Agilent 1260 Infinity Fluorescence Detector

User Manual

Agilent Technologies

Notices

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In This Guide

This manual covers

- the Agilent 1260 Infinity Fluorescence Detector (G1321B).
- the Agilent 1200 Series Fluorescence Detector (G1321A) (obsolete).

1 Introduction to the Fluorescence Detector

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

2 Site Requirements and Specifications

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

3 Installing the Module

This chapter gives information about the preferred stack setup for your system and the installation of the module.

4 Using the Fluorescence Detector

This chapter guides you how to start the work with the detector.

5 Optimizing 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 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

8 Test Functions

This chapter describes the detector’s built in test functions.

9 Maintenance

This chapter provides general information on maintenance of the detector.

10 Parts for Maintenance

This chapter provides information on parts for maintenance.

11 Identifying Cables

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1
Introduction to the Fluorescence Detector

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

Introduction to the Detector

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

- flash lamp for highest intensity and lowest detection limit
- multi-wavelength mode for on-line spectra
- spectra acquisition and simultaneous multi-signal detection
- optional cuvette is available and can be used for off-line measurements
- easy front access to flow cell for fast replacement and
- built-in wavelength accuracy verification.

For specifications, see “Performance Specifications” on page 36

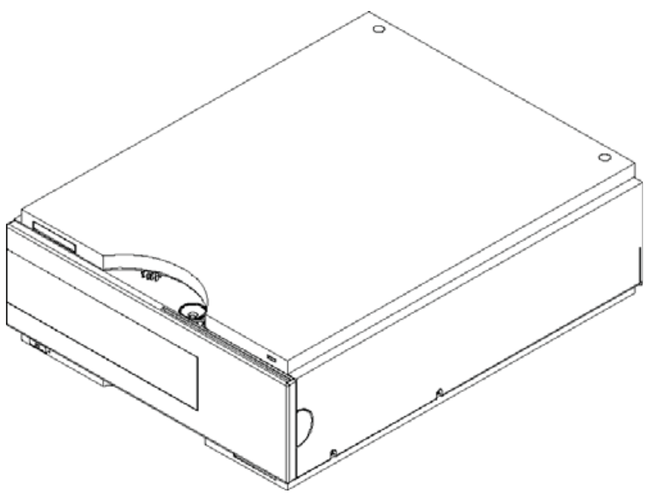


Figure 1 The Agilent 1260 Infinity Fluorescence Detector

How the Detector Operates

Luminescence Detection

Luminescence, the emission of light, occurs when molecules change from an excited state to their ground state. Molecules can be excited by different forms of energy, each with its own excitation process. For example, when the excitation energy is light, the process is called *photoluminescence*.

In basic cases, the emission of light is the reverse of absorption, see Figure 2 on page 11. With sodium vapor, for example, the absorption and emission spectra are a single line at the same wavelength. The absorption and emission spectra of organic molecules in solution produce bands instead of lines.

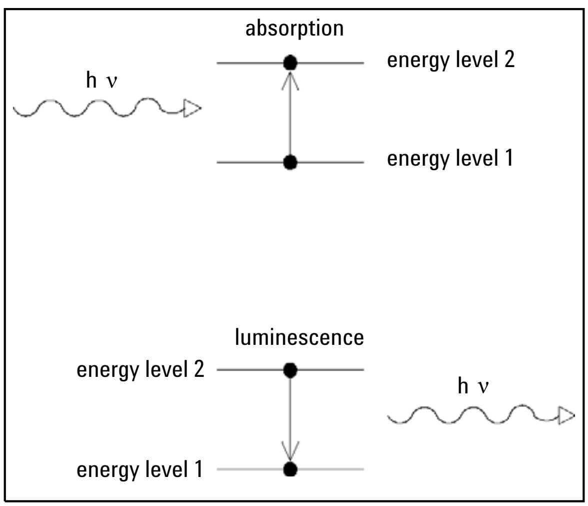


Figure 2 Absorption of Light Versus Emission of Light

When a more complex molecule transforms from its ground energy state into an excited state, the absorbed energy is distributed into various vibrational and rotational sub-levels. When this, same molecule returns to the ground state, this vibrational and rotational energy is first lost by relaxation without any radiation. Then the molecule transforms from this energy level to one of the vibrational and rotational sub-levels of its ground state, emitting light, see Figure 3 on page 12. The characteristic maxima of absorption for a substance is its λ_{EX}, and for emission its λ_{EM}.

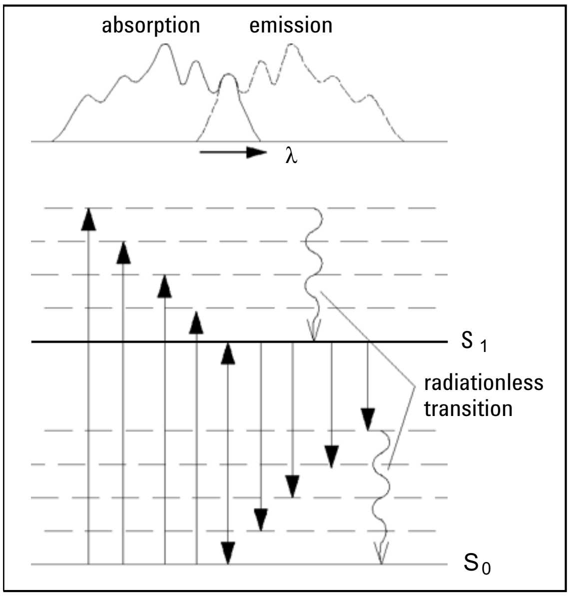


Figure 3 Relationship of Excitation and Emission Wavelengths

Photoluminescence is the collective name for two phenomena, *fluorescence* and *phosphorescence*, which differ from each other in one characteristic way--the delay of emission after excitation. If a molecule emits light 10^{-9} to 10^{-5} seconds after it was illuminated then the process was fluorescence. If a molecule emits light longer than 10^{-3} seconds after illumination then the process was phosphorescence.

Phosphorescence is a longer process because one of the electrons involved in the excitation changes its spin, during a collision with a molecule of solvent, for example. The excited molecule is now in a so-called triplet state, T, see Figure 4 on page 13.

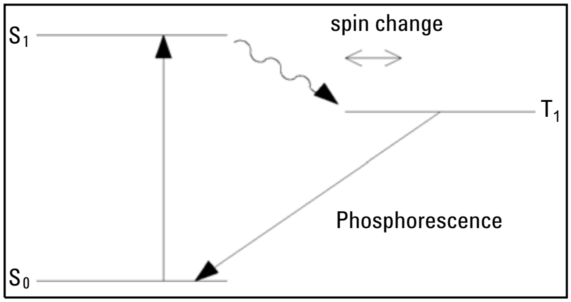


Figure 4 Phosphorescence Energy Transitions

The molecule must change its spin back again before it can return to its ground state. Since the chance of colliding with another molecule with the necessary spin for change is slight, the molecule remains in its triplet state for some time. During the second spin change the molecule loses more energy by relaxing without radiation. The light which is emitted during phosphorescence therefore has less energy and is at a longer wavelength than fluorescence.

Formula: $E = h \times {}^{\lambda-1}$

In this equation:

E is energy

h is Planck's constant

λ is the wavelength

Raman Effect

The Raman effect arises when the incident light excites molecules in the sample which subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This inelastically scattered light is called Raman scatter. It results from the molecule changing it's molecular motions.

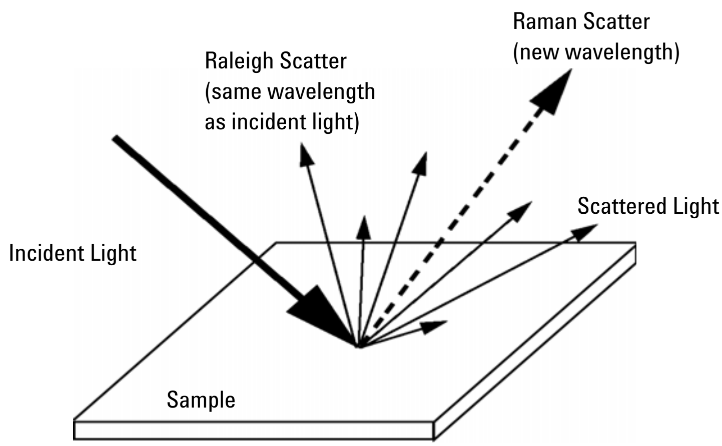


Figure 5 Raman

The energy difference between the incident light (E_i) and the Raman scattered light (E_s) is equal to the energy involved in changing the molecule's vibrational state (i.e. getting the molecule to vibrate, E_v). This energy difference is called the Raman shift.

$E_v = E_i - E_s$

Several different Raman shifted signals will often be observed; each being associated with different vibrational or rotational motions of molecules in the sample. The particular molecule and its environment will determine what Raman signals will be observed (if any).

A plot of Raman intensity versus Raman shift is a Raman spectrum.

Optical Unit

All the elements of the optical system, shown in Figure 6 on page 16, including Xenon flash lamp, excitation condenser, excitation slit, mirror, excitation grating, flow cell, emission condenser, cut-off filter, emission slit, emission grating and photo-multiplier tube are housed in the metal casting inside the detector compartment. The fluorescence detector has grating/grating optics, enabling the selection of both excitation and emission wavelengths. The flow cell can be accessed from the front of the fluorescence detector.

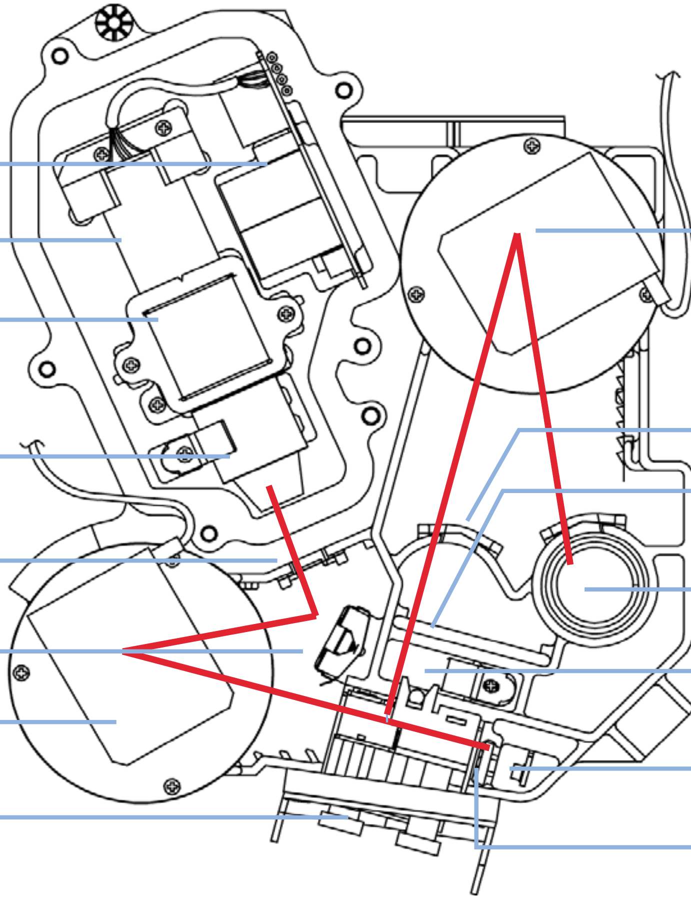


Figure 6 Optical Unit

The radiation source is a xenon flash-lamp. The 3 µs flash produces a continuous spectrum of light from 200 nm to 900 nm. The light output distribution can be expressed as a percentage in 100 nm intervals, see Figure 7 on page 17. The lamp can be used for some 1000 hours depending on the sensitivity requirements. You can economize during automatic operation using keyboard setpoints, so the lamp flashes during your analysis only. The lamp can be used until it no longer ignites, but the noise level may increase with usage.

UV degradation, especially below 250 nm is significantly higher compared to Visible wavelength range. Generally the "LAMP ON during run" - setting or using "economy mode" will increase lamp life by a magnitude.

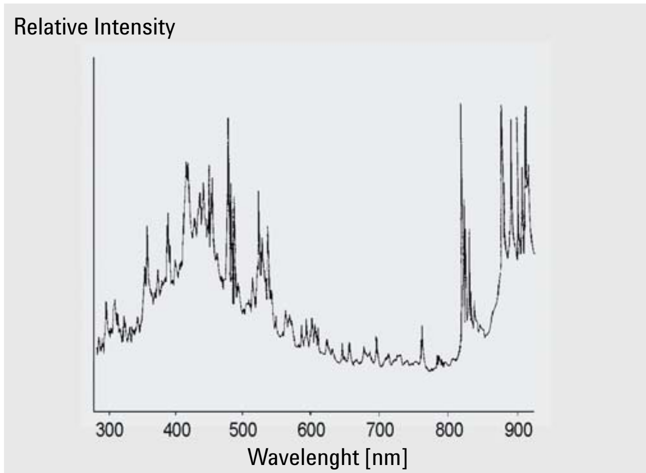


Figure 7 Lamp Energy Distribution (vendor data)

The radiation emitted by the lamp is dispersed and reflected by the excitation monochromator grating onto the cell entrance slit.

