method (in case of an error).
- **Analog Output Range:** can be set to either 100 mV or 1 V full scale, see also ["Analog Output Settings"](#) on page 61.
- **Automatic Turn On:** lamps can be programmed (detector must be on for this).
- **Help:** online help.

Figure 18 Detector control settings

Online Spectra

- 1 To view the online spectra during the run select *Online Spectra*.

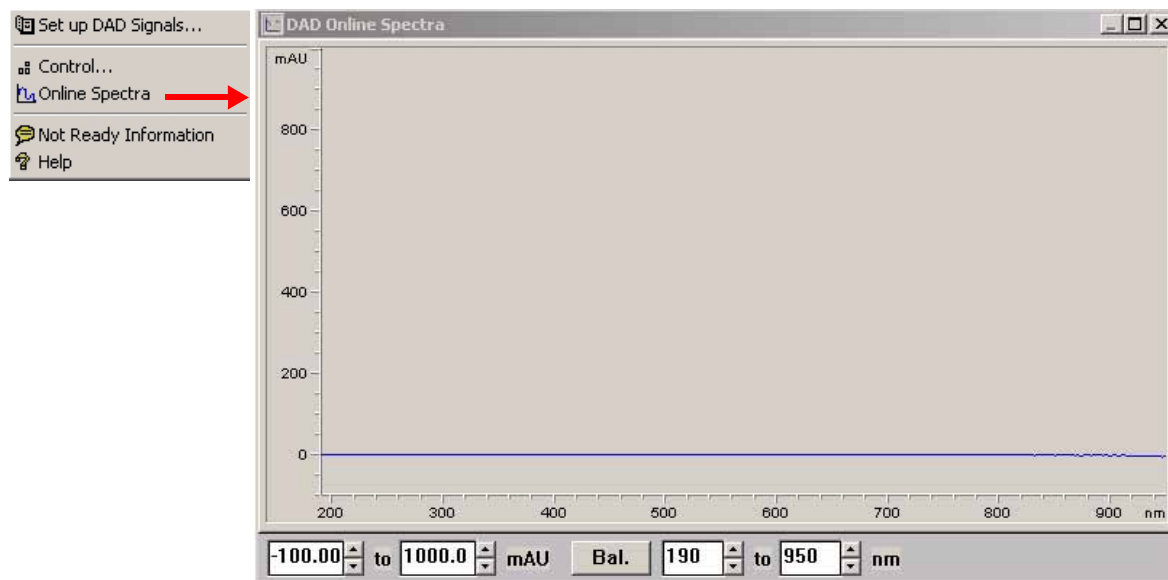


Figure 19 Online Spectra Window

- 2 Change the absorbance and wavelength range according your needs.

Analog Output Settings

To change the Output Range of the analog outputs see “Control Settings” on page 59.

- 1 To change the offset and the attenuation select *Analog Outputs*.
- 2 Change the ranges for absorbance and wavelength according your needs.

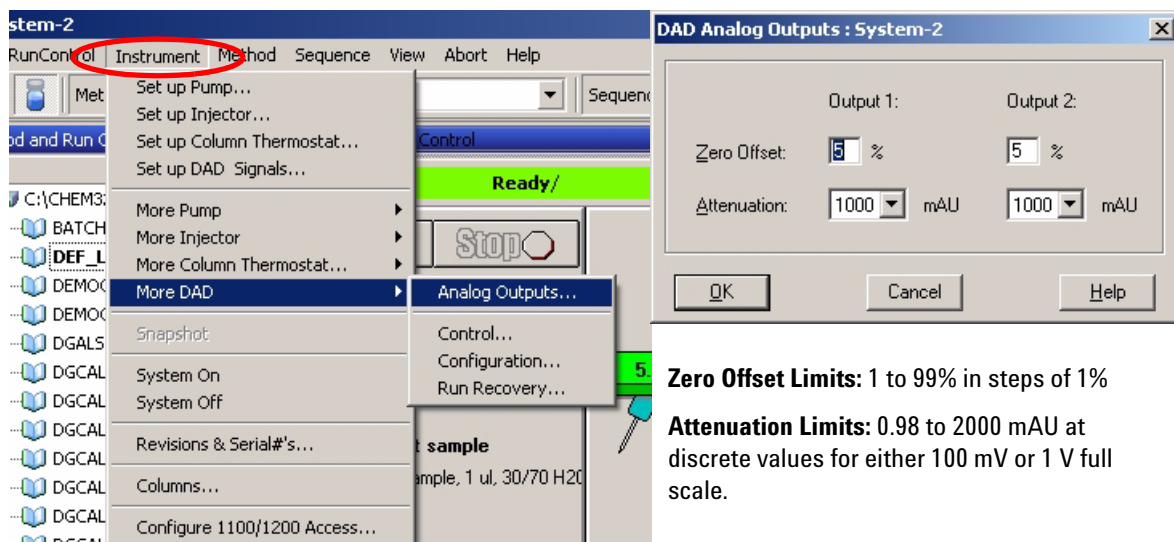


Figure 20 Analog Output Settings

- 3 Change the values if required.

Spectrum Settings

To change the Spectra settings open.

- 1 To change the Spectra settings select *Setup Detector Signals*.
- 2 In the section Spectrum click on the drop-down list and chose a parameter.
[Table 9](#) on page 63 shows the possible parameters.
- 3 Change the Range, Step width and Threshold according to your needs.

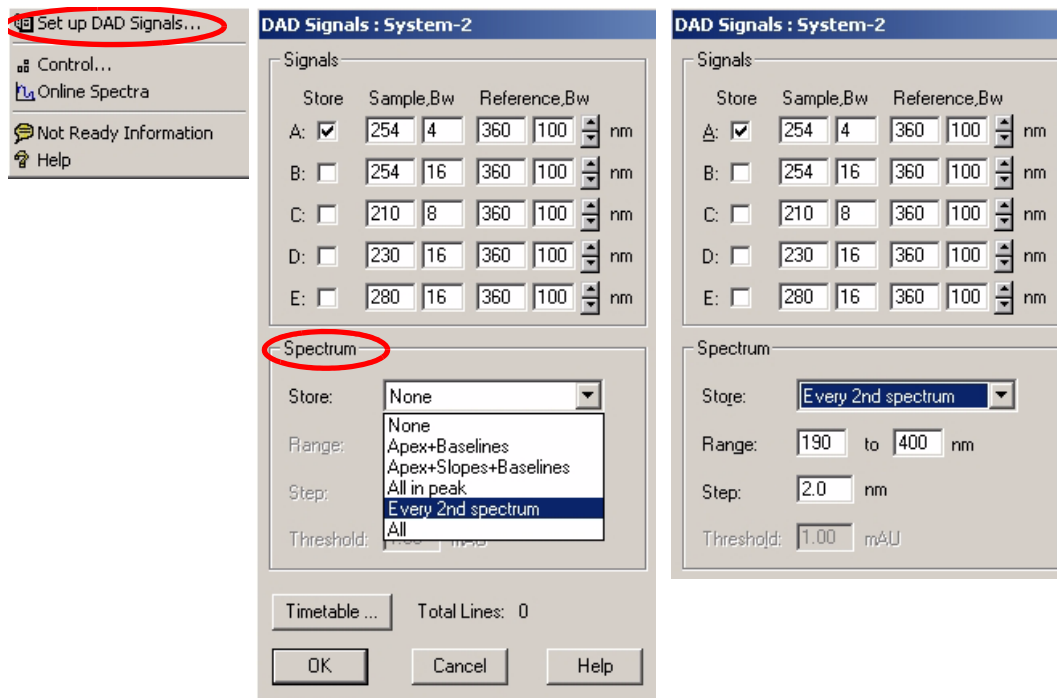


Figure 21 Spectra Settings

Table 9 Spectrum Settings

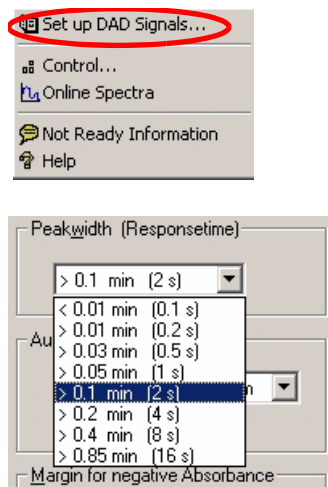
Store	Defines at which points on “signal A” spectra will be taken and saved. Signal A is used to control the “peak controlled spectra acquisition”; the other signals have no influence on spectra acquisition.
None	No spectra are taken.
Apex + Baselines	Spectra are taken at the apex and baselines of the peak.
Apex + Slopes + Baselines	Spectra are taken at the apex, baselines, upslope, and downslope of the peak.
All in Peak	All spectra within the peak are taken.
NOTE	The three spectra acquisition types mentioned above are also referred to as peak-controlled spectra acquisition. The peak detection is done by the detector firmware based on the threshold and peakwidth parameters you set for the DAD. If you want to use peak-controlled spectra storage, make sure that you set these parameters to recognize all the peaks of interest. The integration algorithm also includes peak detection based on the threshold and peakwidth parameters set in the integration events.
Every 2nd spectrum	Spectra are taken continuously as for All, but only every second spectrum is stored; other spectra are discarded. This reduces the amount of data storage necessary.
All	Spectra are taken continuously depending on the setting of the Peakwidth. Eight spectra are acquired per Peakwidth. The acquisition time for one spectrum is slightly less than the Peakwidth divided by 8, that is, greater than or equal to 0.01s and less than or equal to 2.55s.
NOTE	If there are no peaks in Signal A, there are no spectra. You cannot process spectra present in other signals.
Range	Range defines the wavelength range for spectral storage. Limits: 190 to 950 nm in steps of 1 nm for both low and high values. The high value must be greater than the low value by at least 2 nm.
Step	Step defines the wavelength resolution for spectral storage. Limits: 0.10 to 100.00 nm in steps of 0.1 nm.
Threshold:	The threshold is the height in mAU of the smallest expected peak. The peak detector ignores any peaks which are lower than the threshold value and does not save spectra. Limits: 0.001 to 1000.00 mAU in steps of 0.001 mAU. Usable for modes Apex + Baselines, Apex + Slopes + Baselines and All in Peak

Peakwidth Settings

NOTE

- Do not use peak width shorter than necessary

- 1 To change the Peakwidth settings select *Setup Detector Signals*.
- 2 In the section Peakwidth (Responsetime) click on the drop-down list.
- 3 Change the Peakwidth according to your needs.



Peakwidth enables you to select the peak width (response time) for your analysis. The peak width is defined as the width of a peak, in minutes, at half the peak height. Set the peak width to the narrowest expected peak in your chromatogram. The peak width sets the optimum response time for your detector. The peak detector ignores any peaks that are considerably narrower, or wider, than the peak width setting. The response time is the time between 10% and 90% of the output signal in response to an input step function. When the All spectrum storage option is selected, then spectra are acquired continuously depending on the setting of the peak width. The time specified by the peak width is used as a factor in the acquisition of spectra. The acquisition time for one spectrum is slightly less than the peak width divided by 8, that is the acquisition time is between 0.1 seconds (20 Hz) and 3.2 seconds.

Limits: When you set the peak width (in minutes), the corresponding response time is set automatically and the appropriate data rate for signal and spectra acquisition is selected as shown in [Table 10](#) on page 65.

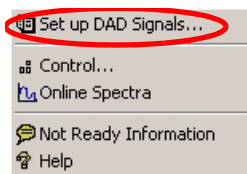
Figure 22 Peakwidth Setting

Table 10 Peak Width — Response Time — Data Rate

Peak Width (min)	Response Time (sec)	Data Rate (Hz)
<0.01	0.1	20
>0.01	0.2	20
>0.03	0.5	10
>0.05	1.0	5
>0.10	2.0	2.5
>0.20	4.0	1.25
>0.40	8.0	0.62
>0.85	16.0	0.31

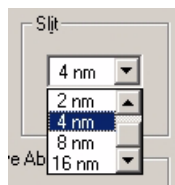
Slit Settings

- 1 To change the Slit settings select *Setup Detector Signals*.
- 2 In the section Slit click on the drop-down list.
- 3 Change the Slit width according to your needs.



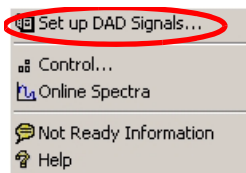
The Slit group allows you to select the optical bandwidth of the detector; the narrower the slit, the smaller the optical bandwidth of the instrument, but the lower its sensitivity. The smaller the optical bandwidth the higher the spectral resolution.

To set the slit width, display the drop-down list and select an appropriate slit width from the list.

**Figure 23** Slit Settings

Margin for Negative Absorbance Settings

- 1 To change the settings select *Setup Detector Signals*.
- 2 In the section Margin for Negative Absorbance change the value according to your needs.



Use this field to modify the detector's signal handling to increase the margin for negative absorbance. Use this option if, for example, your solvent gradient produces a decreasing baseline absorbance, and for GPC analyses.

Limits: 100 to 4000 mAU.

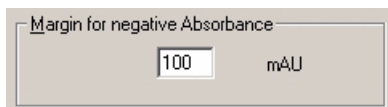


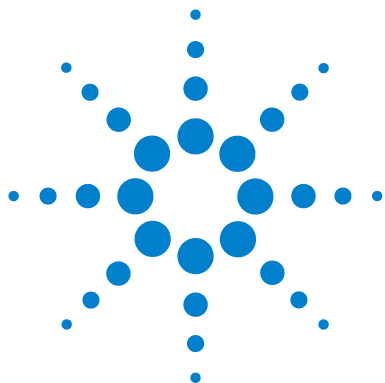
Figure 24 Margin for Negative Absorbance

NOTE

The higher the value the greater the baseline noise. Set this value only if you expect negative absorbance greater than -100 mAU.

Optimizing the Detector

Additional theoretical information can be found in chapter [“How to optimize the Detector”](#) on page 67:



5 How to optimize the Detector

Optimizing the Detector Performance	68
Optimization Overview	69
Optimizing for Sensitivity, Selectivity, Linearity and Dispersion	71
Optimizing Selectivity	82

This chapter provides information on how to optimize the detector.



Optimizing the Detector Performance

The detector has a variety of parameters that can be used to optimize performance. Depending on whether signal or spectral data need to be optimized, different settings are recommended. The following sections describe optimization for:

- signal sensitivity, selectivity and linearity,
- spectral sensitivity and resolution (DAD only), and
- disk space required for storing data.

NOTE

The information in this chapter should be seen as a basic introduction to diode array detector technics. Some of these technics may not be available in the instrument firmware or the user interfaces (Control Module and/or Agilent Chemstation).

How to Get the Best Detector Performance

The information below will guide you on how to get the best detector performance. Follow these rules as a start for new applications. It gives rules-of-thumb for optimizing detector parameters.

Optimization Overview

Table 11 Optimization Overview

Parameter	Impact
1 Selection of flow cell Choose flow cell according to used column, see Figure 25.	peak resolution versus sensitivity
2 Connection of flow cell - For flow rates from 0.5 ml/min connect column using the zero-dead-volume fittings of the detector. - For small column i.d. (e.g 1 mm) the inlet capillary of the micro flow cell can be connected directly to the column.	chromatographic resolution
3 Setting the peak width (response time) - Use peak width according Figure 25 as starting point. - Set the peak-width close to the width of a narrow peak of interest in your chromatogram.	peak resolution versus sensitivity versus disk space
4 Setting wavelength and bandwidth - Sample wavelength: - Never miss a peak by the use of a browser wavelength like 250 nm with 100 nm bandwidth. - Select specific wavelength with reduced bandwidth if you need selectivity, e.g. 250,10 nm and 360,100 nm as reference wavelength. - Set the sample wavelength to a peak or valley in the spectrum to get best linearity for high concentrations. - Reference wavelength: - Select the reference wavelength with broad bandwidth (30...100 nm) wavelength range where your analytes have little or no absorbance (e.g. sample at 254 nm, reference at 320 nm).	- sensitivity versus selectivity - sensitivity versus linearity - baseline drift due to RI effects.

Table 11 Optimization Overview, continued

Parameter	Impact
5 Setting the slit width • Use 4 nm slit for normal applications. • Use narrow slit (e.g 1 nm) if your analytes have narrow absorbance bands and for high concentrations. • Use a wide slit (e.g. 16 nm) to detect very low concentrations. • Optimizing spectral acquisition (DAD only) • Select spectra acquisition mode according to your needs. • Set the spectral wavelength range (for colorless samples 190...400 nm is sufficient). • Set step to 4 nm for normal use; set small step (and slit width) if high resolution of spectra with fine structure is wanted.	• spectral resolution, sensitivity and linearity.

Typical column length	Typical peak width	Recommended flow cell					
T ≤ 5 cm	0.025 min	Micro or Semi-nano					High pressure flow cell for pressures above 100 bar
10 cm	0.05 min		Semi-micro flow cell				
20 cm	0.1 min			Standard flow cell			
≥ 40 cm	0.2 min						
	Typical flow rate	0.01 ... 0.2 ml/min	0.2 ... 0.4 ml/min	0.4 ... 0.4 ml/min	1 ... 2 ml/min	0.01 ... 5 ml/min	
	Internal column diameter	0.5 ... 1 mm	2.1 mm	3.0 mm	4.6 mm		

Figure 25 Choosing a Flow Cell in HPLC

Optimizing for Sensitivity, Selectivity, Linearity and Dispersion

Flow Cell Path Length

Lambert-Beer's law shows a linear relationship between the flow cell path length and absorbance.

$$\text{Absorbance} = -\log T = \log \frac{I_0}{I} = \epsilon \cdot C \cdot d$$

where

T is the transmission, defined as the quotient of the intensity of the transmitted light I divided by the intensity of the incident light, I_0 ,

ϵ is the extinction coefficient, which is a characteristic of a given substance under a precisely-defined set of conditions of wavelength, solvent, temperature and other parameters,

C is the concentration of the absorbing species (usually in g/l or mg/l), and d is the path length of the cell used for the measurement.

Therefore, flow cells with longer path lengths yield higher signals. Although noise usually increases little with increasing path length, there is a gain in signal-to-noise ratio. For example, in [Figure 26](#) the noise increased by less than 10 % but a 70 % increase in signal intensity was achieved by increasing the path length from 6 mm to 10 mm.

When increasing the path length, the cell volume usually increases – in our example from 5 – 13 μ l. Typically, this causes more peak dispersion. As [Figure 26](#) demonstrates, this did not affect the resolution in the gradient separation in our example.

As a rule-of-thumb the flow cell volume should be about 1/3 of the peak volume at half height. To determine the volume of your peaks, take the peak width as reported in the integration results multiply it by the flow rate and divide it by 3).

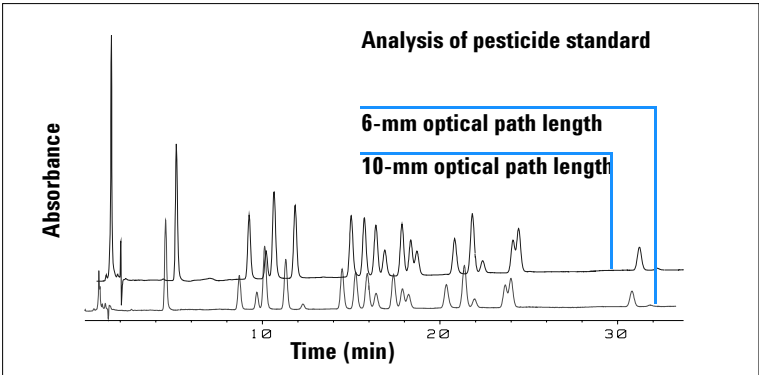


Figure 26 Influence of Cell Path Length on Signal Height

Traditionally LC analysis with UV detectors is based on comparing measurements with internal or external standards. To check photometric accuracy of the Agilent 1200 DAD/MWD it is necessary to have more precise information on path lengths of the flow cells.

The correct response is:
 expected response * correction factor

Please find below the details of the Agilent 1200 DAD/MWD flow cells:

Table 12 Correction factors for Agilent 1200 DAD/MWD flow cells

Flow cell type	Cell volume	Part number	Path length (nominal)	Path length (actual)	Correction factor
Standard flow cell	13 µl	G1315-60022	10 mm	9.80 ± 0.07 mm	10/9.8
Semi-micro flow cell	5 µl	G1315-60025	6 mm	5.80 ± 0.07 mm	6/5.8
Micro flow cell	2 µl	G1315-60024	3 mm	3.00 + 0.05 mm / -0.07 mm	3/3
500 Nanoliter flow cell kit	0.5 µl	G1315-68724	10 mm	10.00 ± 0.02 mm	10/10
80 Nanoliter flow cell kit	0.08 µl	G1315-68716	6 mm	6.00 ± 0.02 mm	6/6
High Pressure flow cell	1.7 µl	G1315-60015	6 mm	5.75 ± 0.07 mm	6/5.75

Peak width (response time)

Response time describes how fast the detector signal follows a sudden change of absorbance in the flow cell. The detector uses digital filters to adapt response time to the width of the peaks in your chromatogram. These filters do not affect peak area nor peak symmetry. When set correctly, such filters reduce baseline noise significantly (see [Figure 27](#)), but reduce peak height only slightly. In addition, these filters reduce the data rate to allow optimum integration and display of your peaks and to minimize disk space required to store chromatograms and spectra.

