Agilent 325 UV/VIS Dual Wavelength Detector

User Manual

Agilent Technologies

Notices

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Book

This manual covers the Agilent 325 UV/VIS Dual Wavelength Detector (G9309A)

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2 Site Requirements and Specifications

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This chapter gives information about the installation of your instrument.

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Contents

1 Introduction

This chapter gives an instrument overview.

Introduction to the System

The Agilent 325 UV/VIS Dual Wavelength Detector is integrated into a Liquid Chromatography System. The detector is controlled remotely by OpenLAB through Ethernet communications. In this situation, all functions of the detector are controlled through the Workstation software.

The detector measures the sample absorbance at the user-selected wavelength. The absorbance is displayed. Wavelength absorbance parameters are time programmable.

Features of the Agilent 325 Detector:

- Stackable module
- Interchangeable flowcells
- Simple lamp replacement
- Comfortable control (OpenLAB)
- Wide detection range (absorbances of up to 70 AU can be measured)

Figure 1 The Agilent 325 UV/VIS Dual Wavelength Detector

System Description

Controls and Lights

The following controls and lights are located on the front of the detector:

- Indicator lights
- Power button

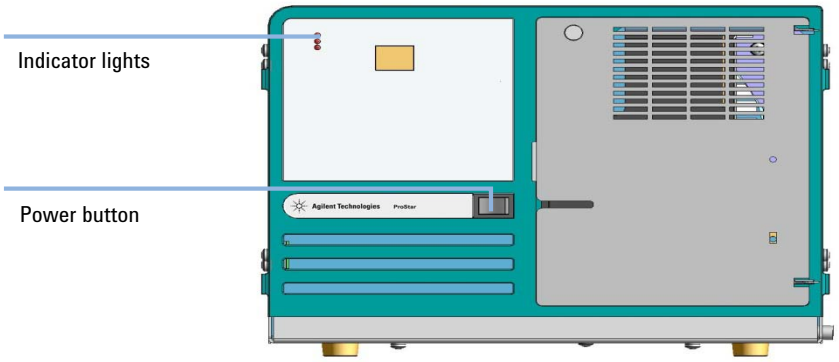


Figure 2 Lights and power button on the front of the detector

Three indicator lights are located at the top left:

- Power
- Lamp
- Ready/Run

At power up, the detector goes through an initialization sequence to check its calibration and verify its overall operation. During this period, the LED's on the front of the detector change color to indicate its current status. The table below defines each status:

Table 1 LED indicator lights

LED	Status	Color
Power	Power up Initializing Power on	Orange Orange flashing Green
Lamp	Initializing Lamp on Fault	Green flashing Green Red
Ready/Run	Not ready Ready/Stopped Method running	Off Green Orange

Press *I* (main power on) or *O* (main power off).

Optics Hardware

The main optics components are:

- UV and visible source lamp assembly
- Beam splitter
- Flowcell assembly
- Monochromator (containing collimators and grating)
- Photodiode detectors

The only user-serviceable optics components are the flowcell and lamp assemblies. These assemblies are located behind the panel on the front right side of the detector. To access the flowcell and lamp assemblies, see "Removing the Front Panel" on page 39. All other optical components are pre-aligned and sealed and must not be readjusted under any circumstance.

Optical Path

The optical path of the Agilent 325 UV/VIS Dual Wavelength Detector is shown in Figure 3 on page 11.

Light coming from the source lamp is passed through a focusing lens before hitting the beam splitter. The sample path then hits mirror N1 and the reference path hits mirrors N2 and N3 . The two beams pass through an entrance mask which shapes the beam to the geometry required before entering the flowcell. Upon leaving the flowcell, they again pass through a mask before entering the monochromator. This mask helps determine the resolution of the detector optics. In the monochromator, the light is directed onto the entry collimating mirror and then onto the grating. The dispersed light hits the exit collimating mirror before leaving the monochromator through the exit mask. From here the beams are focused onto the dual photodiode detectors.

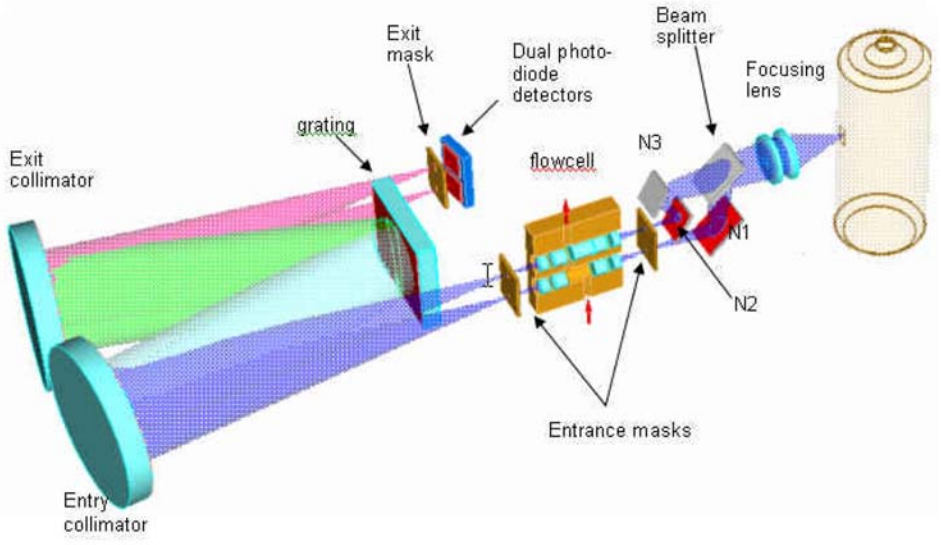


Figure 3 Optical ray trace diagram

Deuterium (UV) Lamp Assembly

The lamp assembly consists of the lamp bulb rigidly cemented into its mounting bracket. The assembly is pre-aligned. Lamp replacement is easy to perform, as no alignment procedures are required. See “Removing the Old Lamp” on page 49 for instructions on how to replace the UV lamp.

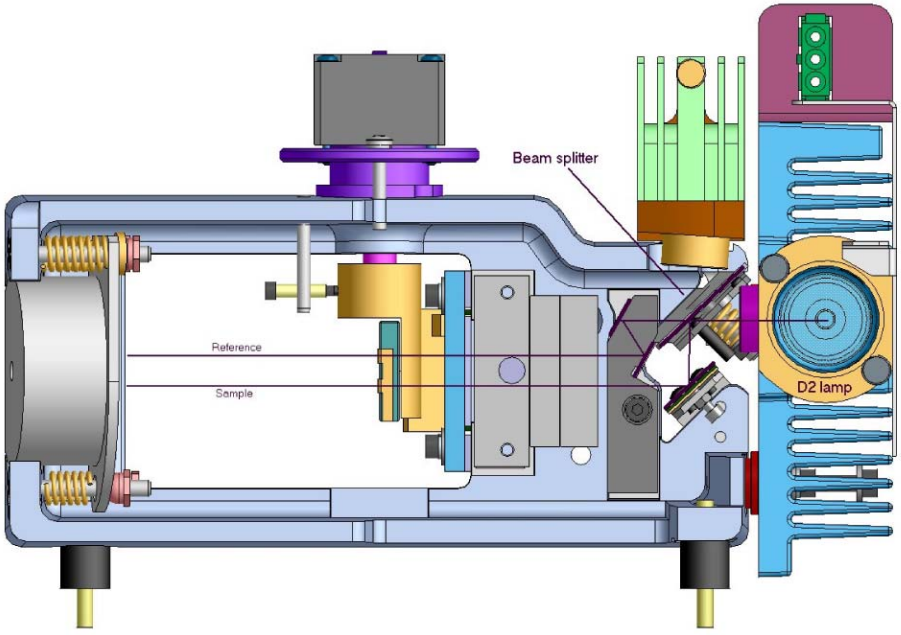


Figure 4 Deuterium lamp optical path

Visible Lamp Assembly

The visible lamp mounts on top of the optics module and is held in place by two screws. These are in a fixed position therefore allowing replacement of the lamp without the need for alignment. See "Removing the Old Lamp" on page 51 for instructions on how to replace the visible lamp.