The holographic concave grating is the main part of the monochromator, dispersing and reflecting the incident light. The surface contains many minute grooves, 1200 of them per millimeter. The grating carries a blaze to show improved performance in the visible range.

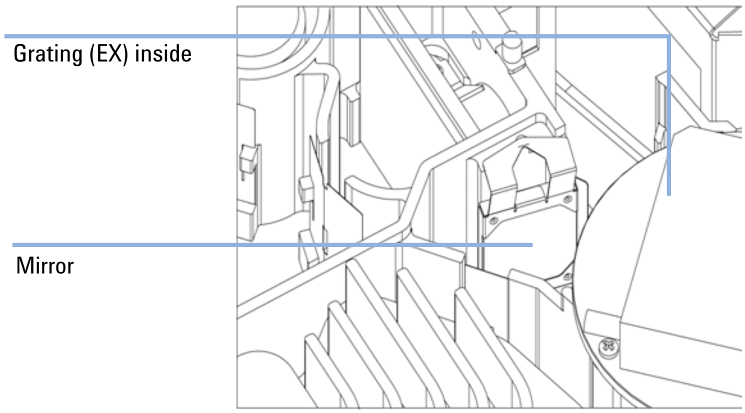


Figure 8 Mirror Assembly

The geometry of the grooves is optimized to reflect almost all of the incident light, in the 1^{st} order and disperse it with about 70 % efficiency in the ultra-violet range. Most of the remaining 30 % of the light is reflected at zero order, with no dispersion. Figure 9 on page 19 illustrates the light path at the surface of the grating.

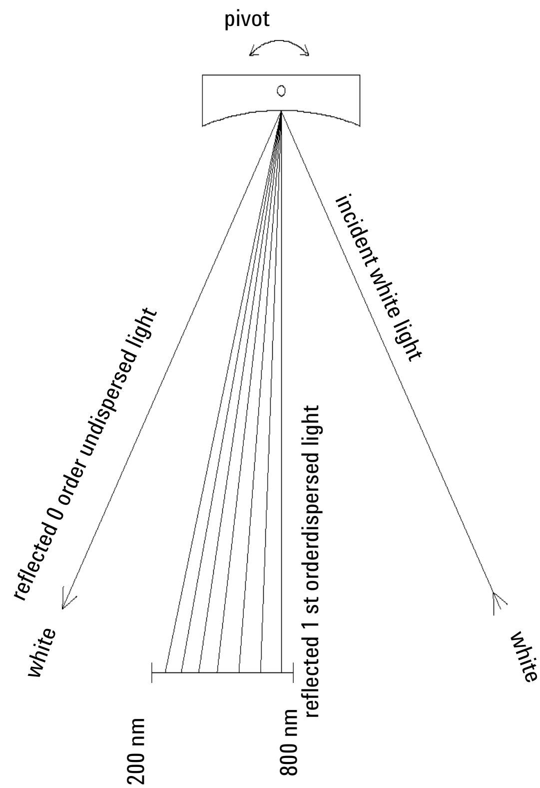


Figure 9 Dispersion of Light by a Grating

The grating is turned using a 3-phase brushless DC motor, the position of the grating determining the wavelength or wavelength range of the light falling onto the flow cell. The grating can be programmed to change its position and therefore the wavelength during a run.

For spectra acquisition and multi-wavelength detection, the grating rotates at 4000 rpm.

The excitation and emission gratings are similar in design, but have different blaze wavelengths. The excitation grating reflects most 1^{st} order light in the ultra-violet range around 250 nm, whereas the emission grating reflects better in the visible range around 400 nm.

The flow cell is a solid fused silica body with a maximum back pressure of 20 bar. Excessive back pressure will result in destruction of the cell. Operating the detector close to waste with low back pressure is recommended. A slit is integrated to the body.

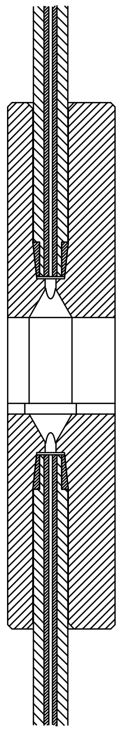


Figure 10 Cross-Section of Flow Cell

The luminescence from the sample in the flow cell is collected at right angles to the incident light by a second lens, and passes through a second slit. Before the luminescence reaches the emission monochromator, a cut-off filter removes light below a certain wavelength, to reduce noise from 1^{st} order scatter and 2^{nd} order stray light, see Figure 9 on page 19.

The selected wavelength of light is reflected onto the slit in the wall of the photo-multiplier compartment of the optical unit. The bandwidth of the emitted light is 20 nm.

On the photocathode, Figure 11 on page 21, incident photons generate electrons. These electrons are accelerated by an electrical field between several arc-shaped dynodes. Depending on the voltage difference between any pair of dynodes, an incident electron may spark-off further electrons which accelerate onto the next dynode. An avalanche effect results: finally so many electrons are generated that a current can be measured. The amplification is a function of the voltage at the dynodes and is microprocessor controlled. You can set the amplification using the PMTGAIN function.

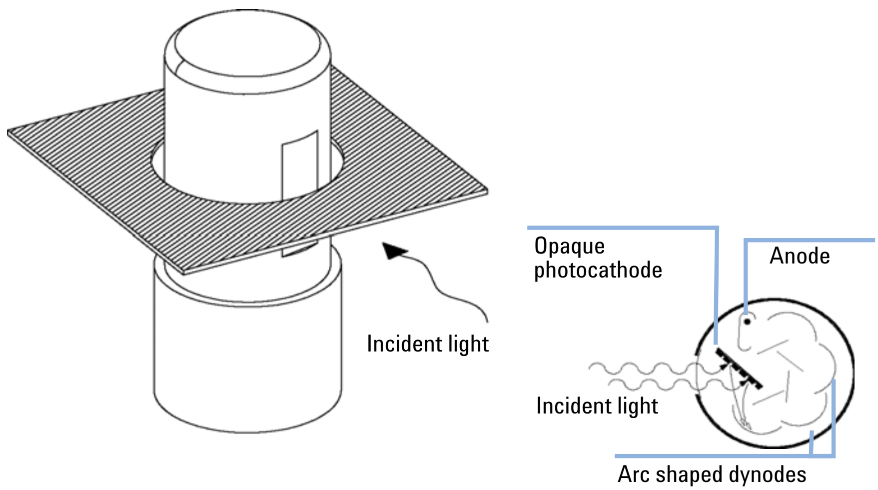


Figure 11 Photo-multiplier Tube

This type of so-called side-on photo-multiplier is compact ensuring fast response, conserving the advantages of the short optical path shown in Figure 6 on page 16.

PMTs are designed for specific wavelength ranges. The standard PMT offers optimum sensitivity from 200 to 600 nm. In the higher wavelength range a red-sensitive PMT can improve performance.

Reference System

A reference diode, located behind the flow cell, measures the excitation (EX) light transmitted by the flow cell and corrects flash lamp fluctuations and long-term intensity drift. Because of a non-linear output of the diode (depending on the EX-wavelength), the measured data are normalized.

A diffuser is located in front of the reference diode (see Figure 6 on page 16). This diffuser is made of quartz, reduces light and allows integral measurement of the light.

Analytical Information From Primary Data

We now know how the primary data from your sample is acquired in the optical unit. But how can the data be used as information in analytical chemistry? Depending on the chemistry of your application, the luminescence measured by the fluorescence detector will have different characteristics. You must decide, using your knowledge of the sample, what mode of detection you will use.

Fluorescence Detection

When the lamp flashes, the fluorescing compounds in your sample will luminesce almost simultaneously, see Figure 12 on page 23. The luminescence is short-lived, therefore the fluorescence detector need only measure over a short period of time after the lamp has flashed.

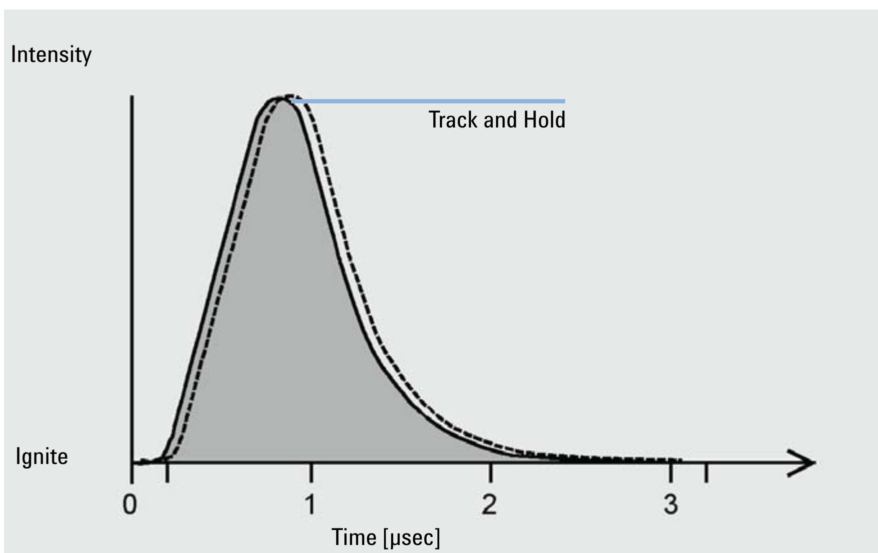


Figure 12 Measurement of Fluorescence

Phosphorescence Detection

An appropriate parameter set will be specified as soon as you chose the phosphorescence detection mode (special setpoints under FLD parameter settings).

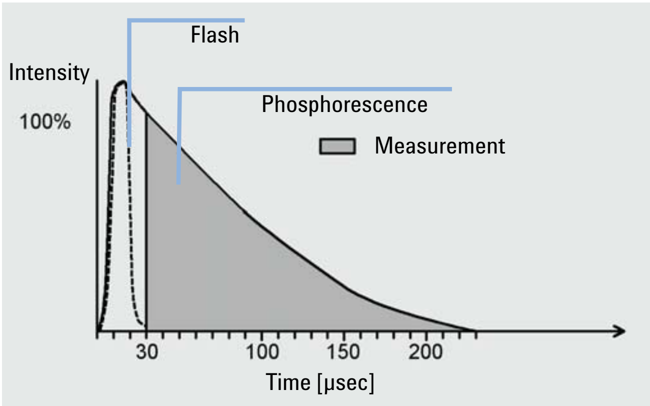


Figure 13 Measurement of Phosphorescence

Processing of Raw Data

If the lamp flashes at single wavelength and high-power, then the fluorescence data rate is 296 Hz. That means that your sample is illuminated 296 times per second, and any luminescence generated by the components eluted from the column is measured 296 times per second.

If the “economy” or multi-wavelength mode is set, then the flash frequency is 74 Hz.

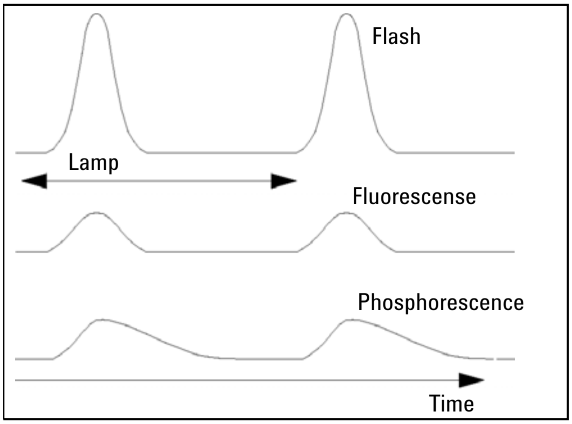


Figure 14 LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

You can improve the signal-to-noise characteristics by disabling the "economy" mode.

NOTE Disabling the "economy" mode will shorten the lifetime of the lamp significantly. Consider lifetime saving by switching off the lamp after the run is completed.

The data resolution is 20 bit at a response time of 4 s (default, which is equivalent to a time constant of 1.8 s and appropriate for standard chromatographical conditions). Weak signals may cause errors in quantification because of insufficient resolution. Check your proposed PMTGAIN. If it is significantly distant from your setting, change your method or check the purity of your solvent.

You can amplify the signal using PMTGAIN. Depending on the PMTGAIN you have set, a multiple of electrons is generated for every photon falling on the photomultiplier. You can quantify large and small peaks in the same chromatogram by adding PMTGAIN changes during the run into a timetable.

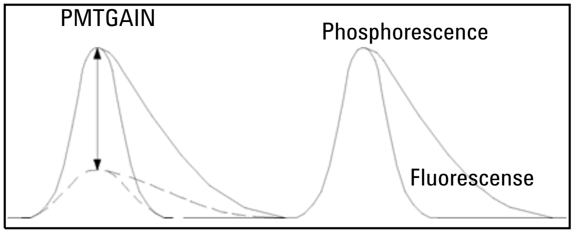


Figure 15 PMTGAIN: Amplification of Signal

Check proposed PMTGAIN. Deviations of more than 2 PMT gains should be corrected in the method.

Each PMTGAIN step is increased approximately by a factor of 2 (range 0 - 18). To optimize your amplification for the peak with the highest emission, raise the PMTGAIN setting until the best signal-to-noise is achieved.