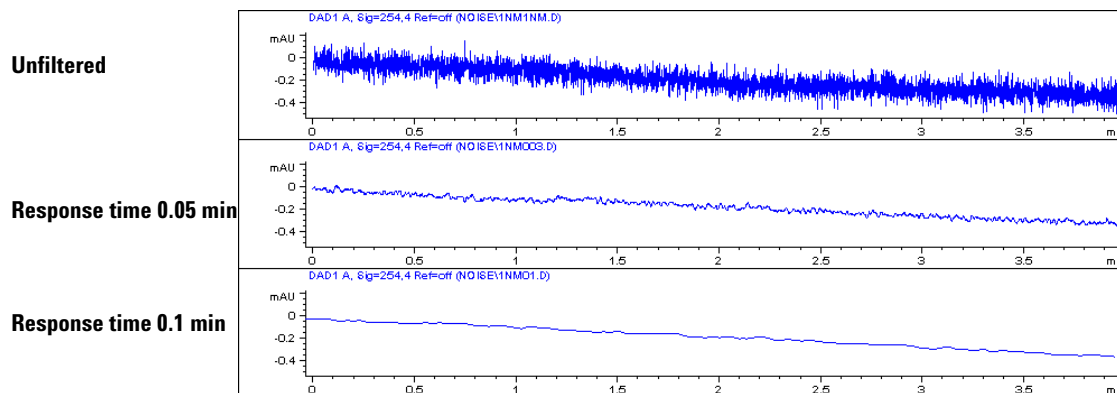


Figure 27 Influence of Response Time on Signal and Noise

[Figure 13](#) lists the filter choices of the detector. To get optimum results, set peak width as close as possible to a narrow peak of interest in your chromatogram. Response time will be approximately 1/3 of the peak width, resulting in less than 5 % peak-height reduction and less than 5 % additional peak dispersion. Decreasing the peak width setting in the detector will result in less than 5 % gain in peak height but baseline noise will increase by a factor of 1.4 for a factor of 2 response-time reduction. Increasing peak width (response time) by factor of two from the recommended setting (over-filtering) will reduce peak height by about 20 % and reduce baseline noise by a factor of 1.4. This gives you the best possible signal-to-noise ratio, but may affect peak resolution.

Table 13 Peak Width — Response Time — Data Rate

Peak Width [minutes]	Response Time [seconds]	Data Rate [Hz]
<0.01	0.1	20
>0.01	0.2	20
>0.03	0.5	10
>0.05	1.0	5
>0.10	2.0	2.5
>0.20	4.0	1.25
>0.40	8.0	0.62
>0.85	16.0	0.31

Sample and Reference Wavelength and Bandwidth

The detector measures absorbance simultaneously at wavelengths from 190 to 950 nm. Two lamps provide good sensitivity over the whole wavelength range. The deuterium discharge lamp provides the energy for the UV range (190 to 400 nm) and the tungsten lamp emits light from 400 to 950 nm for the visible and short wave near infrared.

If you know little about the analytes in your sample, use both lamps and store all spectra over the full wavelength range. This provides full information but fills up your disk space rather quickly. Spectra can be used to check a peak's purity and identity. Spectral information is also useful to optimize wavelength settings for your chromatographic signal.

The detector can compute and store at run time up to 8 signals with these properties:

- sample wavelength, the center of a wavelength band with the width of sample bandwidth (BW), and optionally
- reference wavelength, the center of a wavelength band with the width of reference bandwidth.

The signals comprises a series of data points over time, with the average absorbance in the sample wavelength band minus the average absorbance of the reference wavelength band.

Signal A in the detector default method is set to sample 250,100, reference 360,100, that is, the average absorbance from 200 – 300 nm minus the average absorbance from 300 – 400 nm. As all analytes show higher absorbance at 200 – 300 nm than at 300 – 400 nm, this signal will show you virtually every compound which can be detected by UV absorbance.

Many compounds show absorbance bands in the spectrum. [Figure 28](#) shows the spectrum of anisic acid as an example.

To optimize for lowest possible detectable concentrations of anisic acid, set the sample wavelength to the peak of the absorbance band (that is, 252 nm) and the sample bandwidth to the width of the absorbance band (that is, 30 nm). A reference of 360,100 is adequate. Anisic acid does not absorb in this range.

If you work with high concentrations, you may get better linearity above 1.5 AU by setting the sample wavelength to a valley in the spectrum, like 225 nm for anisic acid.

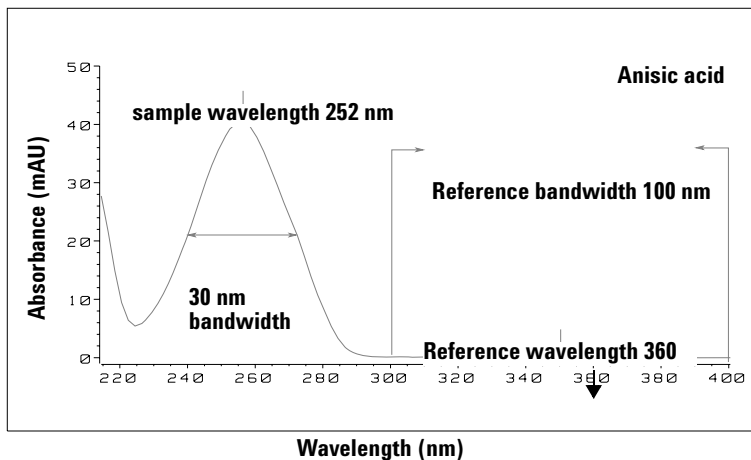


Figure 28 Optimization of Wavelength Setting

A wide bandwidth has the advantage of reducing noise by averaging over a wavelength range – compared to a 4 nm bandwidth, the baseline noise is reduced by a factor of approximately 2.5, whereas the signal is about 75 % of a 4-nm wide band. The signal-to-noise ratio for a 30 nm bandwidth is twice that for a 4-nm bandwidth in our example.

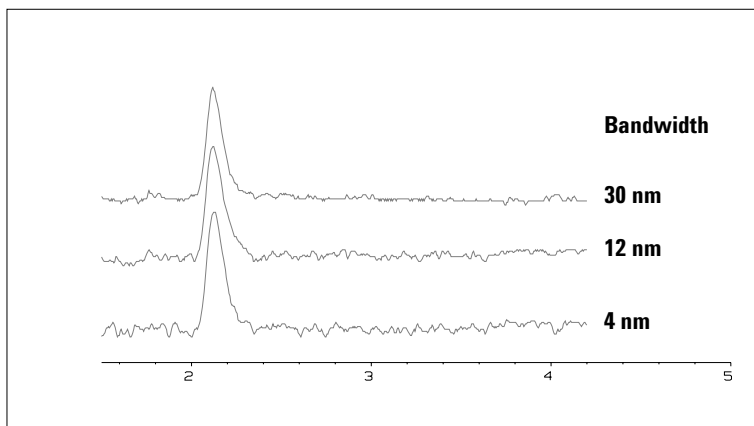


Figure 29 Influence of Bandwidth on Signal and Noise

Because the detector averages absorbance values that are calculated for each wavelength, using a wide bandwidth does not negatively impact linearity.

The use of a reference wavelength is highly recommended to further reduce baseline drift and wander induced by room temperature fluctuations or refractive index changes during a gradient.

An example of the reduction of baseline drifts is shown in [Figure 30](#) for PTH-amino acids. Without a reference wavelength, the chromatogram drifts downwards due to refractive index changes induced by the gradient. This is almost completely eliminated by using a reference wavelength. With this technique, PTH-amino acids can be quantified in the low picomole range even in a gradient analysis.

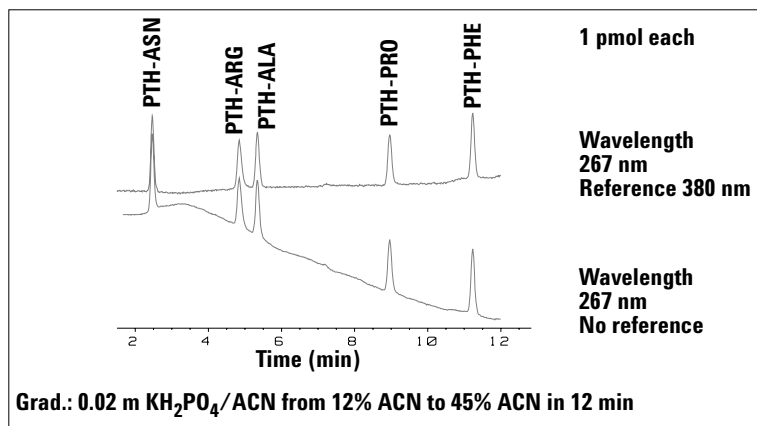


Figure 30 Gradient Analysis of PTH-Amino Acids (1 pmol each), with and without Reference

Slit Width

The detector has a variable slit at the entrance of the spectrograph. This is an effective tool to adapt the detector to changing demand of different analytical problems.

A narrow slit provides spectral resolution for analytes with very fine structures in the absorbance spectrum. An example of such a spectrum is benzene. The five main absorbance bands (fingers) are only 2.5 nm wide and just 6 nm apart from each other.

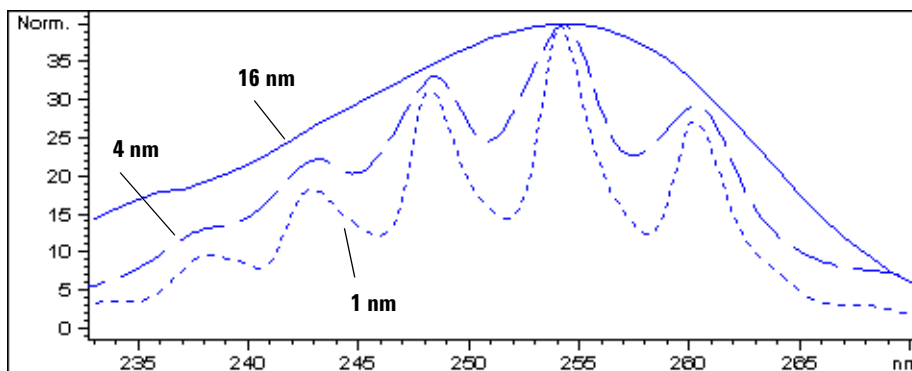


Figure 31 Benzene at 1, 4 and 16 nm slit width

A wide slit uses more of the light shining through the flow cell. This gives lower baseline noise as shown in [Figure 32](#).

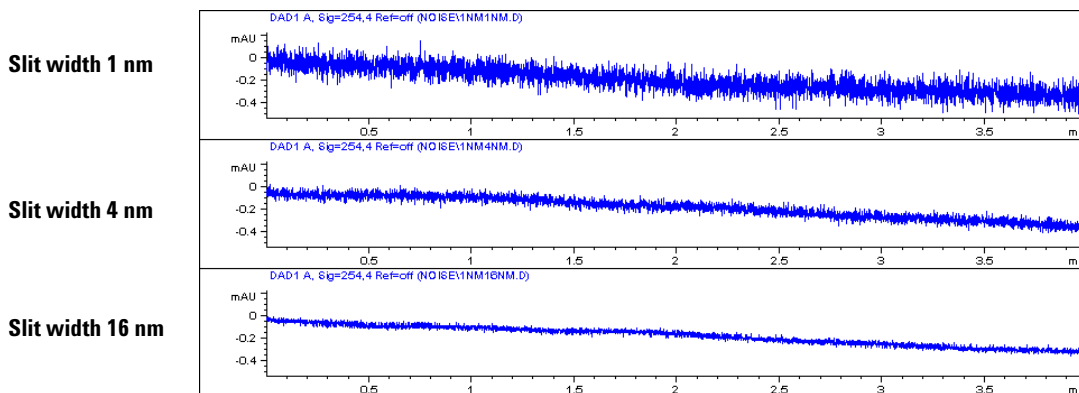


Figure 32 Influence of the Slit Width on Baseline Noise

However, with a wider slit, the spectrograph's optical resolution (its ability to distinguish between different wavelengths) diminishes. Any photodiode receives light within a range of wavelength determined by the slit width. This explains why the fine spectral structure of benzene disappears when using a 16-nm wide slit.

Furthermore, the absorbance is no longer strictly linear with concentration for wavelengths at a steep slope of a compound's spectrum.

Substances with fine structures and steep slopes like benzene are very rare.

In most cases the width of absorbance bands in the spectrum is more like 30 nm as with anisic acid (see [Figure 28](#)).

In most situations, a slit width of 4 nm will give the best results.

Use a narrow slit (1 or 2 nm) if you want to identify compounds with fine spectral structures or if you need to quantify at high concentrations (> 1000 mAU) with a wavelength at the slope of the spectrum. Signals with a wide bandwidth can be used to reduce baseline noise. Because (digital) bandwidth is computed as average of absorbance, there is no impact on linearity.

Use a wide (8 or 16 nm) slit when your sample contains very small concentrations. Always use signals with bandwidth at least as wide as the slit width.

Optimizing Spectral Acquisition (DAD only)

Storage of all spectra consumes a lot of disk space. It is very useful to have all spectra available during optimization of a method or when analyzing unique samples. However when running many samples of the same type, the large size of data files with all spectra may become a burden. The detector provides functions to reduce the amount of data, yet retaining the relevant spectral information.

Range

Only the wavelength range where the compounds in your sample absorb contains information that is useful for purity checks and library searches. Reducing the spectrum storage range saves disk space.

Step

Most substances have broad absorbance bands. Display of spectra, peak purity and library search works best if a spectrum contains 5 to 10 data points per width of the absorbance bands. For anisic acid (the example used before) a step of 4 nm would be sufficient. However a step of 2 nm gives a more optimal display of the spectrum.

Threshold

Sets the peak detector. Only spectra from peaks higher than threshold will be stored when a peak-controlled storage mode is selected.

Margin for Negative Absorbance

The detector adjusts its gain during *balance* such that the baseline may drift slightly negative (about -100 mAU). In some special case, for example, when gradient with absorbing solvents are used, the baseline may drift to more negative values.

Only for such cases, increase the margin for negative absorbance to avoid overflow of the analog-to-digital converter.

Optimizing Selectivity

Quantifying Coeluting Peaks by Peak Suppression

In chromatography, two compounds may often elute together. A conventional dual-signal detector can only detect and quantify both compounds independently from each other if their spectra do not overlap. However, in most cases this is highly unlikely.

With a dual-channel detector based on diode-array technology, quantifying two compounds is possible even when both compounds absorb over the whole wavelength range. The procedure is called peak suppression or signal subtraction. As an example, the analysis of hydrochlorothiazide in the presence of caffeine is described. If hydrochlorothiazide is analyzed in biological samples, there is always a risk that caffeine is present which might interfere chromatographically with hydrochlorothiazide. As the spectra in [Figure 33](#) show, hydrochlorothiazide is best detected at 222 nm, where caffeine also shows significant absorbance. It would therefore be impossible, with a conventional variable wavelength detector, to detect hydrochlorothiazide quantitatively when caffeine is present.

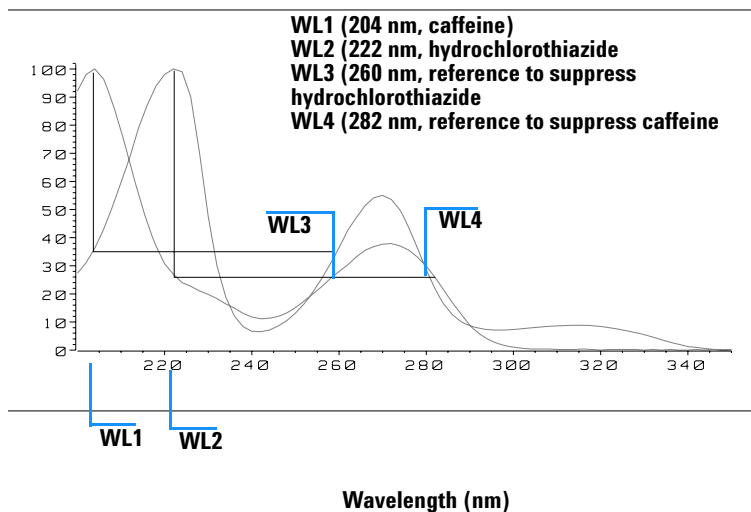


Figure 33 Wavelength Selection for Peak Suppression

With a UV-visible detector based on a diode array and the correct choice of a reference wavelength setting, quantitative detection is possible. To suppress caffeine, the reference wavelength must be set to 282 nm. At this wavelength, caffeine shows exactly the same absorbance as at 222 nm. When the absorbance values are subtracted from each other, any indication of the presence of caffeine is eliminated. In the same way, hydrochlorothiazide can be suppressed if caffeine is to be quantified. In this case the wavelength is set to 204 nm and the reference wavelength to 260 nm. [Figure 34](#) shows the chromatographic results of the peak suppression technique.

The trade-off for this procedure is a loss in sensitivity. The sample signal decreases by the absorbance at the reference wavelength relative to the signal wavelength. Sensitivity may be decreased by as much as 10–30 %.

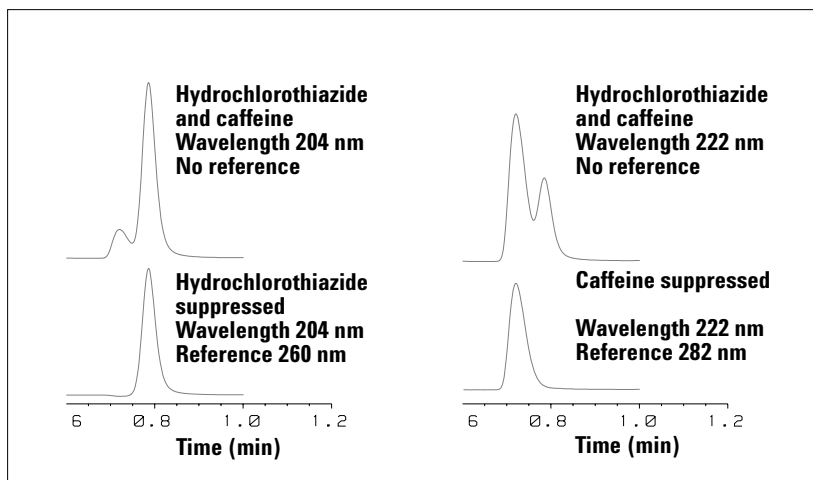


Figure 34 Peak Suppression Using Reference Wavelength

Ratio Qualifiers for Selective Detection of Compound Classes

Ratio qualifiers can be used where, in a complex sample, only one particular class needs to be analyzed – a parent drug and its metabolites in a biological sample, for example. Another example is the selective analysis of derivatives after pre or postcolumn derivatization. Specifying a signal ratio that is typical for the sample class is one way of selectively plotting only those peaks that are of interest. The signal output remains at zero so long as the ratio is out of the user-specified ratio range. When the ratio falls within the range, the signal output corresponds to the normal absorbance, giving single, clear peaks on a flat baseline. An example is shown in [Figure 35](#) and [Figure 36](#).

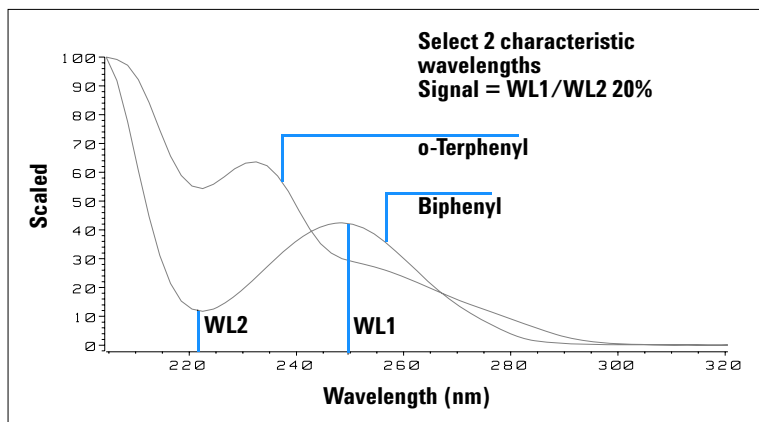


Figure 35 Wavelength Selection for Ratio Qualifiers

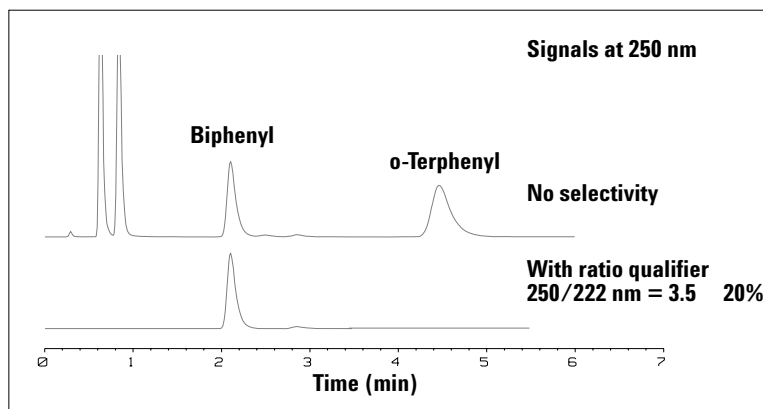
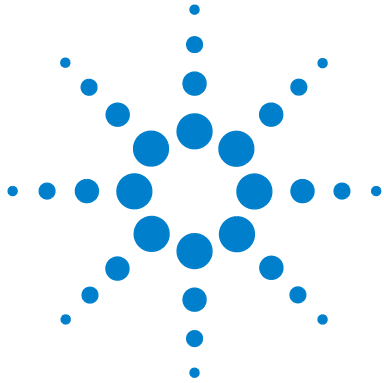


Figure 36 Selectivity by Ratio Qualifiers

In a four-component mixture, only biphenyl was recorded. The other three peaks were suppressed because they did not meet the ratio-qualifier criterion and therefore the output was set to zero. The characteristic wavelengths 249 nm (λ_1) and 224 nm (λ_2) were found from the spectra shown in [Figure 35](#).