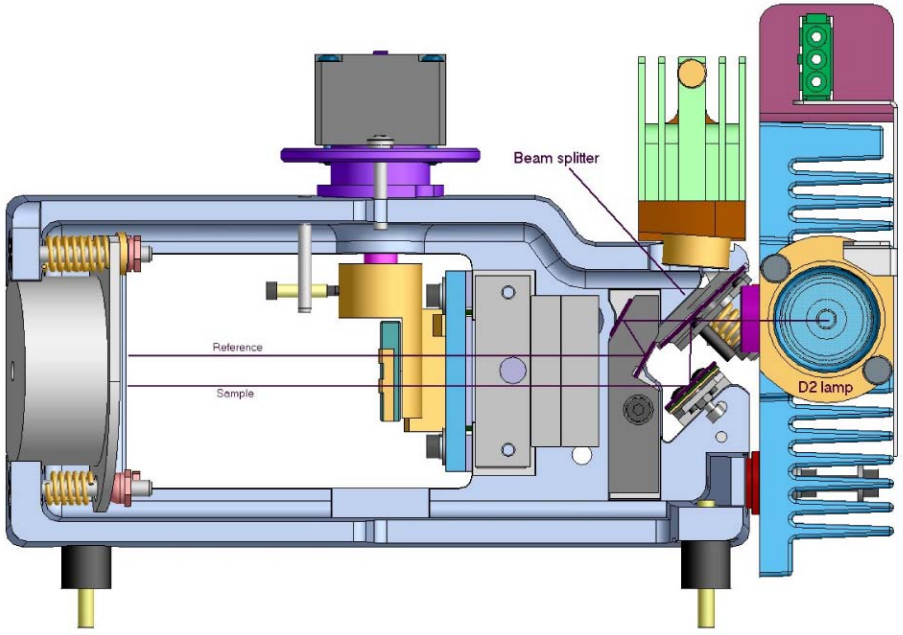


Figure 5 Visible lamp optical path

Beam Splitter

The beam splitter is a silica plate that divides the beam into sample light and reference light.

Entrance and Exit Masks

The masks used throughout the optical system, determine the optical resolution of the detector.

Flowcell Assembly

The function of the flowcell is to direct the focused light from the lamp module through a critically dimensioned sample/solvent flow path and then into the monochromator. Focusing is achieved by quartz optics lenses fitted to the flowcell. The flowcell is shown in Figure 6 on page 14 , where arrows show the liquid flow path.

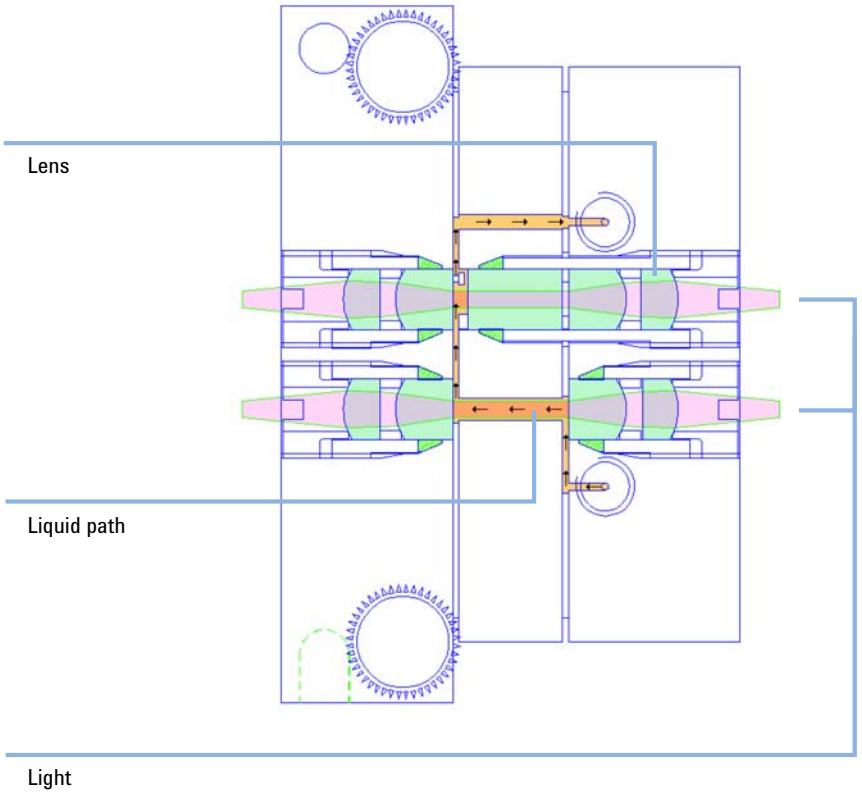


Figure 6 A typicall flowcell (9 mm x 1 mm)

Flowcells are made of titanium and have a maximum working pressure range of approximately 68.95 bar (1000 psi). The lens retainers are made of brass and the gaskets are made of Teflon® (FEP).

The flowcell and its attending hardware are designed for removal, installation and replacement by the user. See “Installing a Flowcell” on page 43 for instructions on how to replace the flowcell.

Whenever the type of flowcell is changed, you must perform a lamp calibration (see “Calibrating the Lamps” on page 53).

Monochromator

The monochromator is an enclosed unit that disperses a beam of light which has been directed through the flowcell, and discriminates within 1 nm, to a specifically selected wavelength. The monochromator is shown in Figure 7 on page 16, indicated by the line.

The monochromator assembly contains the following components:

- An entrance mask (producing a well defined beam of polychromatic UV or visible radiation)
- An entry collimating mirror
- A diffraction grating (dispersing incident radiation into a continuous spectrum)
- A stepping motor (rotating the grating to obtain the desired wavelength of exit radiation)
- An exit collimating mirror
- An exit mask (producing a very narrow bandwidth of light that is passed on to the detectors)

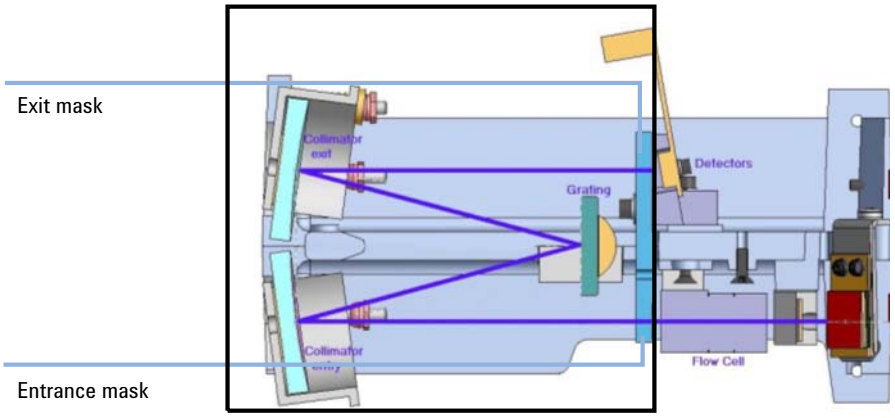


Figure 7 Top view of the Monochromator (optical path)

Table 2 Grating details

Grating size	70 mm x 45 mm
Blaze angle	8.5 ° (UV)
Balze wavelength	250 nm (UV-Vis)
Reciprocal dispersion	0.98 nm/mm (UV-Vis)
Lines per mm	1200 lines/mm (UV-Vis)

Photodiode Detectors

The detector is capable of operating in the 190 – 900 nm range. Dual silicon photodiode detectors provide an output for measurement by the electronics system.

Extended Range Operation

If you chose the 9 mm x 1 mm or 4 mm x 0.15 mm flowcell, extended range is automatically turned on. Use the ratio indicated on the flowcell or use 8 for the 9 mm x 1 mm and 28 for the 4 mm x 0.15 mm.

Normally in HPLC with a long pathlength flowcell, high sample concentrations increase absorbance to the point of saturation. The light path becomes opaque (transmittance near zero) and the signal output on the recorder or integrator is truncated, or flat-topped. Any signal beyond this point is lost until absorbance reduces enough to allow transmittance.

When there are large sample concentrations in HPLC, which result in high absorbance, causing truncated peaks and loss of peak information, the classical remedy is to reduce sample concentration or change to a flowcell with a shorter light path. The flowcell with the shorter light path will be less sensitive but its saturation point will be much higher, thereby allowing higher sample concentration and Extended Range.

The ideal solution for increasing absorbance would be a flowcell with two pathlengths, providing dual path operation, which would switch automatically to the short pathlength when concentration became too high for operation on the long pathlength. The useful dynamic range would be extended by the ratio of the long pathlength to the short pathlength. If the maximum range attainable on a detector with an 8 mm pathlength were 1.5 AU, then a 1 mm flowcell would allow a maximum range of 12 AU relative to the 8 mm path (8 mm x 1.5 mm). As the absorbance decreased, the detector would switch back to operation on the long path for maximum signal-to-noise ratio.

The unique optional dual pathlength flowcells used in the Agilent 325 Detector provide seamless automatic Extended Range. The dual pathlength flowcell performs as two flowcells in one, allowing HPLC work well beyond the normal +LIMIT value (the highest absorbance in normal range). As absorbance continues to increase, the Agilent 325 Detector automatically switches to operation on the short pathlength. The useful range is extended to the extended +LIMIT, which is the normal +LIMIT multiplied by the flowcell ratio. Extended Range operation is shown in Figure 8 on page 18.