After the photons are converted and multiplied into an electronic signal, the signal (at present analog) is tracked and held beyond the photo-multiplier. After being held, the signal is converted by an A-to-D converter to give one raw data point (digital). Eleven of these data points are bunched together as the first step of data processing. Bunching improves your signal-to-noise ratio.

The bunched data, shown as larger black dots in Figure 16 on page 27, is then filtered using a boxcar filter. The data is smoothed, without being reduced, by taking the mean of a number of points. The mean of the same points minus the first plus the next, and so on, is calculated so that there are the same number of bunched and filtered points as the original bunched points. You can define the length of the boxcar element using the RESPONSETIME function: the longer the RESPONSETIME, the greater the number of data points averaged. A four-fold increase in RESPONSETIME (for example, 1 sec to 4 sec) doubles the signal-to-noise ratio.

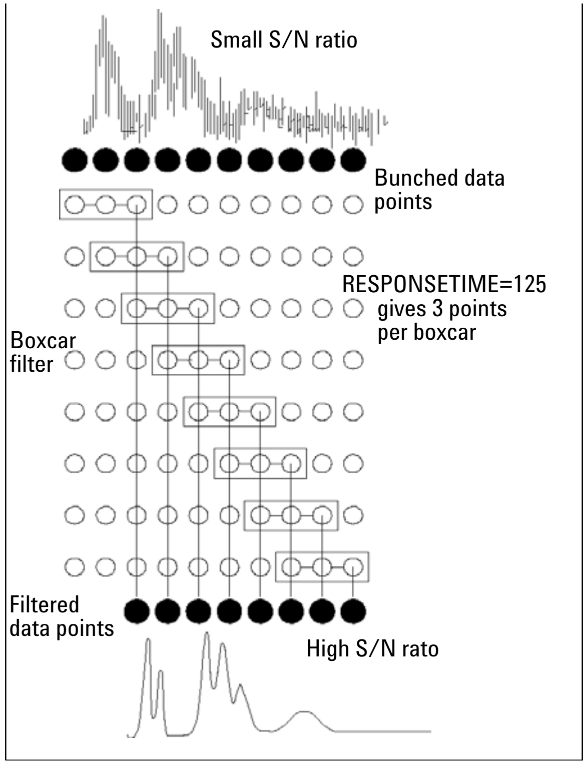


Figure 16 RESPONSETIME: Signal-to-Noise Ratio

Bio-inert Materials

For the Agilent 1260 Infinity Bio-inert LC system, Agilent Technologies uses highest quality materials in the flow path (also referred to as wetted parts), which are widely accepted by life scientists, as they are known for optimum inertness to biological samples and ensure best compatibility with common samples and solvents over a wide pH range. Explicitly, the complete flow path is free of stainless steel and free of other alloys containing metals such as iron, nickel, cobalt, chromium, molybdenum or copper, which can interfere with biological samples. The flow downstream of the sample introduction contains no metals whatsoever.

Table 1 Bio-inert materials used in Agilent 1260 Infinity Systems

Module	**Materials**
Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)	Titanium, gold, platinum-iridium, ceramic, ruby, PTFE, PEEK
Agilent 1260 Infinity Bio-inert High-Performance Autosampler (G5667A)	Upstream of sample introduction: • Titanium, gold, PTFE, PEEK, ceramic Downstream of sample introduction: • PEEK, ceramic
Agilent 1260 Infinity Bio-inert Manual Injector (G5628A)	PEEK, ceramic
Agilent 1260 Infinity Bio-inert Analytical Fraction Collector (G5664A)	PEEK, ceramic, PTFE
Bio-inert Flow Cells:	
Standard flow cell bio-inert, (G5615-60022) (*for Agilent 1260 Infinity Diode Array Detectors DAD G1315C/D*)	PEEK, ceramic, sapphire, PTFE
Max-Light Cartridge Cell Bio-inert ((G5615-60018) and Max-Light Cartridge Cell Bio-inert ((G5615-60017) (*for Agilent 1200 Infinity Series Diode Array Detectors DAD G4212A/B*)	PEEK, fused silica
Bio-inert flow cell, (G5615-60005) (*for Agilent 1260 Infinity Fluorescence Detector FLD G1321B*)	PEEK, fused silica, PTFE

Table 1 Bio-inert materials used in Agilent 1260 Infinity Systems

Module	Materials
Bio-inert heat-exchanger G5616-60050 (*for Agilent 1290 Infinity Thermostatted Column Compartment G1316C*)	PEEK (steel-cladded)
Bio-inert Valve heads	G4235A, G5631A, G5639A: PEEK, ceramic (Al_2O_3 based)
Bio-inert Connection capillaries	Upstream of sample introduction: • Titanium Downstream of sample introduction: • Agilent uses stainless-steel-cladded PEEK capillaries, which keep the flow path free of steel and provide pressure stability to more than 600 bar.

NOTE To ensure optimum bio-compatibility of your Agilent 1260 Infinity Bio-inert LC system, do not include non-inert standard modules or parts to the flow path. Do not use any parts that are not labeled as Agilent “Bio-inert”. For solvent compatibility of these materials, see “Solvent information for parts of the 1260 Infinity Bio-inert LC system” on page 93.

2
Site Requirements and Specifications

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

Site Requirements

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

Power Considerations

The module power supply has wide ranging capability. It accepts any line voltage in the range described in Table 2 on page 35. Consequently there is no voltage selector in the rear of the module. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

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.

WARNING

The module is partially energized when switched off, as long as the power cord is plugged in.

Repair work at the module can lead to personal injuries, e.g. electrical shock, when the cover is opened and the module is connected to power.

→ Always unplug the power cable before opening the cover.

→ Do not connect the power cable to the instrument while the covers are removed.

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.

Bench Space

The module dimensions and weight (see Table 2 on page 35) allow you to place the module 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 shall carry a complete HPLC system, make sure that the bench is designed to bear the weight of all modules.

The module should be operated in a horizontal position.

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 2 Physical Specifications

Type	Specification	Comments
Weight	11.5 kg (26 lbs)	
Dimensions (height × width × depth)	140 x 345 × 435 mm (7 x 13.5 × 17 inches)	
Line voltage	100 – 240 VAC, ± 10 %	Wide-ranging capability
Line frequency	50 or 60 Hz, ± 5 %	
Power consumption	180 VA / 70 W / 239 BTU	Maximum
Ambient operating temperature	0 - 40 °C (32 - 104 °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 (6562 ft)	
Non-operating altitude	Up to 4600 m (15091 ft)	For storing the module
Safety standards: IEC, CSA, UL	Installation category II, Pollution degree 2	For indoor use only.

Performance Specifications

Table 3 Performance Specifications Agilent 1260 Infinity Fluorescence Detector (G1321B)

Type	Specification	Comments
Detection type	Multi-signal fluorescence detector with rapid on-line scanning capabilities and spectral data analysis	
Performance specifications	Single wavelength operation: • RAMAN (H_2O) > 500 (noise reference measured at signal) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell • RAMAN (H_2O) > 3000 (noise reference measured at dark value) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell Dual wavelength operation: RAMAN (H_2O) > 300 Ex 350 nm, Em 397 nm and Ex 350 nm, Em 450 nm, standard flow cell.	see note below this table see Service Manual for details
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W, lifetime 4000 h	
Pulse frequency	296 Hz for single signal mode 74 Hz for economy mode	
Maximum data rate	74 Hz	
Excitation monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 300 nm	

Table 3 Performance Specifications Agilent 1260 Infinity Fluorescence Detector (G1321B)

Type	Specification	Comments
Emission monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 400 nm	
Reference system	in-line excitation measurement	
Timetable programing	up to 4 signal wavelengths, response time, PMT Gain, baseline behavior (append, free, zero), spectral parameters	
Spectrum acquisition	Excitation or Emission spectra Scan speed: 28 ms per datapoint (e.g. 0.6 s/spectrum 200 – 400 nm, 10 nm step) Step size: 1 – 20 nm Spectra storage: All	
Wavelength characteristic	Repeatability +/- 0.2 nm Accuracy +/- 3 nm setting	
Flow cells	Standard: 8 µL volume and 20 bar (2 MPa) pressure maximum, fused silica block Optional: • Fluorescence cuvette for offline spectroscopic measurements with 1 mL syringe, 8 µL volume • Bio-inert: 8 µL volume and 20 bar (2 MPa) pressure maximum, (pH 1–12) • Micro: 4 µL volume and 20 bar (2 MPa) pressure maximum	
Control and data evaluation	Agilent ChemStation for LC, Agilent Instant Pilot G4208A with limited spectral data analysis and printing of spectra	

Table 3 Performance Specifications Agilent 1260 Infinity Fluorescence Detector (G1321B)

Type	Specification	Comments
Analog outputs	Recorder/integrator: 100 mV or 1 V, output range > 100 LU, two outputs	100 LU is the recommended range, see *"FLD Scaling Range and Operating Conditions"*
Communications	Controller-area network (CAN), RS-232C, LAN, APG Remote: ready, start, stop and shut-down signals	
Safety and maintenance	Extensive diagnostics, error detection and display (through Instant Pilot G4208A 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, using the Raman band of water.	
Housing	All materials recyclable.	
Environment	0 – 40 °C constant temperature at <95 % humidity (non-condensing)	
Dimensions	140 mm x 345 mm x 435 mm (5.5 x 13.5 x 17 inches) (height x width x depth)	
Weight	11.5 kg (25.5 lbs)	

Table 4 Performance Specifications Agilent 1200 Series Fluorescence Detector (G1321A)

Type	Specification	Comments
Detection type	Multi-signal fluorescence detector with rapid on-line scanning capabilities and spectral data analysis	
Performance specifications	Single wavelength operation: • RAMAN (H_2O) > 500 (noise reference measured at signal) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell Dual wavelength operation: RAMAN (H_2O) > 300 Ex 350 nm, Em 397 nm and Ex 350 nm, Em 450 nm, standard flow cell.	see note below this table see Service Manual for details
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W, lifetime 4000 h	
Pulse frequency	296 Hz for single signal mode 74 Hz for economy mode	
Maximum data rate	18 Hz	
Excitation monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 300 nm	
Emission monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 400 nm	
Reference system	in-line excitation measurement	
Timetable programing	up to 4 signal wavelengths, response time, PMT Gain, baseline behavior (append, free, zero), spectral parameters	

Table 4 Performance Specifications Agilent 1200 Series Fluorescence Detector (G1321A)

Type	Specification	Comments
Spectrum acquisition	Excitation or Emission spectra Scan speed: 28 ms per datapoint (e.g. 0.6 s/spectrum 200 – 400 nm, 10 nm step) Step size: 1 – 20 nm Spectra storage: All	
Wavelength characteristic	Repeatability +/- 0.2 nm Accuracy +/- 3 nm setting	
Flow cells	Standard: 8 µL volume and 20 bar (2 MPa) pressure maximum, fused silica block Optional: • Fluorescence cuvette for offline spectroscopic measurements with 1 mL syringe, 8 µL volume • Bio-inert: 8 µL volume and 20 bar (2 MPa) pressure maximum, (pH 1–12) • Micro: 4 µL volume and 20 bar (2 MPa) pressure maximum	
Control and data evaluation	Agilent ChemStation for LC, Agilent Instant Pilot G4208A with limited spectral data analysis and printing of spectra	
Analog outputs	Recorder/integrator: 100 mV or 1 V, output range > 100 LU, two outputs	100 LU is the recommended range, see *"FLD Scaling Range and Operating Conditions"*
Communications	Controller-area network (CAN), RS-232C, LAN, APG Remote: ready, start, stop and shut-down signals	

Table 4 Performance Specifications Agilent 1200 Series Fluorescence Detector (G1321A)

Type	Specification	Comments
Safety and maintenance	Extensive diagnostics, error detection and display (through Instant Pilot G4208A 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, using the Raman band of water.	
Housing	All materials recyclable.	
Environment	0 – 40 °C constant temperature at <95 % humidity (non-condensing)	
Dimensions	140 mm x 345 mm x 435 mm (5.5 x 13.5 x 17 inches) (height x width x depth)	
Weight	11.5 kg (25.5 lbs)	

3
Installing the Module

This chapter gives information about the preferred stack setup for your system and the installation of the module.

Unpacking the Module

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 instrument may have been damaged during shipment.

CAUTION

"Defective on arrival" problems

If there are signs of damage, please do not attempt to install the module. Inspection by Agilent is required to evaluate if the instrument is in good condition or damaged.

→ Notify your Agilent sales and service office about the damage.

→ An Agilent service representative will inspect the instrument at your site and initiate appropriate actions.

Delivery Checklist

Ensure all parts and materials have been delivered with your module. The delivery checklist is shown below.

For parts identification please check the illustrated parts breakdown in "Parts for Maintenance" on page 183

Please report any missing or damaged parts to your local Agilent Technologies sales and service office.