The ratio range was set at 2 – 2.4 ($2.2 \pm 10\%$). Only when the ratio between 249 and 224 nm was within this range, is the signal plotted. Of all four peaks, only the third fulfilled the criterion ([Figure 36](#)). The others were not plotted.



6 Troubleshooting and Diagnostics

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User Interfaces	91
Agilent LC Diagnostic Software	92

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.



Overview of the Detector's Indicators and Test Functions

Status Indicators

The detector is provided with two status indicators which indicate the operational state (prerun, run, and error states) of the detector. The status indicators provide a quick visual check of the operation of the detector (see [page 51](#)).

Error Messages

In the event of an electronic, mechanical or hydraulic failure, the detector generates an error message in the user interface. For each message, a short description of the failure, a list of probable causes of the problem, and a list of suggested actions to fix the problem are provided (see [“Error Information” in the Service Manual](#)).

Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components (see [“Test Functions” in the Service Manual](#)).

Wavelength Verification / Recalibration

Wavelength recalibration is recommended after exchange of lamps and flow cells, maintenance of flow cells, repair of internal components, and on a regular basis to ensure correct operation of the detector. The detector uses the deuterium alpha and beta emission lines for wavelength calibration (see [“Wavelength Verification and Recalibration”](#) on page 138).

Diagnostic Signals

The detector has several signals (internal temperatures) that can be used for diagnosing baseline problems (see [“Diagnostic Signals” in the Service Manual](#)).

Status Indicators

Two status indicators are located on the front of the detector. The lower left indicates the power supply status, the upper right indicates the detector status.

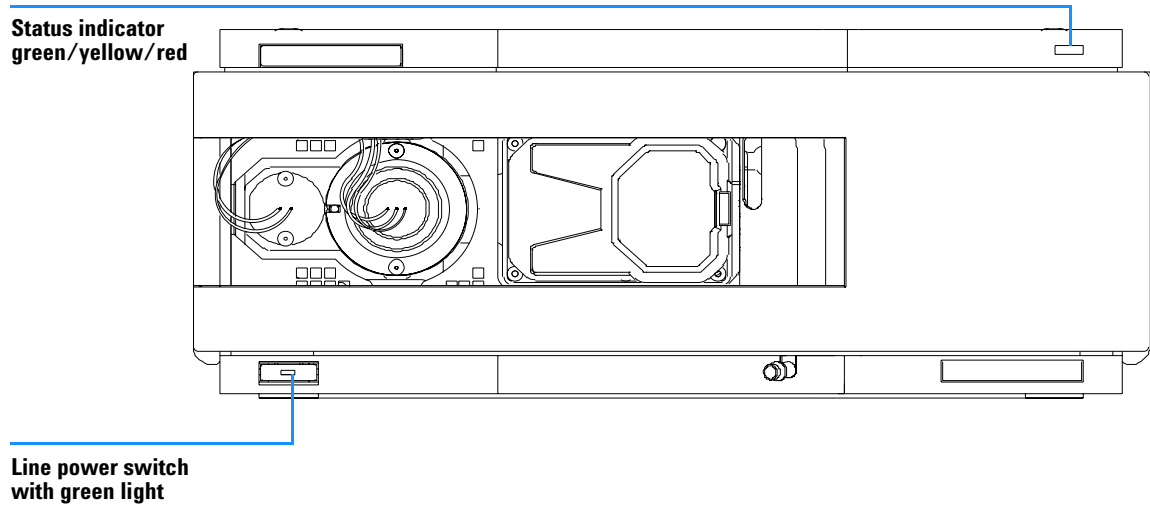


Figure 37 Location of Status Indicators

Power Supply Indicator

The power supply indicator is integrated into the main power switch. When the indicator is illuminated (*green*) the power is *ON*.

Detector Status Indicator

The detector status indicator indicates one of four possible detector conditions:

- When the status indicator is *OFF* (and power switch light is on), the detector is in a *prerun* condition, and is ready to begin an analysis.
- A *green* status indicator, indicates the detector is performing an analysis (*run* mode).
- A *yellow* indicator indicates a *not-ready* condition. The detector is in a not-ready state when it is waiting for a specific condition to be reached or completed (for example, immediately after changing a set point), or while a self-test procedure is running.
- An *error* condition is indicated when the status indicator is *red*. An error condition indicates the detector has detected an internal problem which affects correct operation of the detector. Usually, an error condition requires attention (e.g. leak, defective internal components). An error condition always interrupts the analysis.
- A *red-blinking* indicator indicates that the module is in resident mode (e.g. during update of main firmware).

User Interfaces

Depending on the user interface the available tests vary. All test descriptions are based on the Agilent ChemStation as user interface. Some descriptions are only available in the Service Manual.

Table 14 Test Functions available vs. User Interface

Test	ChemStation	Instant Pilot G4208A	Control Module G1323B
Selftest	Yes	No	No
Filter	Yes	No	No
Slit	Yes	No	Yes
D/A Converter	Yes	No	No
Test Chromatogram	Yes (C)	No	Yes
Wavelength Calibration	Yes	Yes (M)	Yes
Lamp Intensity	Yes	Yes (D)	Yes
Holmium	Yes	Yes (D)	Yes
Cell	Yes	Yes (D)	No
Dark Current	Yes	Yes (D)	No

C via command
M section Maintenance
D section Diagnose

NOTE

The Agilent Control Module (G1323B) does not do any calculations. So there will be no reports generated with passed/failed information.

Agilent LC Diagnostic Software

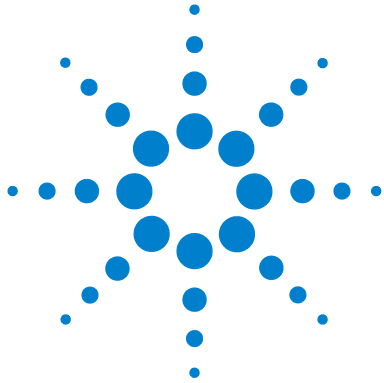
The Agilent LC diagnostic software is an application independent tool that provides troubleshooting capabilities for the Agilent 1200 Series modules. It provides for all 1200 Series LC the possibility of a first guided diagnostic for typical HPLC symptoms and a status report stored as Adobe Acrobat pdf or as a printable file to assist users evaluating the instrument state.

At the introduction, following modules will be fully supported by the software, including module tests and calibrations as well as injector steps and maintenance positions.

- Agilent 1200 Series binary pump SL (G1312B)
- Agilent 1200 Series high performance autosampler SL (G1367B)
- Agilent 1200 Series thermostatted column compartment SL (G1316B)
- Agilent 1200 Series diode array detector SL (G1315C)

With further releases of the diagnostic software all Agilent 1200 Series HPLC modules will be fully supported.

This diagnostic software provides tests and diagnostic features that may differ from the descriptions in this manual. For details refer to the help files provided with the diagnostic software.



7 Maintenance and Repair

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Warnings and Cautions	95
Cleaning the Detector	96
Using the ESD Strap	97

This chapter provides general information on maintenance and repair of the detector.



Introduction into Repairing the Detector

Simple Repairs

The detector is designed for easy repair. The most frequent repairs such as lamp change and flow cell change can be done from the front of the detector with the detector in place in the system stack. These repairs are described in [“Maintenance”](#) on page 99.

Exchanging Internal Parts

Some repairs may require exchange of defective internal parts. Exchange of these parts requires removing the detector from the stack, removing the covers, and disassembling the detector. The security lever at the power input socket prevents that the detector cover is taken off when line power is still connected. These repairs are described in [the Service Manual](#).

Warnings and Cautions

WARNING

To prevent personal injury, the power cable must be removed from the instrument before opening the detector cover. Do not connect the power cable to the detector while the covers are removed.

WARNING

To prevent personal injury, be careful when getting in contact with sharp metal areas.

WARNING

When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

CAUTION

Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection when handling electronic boards and components (see [“Using the ESD Strap”](#) on page 97).

WARNING

Eye damage may result from directly viewing the light produced by the deuterium lamp used in this product. Always turn the deuterium lamp off before removing it.



Cleaning the Detector

The detector case should be kept clean. Cleaning should be done with a soft cloth slightly dampened with water or a solution of water and mild detergent. Do not use an excessively damp cloth allowing liquid to drip into the detector.

WARNING

Do not let liquid drip into the detector. It could cause shock hazard and it could damage the detector.

Using the ESD Strap

Electronic boards are sensitive to electronic discharge (ESD). In order to prevent damage, always use an ESD strap when handling electronic boards and components.

- 1 Unwrap the first two folds of the band and wrap the exposed adhesive side firmly around your wrist.
- 2 Unroll the rest of the band and peel the liner from the copper foil at the opposite end.
- 3 Attach the copper foil to a convenient and exposed electrical ground.

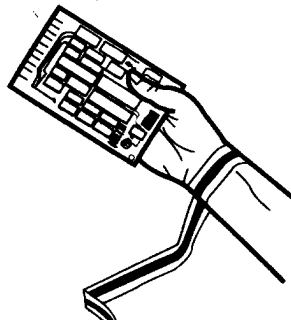
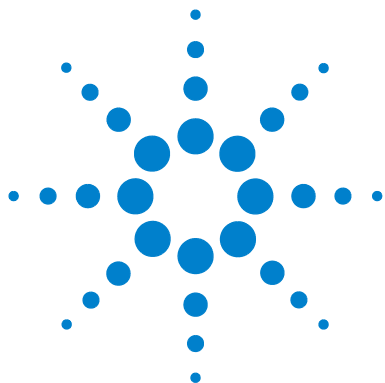


Figure 38 Using the ESD Strap



8 Maintenance

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This chapter describes the maintenance of the detector and the required tests.



Overview of Maintenance

The following pages describe maintenance (simple repairs) of the detector that can be carried out without opening the main cover.

Table 15 Simple Repairs

Procedure	Typical Frequency	Notes
Deuterium lamp or tungsten lamp exchange	If noise and/or drift exceeds your application limits or lamp does not ignite.	An intensity test should be performed after replacement.
Flow cell exchange	If application requires a different flow cell type.	A holmium or wavelength calibration test should be performed after replacement.
Flow cell parts Cleaning or exchange	If leaking or if intensity drops due to contaminated flow cell windows.	A pressure tightness test should be done after repair.
Holmium oxide filter Cleaning or exchange	If contaminated.	A holmium or wavelength calibration test should be performed after replacement.
Leak sensor drying	If leak has occurred.	Check for leaks.
Leak handling System replacement	If broken or corroded.	Check for leaks.

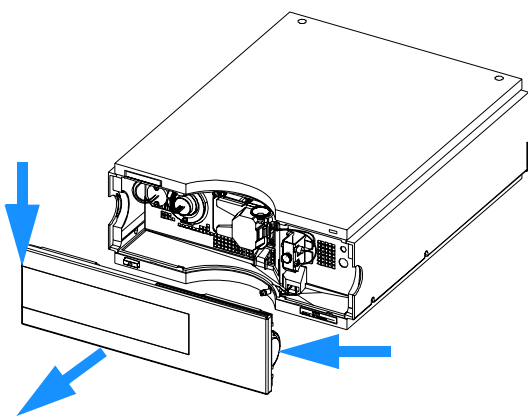
Exchanging a Lamp

When required	If noise or drift exceeds application limits or lamp does not ignite
Tools required	Screwdriver POZI 1 PT3
Parts required	Longlife Deuterium lamp 2140-0813 (without black cover) Longlife Deuterium lamp 5181-1530 (with black cover) Tungsten lamp G1103-60001
Preparations	Turn the lamp(s) off.

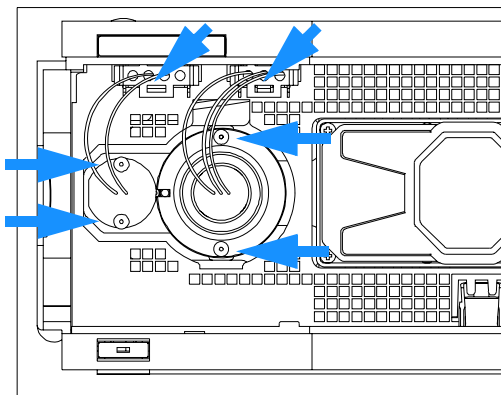
CAUTION

If the detector has been in use, the lamp may be hot. If so, wait five minutes until lamp cools down.

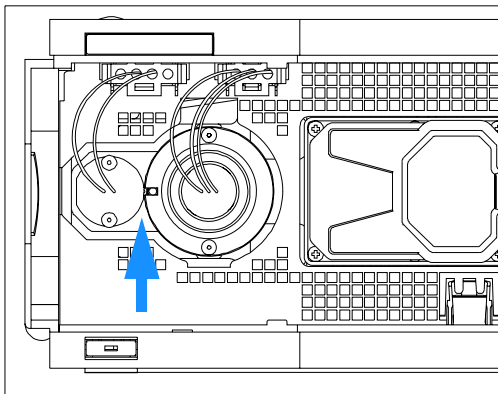
- 1** Press the release buttons and remove the front cover to have access to the lamp area.



- 2** Disconnect lamp from the connector and unscrew the lamp and remove the lamp. Do not touch the glass bulb with your fingers.

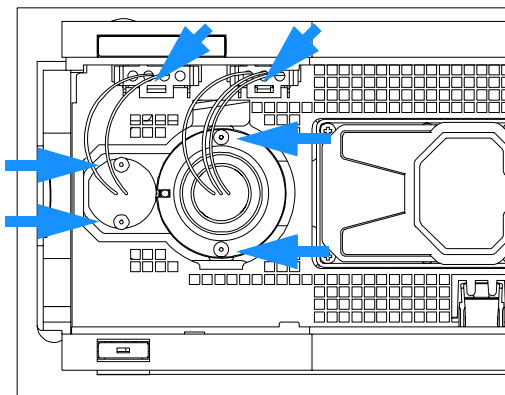


- 3** When replacing the vis-lamp, assure that the vis-lamp is inserted as shown (flat edge towards the deuterium lamp - opposite as in the figure).

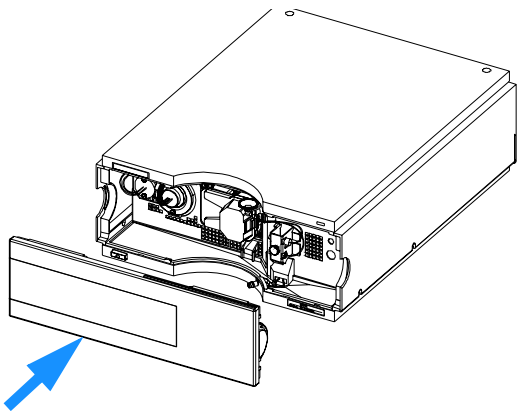


- 4** Insert the lamp. Fix the screws and reconnect the lamp to connector.

5



- 6** Replace the front cover.



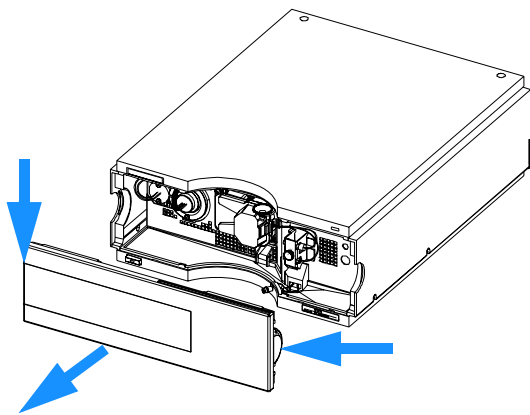
Next steps:

- 7** Reset the lamp counter as described in the user interface documentation.
- 8** Turn the lamp ON and give the lamp 10 minutes to warm up.
- 9** Perform a [“Wavelength Verification and Recalibration”](#) on page 138 or a [“Holmium Oxide Test”](#) on page 139 to check the correct positioning of the UV-lamp.
- 10** Perform an intensity test, see [“Intensity Test”](#) on page 136.

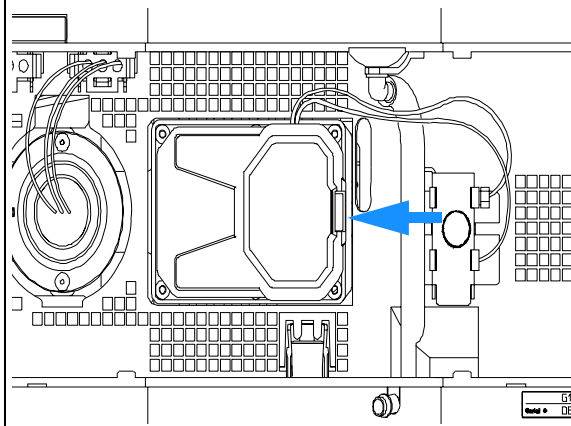
Exchanging a Flow Cell

When required	If an application needs a different type of flow cell or the flow cell needs repair.
Tools required	Two 1/4 inch wrenches for capillary connections
Parts required	Standard flow cell, 10 mm, 13 µl, 120 bar, G1315-60022 Semi-micro flow cell, 6 mm, 5 µl, 120 bar, G1315-60025 Micro flow cell, 3 mm, 2 µl, 120 bar, G1315-60024 High pressure flow cell, 6 mm, 1.7 µl, 400 bar, G1315-60015 Nano flow cell, refer to "Nano Flow Cell - Replacing or Cleaning" on page 123
Preparations	Turn the lamp(s) off. Remove the front cover.

1 Press the release buttons and remove the front cover to have access to the flow cell area.



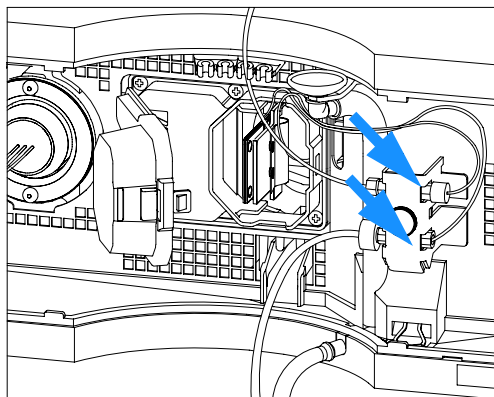
2 Press the release button and open the flow cell door.



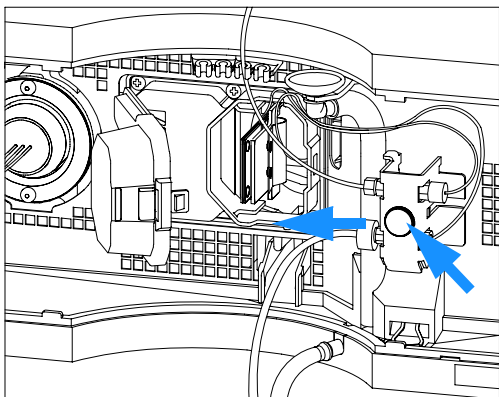
Note:

Depending on the system setup, the inlet capillary might be routed directly from the module above or below to the cell and not to the capillary holder.

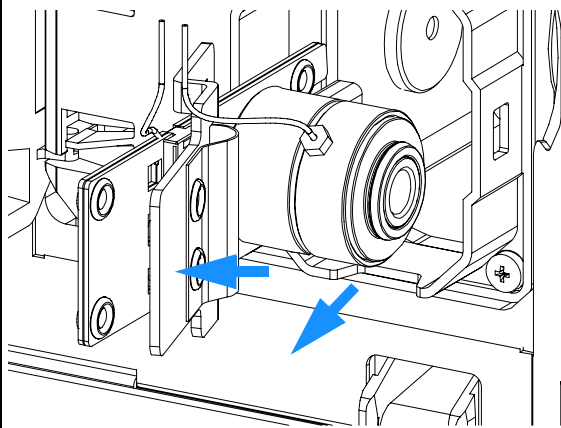
- 3** Disconnect the cell inlet and the cell outlet capillary and the waste capillary from the capillary holder.



- 4** Loosen the thumb screw and remove the flow cell outlet capillary (bottom) with the union.



- 5** Remove the flow cell while pressing the flow cell holder.