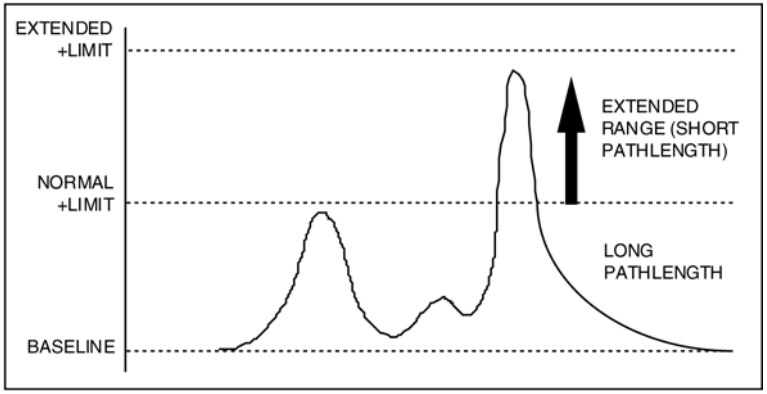


Figure 8 Extended Range operation

The detector constantly samples both beams and records offset constants, even in analytical operation. Because of this, the detector has in memory the necessary constants to mathematically scale and seamlessly extend the long path response with the short path response. During Extended Range operation, the long pathlength is constantly sampled, and as absorbance decreases there is a seamless switch back to dual beam operation in normal range.

A comparison of two chromatograms, one from the Agilent 325 Detector and the other from a typical UV detector using the same conditions, is shown in Figure 9 on page 19.

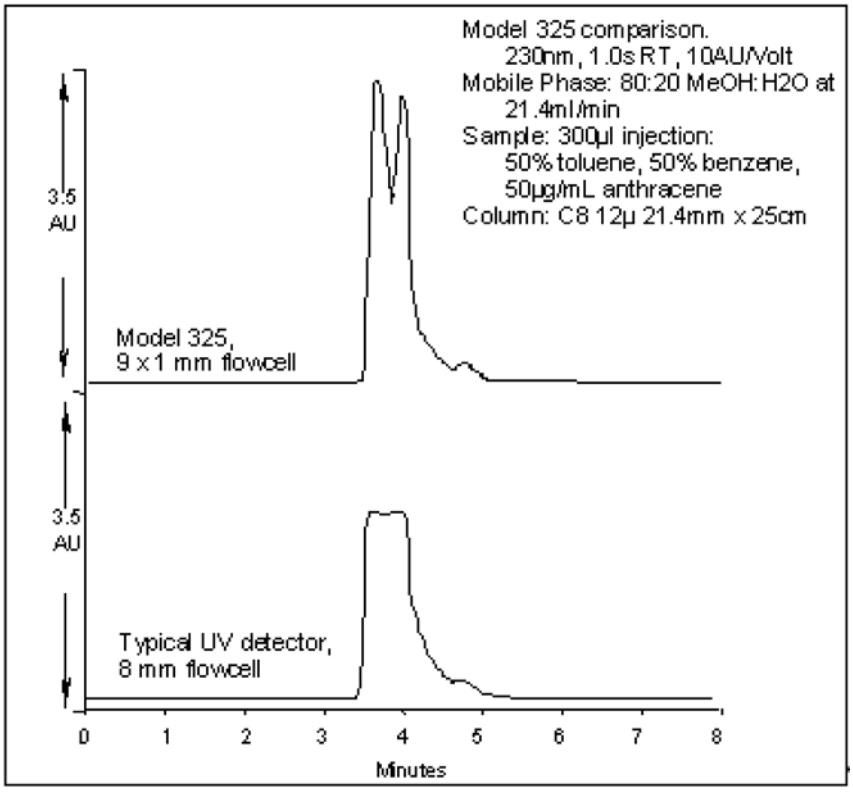


Figure 9 Automatic Extended Range

Hydraulic Connections - Flowcells

Hydraulic connections are located at the front of the Agilent 325 Detector.

The only line installed by the user where dead volume and low holdup are critical is the line from the column exit to the flowcell inlet port. This line should be as short as possible.

The Agilent 325 Detector can be fitted with any one of four flowcells (ordered separately). Each one has an inlet and outlet connection and quartz optics cell window. Your chosen flowcell type is packed internally in the detector. The four flowcell types are outlined in Table 3 on page 20.

Table 3 Compatible flowcells

Flowcell type	Flowcell p/n	Pathlength[1]	Column ID	Flow rate	Maximum pressure
Analytical	210181800	9 mm x 0 mm	2 – 8 mm	0.0001 – 10 mL/min	69 bar (1000 psi)
Preparative[2]	210181900	9 mm x 1 mm	4 – 76 mm	1 – 500 mL/min	69 bar (1000 psi
Scale Up[2]	210224200	4 mm x 0.25 mm	4 – 76 mm	10 – 200 mL/min	69 bar (1000 psi
Super Prep[2]	210182000	4 mm x 0.15 mm	8 – 152 mm	30 – 1200 mL/min	69 bar (1000 psi
Micro-analytical	210182100	4 mm x 0 mm	1 – 4 mm	0 – 20 mL/min	69 bar (1000 psi

[1] A pathlength of a mm x b mm means that the sample light path has a pathlength of a mm, and the reference light path has a pathlength of b mm. A reference light path of zero means there is no fluid in the cell - air acts as the reference.

[2] Extended range cell

NOTE It is important for the extended range cells to run at least at the minimum flow rate.

Detector Outlet Back Pressure Restrictor

The Back pressure restrictor (p/n 110743300) should be added to all flow cells, unless a fraction collector is installed after the detector. The back pressure restrictor assembly that was supplied with your detector should be threaded into the outlet line from the flowcell. The back pressure restrictor applies approximately 2.76 bar (40 psi) back pressure on the flowcell. This prevents outgassing and bubbles from forming or being trapped in the flowcell, which can cause an unstable baseline.

Note the arrow stamped on the restrictor body. This arrow must point away from the flowcell outlet port, and toward the waste receiver. The threaded plastic fittings should be finger tightened only enough to prevent leaks.

About 122 cm (48") of 1.6 mm (1/16") tubing is supplied at both the inlet and outlet of the restrictor. The inlet tubing is provided with a 1.6 mm (1/16") plastic tubing fitting for connecting to the flowcell outlet port. Either the outlet tubing can be directed to the waste container, or the tubing removed, and the restrictor itself dropped to the bottom of the waste bottle.

The restrictor pressure setting is not adjustable. If the restrictor fails or becomes plugged, replace the existing cartridge with a new 2.76 bar (40 psi) replacement.

2
Site Requirements and Specifications

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

Site Requirements

Power Considerations

The instrument power supply has wide ranging capability. It accepts any line voltage in the range described in *Physical Specifications*.

WARNING

Hazard of electrical shock or damage of your instrumentation

can result, if the devices are connected to a line voltage higher than specified.

➔ Connect your instrument to the specified line voltage only.

CAUTION

Inaccessible power plug.

In case of emergency it must be possible to disconnect the instrument from the power line at any time.

➔ Make sure the power connector of the instrument can be easily reached and unplugged.

➔ Provide sufficient space behind the power socket of the instrument to unplug the cable.

Power Cords

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

WARNING

Absence of ground connection or use of unspecified power cord

The absence of ground connection or the use of unspecified power cord can lead to electric shock or short circuit.

➔ 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

Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

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

WARNING

Unintended use of supplied power cords

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

➔ Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.

Condensation

CAUTION

Condensation within the module

Condensation will damage the system electronics.

➔ Do not store, ship or use your module under conditions where temperature fluctuations could cause condensation within the module.

➔ If your module was shipped in cold weather, leave it in its box and allow it to warm slowly to room temperature to avoid condensation.

Physical Specifications

Table 4 Specifications Agilent 325 Detector

Type	Specification	Comments
Rated Voltage	100 – 240 VAC ±10 %, 50 /60 Hz ±1 Hz single phase	
Power consumption	130 VA	Maximum
Ambient operating temperature	10 – 35 °C (50 – 95 °F)	
Ambient non-operating temperature	-20 – 65 °C (-4 – 149 °F)	
Ambient laboratory temperature	20 – 25 °C (68 – 77 F) ± 2 °C (35.6 F)	
Humidity	5 – 95 %	
Operating altitude	2000 m (6562 feet)	
Weight	15.5 kg (34.2 lbs)	
Weight (Packed)	21 kg (46.3 lbs)	
Size (height x width x depth)	212 x 296 x 475 mm (8.3 x 11.7 x 18.7 in)	
Packaged Size (height x width x depth)	385 x 460 x 775 mm (15.2 x 18.1 x 30.5 in)	
Safety standards: IEC, CSA, UL	Overvoltage Category II, Pollution Degree 2	For indoor use only.