Table 5 Detector Checklist

Description	Quantity
Detector	1
Power cable	1
CAN cable	1
Flow cell	1 (built-in)
Optional flow cell/cuvette	as ordered
User Manual	on Documentation CD (part of the shipment - not module specific)
Accessory kit (see "Standard Accessory Kit" on page 186)	1

Optimizing the Stack Configuration

If your module is part of a complete Agilent 1260 Infinity Liquid Chromatograph, you can ensure optimum performance by installing the following configurations. These configurations optimize the system flow path, ensuring minimum delay volume.

One Stack Configuration

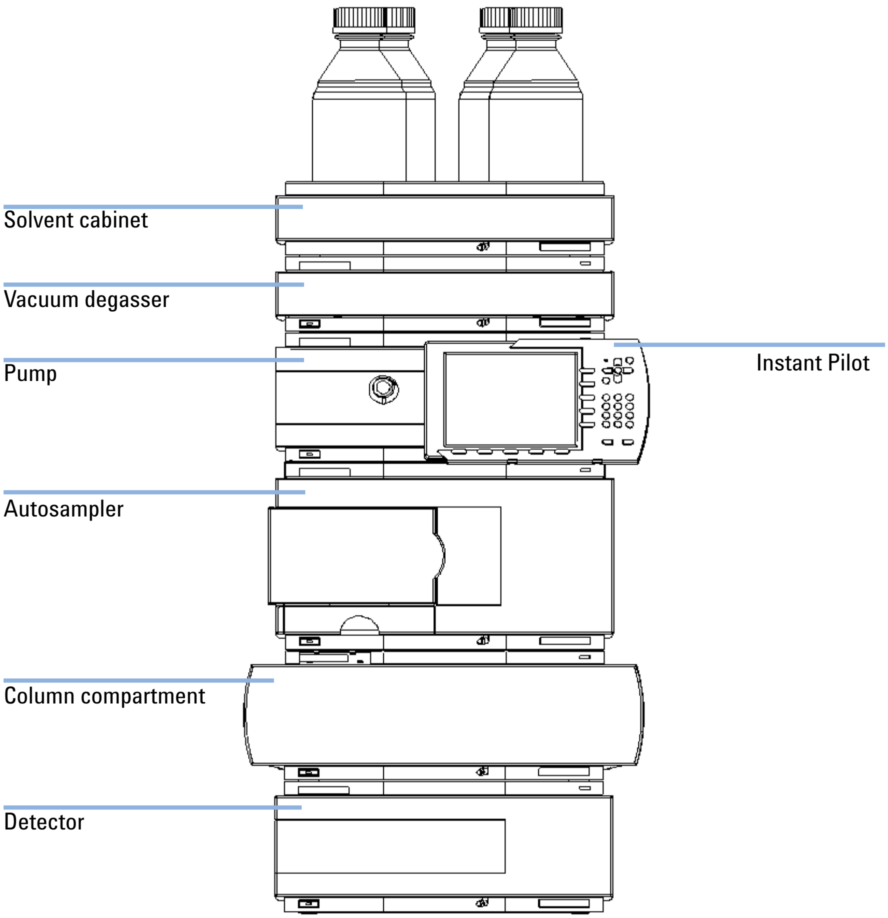


Figure 17 Recommended Stack Configuration (Front View)

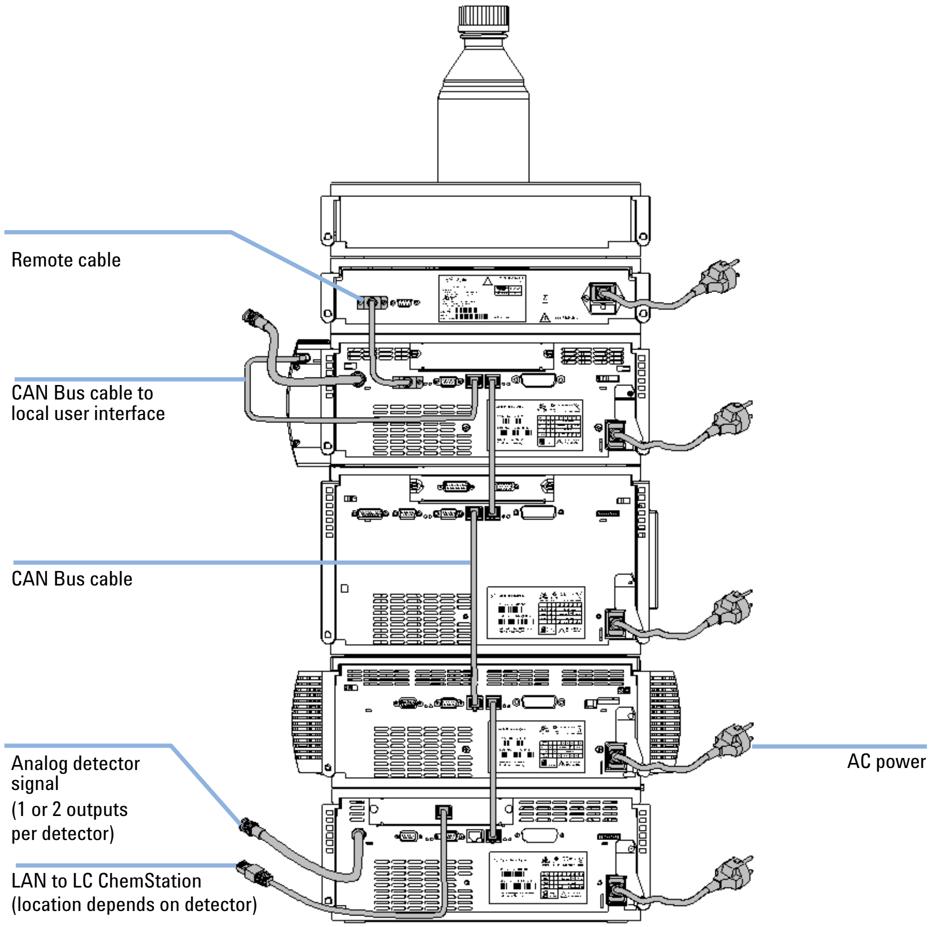


Figure 18 Recommended Stack Configuration (Rear View)

Two Stack Configuration

To avoid excessive height of the stack when the autosampler thermostat is added to the system it is recommended to form two stacks. Some users prefer the lower height of this arrangement even without the autosampler thermostat. A slightly longer capillary is required between the pump and autosampler. (See Figure 19 on page 49 and Figure 20 on page 50).

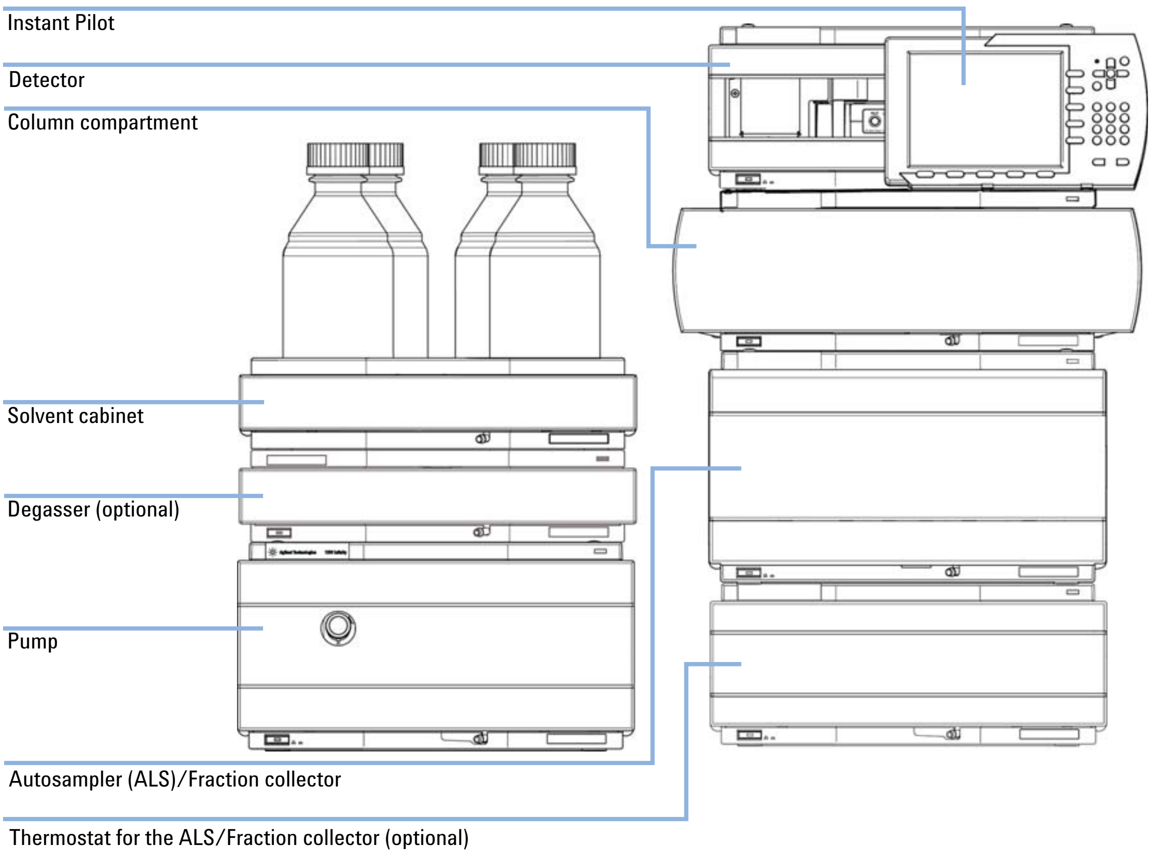


Figure 19 Recommended Two Stack Configuration for 1260 Infinity (Front View)

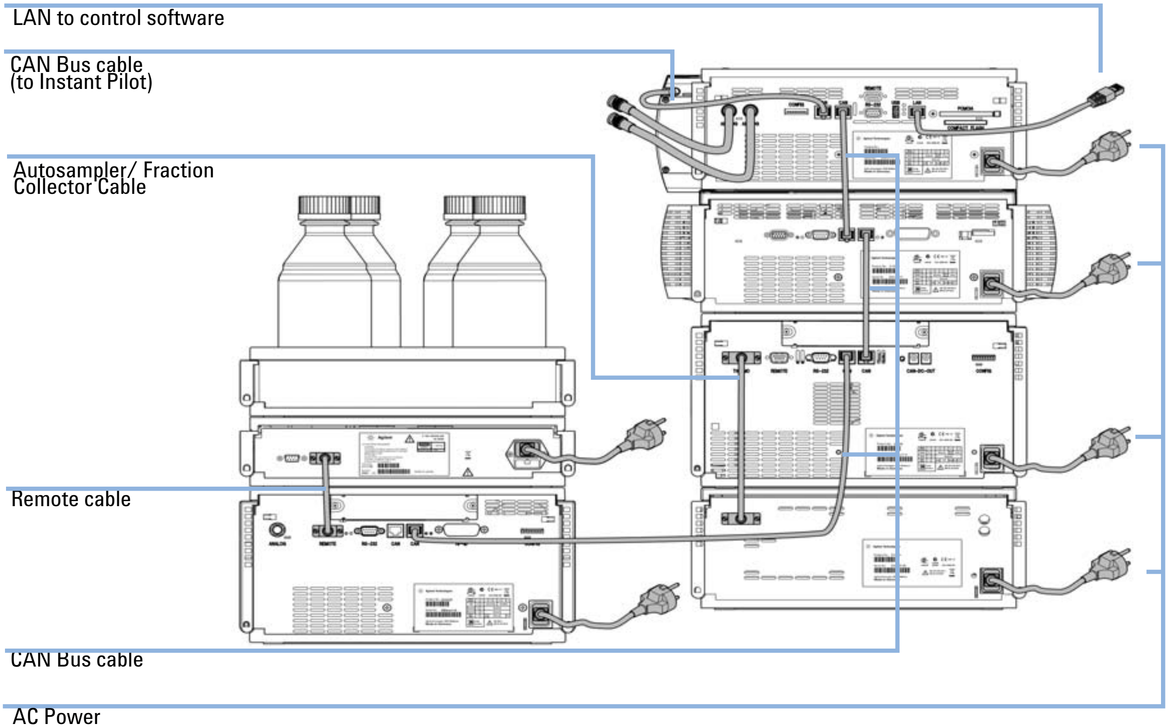


Figure 20 Recommended Two Stack Configuration for 1260 Infinity (Rear View)

Installing the Module

Parts required	**Description**
	Power cord
	For other cables see "Cable Overview" on page 190.
Software required	Agilent Data System and/or Instant Pilot G4208A.
Preparations	Locate bench space
	Provide power connections
	Unpack the detector

WARNING

Module is partially energized when switched off, as long as the power cord is plugged in.

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened and the module is connected to power.

- → Make sure that it is always possible to access the power plug.
- → Remove the power cable from the instrument before opening the cover.
- → Do not connect the power cable to the Instrument while the covers are removed.

1 Install the LAN interface board in the detector (if required), see "Replacing the Interface Board" on page 179.
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.