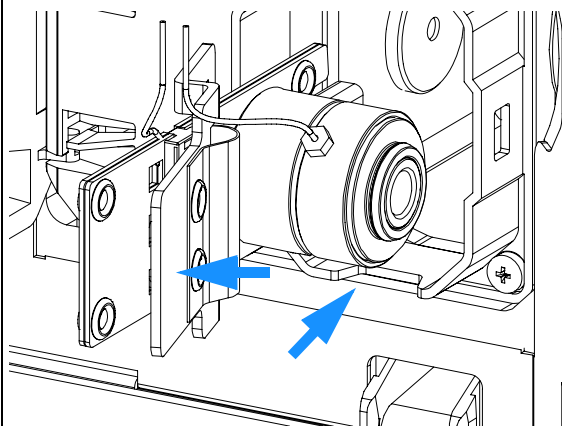


Note:

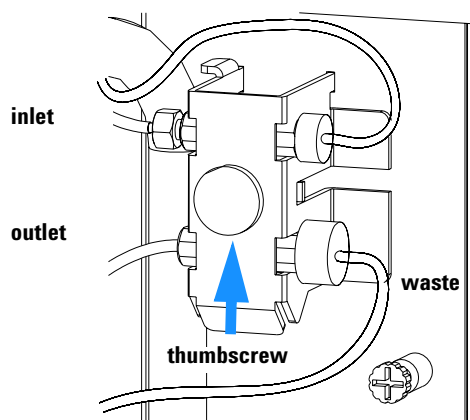
The label attached to the flow cell provides information on part number, path length, volume and maximum pressure.

If you want to replace flow cell parts, see [“Maintenance of Standard, Semi-Micro or Micro Flow Cell”](#) on page 106, [“Maintenance of High Pressure Flow Cell”](#) on page 111 or [“Nano Flow Cell - Replacing or Cleaning”](#) on page 123.

6 Insert the flow cell while pressing the flow cell holder.



7 Insert the flow cell capillaries into the union holder (top is inlet, bottom is outlet). Tighten the thumb screw and Reconnect the waste tubing (bottom) to the union.

**Note:**

To check for leaks, establish a flow and observe the flow cell (outside of the cell compartment) and all capillary connections.

Next steps:

- 8** Perform a [“Wavelength Verification and Recalibration”](#) on page 138 or a [“Holmium Oxide Test”](#) on page 139 to check the correct positioning of the flow cell.
- 9** Replace the front cover.

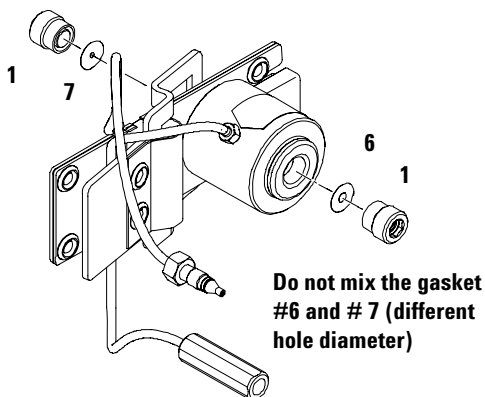
Maintenance of Standard, Semi-Micro or Micro Flow Cell

When required	If the flow cell needs repair due to leaks or contaminations (reduced light throughput)
Tools required	Two 1/4 inch wrenches for capillary connections hexagonal key 4 mm Tooth picks
Parts required	For parts, see “Standard Flow Cell” on page 146, “Semi-Micro Flow Cell” on page 148 or “Micro Flow Cell” on page 150.
Preparations	Turn the flow off. Remove the front cover. Remove the flow cell, see “Exchanging a Flow Cell” on page 103.

NOTE

The gaskets used in the standard and semi-micro/micro flow cell are different.

- 1** Use a 4 mm hex key to unscrew the window assembly and remove the gasket from the cell body.



Note:

If you want to replace the gasket only, continue with step 7.

Do not mix the gasket #6 and #7. They are different for standard and semi-mirco flow cell.

- 2** Use a tooth pick to remove the quartz window from the window assembly.

Note:

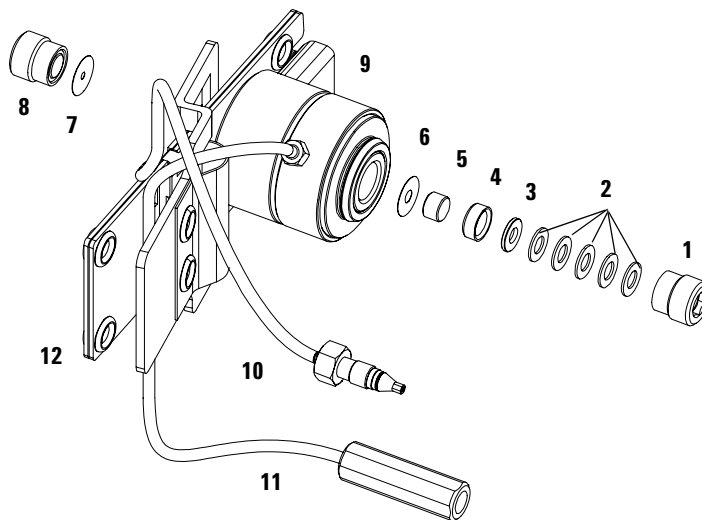
If the washers fall out of the window assembly, they must be inserted in the correct order with the Teflon® ring to prevent any leaks from the flow cell window.

Orientation of Flow Cell Parts

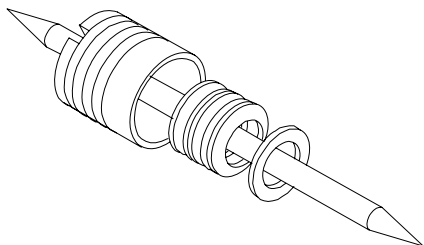
NOTE

Gaskets # 6 and #7 have different hole diameters

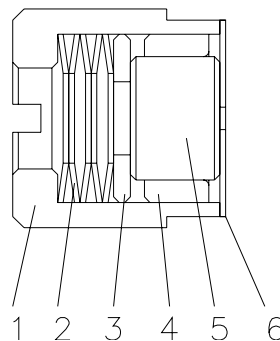
- 1 - window screw
- 2 - spring washers
- 3 - compression washer
- 4 - window holder
- 5 - quartz window
- 6 - gasket (light in)
- 7 - gasket (light out)
- 8 - window screw (contains items 2, 3, 4 and 5)
- 9 - flow cell body
- 10 - inlet capillary
- 11 - outlet capillary
- 12 - holder



- 3** Assemble the washers and the window assembly in correct order.



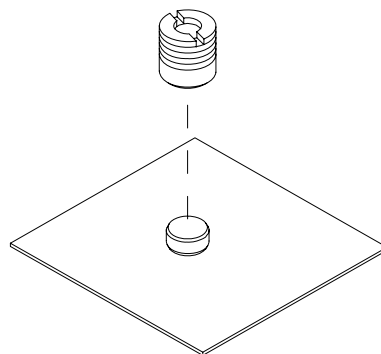
- 4** Correct orientation of spring washers [2] is required.



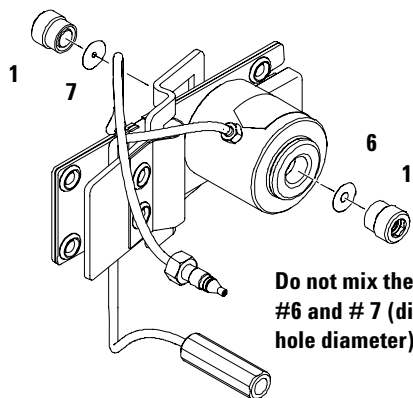
- 5 Press the Teflon® ring into the window assembly.



- 6 Press the window assembly onto the new or cleaned quartz window.



- 7 Insert a new gasket [6, 7] and the window assembly [1] into the cell body. Assure, that the gasket is on the bottom.



- 8 Using a 4-mm hex key, tighten the window screw hand tight plus a quarter turn.

Next steps:

- 10** Reconnect the capillaries, see [“Exchanging a Flow Cell”](#) on page 103.
- 11** Perform a leak test.
- 12** Insert the flow cell.
- 13** Replace the front cover
- 14** Perform a [“Wavelength Verification and Recalibration”](#) on page 138 or a [“Holmium Oxide Test”](#) on page 139 to check the correct positioning of the flow cell.

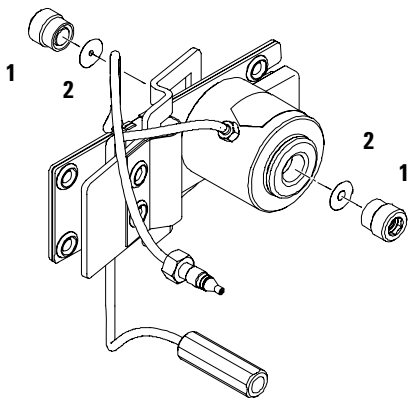
Maintenance of High Pressure Flow Cell

When required	If the flow cell needs repair due to leaks or contaminations (reduced light throughput)
Tools required	Two 1/4 inch wrenches for capillary connections hexagonal key 4 mm Tooth picks
Parts required	For parts see “High Pressure Flow Cell” on page 152
Preparations	Turn the flow off. Remove the front cover. Remove the flow cell, see “Exchanging a Flow Cell” on page 103.

NOTE

All descriptions in this procedure are based on the default orientation of the cell (as it is manufactured). The heat exchanger/capillary and the cell body can be fixed mirror symmetrically to have both capillaries routed to the bottom or to the top (depending on the routing of the capillaries to the column)

- 1** Use a 4 mm hex key to unscrew the window assembly [1] and remove the gasket [2] from the cell body.



If you want to replace the gasket only, continue with step 7 on [page 109](#).

- 2** Use a tooth pick to remove the quartz window from the window assembly.

Note:

If the washers fall out of the window assembly, they must be inserted in the correct order with the Teflon® ring to prevent any leaks from the flow cell window.

Orientation of Flow Cell Parts

1 - window assembly (contains items 2, 3, 4, 5 and 6)

2 - seal ring

3 - quartz window

4 - compression washer

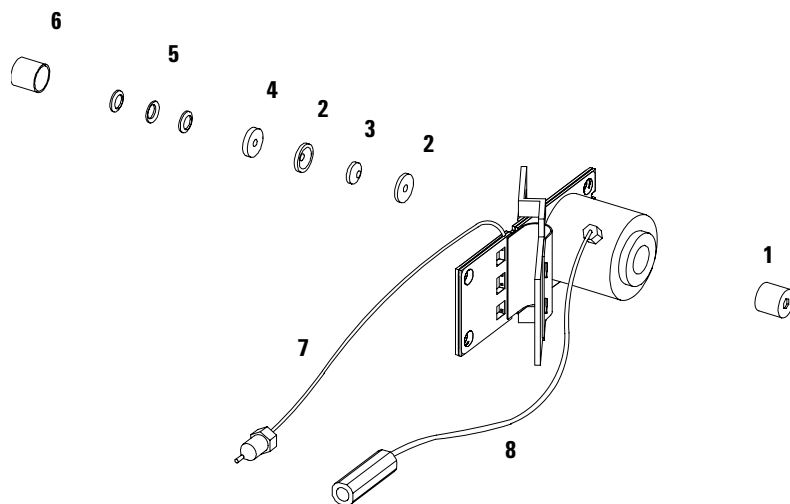
5 - spring washers

4 - window holder

6 - window screw

7 - inlet capillary

8 - outlet capillary



3 Follow the procedure [“Maintenance of Standard, Semi-Micro or Micro Flow Cell”](#) on page 106 for reassembling.

Replacing Capillaries on a Standard Flow Cell

When required	If the capillary is blocked
Tools required	Two 1/4 inch wrenches for capillary connections Wrench 4 mm for capillary connections Screwdriver Pozi 1 PT3
Parts required	For parts see “Standard Flow Cell” on page 146
Preparations	Turn the lamp(s) off. Remove the front cover. Remove the flow cell, see “Exchanging a Flow Cell” on page 103.

NOTE

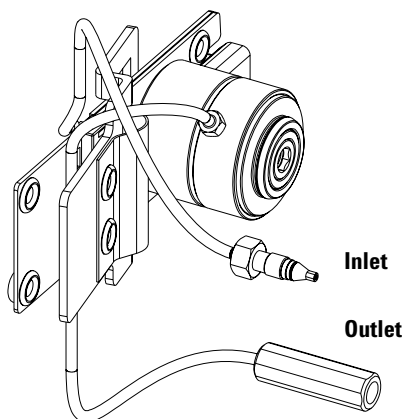
All descriptions in this procedure are based on the default orientation of the cell (as it is manufactured). The heat exchanger/capillary and the cell body can be fixed mirror symmetrically to have both capillaries routed to the bottom or to the top (depending on the routing of the capillaries to the column).

NOTE

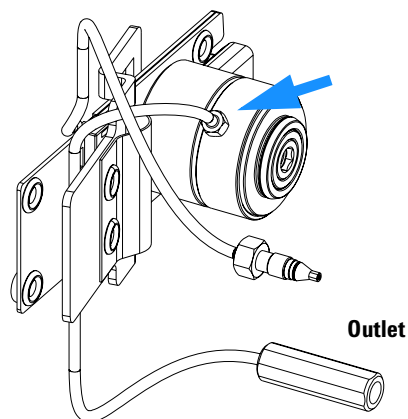
The fittings at the flow cell body are special types for low dead volumes and not compatible with other fittings.

When retightening the fittings, make sure that they are carefully tightened (handtight plus 1/4 turn with a wrench). Otherwise damage of the flow cell body or blockage may result.

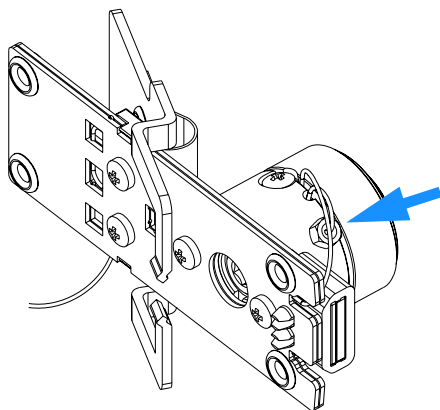
- 1** Identify the inlet and outlet capillaries. To replace the inlet capillary, continue with step 3.



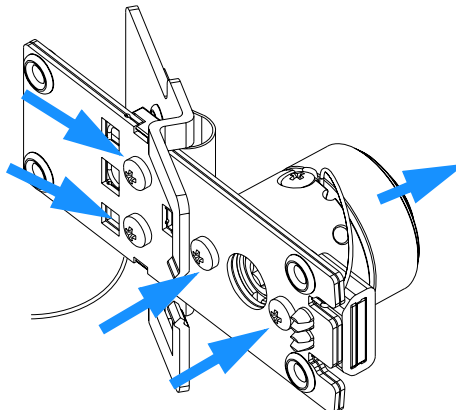
- 2** After replacing the outlet capillary, fix it handtight first. Then do a 1/4 turn with a 4-mm wrench.



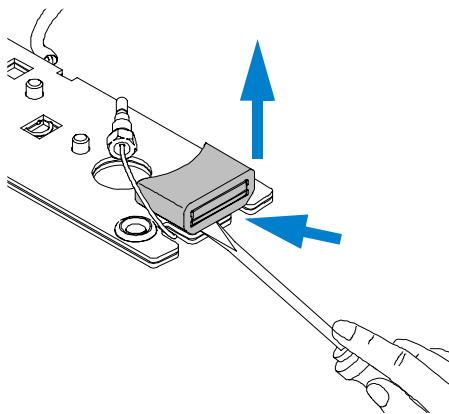
- 3** To replace the inlet capillary, use a 4-mm wrench for the fitting.



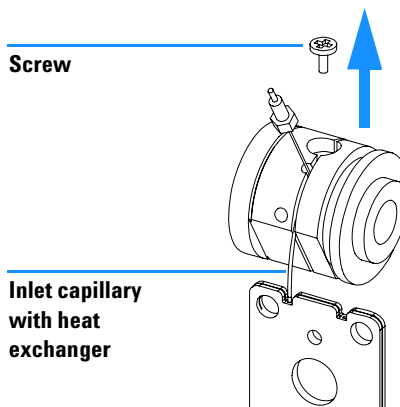
- 4** Unscrew the cell body from the heat exchanger and the heat exchanger from the clamp unit.



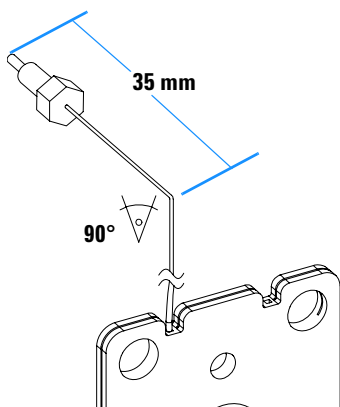
- 5 Use a small flat screw driver to carefully lift off the I.D. tag. Shown is the default orientation. See Note on [page 114](#).



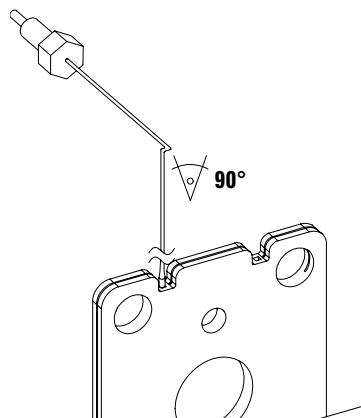
- 6 Unscrew the fixing screw and unwrap the inlet capillary from the groove in the flow cell body.



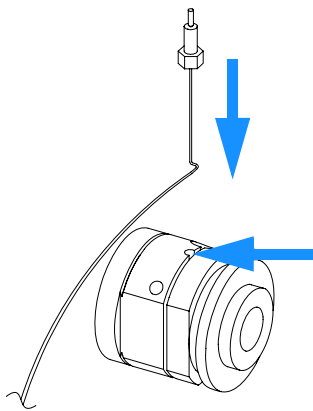
- 7 Take the new inlet capillary and bend it 90° about 35 mm from its end.



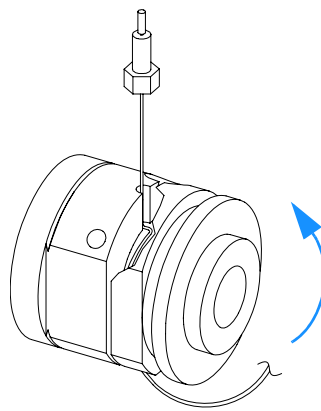
- 8 Bend the capillary again by 90° as shown below.



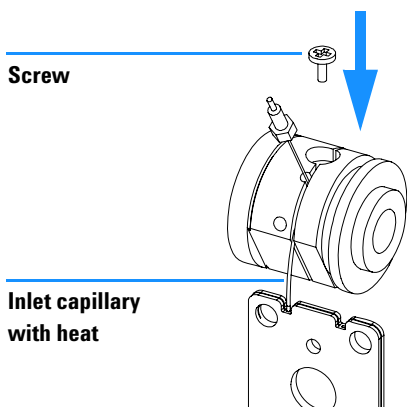
- 9** Insert the capillary into the hole between fixing screw and the inlet fitting..



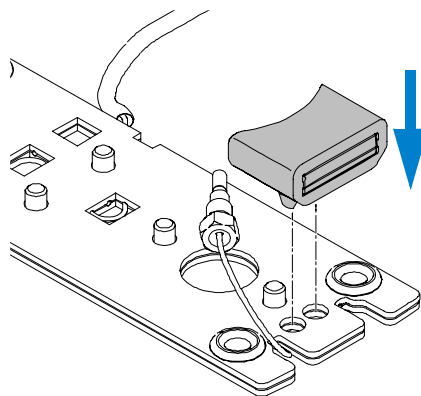
- 10** The capillary lays in the groove and should be tied around the body (in the groove) 5 times.



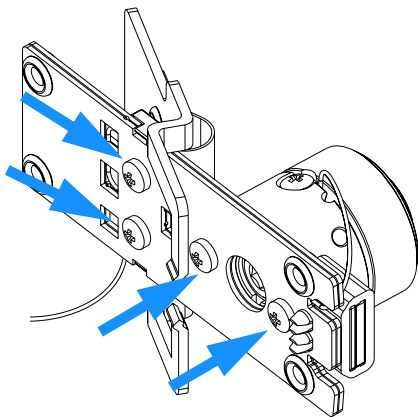
- 11** Insert the fixing screw, so that the capillary cannot leave the groove.



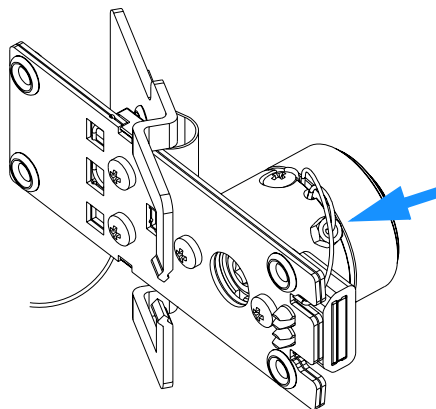
- 12** Carefully insert the I.D. tag into the new heat exchanger. Shown is the default orientation. See Note on [page 114](#).