Performance Specifications

Table 5 Performance specifications Agilent 325 Detector

Type	Specification
Diagnostics	Built-in self-test and diagnostics
Time programming	• Time programmable wavelength • Recorder attenuation • Auto-zero • Response time (programmable at time = zero only) • Peaksense • Timeslice • Pulse and external event relays • Method storage
Wavelength	UV (Deuterium) lamp and visible (quartz halogen) lamp, 190 – 900 nm
Flowcell	Optional • 9 x 0 mm • 9 x 1 mm • 4 x 0 mm • 4 x 0.15 mm • 4 x 0.25 mm
Pressure	69 bar (1000 psi) maximum on flowcells
Response time	0.05 s, 0.5 s, 1.0 s, 2.0 s
Spectral bandwidth	5 nm
Recorder output	1 V FS
Data System output	1 V FS
Peak sensing	The following events will activate the Peak Sense relay and generate event marks on the recorder chart: • Peak Start • Peak End • Valley • Time Slice • Pulse

Table 5 Performance specifications Agilent 325 Detector

Type	Specification
External communication	To other instruments using synchronization cables.
Mains inlet coupler	3/2 A 120/150 VAC 50 – 60 Hz IEC type
Mains power cord	• Australia: 10 A250 VAC complies with AS3112 • USA: 10 A125 VAC complies with NEMA 5-15P • Europe: 6 A250 VACcomplies with CEE7 sheet vii or NFC61.303VA
Rear connectors	• J1 Comm: Ethernet TCP/IP RJ-45 type connector (system connection) • J14 Analog out: female 9-pin D-range connector • J4 Relay out: female 15-pin D-range connector • P9 Sync signals: male 15-pin D-range connector • J10 Sync signals: female 9-pin D-range connector
Contact closure outputs	• 4 time progammable external event relays • 1 peak sense relay • 3 synchronization signals (READY OUT, START OUT, FALUT OUT)
Contact closure inputs	• 3 synchronization signals (READY IN, START IN, FAULT IN) • LAMP OFF and AUTO-ZERO
Bus communication	The Agilent 325 UV/VIS Dual Wavelength Detector will communicate with OpenLAB by means of Ethernet.
Fuses	T3.15AH250V (5 x 20 mm) IEC 127 Sheet 5[1] (5 x 20 mm)

[1] Fuse information on the rear of the instrument is the most up-to-date.

3
Installation

This chapter gives information about the installation of your instrument.

Installation

For details on installation of the module, refer to Agilent 218 Purification System – Setup and Installation Guide (p/n G9300-90300).

4
Using the Detector

This chapter explains the operational parameters of the instrument.

General

For information about using the Agilent 325 UV/VIS Dual Wavelength Detector refer to the help of the Control Software.

5
Troubleshooting and Diagnostics

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

Excessive Noise and/or Drift

Excessive noise and/or drift has several sources:

- A contaminated or leaking flowcell
- A worn out lamp
- A temperature fluctuation of the location where the detector is installed due to air vents or sunshine

NOTE

If the flowcell is the problem, clean it (see "Cleaning the Flowcell" on page 46 for more information). If the cleaning procedure does not rectify the problem, it will be necessary to replace the flowcell assembly (see "Installing a Flowcell" on page 43 for more information). If one of the lamps is the problem source, it must be replaced with a new lamp.

1 If cleaning or replacing the flowcell and/or replacing the lamps does not fix the problem, call your Agilent service representative.

6 Maintenance

This chapter describes the maintenance of the instrument.

General

The Agilent 325 UV/VIS Dual Wavelength Detector requires three maintenance procedures to be performed periodically:

- Changing and cleaning the flowcell
- Replacing the lamp(s)
- Calibrating the lamps

Flowcell removal and replacement will be necessary if a flowcell of different pathlength is desired, or if cleaning procedures do not satisfactorily clean the cell.

Lamp replacement is required when the lamp output deteriorates to the level that it affects the reliability of analytical results.

Whenever a lamp is changed or the flowcell type is changed, perform a lamp calibration. Also perform a periodic calibration once per month.

This chapter also covers cleaning of the instrument.

Warnings and Cautions

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

➔ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.

➔ The volume of substances should be reduced to the minimum required for the analysis.

➔ Do not operate the instrument in an explosive atmosphere.

WARNING

Eye damage by detector light

Eye damage may result from directly viewing the UV-light produced by the lamp of the optical system used in this product.

➔ Always turn the lamp of the optical system off before removing it.

WARNING

Electrical shock

Risk of stroke and other personal injury.

➔ Turn the main power switch to OFF and disconnect the power cord to the detector before starting the lamp replacement procedure.

WARNING

Injury by touching hot lamp housing

If the detector has been in use, the lamp housing may be hot.

➔ Turn off the lamp.

➔ Let the lamp housing cool before removing the lamp.

CAUTION

Lamp failure

Oil or other material on the lamp glass envelope can cause the lamp to explode.

➔ Never touch the glass on the lamp with bare hands.

➔ Always wear gloves when replacing the lamp.

Removing the Front Panel

1 If installed, remove the door from the Agilent 325 Detector (see "Installing and Removing the Door" on page 40 for more information).

2 Remove the panel on the front right side of the detector by unscrewing the captive screw in the top left corner of the panel.

Figure 10 Removing the front panel

Installing and Removing the Door

1. Gently push down on the door and slide the lower hinge pin out of the lower hinge.
2. Lift and slide out the top of the door.

Installing the Door

The module door may be attached to the front of the Agilent 325 Detector to cover the tubing connections to the flowcell.

NOTE If the Agilent 325 Detector is the top module in the stack, the door cap should be installed before installing the door on the module.

Installing the Door Cap

If the Agilent 325 Detector is not at the top of the stack, do not install the cap and proceed to the instructions describing door installation.

1 Stand the door upside down on a flat surface (i.e., with the two ribs towards the bottom).

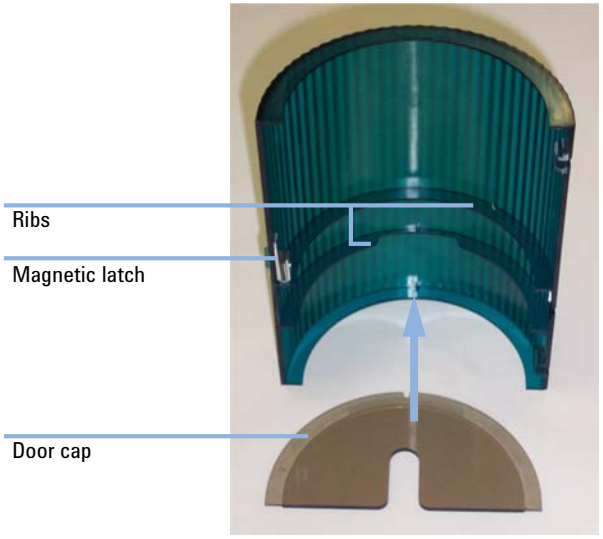


Figure 11 Installing the door cap

2 Take the cap and remove the protective paper exposing the adhesive that will attach the cap to the door.

3 Insert the cap into the door and press the adhesive onto the inside of the door lip. Be sure to keep the door edges and cap edges flush.

Installing the Door

1 Insert the top hinge pin into the top hinge.

2 Gently press down on the top of the door and slide the lower hinge pin into the lower hinge. The door should now pivot on the pins and close. The magnetic door latch should stick to the instrument.

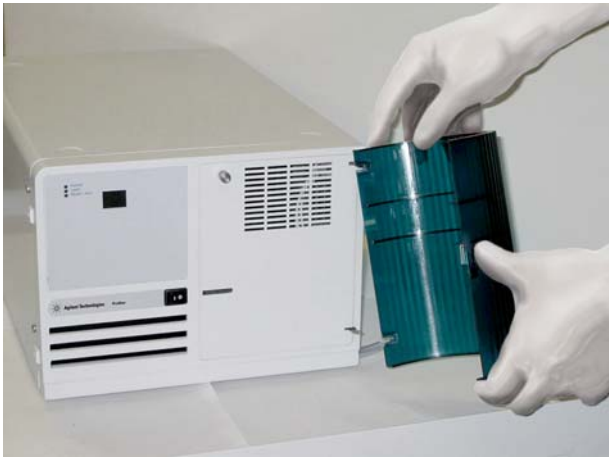


Figure 12 Installing the Agilent 325 Detector door

Installing a Flowcell

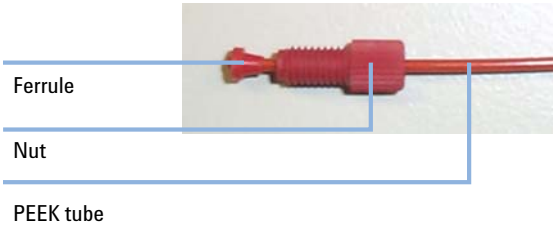
The Agilent 325 UV/VIS Dual Wavelength Detector is not shipped with a flowcell installed. You will need to install the flowcell that you purchased with the detector. Each flowcell comes with a set of recommended nuts and ferrules that may be fitted to tubing.

1/16" tubing is used on all flowcells. However for the 4 mm x 0.15 mm super prep. flowcell it is recommended to use 1/8" tubing at higher flow rates. In this case you can add the Adaptor 1/8" - 1/16" (p/n 1610126800). This will require the 1/8" tubing and the 1/8" flowcell fittings.

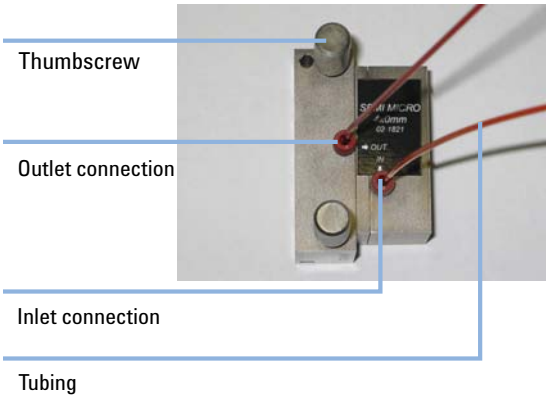
Tubing connections are PEEK™ type, except for the Super Prep flowcell which uses Tefzel® tubing 0.125 mm x 0.062 mm.