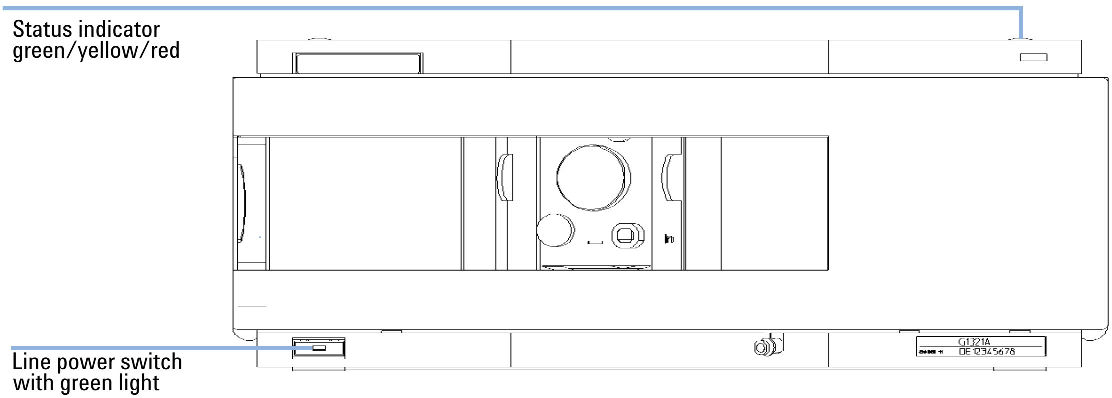


Figure 21 Front View of Detector

4 Connect the power cable to the power connector at the rear of the detector.

5 Connect the CAN cable to other modules.

6 If an Agilent ChemStation is the controller, connect the LAN connection to the LAN interface board in the detector.

NOTE The detector (DAD/MWD/FLD/VWD/RID) is the preferred access point for control via LAN (due to higher data load).

7 Connect the analog cable(s) (optional).

8 Connect the APG remote cable (optional) for non-Agilent 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.

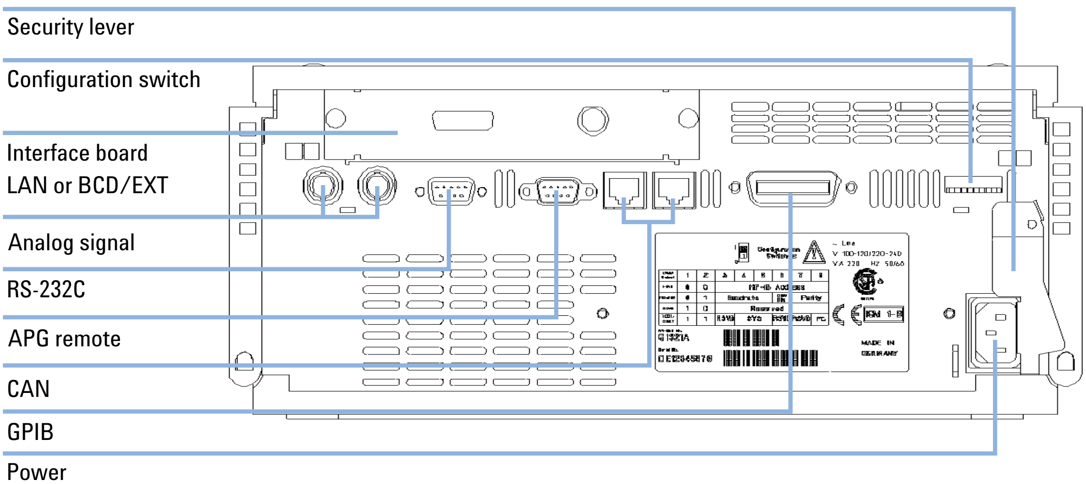


Figure 22 Rear View of Detector

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.

NOTE The detector was shipped with default configuration settings.

NOTE The GPIB interface has been removed with the introduction of the 1260 Infinity modules.

Flow Connections to the Module

BIO inert

For bio-inert modules use bio-inert parts only!

Tools required	Description
	Wrench, 1/4 – 5/16 inch (for capillary connections)

Parts required	p/n	Description
	G1321-68755	Accessory kit

Preparations Detector is installed in the LC system.

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

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

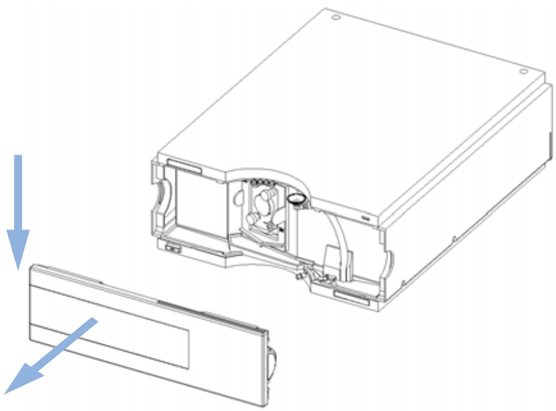
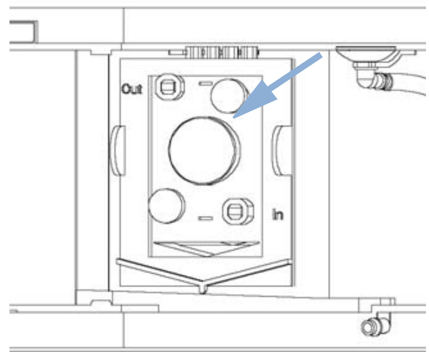
→ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.

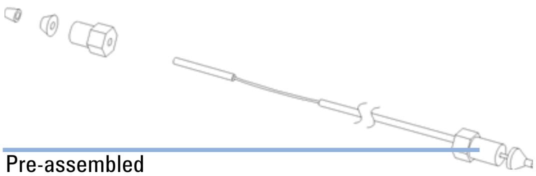
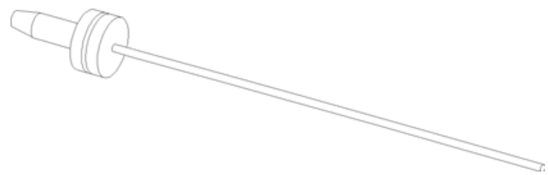
→ The volume of substances should be reduced to the minimum required for the analysis.

→ Do not operate the instrument in an explosive atmosphere.

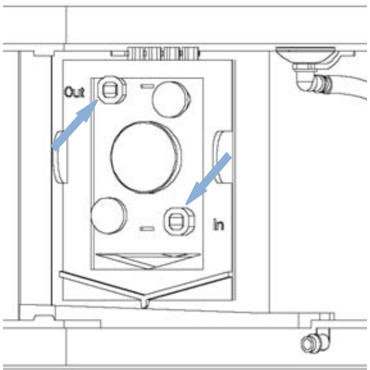
NOTE

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.

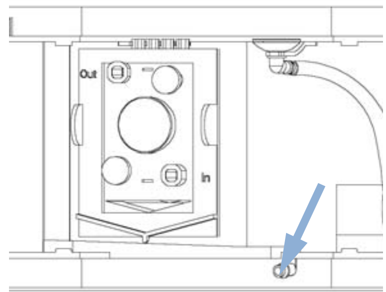
1 Press the release buttons and remove the front cover to gain access to the flow cell area.	2 Locate the flow cell.
	

3 Assemble the column detector capillary from the accessory kit. One side is already factory-assembled.  Pre-assembled	**4** Assemble the waste tubing from the accessory kit.  **NOTE** The fluorescence detector should be the last module in the flow system. An additional detector should be installed before the fluorescence detector to prevent any overpressure to the cell (maximum 20 bar). When working with detector behind the FLD (on own risk) determine the backpressure of this detector first by - removing the column and the last detect and measuring system pressure at the application flow rate. - connecting the last detector (without column and FLD) and measuring the system pressure with flow. - the difference in measured pressure is due to the back pressure generated by the last detector and is the pressure seen by the FLD.

5 Insert the flow cell and install the capillaries to the flow cell (top is outlet, bottom is inlet).

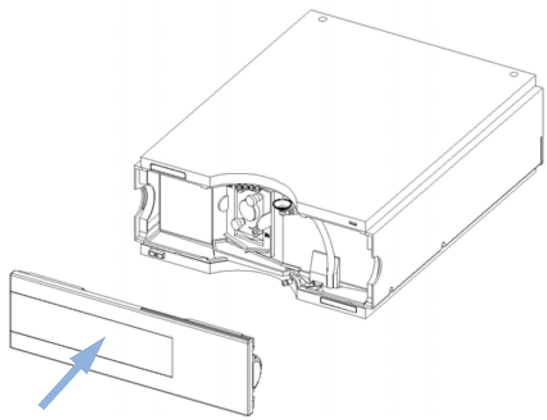


6 Connect the waste tubing to the bottom waste fitting.



7 Establish flow and observe if leaks occur.

8 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 ouside.

4
Using the Fluorescence Detector

This chapter guides you how to start the work with the detector.

Before You Start

Your normal LC grade solvents usually give good results most of the time. But experience shows that baseline noise can be higher (lower signal-to-noise ratio) when impurities are in the solvents.

Flush your solvent delivery system for at least 15 minutes before checking sensitivity. If your pump has multiple channels, you should also flush the channels not in use.

For optimal results refer to “Optimizing the Detector” on page 97.

Getting Started and Checkout

This chapter describes the check out of the Agilent 1260 Infinity Fluorescence Detector using the Agilent isocratic checkout sample.

Starting Your Detector

When If you want to checkout the detector

Parts required

#	p/n	Description
1	5063-6528	Start-up Kit, includes
1		LC cartridge Hypersil ODS, 5 µm, 125 x 4 mm with CIS cartridge holder
1	01080-68704	Agilent isocratic checkout sample This 0.5 mL ampoule contains 0.15 wt.% dimethylphthalate, 0.15 wt.% diethylphthalate, 0.01 wt.% biphenyl, 0.03 wt.% o-terphenyl in methanol.
1	0100-1516	Fitting male PEEK, 2/pk
1	5021-1817	Capillary, 150 mm long, 0.17 mm i.d.

Hardware required LC system with FLD

1 Turn ON the detector.
2 Turn ON the lamp.

   When the lamp is turned on the first time the instrument performs some internal checks and a calibration check which takes about 5 minutes.
3 You are now ready to change the settings of your detector.

Setting the Chromatographic Conditions

1 Set up the system with the following chromatographic conditions and wait until the baseline gets stable.

Table 6 Chromatographic Conditions

Mobile phases	A = water = 35 % B = Acetonitrile = 65 %
Column	OSD-Hypersil column, 125 mm x 4 mm i.d. with 5 µm particles
Sample	Isocratic standard sample, 1:10 diluted in methanol
Flow rate	1.5 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Stop time	4 min
Injection volume	5 µl
Oven temperature (1200)	30 °C
FLD Excitations/Emission Wavelength	EX = 246 nm, EM = 317 nm
FLD PMT Gain	PMT = 10
FLD Response time	4 s

2 Set the FLD setpoints according to Figure 23 on page 63.

In this example additional excitation wavelenghts (B, C, D) are used. This will increase the scan time and may lower the performance.

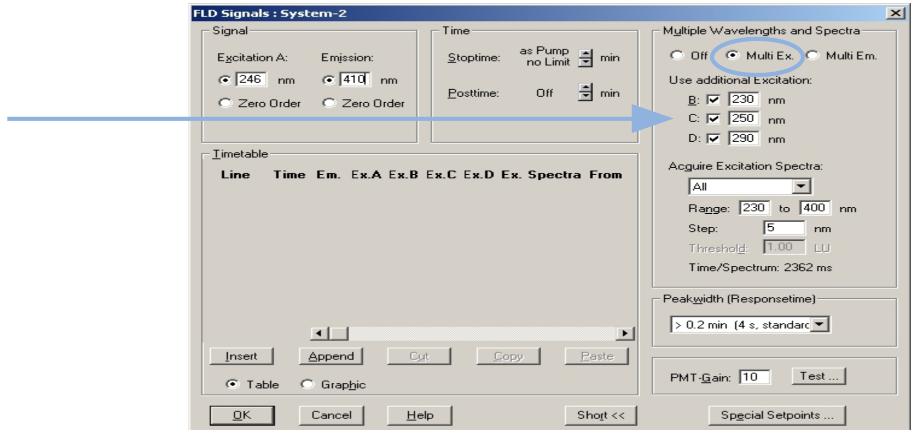


Figure 23 FLD Parameters

3 Start the run.

The resulting chromatograms are shown below :

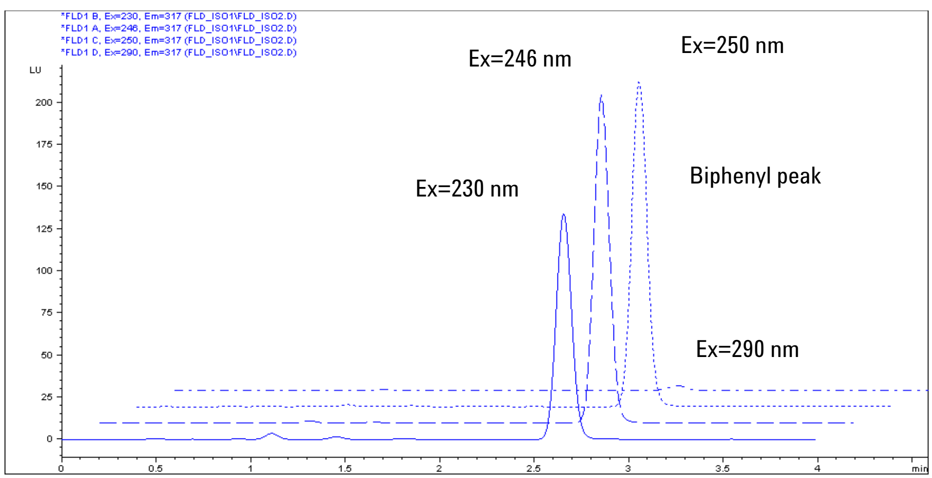


Figure 24 Biphenyl Peak With Different Excitation Wavelengths

The excitation maxima are around 250 nm.