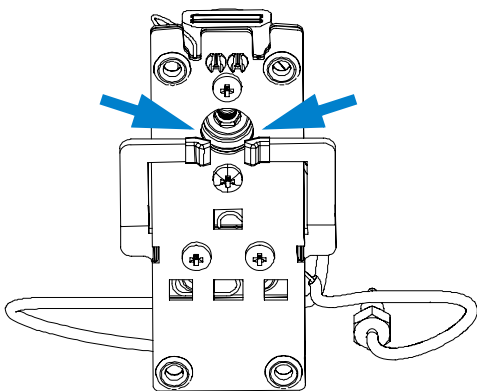
- 13** Fix the heat exchanger to the clamp unit and the flow cell body to the heat exchanger..



- 14** Fix the inlet capillary to the flow cell body handtight first. Then do a 1/4 turn with a 4-mm wrench.



- 15** Check for a centered holder vs. hole. If required adjust with the holder screws.



Next steps:

- 16** Reconnect the capillaries, see [“Exchanging a Flow Cell”](#) on page 103.
17 Perform a leak test.
18 Insert the flow cell.
19 Replace the front cover.
20 Perform a [“Wavelength Verification and Recalibration”](#) on page 138 or a [“Holmium Oxide Test”](#) on page 139 to check the correct positioning of the flow cell.

Replacing Capillaries on a Semi-Micro, Micro and High Pressure Flow Cell

When required	If the capillary is blocked
Tools required	Two 1/4 inch wrenches for capillary connections Wrench 4 mm for capillary connections Screwdriver Pozi 1 PT3
Parts required	For parts see “Semi-Micro Flow Cell” on page 148, “Micro Flow Cell” on page 150 or “High Pressure Flow Cell” on page 152.
Preparations	Turn the lamp(s) off. Remove the front cover. Remove the flow cell, see “Exchanging a Flow Cell” on page 103.

NOTE

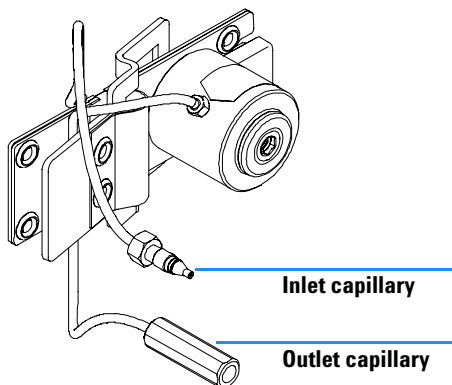
All descriptions in this procedure are based on the default orientation of the cell (as it is manufactured). The heat exchanger/capillary and the cell body can be fixed mirror symmetrically to have both capillaries routed to the bottom or to the top (depending on the routing of the capillaries to the column).

NOTE

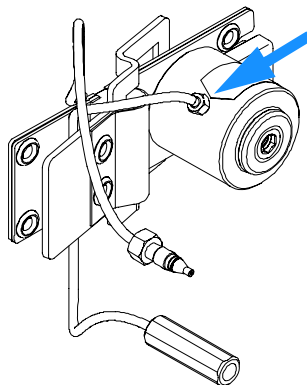
The fittings at the flow cell body are special types for low dead volumes and not compatible with other fittings.

When retightening the fittings, make sure that they are carefully tightened (handtight plus 1/4 turn with a wrench). Otherwise damage of the flow cell body or blockage may result.

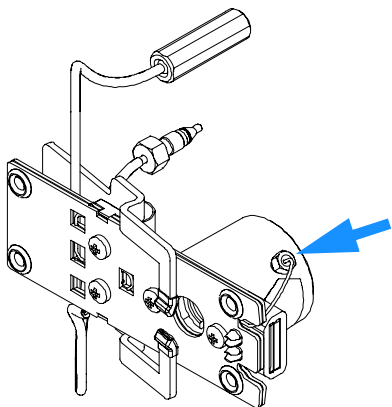
- 1 Identify the inlet and outlet capillaries.



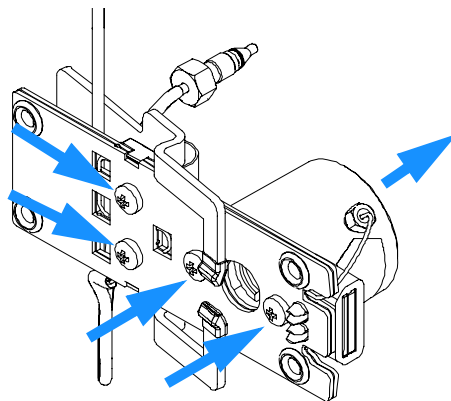
- 2 After replacing the outlet capillary, fix it handtight first. Then do a 1/4 turn with a 4-mm wrench.



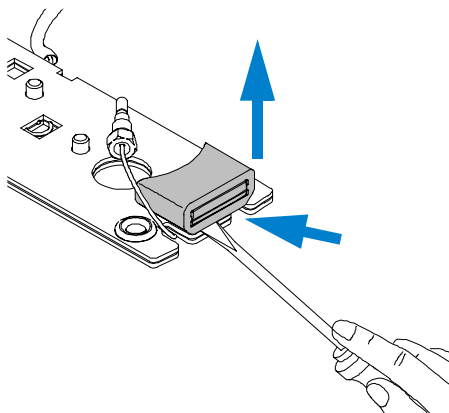
- 3 To replace the inlet capillary, use a 4-mm wrench for the fitting.



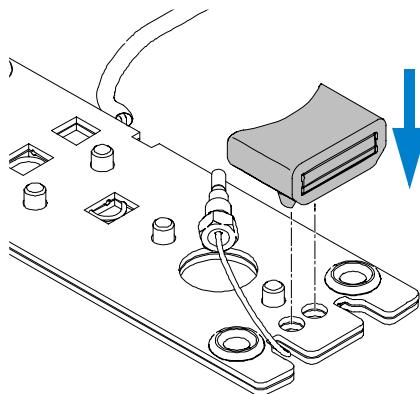
- 4 Unscrew the cell body from the heat exchanger and the heat exchanger from the clamp unit.



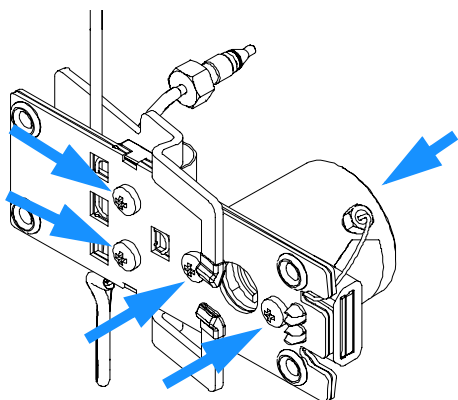
- 5** Use a small flat screw driver to carefully lift off the I.D. tag. Shown is the default orientation. See Note on [page 114](#).



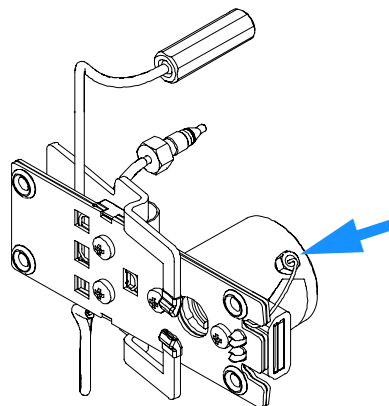
- 6** Carefully insert the I.D. tag into the new heat exchanger. Shown is the default orientation. See Note on [page 114](#).



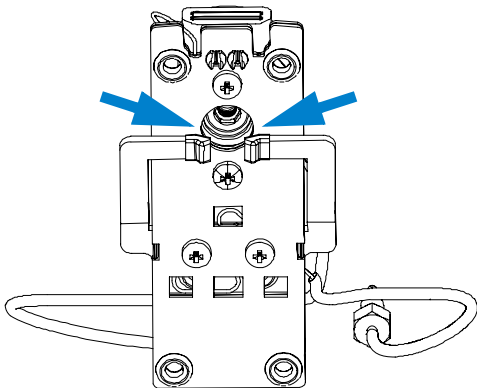
- 7** Fix the new heat exchanger to the clamp unit and the heat exchanger to the cell body.



- 8** Fix the inlet capillary to the flow cell body handtight first. Then do a 1/4 turn with a 4-mm wrench.



- 9 Check for a centered holder vs. hole. If required adjust with the holder screws.



Next steps:

- 10 Reconnect the capillaries, see [“Exchanging a Flow Cell”](#) on page 103.
- 11 Perform a leak test.
- 12 Insert the flow cell.
- 13 Replace the front cover.
- 14 Perform a [“Wavelength Verification and Recalibration”](#) on page 138 or a [“Holmium Oxide Test”](#) on page 139 to check the correct positioning of the flow cell.

Nano Flow Cell - Replacing or Cleaning

When required	If parts are contaminated or leaky.
Tools required	Screwdriver POZI 1 PT3 Two 1/4 inch wrenches for capillary connections
Parts required	For parts identification refer to "Nano Flow Cells" on page 158 (80 nl and 500 nl).
Preparations	Turn the lamp(s) off. Remove the front cover. Remove the flow cell, see "Exchanging a Flow Cell" on page 103.

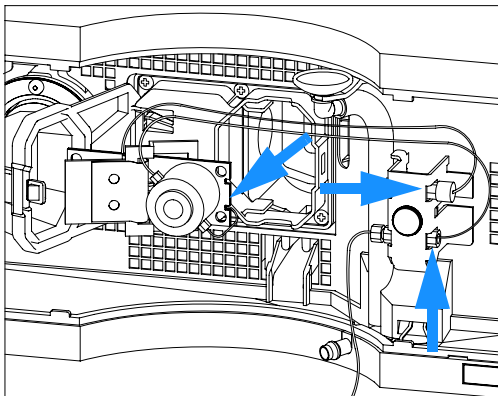
NOTE

For details refer to the technical that comes with the nano-flow cell kit.

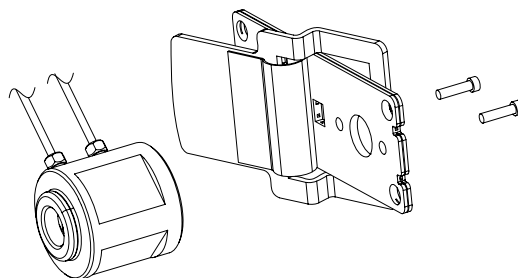
NOTE

The quartz block can be cleaned with alcohol. DO NOT touch the inlet and outlet windows at the quartz block.

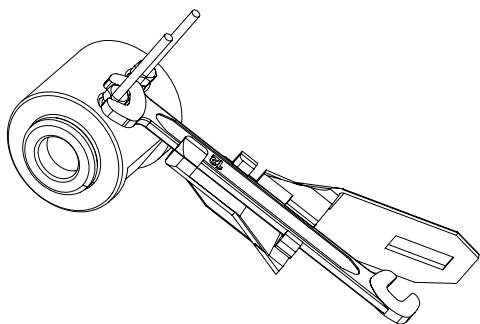
- 1** Disconnect the capillaries from the capillary holder and remove the flow cell.



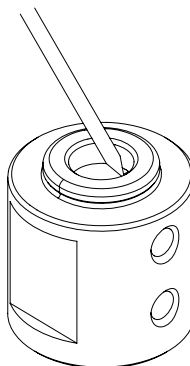
- 2** Unscrew the cell body from the holder.



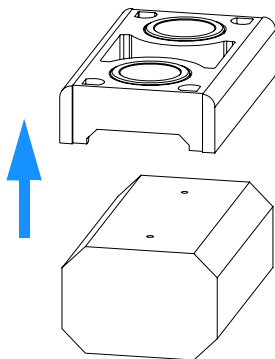
- 3** Unscrew the capillaries from the flow cell. DO NOT use the adapter at this time!



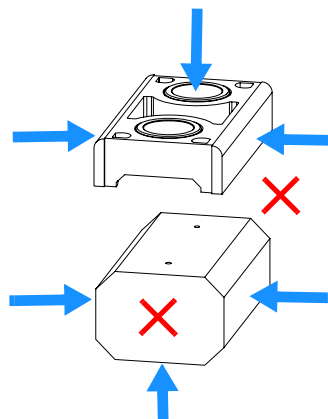
- 4** Using for example a toothpick, press on the plastic part and slide the quartz body out of the cell housing.



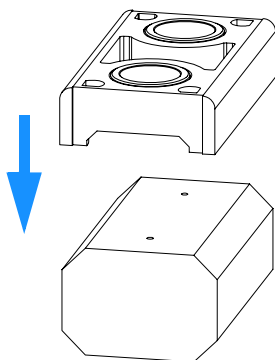
5 The quartz body and the cell seal assembly can be separated for cleaning purpose.



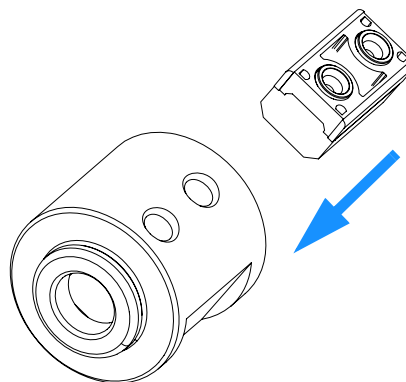
6 This figure shows the correct holding of the quartz body and the cell seal assembly.



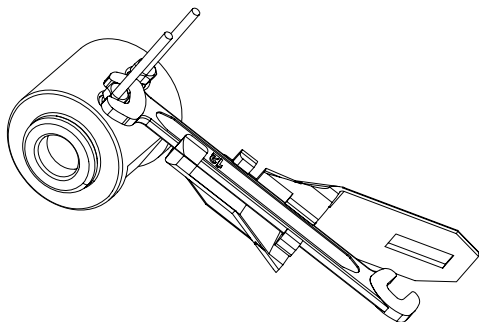
7 Replace the cell seal assembly onto the quartz body. Always use a new seal assembly to exclude damage during disassembling.



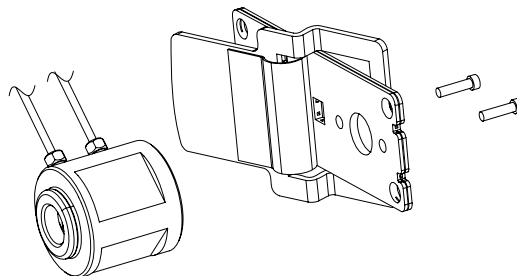
8 Slide the quartz body completely into the cell body to the front stop (use for example a toothpick).



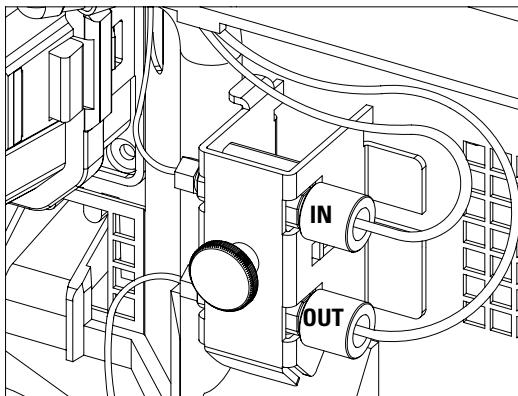
- 9** Insert the flow cell capillaries and tighten them fingertight. Use the wrench and torque adapter as described on [page 127](#) and tighten the fittings alternately.



- 10** Reassemble the flow cell body to the holder.



- 11** Re-install the flow cell and connect the capillaries to the union holder.



Next Steps:

- 12** Perform a leak test with the flow cell outside of the detector.
- 13** If no leak is observed, install the flow cell and you are ready to work.
- 14** Make sure that the flow cell assembly is inserted correctly and fits perfectly in the optical unit (especially when PEEK capillaries are used).

NOTE

The cell body can be fitted in two positions to allow the capillaries routed upwards or downwards (depending on where the column is located). Route the capillaries directly column (inlet) and waste assembly (outlet).

NOTE

With the instrument accessory kit comes a 4-mm wrench and with the Sealing Kit a special adapter. Both together work as a torque wrench with pre-defined torque (maximum allowed torque for the cell fittings is 0.7 Nm). It can be used to tight the capillary fittings at the flow cell body. The wrench has to be plugged into the adapter as shown in [Figure 39](#).

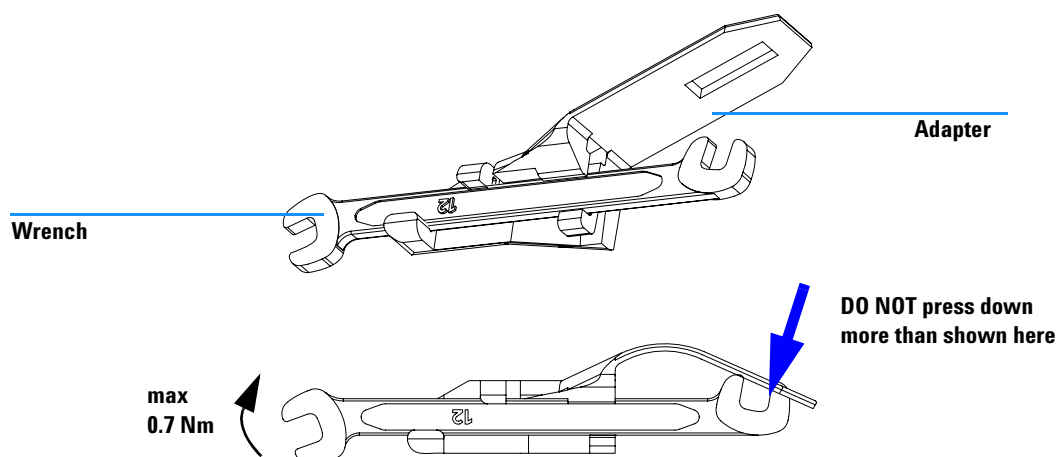


Figure 39 Wrench plus Torque Adapter

Cleaning or Exchanging the Holmium Oxide Filter

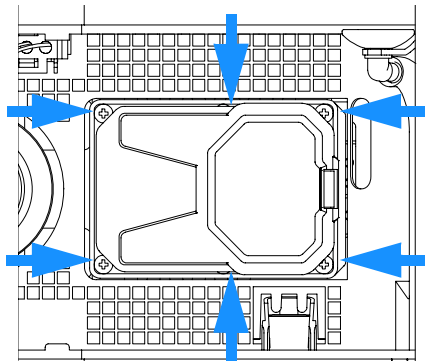
When required	If holmium oxide filter is contaminated
Tools required	Screwdriver POZI 1 PT3 Screwdriver flat blade Two 1/4 inch wrenches for capillary connections A pair of tweezers
Parts required	Holmium oxide filter 79880-22711
Preparations	Turn the lamp(s) off. Remove the front cover. Remove the flow cell, see “Exchanging a Flow Cell” on page 103.

NOTE

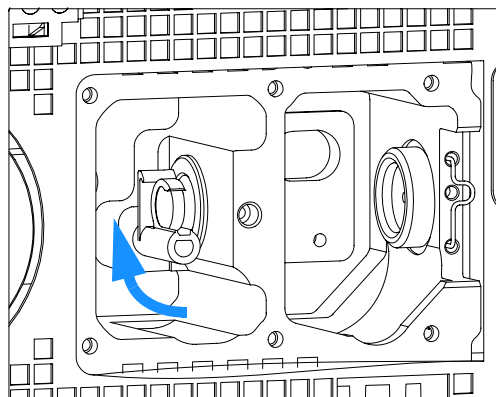
See also [“Declaration of Conformity for HOX2 Filter”](#) on page 176.

The glass tends to build a film on its surface even under normal environmental conditions. This is a phenomenon, which can be found also on the surface of several other glasses and has something to do with the composition of the glass. There is no indication, that the film has an influence on the measurement. Even in the case of a thick film, which scatters the light remarkably, no shift of the peak positions is to be expected. A slight change in the absorbance might be possible. Other components within the light path (lenses, windows, ...) are also changing their behavior over the time.

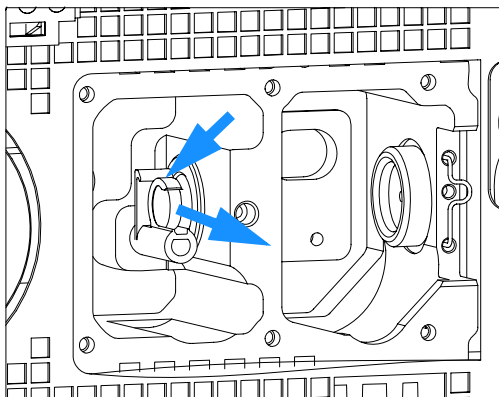
1 Unscrew the six screws and remove the flow cell cover.



2 If not already in this position, move the filter up.



3 While releasing the holder with a screwdriver, carefully remove the holmium oxide filter using a pair of tweezers covered with lint-free cloth or tape.