Parts required	#	p/n	Description
	1	210181800	Flowcell 9 mm x 0 mm, inert (analytical)
OR	1	210181900	Flowcell 9 mm x 1 mm, inert (prep.)
OR	1	210224200	Flowcell 4 mm x 0.25 mm, inert (scale up)
OR	1	210182100	Flowcell 4 mm x 0 mm, inert (micro-analytical)
	1	9910128300	Flow cell replacement fittings
	1		
	1	210182000	Flowcell 4 mm x 0.15 mm,inert (super prep.)
	1	1610126900	Fitting 1/8" tube nut flat bottom
	1	1610126400	Fitting 1/8" tube ferrule, Pack of 10
	1	1610126800	Adaptor 1/8" - 1/16"

1 Fit the nuts and ferrules on the PEEK™ tubes.



2 Screw the two tubes into the inlet and outlet connection of the flowcell. Each flowcell has an inlet and outlet connection.



NOTE

The position of the inlet and outlet connections varies for different flowcells.

3 Remove the front panel (see "Removing the Front Panel" on page 39 for more information).

4 Carefully position the flowcell so that the two thumbscrews are positioned in the threaded holes in the flowcell compartment.

a Push at the center of the flowcell to ensure it is positioned squarely in its housing and that it is not tilted in any way.

NOTE

It is critical to the performance of the detector that the flowcell is inserted correctly. The internal optical components of the flowcell are an integral part of the detector's optical system. If the flowcell is not fitted correctly, it will have an adverse effect on detector performance.

Next Steps:

5 Secure the flowcell by tightening the thumbscrews with your fingers. Alternate tightening the thumbscrews until they are snug.

6 Replace the front panel.

NOTE

For optimum performance, the detector should be operated with the front panel in place. This is because the foam on the inside of the panel stops breezes, which may cause instability and noise, from reaching the flowcell.

NOTE

The flowcell should be removed with the connecting tubing fitted. These must be removed outside the detector compartment.

7 Perform a lamp calibration (see chapter maintenance in Agilent 325 UV/VIS Dual Wavelength Detector - User Manual (p/n G9309-90000)).

Maintaining and Cleaning the Flowcell

Cleaning the Flowcell

When

The frequency of need for cell cleaning depends to a great extent upon the cleanliness maintained during routine operation. In severe cases of contamination, for instance if there is precipitate in the cell, the cell must be replaced.

Tools required

Description
HPLC grade water
Phosphoric acid, 85 %
Methanol
OR Acetonitrile

Parts required

Description
Stainless steel tubing 1.6 mm (1/16") o.d.

CAUTION

Contamination of exposed fittings

Contaminated flowcells can lead to noise and drift problems.

➔ Seal or cap tubings when not connected.

➔ Never apply thread lubricants to compression fittings.

CAUTION

Dismantled flowcell

The flowcell can be damaged.

➔ Do not dismantle the flowcell.

1 Remove all reservoirs containing organic solvent from the system.

2 Replace the column with a clean length of 1.6 mm (1/16") o.d. stainless steel tubing.

3 Fill all of the reservoirs with HPLC grade water and flush the system thoroughly to remove any trace of organic solvents.

4 After the system has been rinsed with water, pump 50 mL of 25 % phosphoric acid at 1 – 2 mL/min through the flowcell.

NOTE The acid is prepared by diluting one part of concentrated phosphoric acid (85 %) with three parts of HPLC grade water.

NOTE Do not allow the acid to remain in the cell for more than one hour.

5 Rinse the flowcell by pumping 100 – 200 mL of HPLC grade water through the system.

NOTE Dispose of the acid in accordance with approved waste disposal procedures.

6 Finally rinse the flowcell with acetonitrile or methanol.

Cleaning the Exterior of the Flowcell

Tools required	Description
	Compressed air
OR	Compressed nitrogen

1 Remove the flowcell from the detector (see "Installing a Flowcell" on page 43 for more information) and carefully blow any dust or contamination from the external windows with a dry, clean soure of compressed air or nitrogen.

2 Replace the flowcell, ensuring that the sample and reference beam windows are not obstructed (see "Installing a Flowcell" on page 43 for more information).

Maintaining of the Flowcell

The high sensitivity of the detector and the low volume of the flowcell make it imperative that a high standard of solvent purity and general system cleanliness be maintained. A contaminated flowcell can lead to noise and drift problems that are often mistakenly attributed to other areas of the system.

To avoid possible degradation in performance, remember the following:

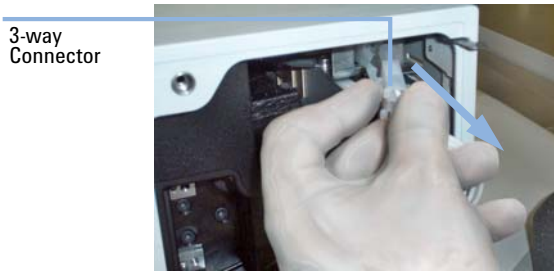
- Store the flowcells sealed in plastic bags if they are not in the detector.
- Set the flowcell with the faceplate upward if they are removed from the detector.
- Avoid touching the inside of the flowcell recess.
- Cover the flowcell opening with a piece of card, or always place a flowcell in the flowcell opening.

Replacing the Deuterium (UV) Lamp

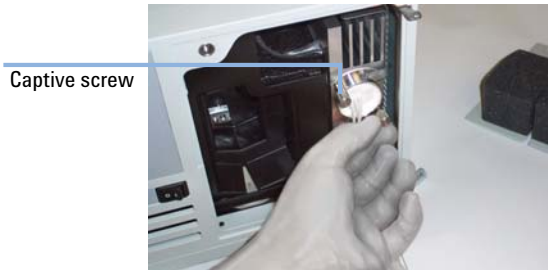
Removing the Old Lamp

1 Remove the front panel (see "Removing the Front Panel" on page 39 for more information).

2 Squeeze the small latch on the 3-way connector and pull the connector out.



3 Undo the first captive screw.

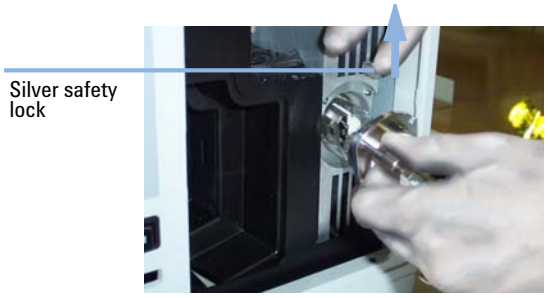


NOTE

Retain screw for re-installation.

4 Undo the second captive screw.

5 Lift up the silver saftey interlock latch on the right and remove the lamp, holding onto the thumbscrews.



Next Steps:

6 Remove the lamp assembly and discard it.

NOTE

No part of the old assembly can be re-used, except for the captive screws.

Installing the New UV Lamp

Your new replacement lamp is delivered to you pre-tested and aligned in a mounting bracket. It is designed for direct replacement and requires no alignment procedures after it has been installed.

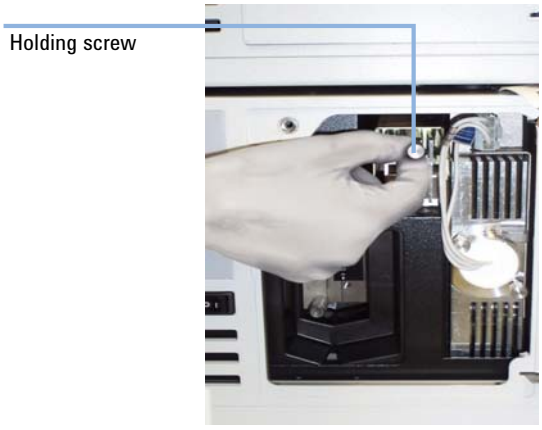
1. Lift the silver interlock latch, and carefully insert the lamp into the lamp housing.
2. Screw in the two thumbscrews.
3. Connect the 3-way connector into the socket.
4. Re-engage the saftey interlock latch by sliding it down.
5. Replace the front panel and screw in the captive screw in the top left corner of the panel.
6. Re-install the door (see “Installing the Door” on page 41 for more information).
7. Perform a lamp calibration (see “Calibrating the Lamps” on page 53 for more information).

Replacing the Visible Lamp

Removing the Old Lamp

1 Remove the front panel (see "Removing the Front Panel" on page 39 for more information).

2 Undo the visible lamp holding screw.



3 Remove the visible lamp housing by holding it via the screw.

This will then expose the lamp, which will still be connected.

Next Steps:

4 Gently grip the visible lamp between your thumb and finger and pull the lamp towards you.

NOTE

The visible lamp has two prongs which slide into two connecting sockets.

5 Remove the lamp and discard.

NOTE

No part of the old assembly can be re-used.