Observe the Maxima via the Isoabsorbance Plot

1 Load the data file (λ_{EX} = 246 nm, λ_{EM} = 317 nm) and open the isoabsorbance plot.

2 The maximum λ_{EX} will be found around 250 nm.

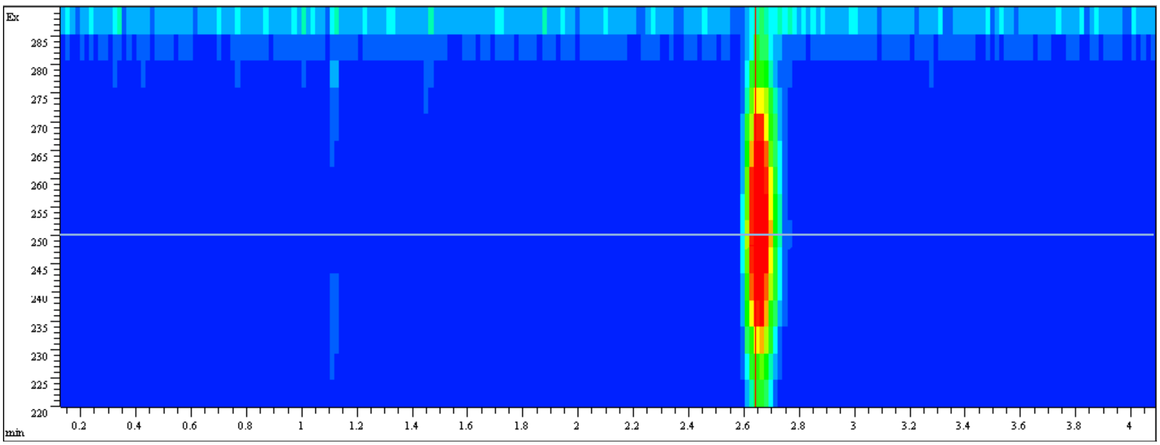


Figure 25 Isoabsorbance Plot

Method Development

Fluorescence detectors are used in liquid chromatography when superior limits of detection and selectivity are required. Thorough method development, including spectra acquisition, is fundamental to achieve good results. This chapter describes three different steps that can be taken with the Agilent 1260 Infinity Fluorescence Detector. Table 7 on page 65 gives an overview of how to benefit from the operation modes during these steps.

Table 7 Steps for thorough method development

	Step 1: Check system	Step 2: Optimize limits of detection and selectivity	Step 3: Set up routine methods
Fluorescence scan	Find impurities (for example, in solvents and reagents)	Determine simultaneously the excitation and emission spectra of a pure compound	
Signal mode		Perform wavelength switching	Use for lowest limits of detection
Spectral mode/multi-wavelength detection		Determine Ex/Em spectra for all separated compounds in a single run Activate up to four wavelengths simultaneously	Collect online spectra, perform library search, determine peak purity Deactivate wavelength switching

Step 1: Check the LC System for Impurities

A critical issue in trace level fluorescence detection is to have an LC system free of fluorescent contamination. Most contaminants derive from impure solvents. Taking a fluorescence scan is a convenient way to check the quality of the solvent in a few minutes. This can be done, for example, by filling the FLD cuvette directly with the solvent for an offline measurement even before the start of a chromatographic run. The result can be displayed as an isofluorescence plot or a three-dimensional plot. Different colors reflect different intensities.

Figure 26 on page 66 shows a sample of slightly impure water which was planned for use as mobile phase. The area where fluorescence of the contaminated water sample can be seen is between the stray light areas: the first- and second-order Raleigh stray light and Raman stray light.

A pure water sample was put into the flow cell. Spectra were recorded at 5 nm step sizes.

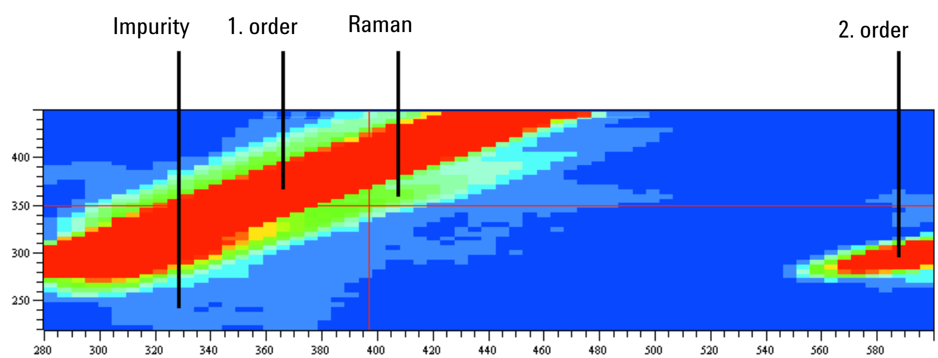


Figure 26 Isofluorescence plot of a mobile phase

Since "excitation" and "emission" wavelength are the same for Raleigh stray light, the area of first-order Raleigh stray light is visible in the left upper area of the diagram. The Raman bands of water are seen below the first-order Raleigh stray light. Since the cut-off filter cuts off light below 280 nm, the second-order Raleigh stray light starts above 560 nm.

Stray light acts in the same way as impurities in that it simulates background noise. In both cases, a higher noise level and therefore a higher limit of detection are obtained. This indicates that high sensitivity measurements should be done away from wavelength settings that have a high stray light background.

Step 2: Optimize Limits of Detection and Selectivity

To achieve optimum limits of detection and selectivity, analysts must find out about the fluorescent properties of the compounds of interest. Excitation and emission wavelengths can be selected for optimum limits of detection and best selectivity. In general, fluorescence spectra obtained with different instruments may show significant differences depending on the hardware and software used.

The traditional approach is to extract an appropriate excitation wavelength from the UV spectrum that is similar to the fluorescence excitation spectrum (see Figure 27 on page 68) and to record the emission spectrum. Then with an optimum emission wavelength determined, the excitation spectrum is acquired.

Excitation spectrum with emission at 440 nm, emission spectrum with excitation at 250 nm of 1 µg/ml quinidine. Detector settings: Step size 5 nm, PMT 12 Response time 4 s.

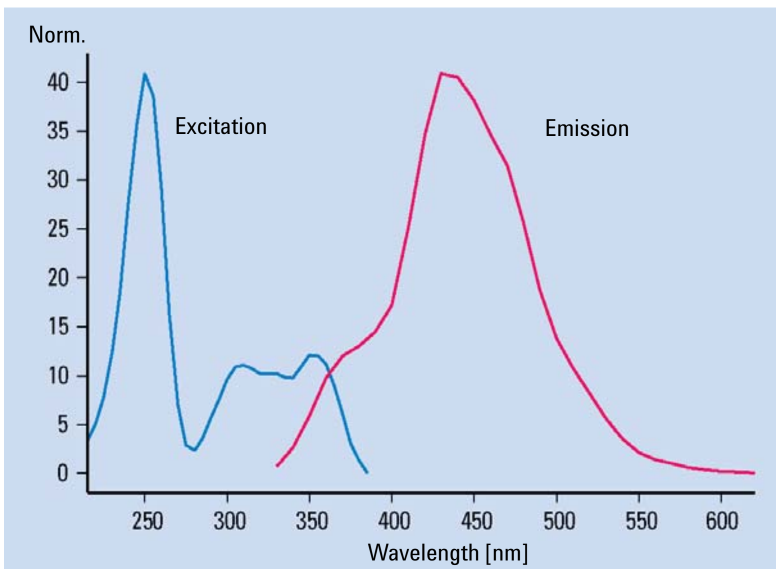


Figure 27 Excitation and emission spectra of quinidine

These tasks have to be repeated for each compound using either a fluorescence spectrophotometer or stop-flow conditions in LC. Usually each compound requires a separate run. As a result, a set of excitation and emission spectrum is obtained (Figure 26 on page 66) for each compound.

Since this is a tedious procedure, it is applicable only when there is a limited number of compounds of interest.

The Agilent 1200 Infinity Series LC offers three different ways to obtain complete information on a compound's fluorescence:

Procedure I - Take a fluorescence scan offline for a single compound as described above for the mobile phase. This is done preferably with a manual FLD cuvette when pure compounds are available.

Procedure II - Use two LC runs with the Agilent 1260 Infinity Fluorescence Detector to separate the compound mix under known conditions and acquire emission and excitation spectra separately.

Procedure III - Use an Agilent 1200 Infinty Series FLD/DAD combination and acquire UV/Visible spectra (equivalent to excitation spectra) with the DAD and emission spectra with the FLD-both in a single run.

Procedure I - Take a fluorescence scan

Because fluorescence spectra traditionally have not been easily available with previous LC fluorescence detectors, standard fluorescence spectrophotometers have been used in the past to acquire spectral information for unknown compounds. Unfortunately this approach limits optimization, as there are differences expected in optical design between an LC detector and a dedicated fluorescence spectrophotometer, or even between detectors. These differences can lead to variations for the optimum excitation and emission wavelengths.

The Agilent 1260 Infinity Fluorescence Detector offers a fluorescence scan that delivers all spectral information previously obtained with a standard fluorescence spectrophotometer, independent of the LC fluorescence detector. Figure 28 on page 71 shows the complete information for quinidine as obtained with the Agilent 1260 Infinity Fluorescence Detector and a manual cuvette in a single offline measurement. The optima for excitation and emission wavelengths can be extracted as coordinates of the maxima in the three dimensional plot. One of the three maxima in the center of the plot can be chosen to define the excitation wavelength. The selection depends on the additional compounds that are going to be analyzed in the chromatographic run and the background noise that may be different upon excitation at 250 nm, 315 nm or 350 nm. The maximum of emission is observed at 440 nm.

Details for Figure 28 on page 71:

All excitation and emission spectra of Quinidine (1 µg/ml) are shown in graphic. Fluorescence intensity is plotted vs excitation and emission wavelengths.

Detector settings: step size 5 nm, PMT 12 , Response time 4 s

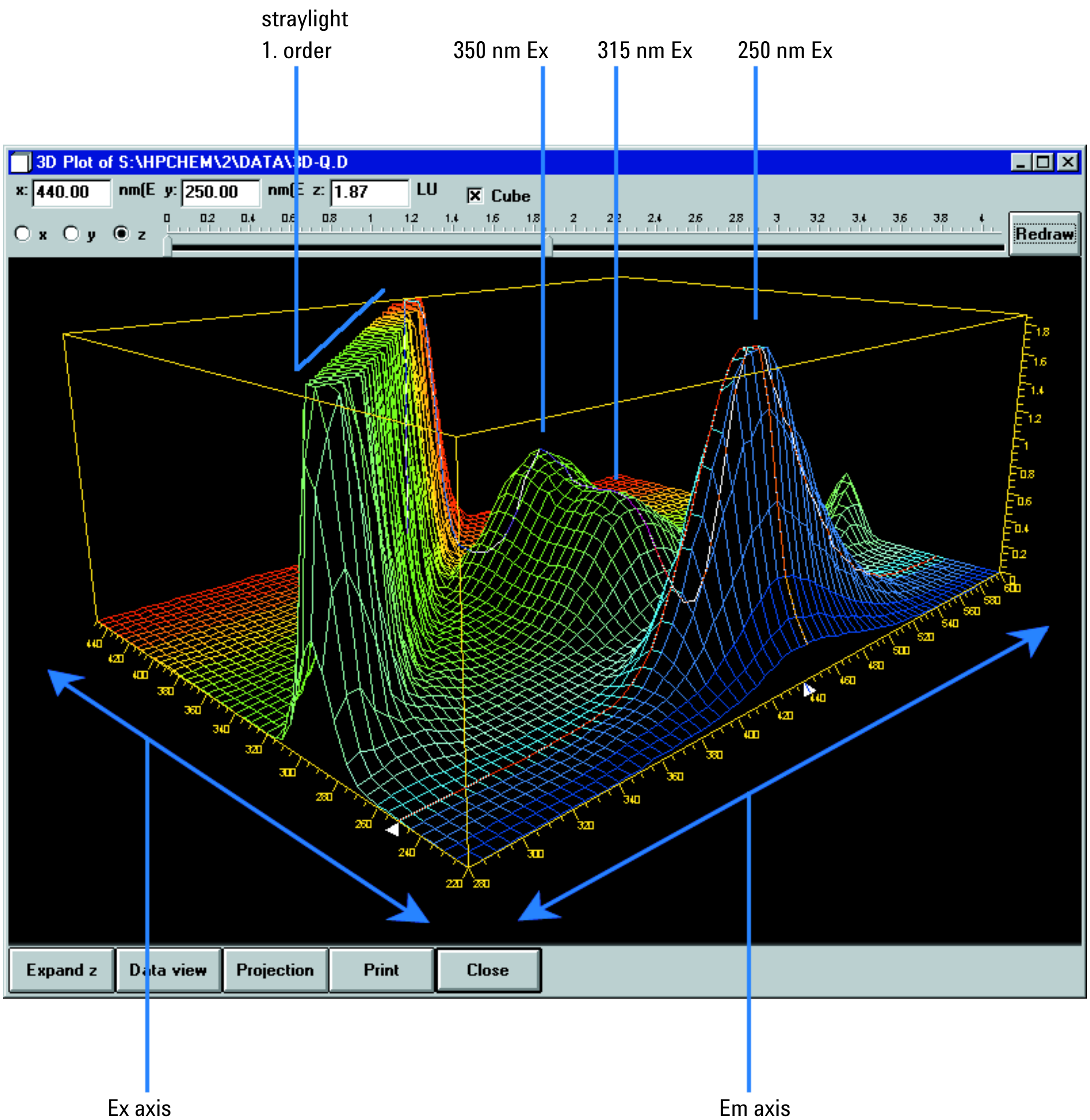


Figure 28 Characterization of a pure compound from a fluorescence scan

Procedure II - Take two LC runs with the FLD

The conditions for the separation of organic compounds such as polyaromatic nuclear hydrocarbons (PNAs) are well described in various standard methods, including commonly used EPA and DIN methods. Achieving the best detection levels requires checking for the optimum excitation and emission wavelengths for all compounds. Yet taking fluorescence scans individually makes this a tedious process. A better approach is to acquire spectra online for all compounds during a run. This speeds up method development tremendously. Two runs are sufficient for optimization.