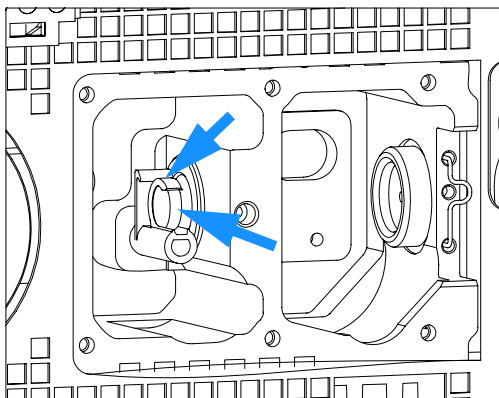


Note:

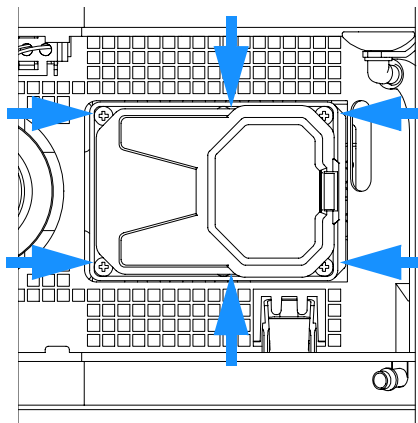
Do not scratch the holmium oxide filter.

The holmium oxide filter can be cleaned with alcohol and a lint-free cloth.

4 While releasing the holder with a screw driver, carefully insert the holmium oxide filter.



5 Replace the flow cell cover and fix the six screws.



Next steps:

- 6** Perform a [“Wavelength Verification and Recalibration”](#) on page 138 to check the proper function of the holmium oxide filter.
- 7** Insert the flow cell, see [“Exchanging a Flow Cell”](#) on page 103.
- 8** Replace the front cover.
- 9** Turn ON the flow.

Correcting Leaks

When required	If a leakage has occurred in the flow cell area or at the heat exchanger or at the capillary connections
Tools required	Tissue Two 1/4 inch wrenches for capillary connections
Parts required	None
Preparations	Remove the front cover.

- 1 Use tissue to dry the leak sensor area and the leak pan.
- 2 Observe the capillary connections and the flow cell area for leaks and correct, if required.

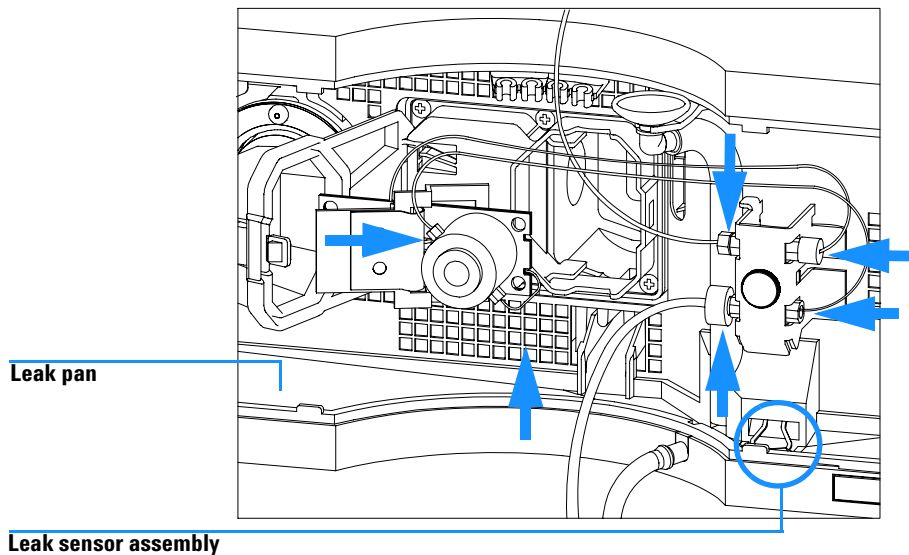


Figure 40 Observing for Leaks

- 3 Replace the front cover.

Replacing Leak Handling System Parts

When required	If the parts are corroded or broken
Tools required	None
Parts required	Leak funnel 5061-3388 Leak funnel holder 5041-8389 Leak tubing (120 mm) 0890-1711
Preparations	Remove the front cover.

- 1 Pull the leak funnel out of the leak funnel holder.
- 2 Pull out the leak funnel with the tubing.
- 3 Insert the leak funnel with the tubing in its position.
- 4 Insert the leak funnel into the leak funnel holder.

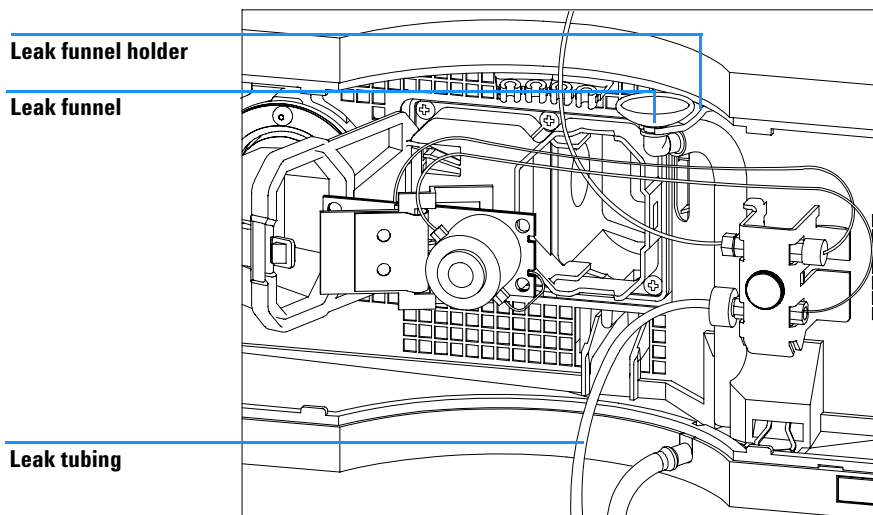


Figure 41 Replacing Leak Handling System Parts

- 5 Replace the front cover.

Replacing the Interface Board

When required

For all repairs inside the detector or for installation of the board

Part required

Interface board (BCD) G1351-68701 with external contacts and BCD outputs, LAN Communication Interface board G1369A or G1369-60001.

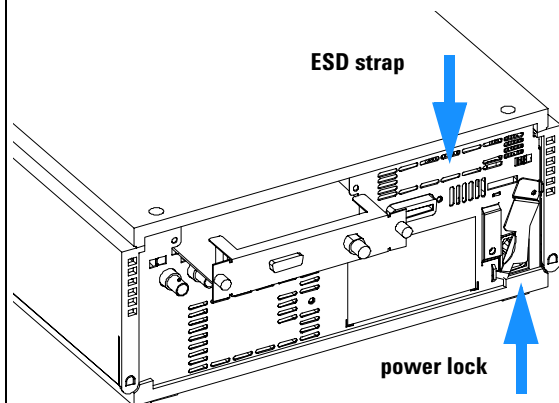
Tools required

None

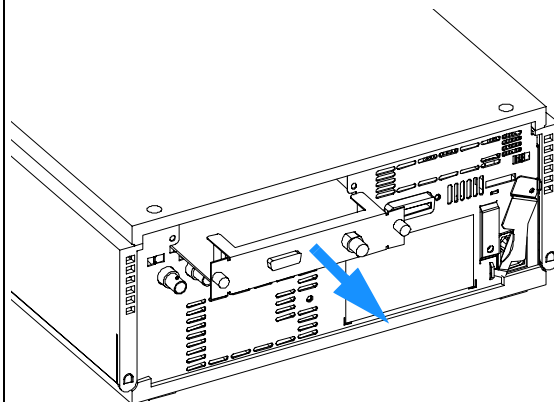
Preparations

- Turn off the lamp.
- Switch off the detector, and disconnect the power cable.
- Assure you have a access to the rear of the module.

1 Install the ESD strap. Move the power lock across the power inlet.



2 If required, unscrew and remove the interface board. Place the board on the ESD kit.



3 If required, insert the interface board and fix the screws.

Next steps:

- Remove the ESD strap.
- Reinstall the module into the stack.

Replacing the Detector's Firmware

When required	If new version solves problems of currently installed version or after exchange of the detector main board (VWM) the version on board is older than previous installed one.
Tools required	LAN/RS-232 Firmware Update Tool, or Instant Pilot G4208A or Control Module G1323B
Parts required	Firmware, tools and documentation from Agilent web site
Preparations	Read update documentation provided with the Firmware Update Tool.

The installation of *older* firmware might be necessary:

- to keep all systems on the same (validated) revision, or
- if third part control software requires a special version.

To upgrade/downgrade the detector's firmware the following steps have to be performed:

- 1 Download the module's firmware, the LAN/RS-232 FW Update Tool Version 2.10 or above and the documentation from the Agilent web

http://www.chem.agilent.com/scripts/cag_firmware.asp.

- 2 Load the firmware into the detector as described in the documentation.

NOTE

The G1315B DAD and the G1365B MWD require firmware revision A.04.x or above (main and resident).

Tests & Calibrations

The following tests are required after maintenance of lamps and flow cells:

- “[Intensity Test](#)” on page 136.
- “[Wavelength Verification and Recalibration](#)” on page 138.
- “[Holmium Oxide Test](#)” on page 139.
- “[Cell Test \(ChemStation only\)](#)” on page 141.

Intensity Test

NOTE

The test is for the standard flow cells (10 mm and 6 mm pathlength) only. The nano-flow cells (80 nl and 500 nl) cannot be run with this test due to its low volume.

The intensity test measures the intensity of the deuterium and tungsten lamps over the full wavelength range (190 – 950 nm). Four spectral ranges are used to evaluate the intensity spectrum. The test is used to determine the performance of the lamps and optics (see also “[Cell Test \(ChemStation only\)](#)” on page 141). When the test is started, the 1-nm slit is moved into the light path automatically, and the gain is set to zero. To eliminate effects due to absorbing solvents, the test should be done with water in the flow cell. The shape of the intensity spectrum is primarily dependent on the lamp, grating, and diode array characteristics. Therefore, intensity spectra will differ slightly between instruments. [Figure 42](#) on page 137 shows a typical intensity test spectrum.

Intensity Test Evaluation

The Agilent ChemStation evaluates four spectral ranges automatically, and displays the limits for each range, the measured intensity counts, and *passed* or *failed* for each spectral range (see [Figure 42](#) on page 137).

NOTE

The evaluation report is only on the Agilent ChemStation available.

Test Failed

Probable Causes

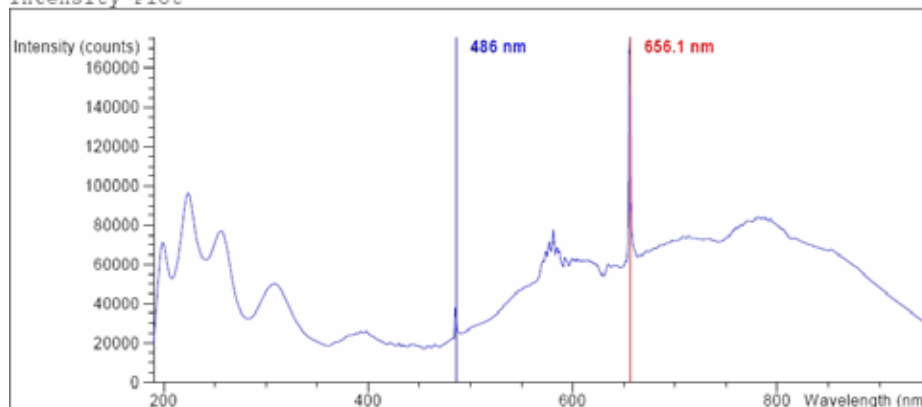
- Absorbing solvent or air bubble in flow cell.
- Dirty or contaminated flow cell.
- Dirty or contaminated optical components (achromat, windows).
- Old or non-Agilent lamp.

Suggested Actions

- ✓ Ensure the flow cell is filled with water, and free from air bubbles.
- ✓ Run the cell test (see “Cell Test (ChemStation only)” on page 141). If the test fails, exchange the flow cell windows.
- ✓ Clean optical components with alcohol and lint-free cloth.
- ✓ Exchange the lamp.

Instrument: G1315B
 Serial Number: DE03010353
 Operator: Wolfgang
 Date: 24.11.2005
 Time: 14:36:20

Intensity Plot



Intensity Test Results

	Specification	Measured	Result
Accumulated UV lamp on time		8.66 h	
Lowest intensity in range 190nm - 220nm:	> 2000 cts	19677 cts	Passed
Lowest intensity in range 221nm - 350nm:	> 5000 cts	20612 cts	Passed
Lowest intensity in range 351nm - 500nm:	> 2000 cts	17300 cts	Passed
Lowest intensity in range 501nm - 950nm:	> 4000 cts	27853 cts	Passed
Highest intensity in range 190nm - 350nm:	< 450000 cts	96368 cts	Passed
Highest intensity in range 700nm - 950nm:	< 300000 cts	83955 cts	Passed
Highest intensity for the D2 alpha line:	< 1200000 cts	175890 cts	Passed

Figure 42 Intensity Test Results (report)

Wavelength Verification and Recalibration

The detector uses the alpha (656.1 nm) and beta (486 nm) emission lines of the deuterium lamp for wavelength calibration. The sharp emission lines enable more accurate calibration than is possible with holmium oxide. When verification is started, the 1-nm slit is moved into the light path automatically, and the gain is set to zero. To eliminate effects due to absorbing solvents, the test should be done with bubble free degassed HPLC water in the flow cell.

If a deviation is found and displayed, it can be recalibrated by pressing Adjust. The deviations are tracked in the Calibration History (diagnosis buffer in the detector).

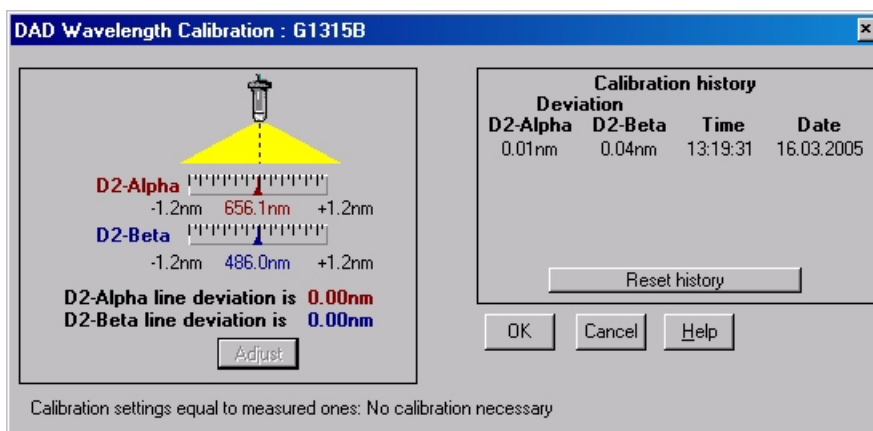


Figure 43 Wavelength Verification and Recalibration

Wavelength calibration should be done

- after maintenance or change of the flow cell,
- lamp exchange, or
- after a major repair, like processor board or optical unit exchange.

After calibration, the holmium oxide test (see “[Holmium Oxide Test](#)” on page 139) provides verification of wavelength accuracy at three additional wavelengths.

Holmium Oxide Test

The holmium oxide test uses three characteristic absorbance maxima of the built-in holmium oxide filter to verify wavelength accuracy (see also [“Wavelength Verification and Recalibration”](#) on page 138). When the test is started, the 1-nm slit is moved into the light path automatically. To eliminate effects due to absorbing solvents, the test should be done with water in the flow cell.

NOTE

See also [“Declaration of Conformity for HOX2 Filter”](#) on page 176.

Holmium Oxide Test Evaluation

Limits:

361.0 nm	360.0 - 362.0 nm (± 1 nm)
453.7 nm	452.7 - 454.7 nm (± 1 nm)
536.7 nm	535.7 - 537.7 nm (± 1 nm)

The test is evaluated by the instrument, and the measured maxima are displayed automatically. The test fails if one or more of the maxima lies outside of the limits (see [Figure 44](#) on page 140).

NOTE

The evaluation report is only on the Agilent ChemStation available.

Test Failed

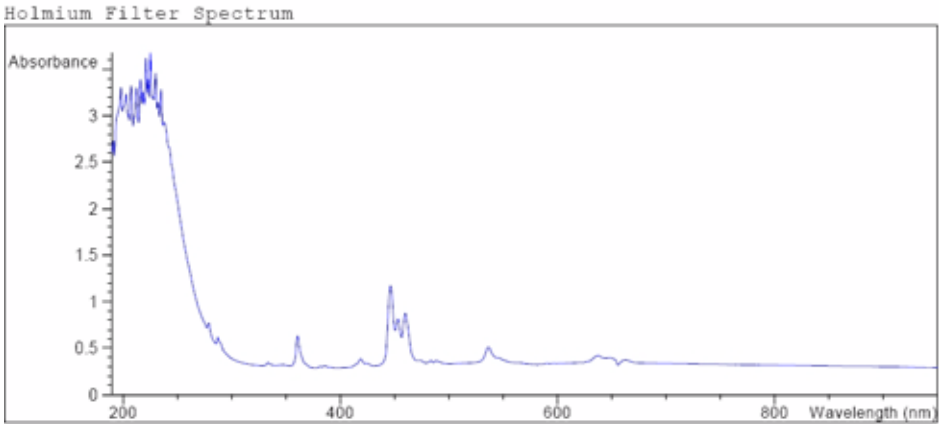
Probable Causes

- Absorbing solvent or air bubble in flow cell.
- Incorrect calibration
- Dirty or contaminated flow cell.
- Dirty or contaminated optical components (achromat, windows).
- Old or non-Agilent lamp.

Suggested Actions

- ✓ Ensure the flow cell is filled with water.
- ✓ Recalibrate (see “Wavelength Verification and Recalibration” on page 138) and repeat the test.
- ✓ Run the cell test (see “Cell Test (ChemStation only)” on page 141). If the test fails, exchange the flow cell windows.
- ✓ Clean optical components with alcohol and lint-free cloth.
- ✓ Exchange the UV lamp.

Instrument: G1315B
Serial Number: DE03010353
Operator: Wolfgang
Date: 24.11.2005
Time: 14:39:01



Holmium Filter Test Results

	Specification	Measured	Result
Wavelength 1: 361.0 nm	360..362 nm	360.6 nm	Passed
Wavelength 2: 453.7 nm	452.7..454.7 nm	453.1 nm	Passed
Wavelength 3: 536.7 nm	535.7..537.7 nm	536.4 nm	Passed

Figure 44 Holmium Oxide Test Results (report)

Cell Test (ChemStation only)

The cell test measures the intensity of the deuterium and tungsten lamps over the full wavelength range (190 – 950 nm), once with the flow cell installed, and once with the flow cell removed. The resulting intensity ratio is a measure of the amount of light absorbed by the flow cell. The test can be used to check for dirty or contaminated flow cell windows. When the test is started, the 1-nm slit is moved into the light path automatically, and the gain is set to zero. To eliminate effects due to absorbing solvents, the test should be done with water in the flow cell.

NOTE

This test should be performed initially with a new detector/flow cell. The values should be kept for later reference/comparison.

Cell Test Evaluation

The Agilent ChemStation calculates the intensity ratio automatically. The intensity ratio (typically between 0.5 and 0.7 for **new standard flow cells**) is dependent on the degree of contamination of the flow cell windows, and on the type of flow cell used.

```
Instrument:      G1315B
Serial Number:  DE3010353
Operator:       Wolfgang
Date:          24.11.2005
Time:          14:42:35
```

	Result	Status
Detector Cell Test		
Expected total time: approx. 45 s.		
Test Procedure:		
1. If cell not in place, install it		done
2. Measuring intensity with cell	51230	done
3. Remove cell		done
4. Measuring intensity without cell	51231	done
5. Calculating intensity ratio	1.0	done

Figure 45 Cell Test Results (report) with no flow cell inserted

NOTE

This test can be used for the standard flow cells only. The nano flow cells will give very low values due to their design.

Test Failed (low ratio value)

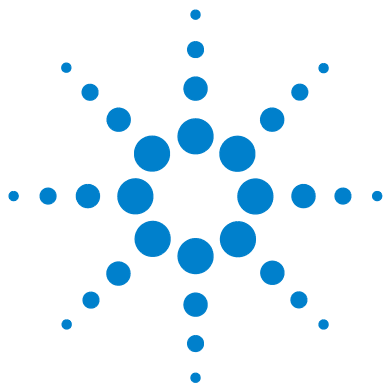
Probable Causes

- Absorbing solvent or air bubble in flow cell.
- Dirty or contaminated flow cell.

Suggested Actions

- ✓ Ensure the flow cell is filled with water, and free from air bubbles.

Exchange the flow cell windows.



9 Parts and Materials for Maintenance

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This chapter provides information on parts for maintenance.