Installing the New Visible Lamp

Your new replacement lamp is delivered to you pre-tested and requires no alignment procedures after it has been installed.

1. Gently grip the lamp between your thumb and forefinger.
2. Insert the two small prongs of the lamp into the two holes in the lamp compartment and gently push the lamp in.
3. Attach the lamp housing by inserting the screw into the hole and tightening the thumbscrew with your fingers.
4. Replace the front panel and screw in the captive screw in the top left corner of the panel.
5. Re-install the door (see “Installing the Door” on page 41 for more information).
6. Perform a lamp calibration (see “Calibrating the Lamps” on page 53 for more information).

Calibrating the Lamps

Lamp calibration minimizes noise by allowing the maximum gain settings to be used. It also improves linearity by re-balancing the 0 %T correction tables as lamps age.

If a calibration is performed with a bubble in the cell, or no cell is fitted, the gain settings will be too high to cope with a cell present and full of water. Readings will be overrange, and will not respond to changes in absorbance. Noise will either be very high, or zero. If the gains are set just too high, chromatograms may show peaks that are cut off at the bottom.

Parts required

	#	p/n	Description
	1	210181800	Flowcell 9 mm x 0 mm, inert (analytical)
OR	1	210181900	Flowcell 9 mm x 1 mm, inert (prep.)
	1	210224200	Flowcell 4 mm x 0.25 mm, inert (scale up)
	1	210182000	Flowcell 4 mm x 0.15 mm,inert (super prep.)
OR	1	210182100	Flowcell 4 mm x 0 mm, inert (micro-analytical)
	1	110743300	Back pressure restrictor

Preparations

Unless a fraction collector is installed after the detector, the Back pressure restrictor (p/n 110743300) assembly that was supplied with your detector should always be threaded into the outlet line from the flowcell.

The back pressure restrictor provides approximately 2.76 bar (40 psi) back pressure on the flowcell. This prevents outgassing and bubbles from forming or being trapped in the flowcell, which can cause an unstable baseline. See "Detector Outlet Back Pressure Restrictor" on page 21 for more information about the Back pressure restrictor (p/n 110743300) .

- Be sure a flow cell is fitted in the detector.
- To keep the flowcell clear of bubbles, pump clean water with 0.2 – 1 mL/min through the cell.
- After turning on the lamp, wait at least half an hour to get the best linearity correction before starting the calibration.

1 In the Toolbar, tap the **Calibration** icon.

2 Tap the **Lamp Calib** button. If the system is in the **Not Ready** state, a message will appear stating that the system must be in the **Ready** state.

NOTE The lamp calibration will now be performed and the process may takes several minutes.

NOTE A lamp calibration can also be performed in OpenLAB.

Cleaning the Instrument

The exterior of the Agilent 325 UV/VIS Dual Wavelength Detector should be kept clean.

Tools required	Description
	Soft, lint free cloth
	Water
	Mild detergent

1 Clean the exterior surfaces with a soft cloth. If necessary, dampen the cloth with water or a mild detergent.

NOTE Do not use organic solvents or abrasive cleaning agents.

7
Parts

This chapter provides information on parts for the instrument.

Parts List

p/n	Description
G9309A	Agilent 325 UV-VIS Dual Wavelength Detector
110728800	Agilent 325 detector door
910206600	Door cap
110743300	Back pressure restrictor
110715400	Assy D_2 lamp (pre-aligned) 325
210186590	Assy PWB sync. interface 325
210187590	Assy PWB relay interface 325
210181800	Flowcell 9 mm x 0 mm, inert (analytical)
210181900	Flowcell 9 mm x 1 mm, inert (prep.)
210224200	Flowcell 4 mm x 0.25 mm, inert (scale up)
210182000	Flowcell 4 mm x 0.15 mm,inert (super prep.)
210182100	Flowcell 4 mm x 0 mm, inert (micro-analytical)
1910010700	Fuse 3.15 A
5610136500	Lamp miniature quartz halogen 325
9910128300	Flow cell replacement fittings
1610126800	Adaptor 1/8" - 1/16"
1610126900	Fitting 1/8" tube nut flat bottom
1610126400	Fitting 1/8" tube ferrule, Pack of 10

8
Cables

This chapter provides information on cables used with the instrument.

Cable Overview

Necessary cables

p/n	Description
392612901	Ethernet cable (for use in a *network*)
5023-0203	Ethernet cable (cross-over, for *standalone* use)
392607969	Inject marker cable
392607975	Next injection cable
393546291	Serial communication ribbon
393597601	Converter RS232 to RS422
7910046300	Serial cable

Optional cables

p/n	Description
110743800	Relay interface cable (for relay interface board, one relay contact per cable)
110744200	Analog signal cable

Cable Connections

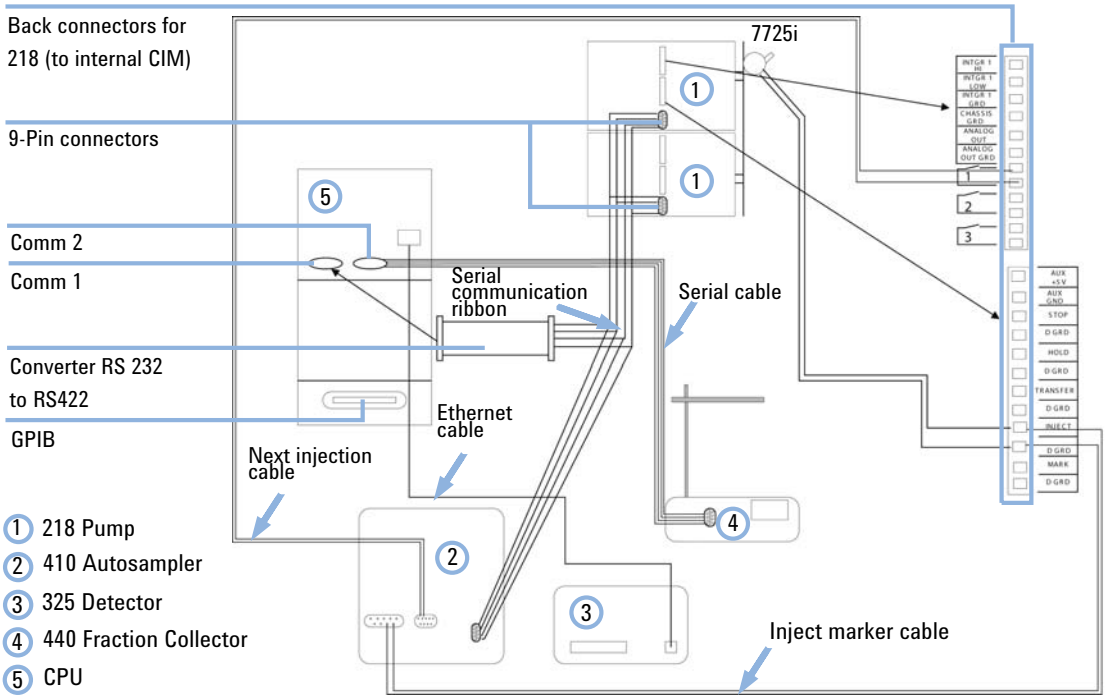


Figure 13 Cable connections for workstation control of Agilent 218 Pumps, Agilent 325 Detector, Agilent 410 Autosampler and Agilent 440 Fraction Collector

Analog Output

For analog output signals, install the optional Analog signal cable (p/n 110744200) into the J14 receptacle. Pin designations are shown below.

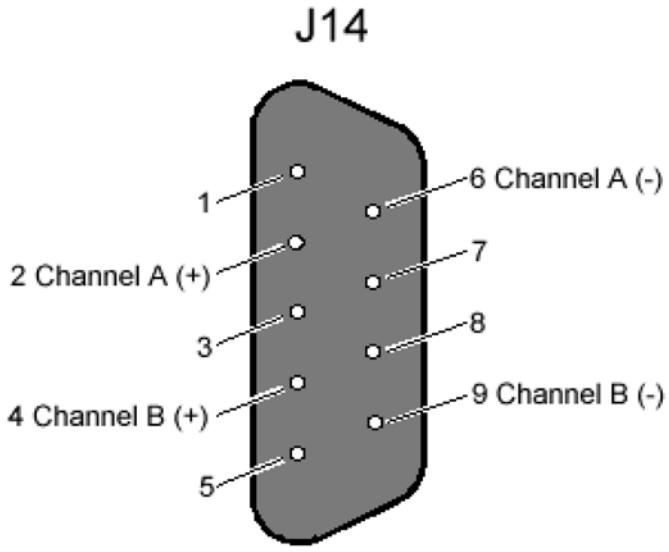


Figure 14 Pin designation for J14

The open ends of the analog output cable have labels with the signal names (Channel A +, Channel A - and Channel B +, Channel B -).

Relay Output

For time programming external events, a contact closure Relay output is available. To configure the Relay output, install the optional Assy PWB relay interface 325 (p/n 210187590) into the J4 receptacle. Pin designations are shown below.