During the *first run*, one wavelength is chosen in the low UV range for the excitation wavelength and one emission wavelength in the spectral range for the emission wavelength. Most fluorophores show strong absorption at these wavelengths and the quantum yield is high. Excitation is sufficient for collecting emission spectra.

Table on page 74 contains all emission spectra obtained in a single run from a mix of 15 PNAs. This set of spectra is used to set up a timetable for optimum emission wavelengths for all compounds.

The individual compound spectra in the isofluorescence plot show that at least three emission wavelengths are needed to detect all 15 PNAs properly:

Table 8 Timetable for PNA analysis

0 min:	350 nm	for naphthalene to phenanthrene
8.2 min:	420 nm	for anthracene to benzo(g,h,i)perylene
19.0 min:	500 nm	for indeno(1,2,3-c,d)pyrene

In the second run, three setpoints for emission wavelengths are entered into the time-program and excitation spectra are recorded, as shown in Figure 30 on page 75. The area of high intensity (red) is caused by stray light when emission spectra overlap with the excitation wavelength. This can be avoided by fitting the spectral range automatically. Excitation at 260 nm is most appropriate for all PNAs.

Table 9 Conditions for Optimization of PNA analysis according to figures below

Column	Vydac, 2.1 x 200 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50 : 50)
Gradient	3 minutes, 60% 14 minutes, 90% 22 minutes, 100%
Flow rate	0.4 ml/min
Column temperature	18 °C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm

This shows the isofluorescence plot of emission spectra for 15 PNAs (5 µg/ml) with a fixed excitation wavelength (260 nm).

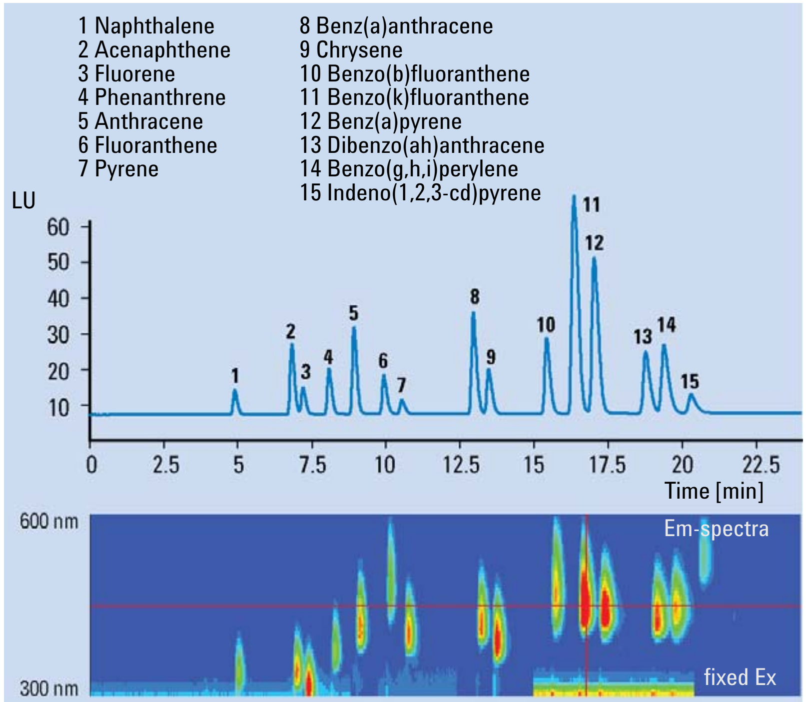


Figure 29 Optimization of the time-program for the emission wavelength

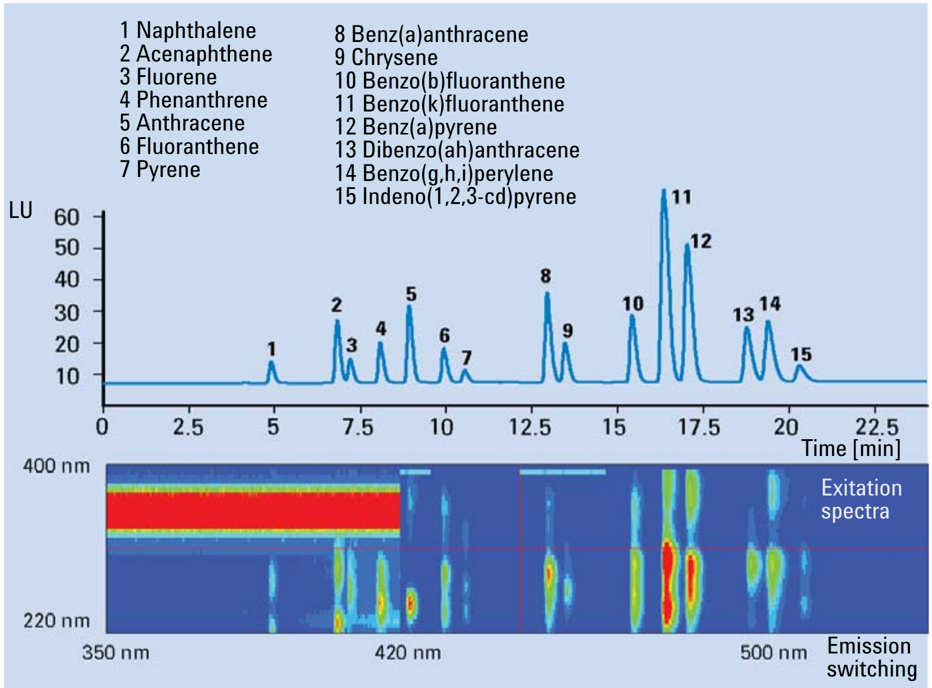


Figure 30 Optimization of the time-program for the excitation wavelength

The obtained data are combined to setup the time-table for the excitation wavelength for best limit of detection and selectivity. The optimized switching events for this example are summarized in Table 10 on page 75.

Table 10 Timetable for the analysis of 15 polynuclear aromatic hydrocarbons

Time [min]	Exitation Wavelength [nm]	Emission Wavelength [nm]
0	260	350
8.2	260	420
19.0	260	500

This timetable gives the conditions for optimum detection based on the results of two chromatographic runs.

Procedure III - Make a single run with a DAD/FLD combination

For most organic compounds, UV-spectra from diode array detectors are nearly identical to fluorescence excitation spectra. Spectral differences are caused by specific detector characteristics such as spectral resolution or light sources.

In practice, combining a diode array detector with a fluorescence detector in series gives the full data set needed to achieve the optimum fluorescence excitation and emission wavelengths for a series of compounds in a single run. With the UV/Visible/excitation spectra available from the diode array detector, the fluorescence detector is set to acquire emission spectra with a fixed excitation wavelength in the low UV range.

The example is taken from the quality control of carbamates. Samples are analyzed for the impurities 2,3-diaminophenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP). Reference samples of DAP and AHP were analyzed with diode array and fluorescence detection. Table on page 77 shows the spectra obtained from both detectors for DAP. The excitation spectrum of DAP is very similar to the UV absorption spectrum from the diode array detector. Table on page 78 shows the successful application of the method to a carbamate sample and a pure mixture of DAP and AHP for reference. The column was overloaded with the non-fluorescent carbamate (2-benzimidazole carbamic acid methylester/MBC) to see the known impurities, AHP and DAP.

This is an impurity of carbamates. The excitation spectrum in a second run shows the equivalence of UV-spectra and fluorescence excitation spectra. An excitation wavelength at 265 nm was used for taking the emission spectrum and an emission wavelength at 540 nm was used for taking the excitation spectrum.

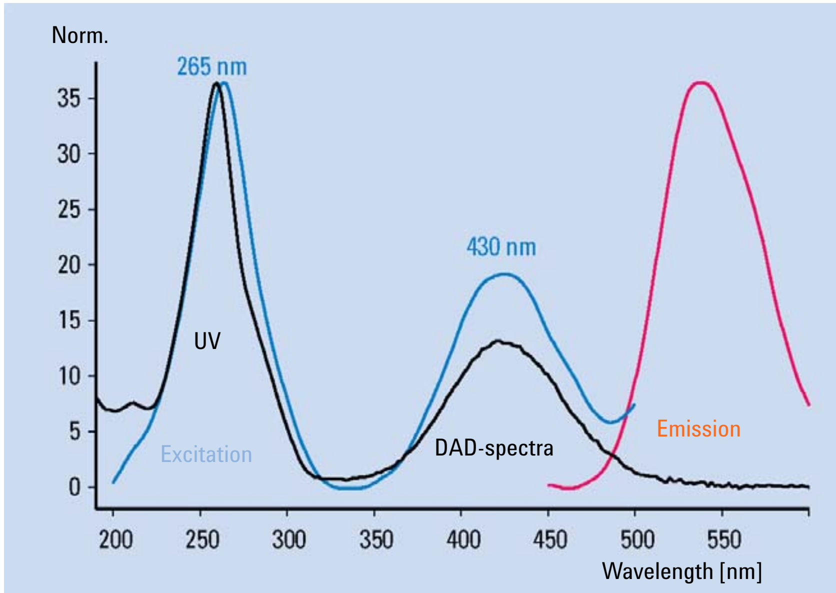


Figure 31 UV-spectrum and fluorescence spectra for 2,3-diaminophenazine (DAP)

The two upper traces are obtained using two different excitation wavelengths.
The lower trace is a pure standard of the known impurities.

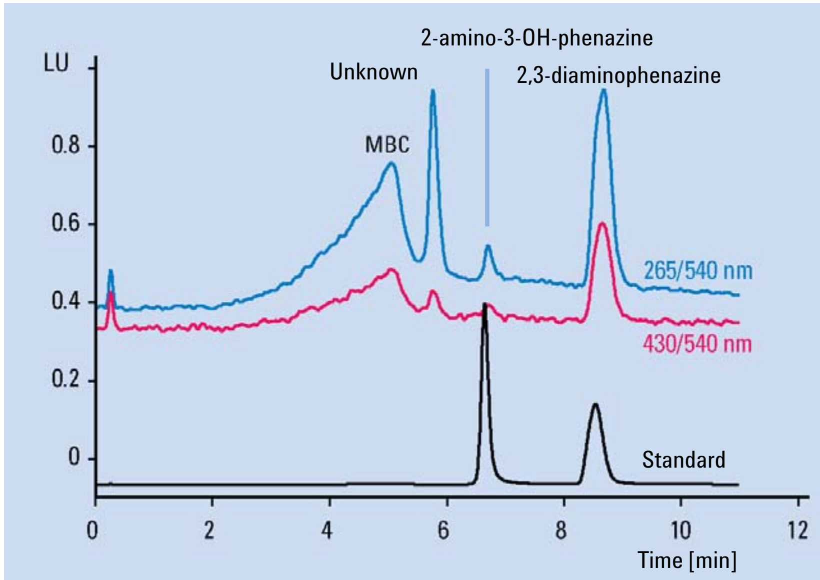


Figure 32 Qualitive analysis of MBC (2-benzimidazole carbamic acid methylester) and impurities

Table 11 Conditions for analysis of DAP and MBC according to figures above

Column	Zorbax SB, 2 x 50 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile
Gradient	0 minutes, 5% 10 minutes, 15%
Flow rate	0.4 ml/min
Column temperature	35 °C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm Ex 265 nm and 430 nm Em 540 nm

Step 3: Set up Routine Methods

In routine analysis, sample matrices can have a significant influence on retention times. For reliable results, sample preparation must be thorough to avoid interferences or LC methods must be rugged enough. With difficult matrices, simultaneous multi-wavelength detection offers more reliability than timetable-controlled wavelength switching. The FLD can, in addition, acquire fluorescence spectra while it records the detector signals for quantitative analysis. Therefore qualitative data are available for peak confirmation and purity checks in routine analysis.