Overview of Maintenance Parts

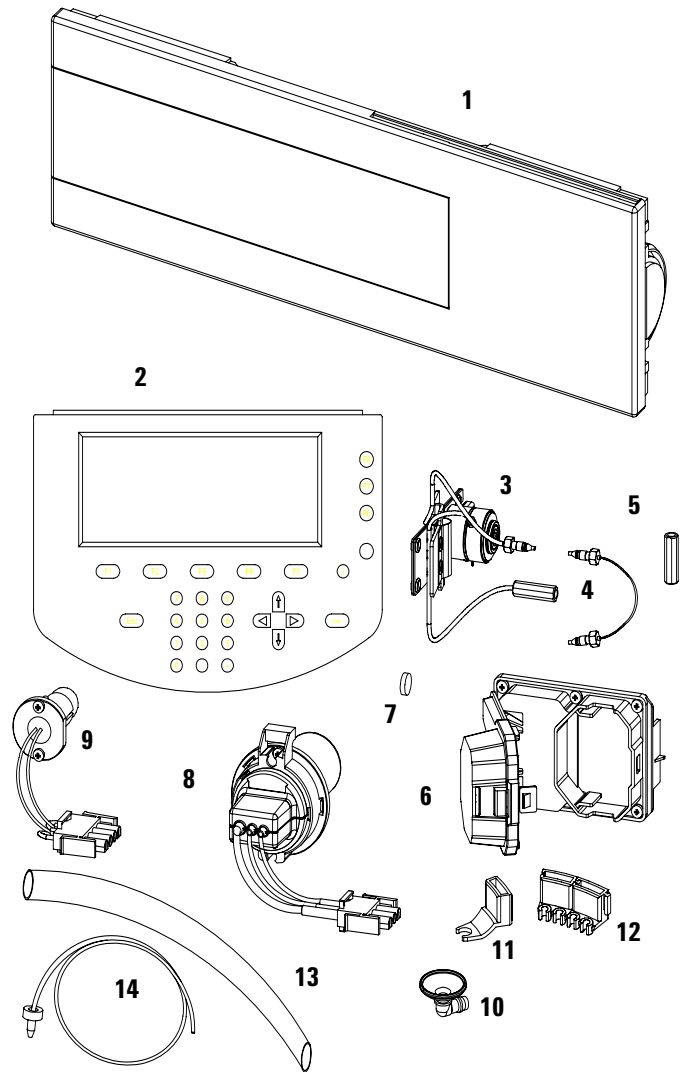


Table 16 Maintenance Parts

Item	Description	Part Number
1	Front cover	5065-9982
2	Control Module G1323B or Instant Pilot G4208A	G1323-67001 G4208-67001
3	Flow Cells with ID tag. For details see “ Standard Flow Cell ” on page 146, “ Semi-Micro Flow Cell ” on page 148, “ Micro Flow Cell ” on page 150, “ High Pressure Flow Cell ” on page 152 or “ Nano Flow Cells ” on page 158	
4	Capillary column – detector 380 mm lg, 0.17 i.d., for details see “ Accessory Kit ” on page 162	G1315-87311
5	Zero dead volume fitting	5022-6515
6	Flow cell door Screw special for flow cell door	G1315-68707 5022-2112
7	Holmium oxide filter	79880-22711
8	Longlife Deuterium lamp	2140-0813
9	Tungsten lamp	G1103-60001
10	Leak funnel	5041-8388
11	Leak funnel holder	5041-8389
12	Clip	5041-8387
13	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
14	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), see “ Accessory Kit ” on page 162	5062-2462
	Cable CAN to Agilent 1200 Series modules (0.5 m)	5181-1516
	Cable CAN to Agilent 1200 Series modules (1 m)	5181-1519
	LAN Communication Interface Board (G1369A)	G1369-60001
	Cross-over network cable (shielded, 3 m long) for point to point connection	5023-0203
	Twisted pair network cable (shielded, 7 m long), for hub connections	5023-0202
	Analog cable (BNC to general purpose, spade lugs)	01046-60105
	Interface board BCD (BCD/external contacts)	G1351-68701

Standard Flow Cell

Table 17 Standard Flow Cell Parts

Item	Description	Part Number
	Standard flow cell assembly, 10 mm, 13 µl, maximum pressure 120 bar (12 MPa), with I.D. tag	G1315-60022
1	Window screw	79883-22402
2	Spring washers, pack of 10	5062-8553
3	Compression washer	79883-28801
4	Window holder	79883-22301
5	Quartz window	1000-0488
6	Gasket BACK (Teflon), 2.3 mm hole, outlet side	See kits below
7	Gasket FRONT (Teflon), 1.3 mm hole, inlet side	See kits below
8	Window assembly, comprises item 1, 2, 3, 4, 5	No part number
9	Capillary IN (0.17 mm, 590 mm lg) including heat exchanger	G1315-87321
10	Capillary OUT (0.17 mm, 200 mm lg)	G1315-87302
11	Clamp unit	G1315-84910
	Screw M 2.5, 4 mm lg for cell body/clamp	0515-1056
	Zero dead volume fitting	5022-2184
	Cell repair kit STD, includes window screw kit, 4mm hexagonal wrench and seal kit	G1315-68712
	Seal kit BACK for STD flow cell, qty=12 of item 6	G1315-68711
	Seal kit FRONT for STD flow cell, qty=12 of item 7	G1315-68710
	Window screw kit, includes 2 quartz windows, 2 compression washers, 2 window holders, 2 window screws and 10 spring washers	79883-68703

NOTE

Gaskets # 6 and #7 have different hole diameters.

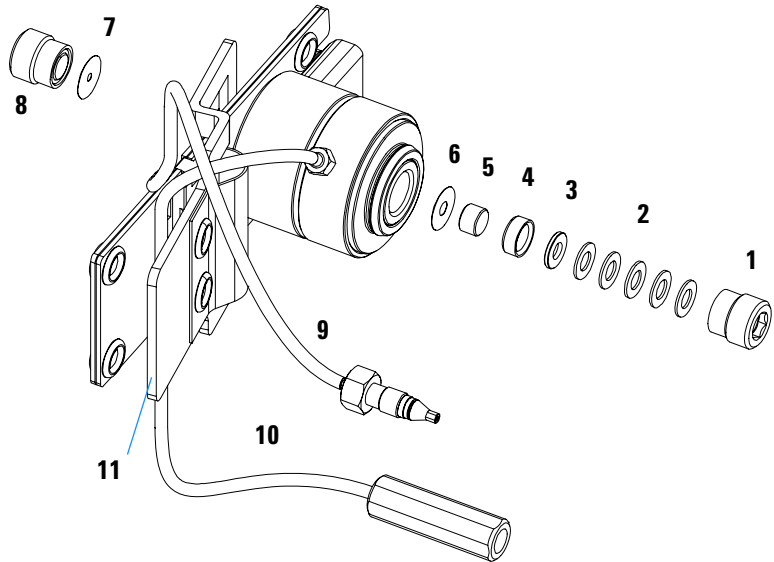


Figure 46 Standard Flow Cell Parts

- 1 - window screw
- 2 - spring washers
- 3 - compression washer
- 4 - window holder
- 5 - quartz window
- 6 - Gasket

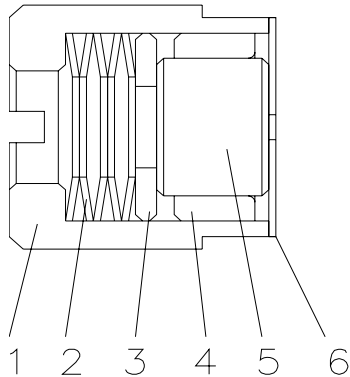


Figure 47 Orientation of Spring Washers.

Semi-Micro Flow Cell

Table 18 Semi-Micro Flow Cell Parts

Item	Description	Part Number
	Semi-micro flow cell assembly, 6 mm, 5 µl, maximum pressure 120 bar (12 MPa), with I.D. tag	G1315-60025
1	Window screw	79883-22402
2	Spring washers, pack of 10	5062-8553
3	Compression washer	79883-28801
4	Window holder	79883-22301
5	Quartz window	1000-0488
6	Gasket BACK (Teflon), 1.8 mm hole, outlet side	See kits below
7	Gasket FRONT (Teflon), 1.3 mm hole, inlet side	See kits below
8	Window assembly, comprises item 1, 2, 3, 4, 5	No part number
9	Capillary IN (0.17 mm, 310 mm lg) including heat exchanger	G1315-87319
10	Capillary OUT (0.12 mm, 200 mm lg)	G1315-87306
10	Capillary OUT (0.17 mm, 200 mm lg)	G1315-87302
11	Clamp unit	G1315-84910
	Screw M 2.5, 4 mm lg for cell body/clamp	0515-1056
	Zero dead volume fitting	5022-2184
	Cell repair kit semi-micro, includes window screw kit, 4 mm hexagonal wrench and seal kits	G1315-68713
	Seal kit BACK for semi-micro flow cell, qty=12 of item 6	79883-68702
	Seal kit FRONT for semi-micro flow cell, qty=12 of item 7	G1315-68710
	Window screw kit, includes 2 quartz windows, 2 compression washers, 2 window holders, 2 window screws and 10 spring washers	79883-68703

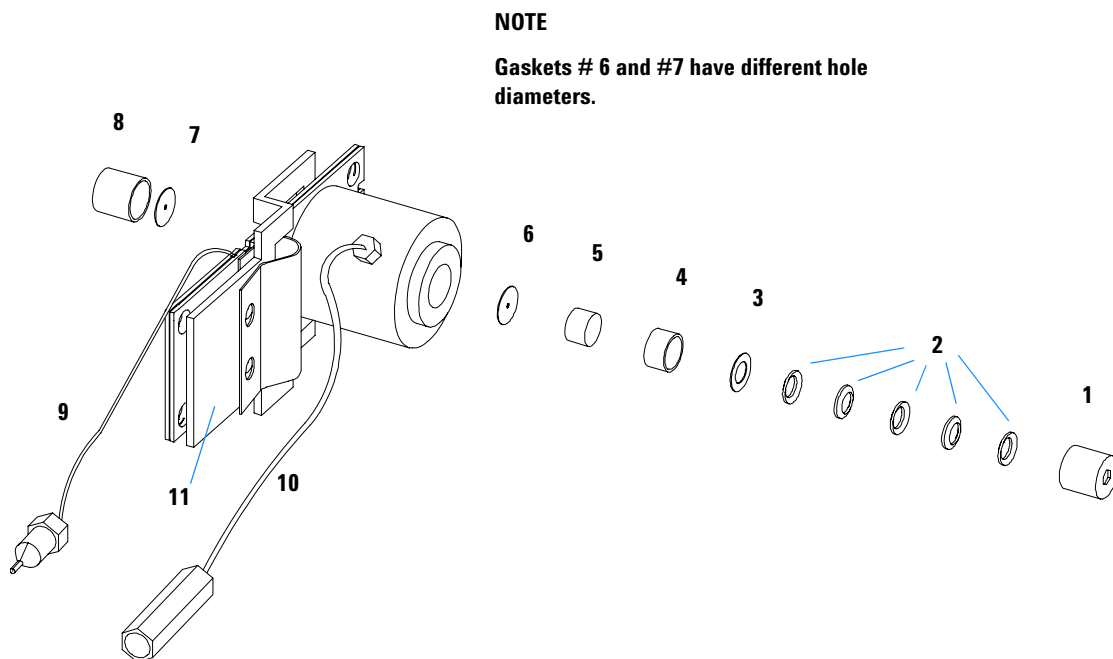


Figure 48 Semi-Micro Flow Cell Parts

- 1 - window screw
- 2 - spring washers
- 3 - compression washer
- 4 - window holder
- 5 - quartz window
- 6 - Gasket

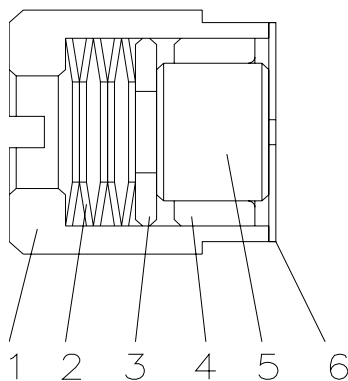


Figure 49 Orientation of Spring Washers.

Micro Flow Cell

Table 19 Micro Flow Cell Parts

Item	Description	Part Number
	Micro flow cell assembly, 3 mm, 2 µl, maximum pressure 120 bar (12 MPa)	G1315-60024
1	Window screw	79883-22402
2	Spring washers, pack of 10	5062-8553
3	Compression washer	79883-28801
4	Window holder	79883-22301
5	Quartz window	1000-0488
6	Gasket BACK (Teflon), 1.8 mm hole, outlet side	See kits below
7	Gasket FRONT (Teflon), 1.3 mm hole, inlet side	See kits below
8	Window assembly, comprises item 1, 2, 3, 4, 5	No part number
9	Capillary IN (0.12 mm, 310 mm lg) including heat exchanger	G1315-87339
10	Capillary OUT (0.12 mm, 200 mm lg)	G1315-87306
10	Capillary OUT (0.17 mm, 200 mm lg)	G1315-87302
11	Clamp unit	G1315-84910
	Screw M 2.5, 4 mm lg for cell body/clamp	0515-1056
	Zero dead volume fitting	5022-2184
	Cell repair kit semi-micro, includes window screw kit, 4 mm hexagonal wrench and seal kits	G1315-68713
	Seal kit BACK for semi-micro flow cell, qty=12 of item 6	79883-68702
	Seal kit FRONT for semi-micro flow cell, qty=12 of item 7	G1315-68710
	Window screw kit, includes 2 quartz windows, 2 compression washers, 2 window holders, 2 window screws and 10 spring washers	79883-68703

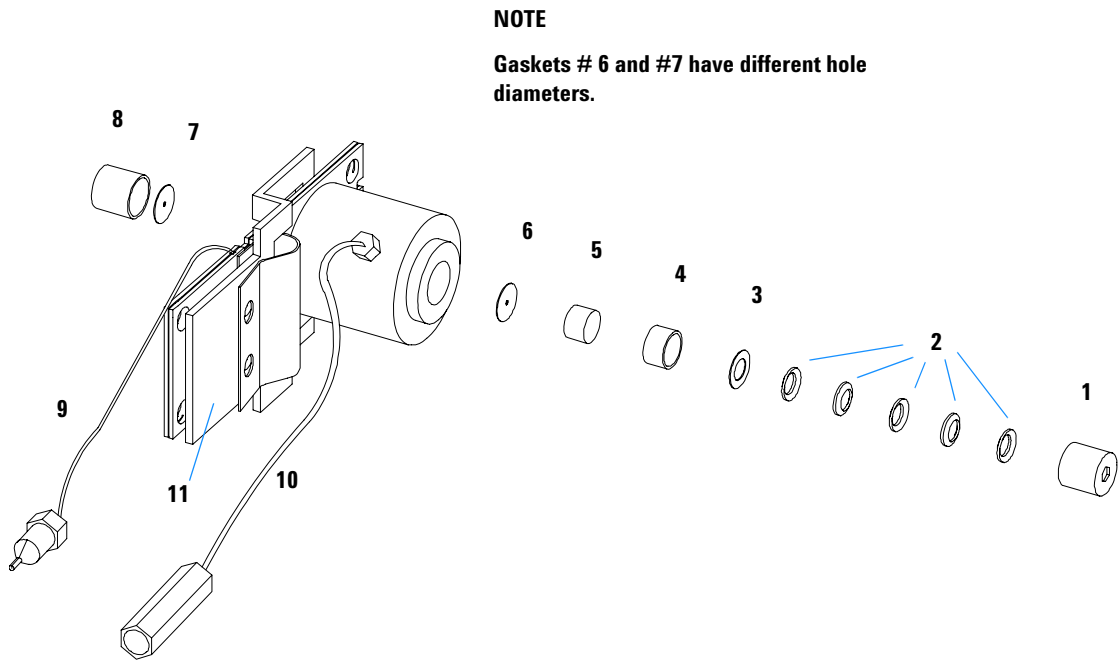


Figure 50 Micro Flow Cell Parts

- 1 - window screw
- 2 - spring washers
- 3 - compression washer
- 4 - window holder
- 5 - quartz window
- 6 - Gasket

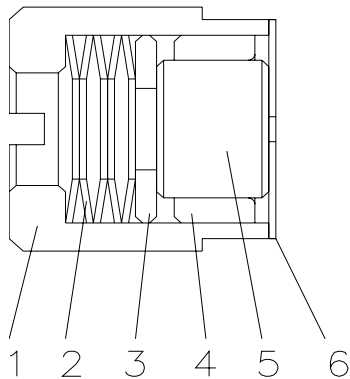


Figure 51 Orientation of Spring Washers.

High Pressure Flow Cell

Table 20 High Pressure Flow Cell Parts

Item	Description	Part Number
	High pressure flow cell assembly, 6 mm, 1.7 µl, maximum pressure 400 bar (40 MPa)	G1315-60015
1	Window assembly, comprises items 2, 3, 4, 5 and 6	
2	Seal ring	79883-27101
3	Quartz window	1000-0953
4	Compression washer	79883-28802
5	Spring washers (pack of 10)	5062-8553
6	Window screw	79883-22404
7	Capillary IN (0.12 mm, 290 mm lg) including heat exchanger	G1315-87325
8	Capillary OUT (0.12 mm, 200 mm lg)	G1315-87306
9	Clamp unit	G1315-84901
	Screw M 2.5, 4 mm lg for cell body/clamp unit	0515-1056
	Capillary (0.12 mm, 150 mm lg)	G1315-87312
	Capillary (0.17 mm, 380 mm lg)	G1315-87311
	High Pressure Cell Repair Kit, includes 1 quartz window, 1 compression washer, 5 spring washers, 2 seal rings	79883-68700

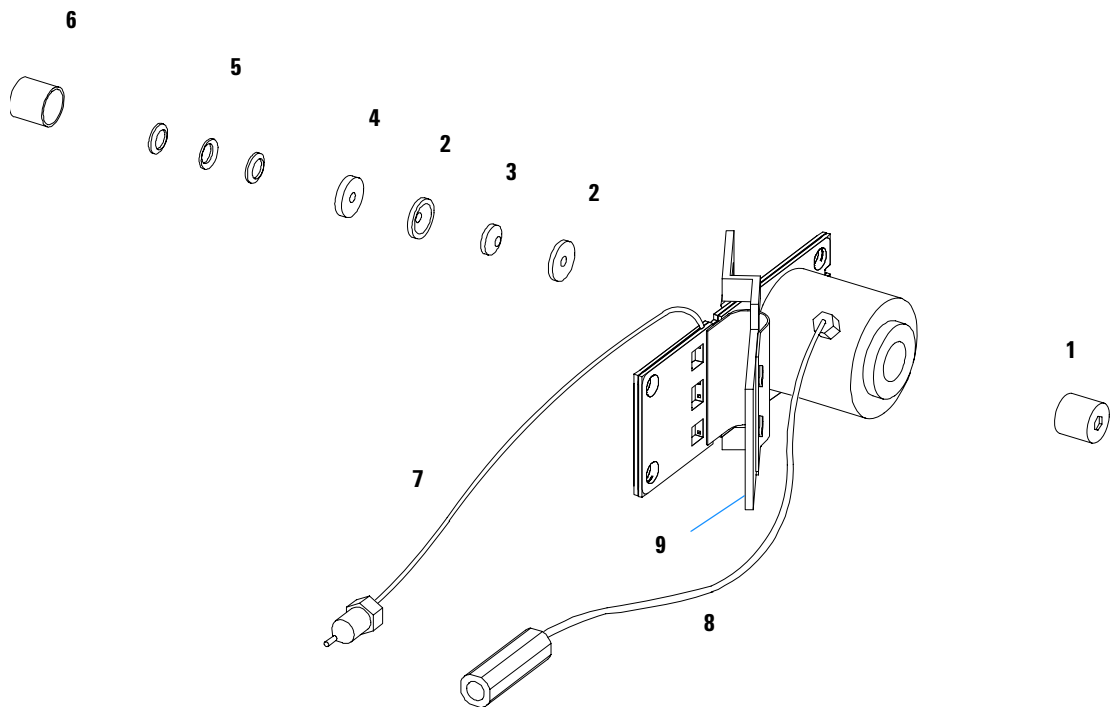


Figure 52 High Pressure Flow Cell Parts

Prep Flow Cell - SST

NOTE

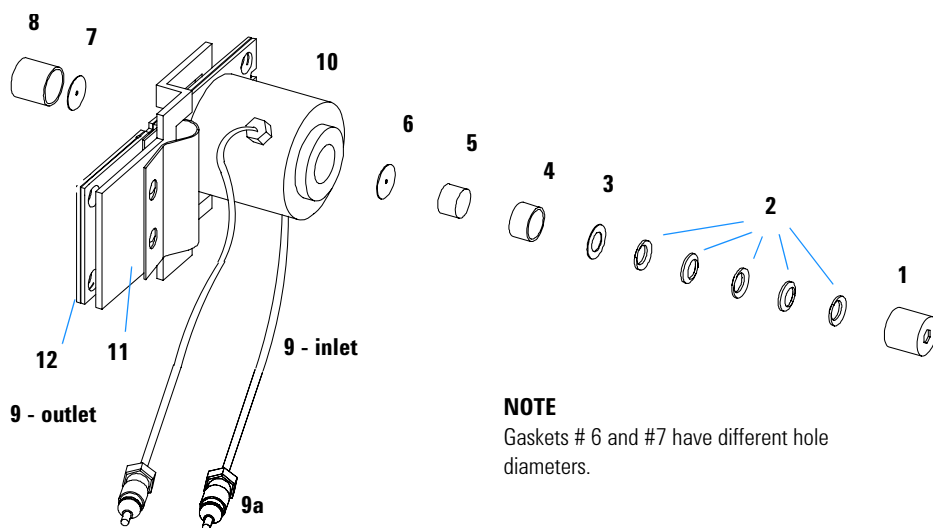
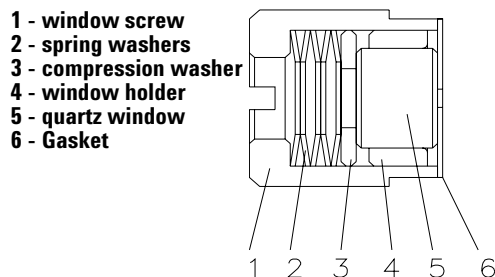
For more details on the Preparative Flow Cells refer to the technical note that comes with the flow cells.