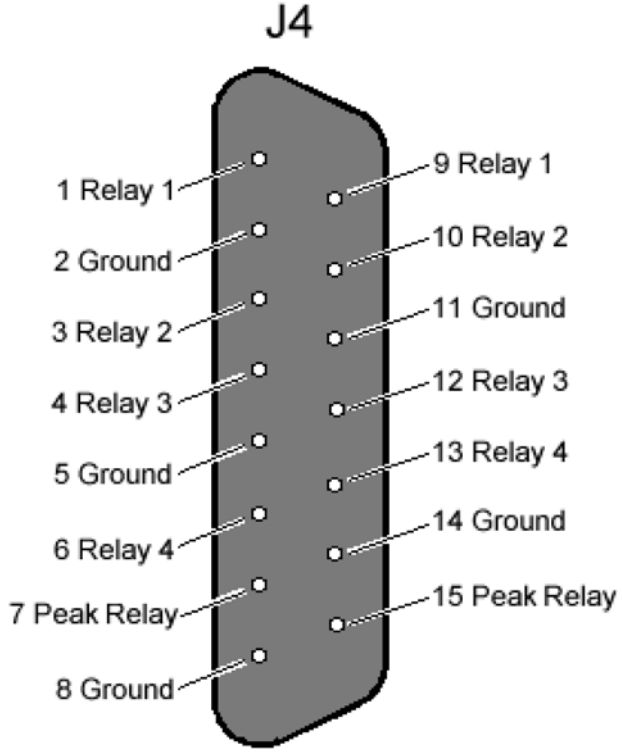


Figure 15 Pin designation for J4

There are four general purpose output relays and one dedicated Peak relay. Each output uses a DIP relay that is capable of handling 500 mA of contact current. At reset or power up, the output relay contacts are set to the default parameters (open). After loading a method they will be set as defined in the method's **time=0 parameters**.

The Peak relay is software programmable for duration, delay and active sense. At power up, the relay contact will be set to the inactive state (as defined by the value of the **active sense** parameter stored in the detector). Upon being triggered, relay activation will occur for the time interval equal to the **Peak Sense duration** parameter as stored in the method.

The Peak relay can be activated from any of the following sources (only one source can be active at any one time):

- Time Slice event – Once time slice has been turned on, it will provide a periodic activation of the Peak Sense relay at an interval defined in **Time Slice period** within the method. Time Slice can be turned on and off by time.
- Pulse event – A single timed programmed activation of the Peak Sense relay as defined in the method.
- Peak sense has been turned on.

To connect open-ended wires to the relay signals available at J4, use the optional Assy PWB relay interface 325 (p/n 210187590) (see Figure 16 on page 64). Simply plug this PWB into the rear panel connector.

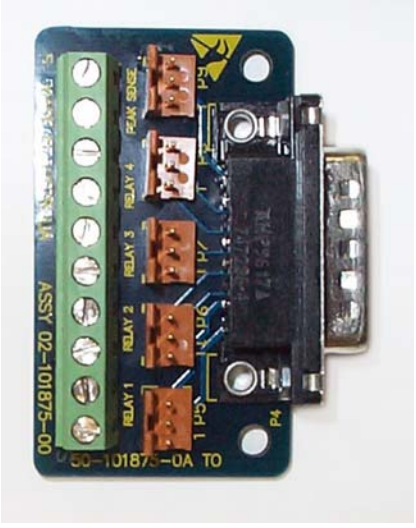


Figure 16 Relay output board

The Relay interface cable (for relay interface board, one relay contact per cable) (p/n 110743800) is used to attach to the 3 pin connectors at the relay output board.

With the 3 pin connectors, the contact closure is between pins 1 and 2 of the plugs. Pin 3 is connected to ground. The relay interface cable has three open-ended wires. The relay contact is connected between the clear and the black wire. The green wire is connected to ground.

Desktop PC Communications

Communication between the detector and a desktop PC occurs by an Ethernet connection. Communication by an Ethernet connection is required to control the detector remotely by OpenLAB. When the Workstation provides HPLC system control, the synchronization cables from P9 and J10 are not used.

To create an Ethernet connection, insert an RJ45 cable included in the ship kit into the J1 receptacle and into the PC. The Ethernet cable that comes with the detector is a cross-over cable, which is appropriate for connecting the detector directly to a PC. Connecting the detector to a network or a hub will usually require a patch cable. A Ethernet cable (for use in a *network*) (p/n 392612901) can be purchased from Agilent or either locally.

Most PCs come pre-configured with an Ethernet connection, which is usually built into the motherboard, or with an Ethernet network card installed. However, if you have a PC that has no network interface, you will need to install and configure a Network Interface Card (PCI bus). The PC must have a spare PCI slot for the installation of this device. You are also responsible for setting up and maintaining any LAN configuration where a detector may be used. All network issues are to be dealt with by the user.

Synchronization Signals

The synchronization signals at P9 and J10 are used to synchronize the operation of a group of instruments that are not interfaced to OpenLAB. The synchronization signals come in four pairs and define how the detector will operate in a HPLC system. These signals are important for controlling timing and synchronization of the detector with the other devices in the system. Synchronization signals are closely tied to the detector states and transitions. P9 and J10 pin designations are shown in Figure 17 on page 67.

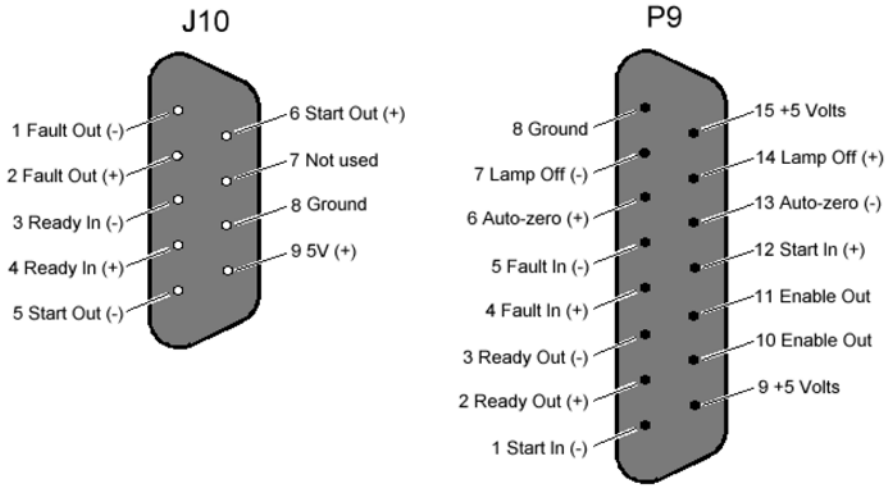


Figure 17 Pin designations for J10 and P9

P9 provides connections when the Agilent 325 UV/VIS Dual Wavelength Detector acts as a “slave” and receives control from another module. J10 provides connections when the detector acts as a “master” and sends control to another device.

An input is activated or said to be present when its two signal wires are connected together. This can be done with a relay contact closure. If the inputs are driven from another instrument with optical isolators or other polarized devices, then attention must be paid to the polarity of the signal wire

connections. The positive (+) output signal must be connected to the positive (+) input signal and the negative (-) output to the negative (-) input.

The color coding and physical design of the cable connectors ensure that correct signals and polarity are matched.

The outputs are optical isolators and simulate a relay contact closure when they are activated (see Figure 18 on page 68). The minimum requirement for an input signal to be detected is 200 ms.

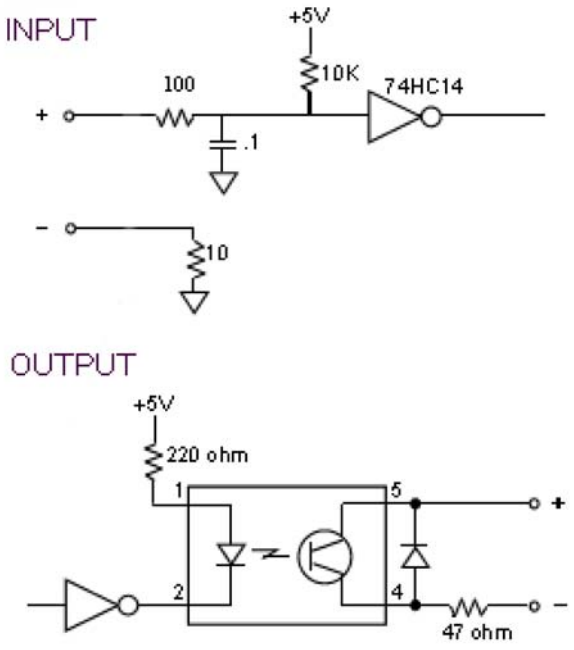


Figure 18 Input/output schematics

Table 6 Signal Description J10 and P9

Signal	Description
Enable Out	A non-polarized constantly active output (a short). This output can be used to activate Enable In on the next instrument.
Ready In	When Enable Ready In is set (software switch), this polarized input signal must be present before the Agilent 325 Detector can go to the Ready state. Specifically, when the Agilent 325 Detector is in the NOT Ready Lamp On state, on receiving a Ready In signal, a monitor period will occur after which the Agilent 325 Detector goes to the Ready state. It must stay active until the Agilent 325 Detector starts. Ready In will be ignored in all other states.
Ready Out	This polarized output signal indicates that the Agilent 325 Detector is in the Ready state and is ready to start a time program.
Start In	This polarized edge triggered input signal will start the active method if the Agilent 325 Detector is in the Ready state.
Start Out	This polarized output signal will be activated for 600 ms when the Agilent 325 Detector starts a time program.
Fault In	This polarized edge triggered input signal informs the Agilent 325 Detector that a fault condition exists in another instrument in the system. The Agilent 325 Detector halts the time program and sends a Fault Out signal. The lamp can be programmed to either remain on or turn off upon receiving a fault signal.
Fault Out	This polarized output signal will activate for 600 ms when either of the following conditions occurs: • The Agilent 325 Detector discovers an internal fault condition that warrants aborting the run. • The Agilent 325 Detector receives a Fault In signal and it has no internal fault condition itself.
Auto-zero	This edge-triggered contact closure causes an auto-zero adjustment.
Lamp off	This edge-triggered contact closure switches the lamp off. It is possible to turn the lamp back on manually if the contact is still closed.

An optional Assy PWB sync. interface 325 (p/n 210186590) is available to interface between the Agilent 325 UV/VIS Dual Wavelength Detector synchronization signals and other devices. This board is inserted into the P9 and J10 connectors and connects to a terminal strip on the adapter board. This terminal strip accepts bare wire leads from cables connecting other devices. These cables may originate from the other device, or a dedicated cable can be used, if available for the particular application.

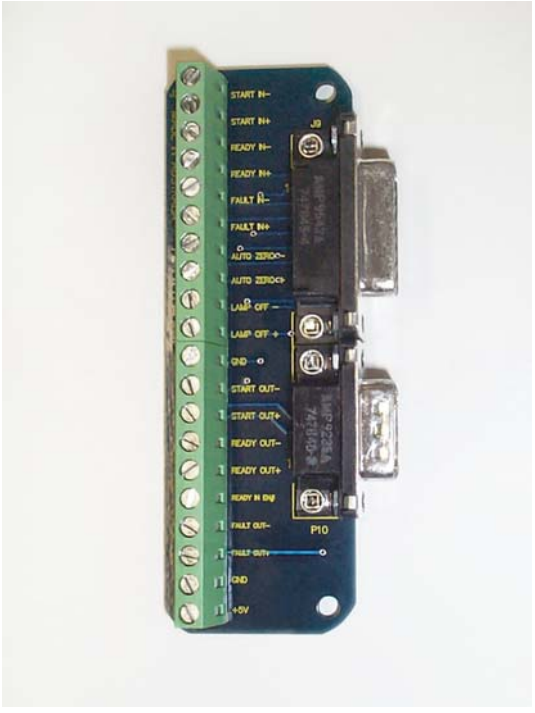


Figure 19 The I/O adapter board

9 Appendix

This chapter provides addition information on safety, legal and web.

General Safety 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. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

➔ The operator of this instrument is advised to use the equipment in a manner as specified in this manual.

Symbols

Table 7 Warning symbols

 Electrical shock	 Chemical hazard	 Hot surface
 Eye hazard	 Fire hazard	 Explosion hazard
 Radiation Source	 Moving parts	 Heavy weight (danger to hands)
 Heavy weight (danger to feet)	 Attention[1]	

[1] The symbol may be used on warning labels attached to the instrument. When you see this symbol, refer to the relevant operation or service manual for the correct procedure referred to by that warning label.

Table 8 Information symbols

Symbol	Description
I	Mains power on
0	Mains power off
	Single phase alternating current
	Fuse
	When attached to the rear of the instrument, indicates that the product complies with the requirements of one or more EU directives.
	When attached to the rear of the product, indicates that the product has been certified (evaluated) to CSA 61010.1 and UL 61010-1.

Solvent Hazards

WARNING

Explosion, fire, asphyxiation

This instrument is not explosion-proof.

Certain solvents may cause weakening and leaks of tubings or fitthings with possible bursting.

Even small leaks in solvent supply systems can be dangerous.

- ➔ Only use solvents compatible with the HPLC system tubings and fittings.
- ➔ Employ static measuring and static discharge devices to safeguard against the buildup of static electricity.
- ➔ In unattended operation, do not use organic solvents having an ignition point below 70 °C.
- ➔ Do not bring a heat or flame source near the instrument.
- ➔ The area in which solvents are stored and the area surrounding the instrument must be adequately ventilated to prevent accumulations of gas.
- ➔ Always check the condition of the instrument (leakage of solvent or waste solution, leakage of solvent inside the instrument). If an abnormality is found, stop operation immediately.
- ➔ When using flammable chemicals, be careful about possible ignition due to static electricity. To prevent the build-up of static electricity, use a conductive container for waste.
- ➔ Use only approved regulator and hose connectors (refer to the supplier's instructions).
- ➔ Keep solvents cool and properly labeled. Ensure that you have the correct solvent before connecting it to the instrument.

WARNING

Inflammation or injury due to toxic, corrosive or stimulative solvent

- ➔ Do not contact toxic, corrosive or stimulative solvent.
- ➔ For details of the properties of each solvent and how to handle it, refer to the relevant Material Safety Data Sheets (MSDS).
- ➔ Be sure to handle each solvent properly.
- ➔ Wear proper personal protective clothes (e.g., safety goggles) so that a solvent will not come into direct contact with the skin.
- ➔ Ventilate the laboratory room adequately to prevent accidental inhalation of harmful solvent vapor.

WARNING

Cuts

- ➔ When working with glass or quartz parts take care to prevent breakage.

Other Precautions

Airflow to the cooling fans of the liquid chromatograph must be unobstructed. Do not block the ventilation grills on the liquid chromatograph and accessories.

Consult the manuals supplied with your PC, monitor and for their specific ventilation requirements.

High Pressure Hazards

WARNING

High velocity stream of volatile and/or toxic liquids.

If a line ruptures, a relief device opens, or a valve opens accidentally under pressure, potentially hazardous high liquid pressures can be generated by the pump.

- ➔ Wear personal protective equipment when you inject samples or perform routine maintenance.
- ➔ Never open a solvent line or valve under pressure. Stop the pump first and let the pressure drop to zero.
- ➔ Always keep the doors and covers closed during operation.
- ➔ Read and adhere to all Notes, Cautions, and Warnings in the manual.

Ultraviolet Radiation

WARNING

Irritation to the skin, eyes and upper respiratory system

- ➔ Ensure that protective lamp covers of variable and fixed wavelength detectors are in place during operation.
- ➔ Do not look directly into detector fluid cells or at the UV light source. When inspecting the light source or fluid cell, always use protective eye covering such as borosilicate glass or polystyrene.
- ➔ Ventilate the area surrounding the detector such that the concentration of ozone does not exceed the maximum permissible level. All venting must be to outside air, never within the building.

Ozon generation

Ozone can be generated by radiation from the source lamps. The maximum permissible exposure level is 0.1 ppm (0.2 mg/m^3).

The Waste Electrical and Electronic Equipment Directive

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 starting with 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.

NOTE

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.

Batteries Information

WARNING **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.**

Danger of explosion if battery is incorrectly replaced.

➔ Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

➔ Replace only with the same or equivalent type recommended by the equipment manufacturer.

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 appararleverandoren.

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

Radio Interference

Cables supplied by Agilent Technologies are screened to provide optimized protection against radio interference. All cables are in compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with unscreened cables, 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.

CE Compliance

Your Agilent 700 Series ICP-OES instrument has been designed to comply with the requirements of the Electromagnetic Compatibility (EMC) Directive and the Low Voltage (electrical safety) Directive (commonly referred to as the LVD) of the European Union. Agilent has confirmed that each product complies with the relevant Directives by testing a prototype against the prescribed EN (European Norm) standards.

Proof that a product complies with these directives is indicated by:

- the CE Marking appearing on the rear of the product, and
- the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the directives listed above, and shows the EN standards to which the product was tested to demonstrate compliance.

Electromagnetic Compatibility

EN55011/CISPR11

Group 1 ISM equipment: group 1 contains all ISM equipment in which there is intentionally generated and/or used conductively coupled radio- frequency energy which is necessary for the internal functioning of the equipment itself.

Class A equipment is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

Operation is subject to the following two conditions:

- This device may not cause harmful interference.
- This device must accept any interference received, including interference that may cause undesired operation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

1. Relocate the radio or antenna.
2. Move the device away from the radio or television.
3. Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
4. Make sure that all peripheral devices are also certified.
5. Make sure that appropriate cables are used to connect the device to peripheral equipment.
6. Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.
7. Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

ICES/NMB-001

This ISM device complies with Canadian ICES- 001. Cet appareil ISM est conforme à la norme NMB-001 du Canada.

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For the latest information on products and services visit our worldwide web site on the Internet at:

http://www.agilent.com

Select Products/Chemical Analysis

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

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In This Book

The manual describes the following:

- Introduction
- Site requirements
- Installation
- Usage
- Troubleshooting and diagnostics
- Error information
- Maintenance and repair
- Parts
- Safety

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