Table 21 Prep Flow Cell - SST Parts

Item	Description	Part Number
	Prep Flow Cell SST - 3 mm, 4 µl, 120 bar (12 MPa), with I.D. Tag completely assembled, includes all items below	G1315-60016
1	Window screw	79883-22402
2	Spring washers, pack of 10	5062-8553
3	Compression washer	79883-28801
4	Window holder	79883-22301
5	Quartz window	1000-0488
6	Gasket BACK (FEP Teflon), 2.3 mm hole, outlet side Seal kit BACK for STD flow cell, qty=12	G1315-68711
7	Gasket FRONT (FEP Teflon), 1.3 mm hole, inlet side Seal kit FRONT for STD flow cell, qty=12	G1315-68710
8	Window assembly, comprises item 1, 2, 3, 4, 5	No part number
	Window screw kit, includes 2 quartz windows, 2 compression washers, 2 window holders, 2 window screws and 10 spring washers	79883-68703
	Cell repair kit semi-micro, includes window screw kit, 4 mm hexagonal wrench and seal kits	G1315-68713
9	Capillary SST, 250 mm length, 0.5 mm i.d., o.D. 0.9 mm with fittings for flow cell assembled	G1315-87305
9a	Fitting FRONT, Fitting BACK, Fitting 1/16", re-order 10/pk	5062-2418
10	Cell body	G1315-27706

Table 21 Prep Flow Cell - SST Parts, continued

Item	Description	Part Number
11	Handle for clamp unit	G1315-84901
12	Clamp unit	G1315-84902
13	Screw M 2.5, 4 mm lg for cell body/clamp	0515-1056

**Figure 53** Prep Flow Cell - SST Parts**Figure 54** Orientation of Spring Washers.

Prep Flow Cell - Quartz

NOTE

For more details on the Preparative Flow Cells refer to the technical note that comes with the flow cells.

Table 22 Prep Flow Cell - Quartz Parts

Item	Description	Part Number
	Prep Flow Cell Quartz, 0.3 mm, 20 bar (2 MPa) completely assembled, includes all items below	G1315-60017
	Prep Flow Cell Quartz, 0.06 mm, 20 bar (2 MPa) completely assembled, includes all items below	G1315-60018
1	PTFE tubing 2 m length, 0.8 mm i.d., o.D. 1.6 mm with fittings for flow cell assembled (see note below)	G1315-67301
1	PTFE tubing 80 cm length, 0.5 mm i.d., o.D. 1.6 mm with fittings for flow cell assembled (see note below)	G1315-67302
2	Fitting - fingertight (2/pk)	0100-1516
3	Cell housing	G1315-27705
4	Handle for clamp unit	G1315-84901
5	Clamp unit	G1315-84902
6	Screw M 2.5, 4 mm lg for cell body/clamp	0515-1056
7	Quartz body - Prep Cell 0.3 mm	G1315-80004
7	Quartz body - Prep Cell 0.06 mm	G1315-80003

NOTE

The flow cell comes with two tubings 0.8 mm i.d. and one 0.5 mm i.d. so that the combination at the flow cell could be either 0.8/0.8 or 0.5/0.8 (inlet/outlet)

Standard is 0.8/0.8. Depending on the system pressure (< 30 ml/min) or bandbroadening, the inlet tubing might be changed to 0.5 mm.

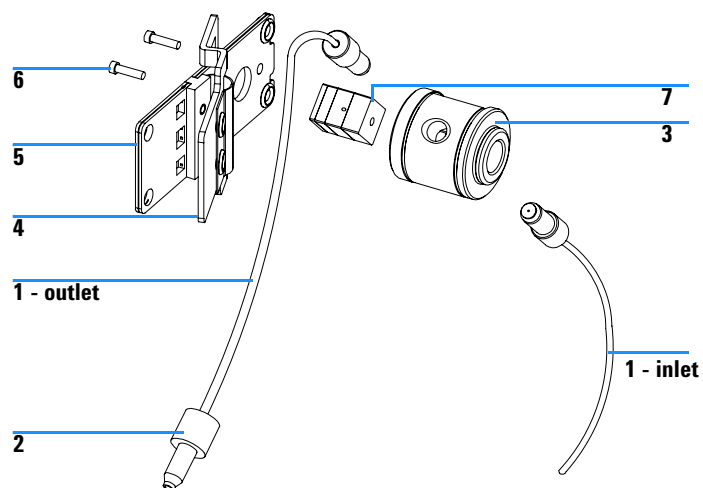


Figure 55 Prep Flow Cell - Quartz Parts

Nano Flow Cells

The following kits are available:

Table 23 Nano-flow cell kits

Description	Part number
500 nl Flow Cell Kit includes Flow cell assembly (10 mm, 500 nl, 5 MPa) completely assembled (includes items 1, 2, 3, 4, 10, 11, 12, 13, 14, 15, and 16)	G1315-68724
80 nl Flow Cell Kit includes Flow cell assembly (10 mm, 500 nl, 5 MPa) completely assembled (includes items 1, 2, 3, 4, 10, 11, 12, 13, 14, 15, and 16)	G1315-68716

Figure 56 shows all parts delivered with the nano-flow cell kits.

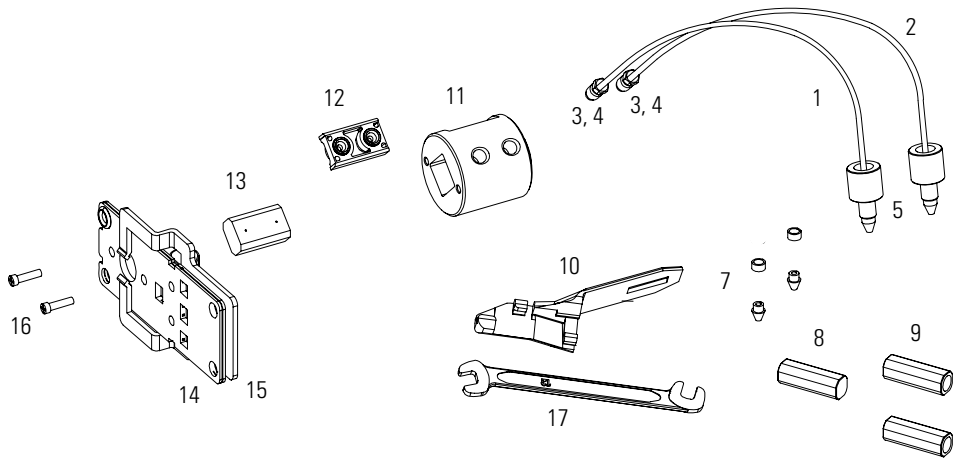


Figure 56 Content of kits

Table 24 lists the generic parts for both nano-flow cells:

Table 24 Generic Parts

Item	Description	Part Number
3	Fitting Screw (for 4 mm wrench), QTY=2 (reorder 10/pk)	5063-6593
4	Cell ferrules are factory installed	
5	PEEK fitting 1/32" (not attached to capillaries), (reorder 10/pk)	5065-4422
7	Litetouch ferrules LT-100, (1/32" Ferrule and SS lock ring), QTY=2 (reorder 10/pk)	5063-6592
8	Union Adjustment Tool, used for item #7	5022-2146
9	ZDV SS Union, no fitting, QTY=2	5022-2184
10	Torque Adapter	G1315-45003
14	Handle for clamp unit	G1315-84902
15	Clamp unit	G1315-84910
16	Screw M 2.5, 4 mm long for cell body/clamp	0515-1056
17	Wrench open end 4 mm (supplied with standard accessory kit G1315-68705 of your detector)	8710-1534

Table 25 lists the specific parts for the 500 nl flow cell.

Table 25 Specific 500 nl Flow Cell Parts

Item	Description	Part Number
	500 nl Flow Cell Kit	G1315-68724
1	PEEK coated fused silica capillary Inlet (100 µm) pre-mounted to cell , includes Inlet capillary, 300 mm long, 100 µm i.d. with pre-fixed ferrules (#4) and fittings (#3), plus one PEEK Fitting FT (#5)	G1315-87333
2	PEEK coated fused silica capillary Outlet (100 µm) pre-mounted to cell , includes Outlet capillary, 120 mm long, 100 µm i.d. with pre-fixed ferrules (#4) and fitting (#3), plus one PEEK Fitting FT (#5)	G1315-87338
1	PEEK coated fused silica capillary Inlet (50 µm) alternative , includes Inlet capillary, 400 mm long, 50 µm i.d. with pre-fixed ferrules (#4) and fittings (#3), plus one PEEK Fitting FT (#5)	G1315-87323
2	PEEK coated fused silica capillary Outlet (50 µm), alternative , includes Outlet capillary, 120 mm long, 50 µm i.d. with pre-fixed ferrules (#4) and fitting (#3), plus one PEEK Fitting FT (#5)	G1315-87328
11	Cell Housing (500 nl)	G1315-27703
12	Cell Seal Assembly (500 nl)	G1315-87101
13	Quartz Body (500 nl)	G1315-80001
	Sealing Kit, includes items #10, #12 (QTY=2) and #7 (QTY=5)	G1315-68715

Table 26 lists the specific parts for the 80 nl flow cell.

Table 26 Specific 80 nl Flow Cell Parts

Item	Description	Part Number
	80 nl Flow Cell Kit	G1315-68716
1	PEEK coated fused silica capillary Inlet (50 µm) pre-mounted to cell , includes Inlet capillary, 400 mm long, 50 µm i.d. with pre-fixed ferrules (#4) and fittings (#3), plus one PEEK Fitting FT (#5)	G1315-87323
2	PEEK coated fused silica capillary Outlet (50 µm) pre-mounted to cell , includes Outlet capillary, 120 mm long, 50 µm i.d. with pre-fixed ferrules (#4) and fitting (#3), plus one PEEK Fitting FT (#5)	G1315-87328
1	PEEK coated fused silica capillary Inlet (25 µm) alternative , includes Inlet capillary, 200 mm long, 25 µm i.d. with pre-fixed ferrules (#4) and fittings (#3), plus one PEEK Fitting FT (#5)	G1315-87313
2	PEEK coated fused silica capillary Outlet (25 µm) alternative , includes Outlet capillary, 600 mm long, 25 µm i.d. with pre-fixed ferrules (#4) and fitting (#3), plus one PEEK Fitting FT (#5)	G1315-87318
11	Cell Housing (80 nl)	G1315-27704
12	Cell Seal Assembly (80 nl)	G1315-42301
13	Quartz Body (80 nl)	G1315-80002
	Sealing Kit 80 nl cell, includes items #10, #12 (QTY=2), #7 (QTY=5) and Lite Touch Sleeves (QTY=5)	G1315-68725

Accessory Kit

This kit contains some accessories and tools needed for the installation and repair of the detector.

Table 27 Accessory Kit Parts

Item	Description	Part Number
	Accessory kit	G1315-68705
	Corrugated tubing (to waste), re-order 5 m	5062-2463
1	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462
2	Fitting male PEEK, Qty=1	0100-1516
3	Capillary column – detector 380 mm lg, 0.17 i.d. includes items 4, 5 and 6 (not assembled)	G1315-87311
4	Ferrule front SST, qty=2	0100-0043
5	Ferrule back SST, qty=2	0100-0044
6	Fitting SST, qty=2	79814-22406
	Hex key set 1 – 5 mm	8710-0641
	Wrench open end 1/4 – 5/16 inch	8710-0510
	Wrench open end 4 mm	8710-1534

For item number refer to [Figure 57](#) and [Figure 58](#).

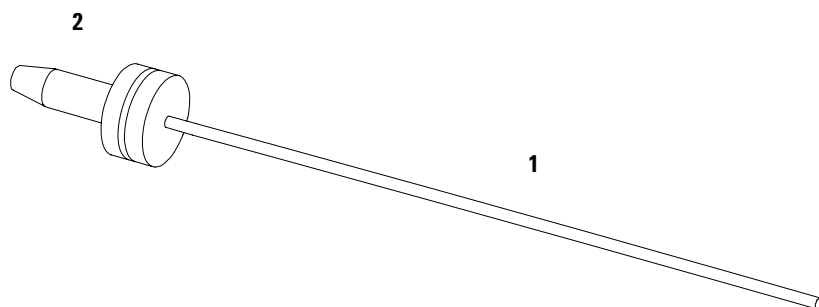


Figure 57 Waste Tubing Parts

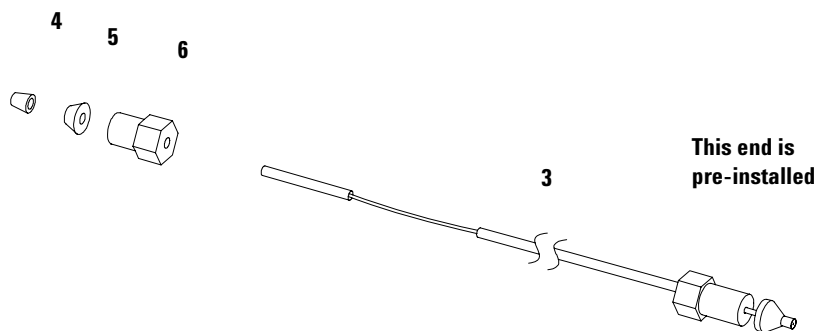
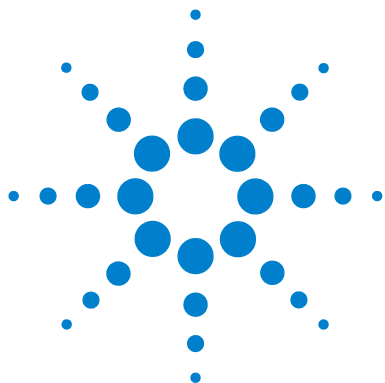


Figure 58 Inlet Capillary (Column-Detector) Parts



A Appendix

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This chapter provides safety and other general information.



General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Aligent Technologies assumes no liability for the customer's failure to comply with these requirements.

General

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

This instrument is designed and certified as a general purpose laboratory instrument for research and routine application only. It is not certified for in-vitro or medical applications.

Operation

Before applying power, comply with the installation section. Additionally the following must be observed.

Do not remove instrument covers when operating. Before the instrument is switched on, all protective earth terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective earth via a ground socket. Any interruption of the protective earth grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, and so on) are used for replacement. The use of repaired fuses and the short-circuiting of fuseholders must be avoided.

CAUTION

The operator of this instrument is advised that if the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

Some adjustments described in the manual, are made with power supplied to the instrument, and protective covers removed. Energy available at many points may, if contacted, result in personal injury.

Any adjustment, maintenance, and repair of the opened instrument under voltage should be avoided as much as possible. When inevitable, this should be carried out by a skilled person who is aware of the hazard involved. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not replace components with power cable connected.

Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Capacitors inside the instrument may still be charged, even though the instrument has been disconnected from its source of supply. Dangerous voltages, capable of causing serious personal injury, are present in this instrument. Use extreme caution when handling, testing and adjusting.

When working with solvents please observe appropriate safety procedures (e.g. goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet by the solvent vendor, especially when toxic or hazardous solvents are used.

Safety Symbols

Table 28 shows safety symbols used on the instrument and in the manuals.

Table 28 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	Indicates eye damage may result from directly viewing the light produced by the deuterium lamp used in this product.

WARNING

A warning alerts you to situations that could cause physical injury or damage to the equipment. Do not proceed beyond a warning until you have fully understood and met the indicated conditions.

CAUTION

A caution alerts you to situations that could cause a possible loss of data. Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Lithium Batteries Information

WARNING

Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer. Lithium batteries may not be disposed-off into the domestic waste.

Transportation of discharged Lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed. Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

WARNING

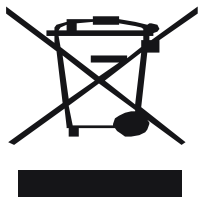
Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering. Udskiftning må kun ske med batteri af samme fabrikat og type. Lever det brugte batteri tilbage til leverandøren.

WARNING

Lithiumbatteri - Eksplosionsfare. Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten. Brukt batteri returneres apparatleverandøren.

NOTE

Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggooien maar inleveren als KCA.



The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC)

Abstract

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC), adopted by EU Commission on 13 February 2003, is introducing producer responsibility on all Electric and Electronic appliances from 13 August 2005.

NOTE



This product complies with the WEEE Directive (2002/96/EC) marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

Product Category:

With reference to the equipment types in the WEEE Directive Annex I, this product is classed as a “Monitoring and Control instrumentation” product.

Do not dispose off in domestic household waste

To return unwanted products, contact your local Agilent office, or see www.agilent.com for more information.

Radio Interference

Never use cables other than the ones supplied by Aligent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with equipment unscreened cables and/or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 18 January 1991.

This product has a sound pressure emission (at the operator position) < 70 dB.

- Sound Pressure $L_p < 70$ dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

UV-Radiation

NOTE

This information is only valid for UV-lamps without cover (e.g. 2140-0590 and 2140-0813).

Emissions of ultraviolet radiation (200-315 nm) from this product is limited such that radiant exposure incident upon the unprotected skin or eye of operator or service personnel is limited to the following TLVs (Threshold Limit Values) according to the American Conference of Governmental Industrial Hygienists:

Table 29 UV-Radiation Limits

Exposure/day	Effective Irradiance
8 hours	0.1 $\mu\text{W}/\text{cm}^2$
10 minutes	5.0 $\mu\text{W}/\text{cm}^2$

Typically the radiation values are much smaller than these limits:

Table 30 UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50 cm distance	Average 0.016 $\mu\text{W}/\text{cm}^2$
Lamp installed, 50 cm distance	Maximum 0.14 $\mu\text{W}/\text{cm}^2$

Solvent Information

Observe the following recommendations on the use of solvents.

Flow Cell

Avoid the use of alkaline solutions (pH > 9.5) which can attack quartz and thus impair the optical properties of the flow cell.

Prevent any crystallization of buffer solutions. This will lead into a blockage/damage of the flow cell.

If the flow cell is transported while temperatures are below 5 degree C, it must be assured that the cell is filled with alcohol.

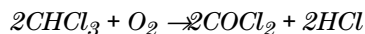
Aqueous solvents in the flow cell can built up algae. Therefore do not leave aqueous solvents sitting in the flow cell. Add small % of organic solvents (e.g. Acetonitrile or Methanol ~5%).

Solvents

Brown glass ware can avoid growth of algae.

Always filter solvents, small particles can permanently block the capillaries. Avoid the use of the following steel-corrosive solvents:

- Solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on).
- High concentrations of inorganic acids like nitric acid, sulfuric acid especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer which are less corrosive against stainless steel).
- Halogenated solvents or mixtures which form radicals and/or acids, for example:



This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.

- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether) such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1-% solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylene diamine tetra-acetic acid).
- Mixtures of carbon tetrachloride with 2-propanol or THF.

Declaration of Conformity for HOX2 Filter

Declaration of Conformity				
We herewith inform you that the Holmium Oxide Glass Filter (Type Hoya HY-1) (Part No. 79880-22711) meets the following specification of absorbance maxima positions:				
Product Number	Series	Measured Wavelength *	Wavelength Accuracy	Optical Bandwidth
79883A	1090	361.0 nm	+/- 1 nm	2 nm
79854A	1050	418.9 nm		
G1306A	1050	453.7 nm		
G1315A	1100	536.7 nm		
G1315B/C	1100 / 1200			
G1600				
79853C	1050	360.8nm 418.5nm 536.4nm	+/- 2 nm	6 nm
G1314A/B/C	1100 / 1200	360.8nm 418.5nm 536.4nm	+/- 1 nm	6 nm

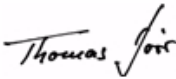
*) The variation in Measured Wavelength depends on the different Optical Bandwidth.

Agilent Technologies guarantees the traceability of the specified absorbance maxima to a National Institute of Standards & Technology (NIST) Holmium Oxide Solution Standard with a lot-to-lot tolerance of ± 0.3 nm.

The wavelength calibration filter built into the Agilent Technologies UV-VIS detectors is made of this material and meets these specifications. It is, therefore, suitable for wavelength calibration of these detectors within the specified wavelength accuracy of the respective detector over its wavelength range.

January 13, 2006

(Date)



(Engineering Manager)



(Quality Manager)

P/N 89550-90501 	Revision: E Effective by: Jan 13, 2006	 Agilent Technologies
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<http://www.agilent.com>

Select **“Products”** - **“Chemical Analysis”**

It will provide also the latest firmware of the Agilent 1200 Series modules for download.

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In This Book

This manual contains technical reference information about the Agilent 1200 Series diode array detectors.

The manual describes the following:

- introduction and specifications,
- installation,
- using and optimizing,
- troubleshooting,
- maintenance and repair,
- parts identification,
- safety and related information.

© Agilent Technologies 2006

Printed in Germany
Edition 02/2006



G1315-90006



Agilent Technologies