Multi wavelength detection

Time-programmed wavelength switching traditionally is used to achieve low limits of detection and high selectivity in routine quantitative analysis. Such switching is difficult if compounds elute closely and require a change in excitation or emission wavelength. Peaks can be distorted and quantitation made impossible if wavelength switching occurs during the elution of a compound. Very often this happens with complex matrices, influencing the retention of compounds.

In spectral mode, the FLD can acquire up to four different signals simultaneously. All of them can be used for quantitative analysis. Apart from complex matrices, this is advantageous when watching for impurities at additional wavelengths. It is also advantageous for reaching low limits of detection or increasing selectivity through optimum wavelength settings at any time. The number of data points acquired per signal is reduced and thus limits of detection may be higher, depending on the detector settings compared to the signal mode.

PNA analysis, for example, can be performed with simultaneous multi wavelength detection instead of wavelength-switching. With four different wavelengths for emission, all 15 PNAs can be monitored (Table on page 81).

Table 12 Conditions for simultanoeus multi wavelength detection for PNA-analysis (see figure below)

Column	Vydac, 2.1 x 250 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50 : 50)
Gradient	3 min, 60 % 14.5 min, 90 % 22.5 min, 95 %
Flow rate	0.4 mL/min
Column temperature	22 °C
Injection volume	2 µL
FLD settings	PMT 12 , response time 4 s

The upper trace was received with traditional wavelength switching.

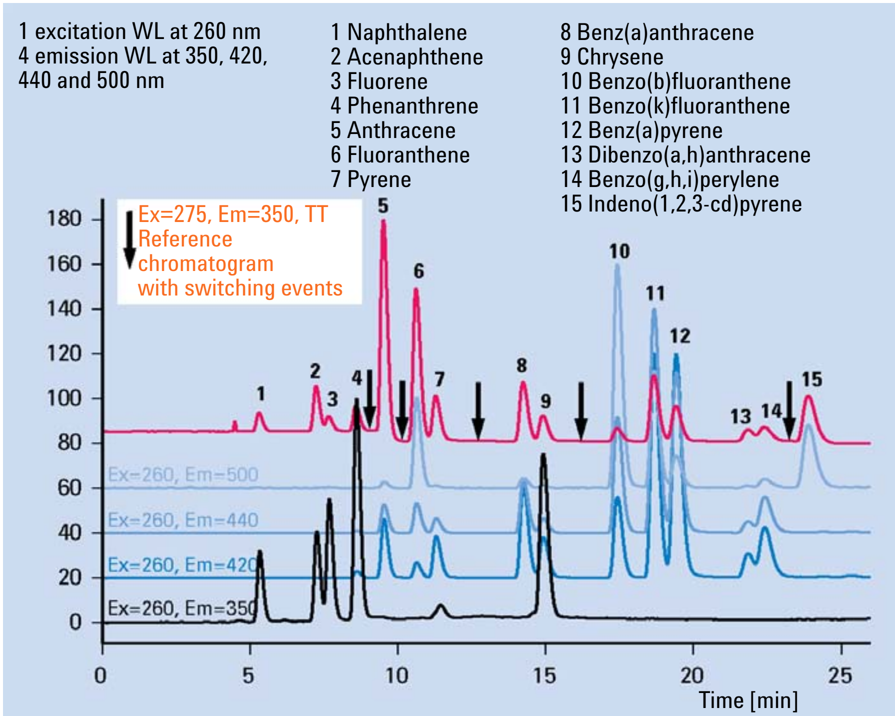


Figure 33 Simultaneous multi wavelength detection for PNA-analysis

Previously, only diode array detectors and mass spectrometric detectors could deliver spectral information on-line to confirm peak identity as assigned by retention time.

Now, fluorescence detectors provide an additional tool for automated peak confirmation and purity control. No additional run is necessary after the quantitative analysis.

During method development, fluorescence excitation and emission spectra are collected from reference standards and entered into a library-at the choice of the method developer. All spectral data from unknown samples can then be compared automatically with library data. Table 13 on page 82 illustrates this principle using a PNA analysis. The match factor given in the report for each peak indicates the degree of similarity between the reference spectrum and the spectra from a peak. A match factor of 1,000 means identical spectra.

In addition, the purity of a peak can be investigated by comparing spectra obtained within a single peak. When a peak is calculated to be within the user-defined purity limits, the purity factor is the mean purity value of all spectra that are within the purity limits.

The reliability of the purity and the match factor depends on the quality of spectra recorded. Because of the lower number of data points available with the fluorescence detector in general, the match factors and purity data obtained show stronger deviations compared to data from the diode array detector, even if the compounds are identical.

Table 13 on page 82 shows an automated library search based on the emission spectra from a PNA reference sample.

Table 13 Peak confirmation using a fluorescence spectral library

Meas. RetTime [min]	Library [min]	CalTbl [min]	Signal	Amount [ng]	Purity Factor	#	Match	Libary Name
4.859	4.800	5.178	1	1.47986e-1	-	1	993	Naphthalene@em
6.764	7.000	7.162	1	2.16156e-1	-	1	998	Acenaphthene@em
7.137	7.100	7.544	1	1.14864e-1	-	1	995	Fluorene@em
8.005	8.000	8.453	1	2.56635e-1	-	1	969	Phenanthrene@em
8.841	8.800	9.328	1	1.76064e-1	-	1	993	Anthracene@em
9.838	10.000	10.353	1	2.15360e-1	-	1	997	Fluoranthene@em
10.439	10.400	10.988	1	8.00754e-2	-	1	1000	Pyrene@em
12.826	12.800	13.469	1	1.40764e-1	-	1	998	Benz(a)anthracene@em
13.340	13.300	14.022	1	1.14082e-1	-	1	999	Chrysene@em
15.274	15.200	16.052	1	6.90434e-1	-	1	999	Benzo(b)fluoranthene@em
16.187	16.200	17.052	1	5.61791e-1	-	1	998	Benzo(k)fluoranthene@em
16.865	16.900	17.804	1	5.58070e-1	-	1	999	Benz(a)pyrene@em
18.586	18.600	19.645	1	5.17430e-1	-	1	999	Dibenz(a,h)anthracene@em
19.200	19.100	20.329	1	6.03334e-1	-	1	995	Benzo(g,h,i)perylene@em
20.106	20.000	21.291	1	9.13648e-2	-	1	991	Indeno(1,2,3-c,d)pyrene@em

Example: Optimization for Multiple Compounds

Example: Optimization for Multiple Compounds

Using PNAs as a sample, this example uses the described scanning functions.

Setting the Chromatographic Conditions

This example uses the following chromatographic conditions (the detector settings are shown in Figure 34 on page 85).

Table 14 Chromatographic Conditions

Mobile phases	A = water = 50 % B = Acetonitrile = 50 %
Column	Vydac-C18-PNA, 250 mm x 2.1 mm i.d. with 5 µm particles
Sample	PAH 0.5 ng
Flow rate	0.4 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Time Table	at 0 min % B=50
	at 3 min % B=60
	at 14.5 min % B=90
	at 22.5 min % B=95
Stop time	26 min
Post time	8 min
Injection volume	1 µl
Oven temperature (1200)	30 °C
FLD PMT Gain	PMT = 15
FLD Response time	4 s

Select a Excitation wavelength in the low UV (230...260 nm). This will cover nearly all fluorescence in your sample.

DO NOT select additional emission wavelengths (B, C, D).
Doing so will increase the scan time and will lower the performance.

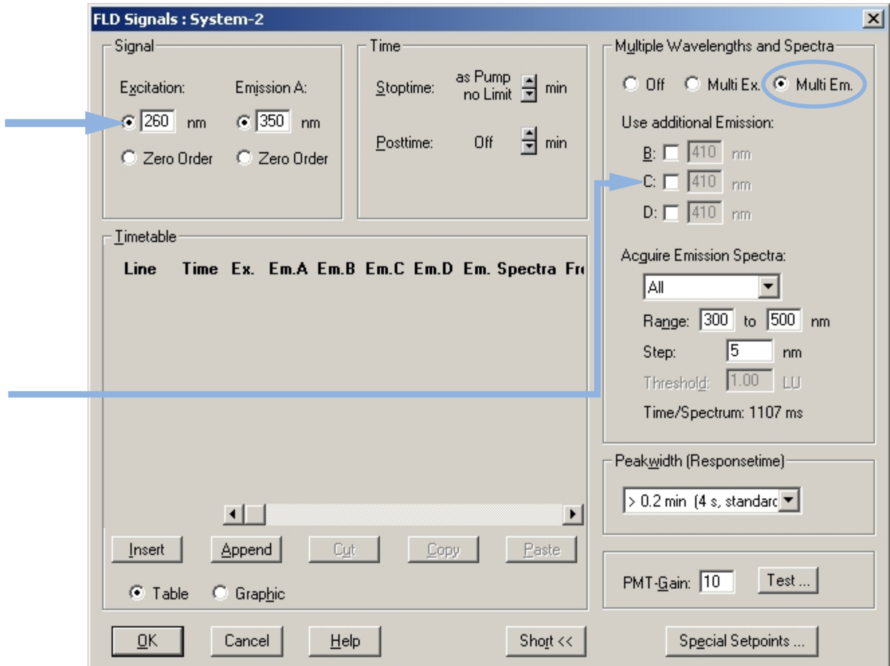


Figure 34 Detector Settings for Emission Scan

1 Wait until the baseline stabilizes. Complete the run.

2 Load the signal. (In this example just the time range of 13 min is displayed).

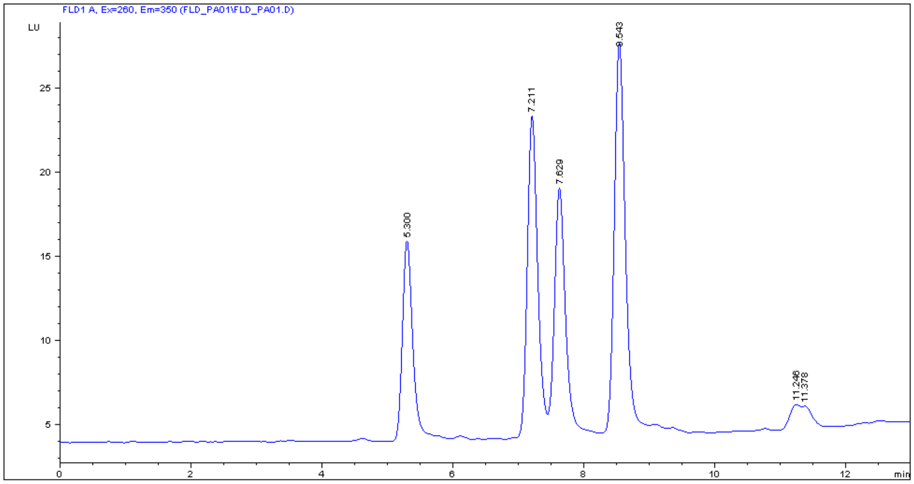


Figure 35 Chromatogram from Emissions Scan

3 Use the isoabsorbance plot and evaluate the optimal emission wavelengths, shown in the table below.

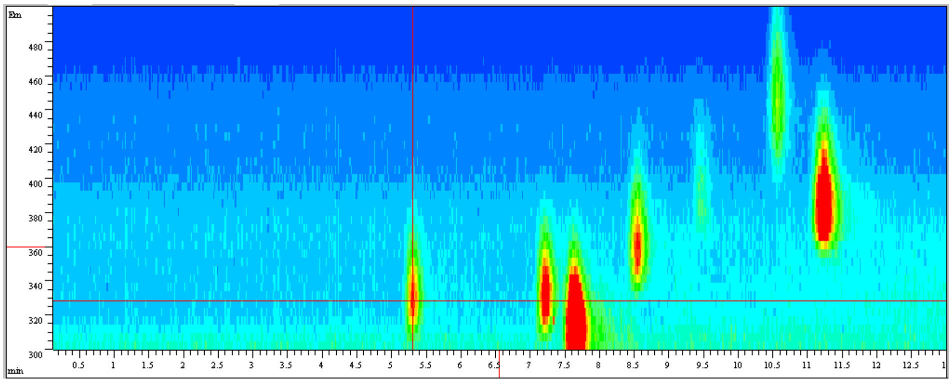


Figure 36 Isoabsorbance Plot from Emission Scan

Table 15

Peak #	Time	Emission Wavelength
1	5.3 min	330 nm
2	7.2 min	330 nm
3	7.6 min	310 nm
4	8.6 min	360 nm
5	10.6 min	445 nm
6	11.23 min	385 nm

4 Using the settings and the timetable (from previous page), do a second run for the evaluation of the optimal excitation wavelength. See Figure 37 on page 88.

DO NOT select additional excitation wavelengths (B, C, D).
Doing so will increase the scan time and will lower the performance.

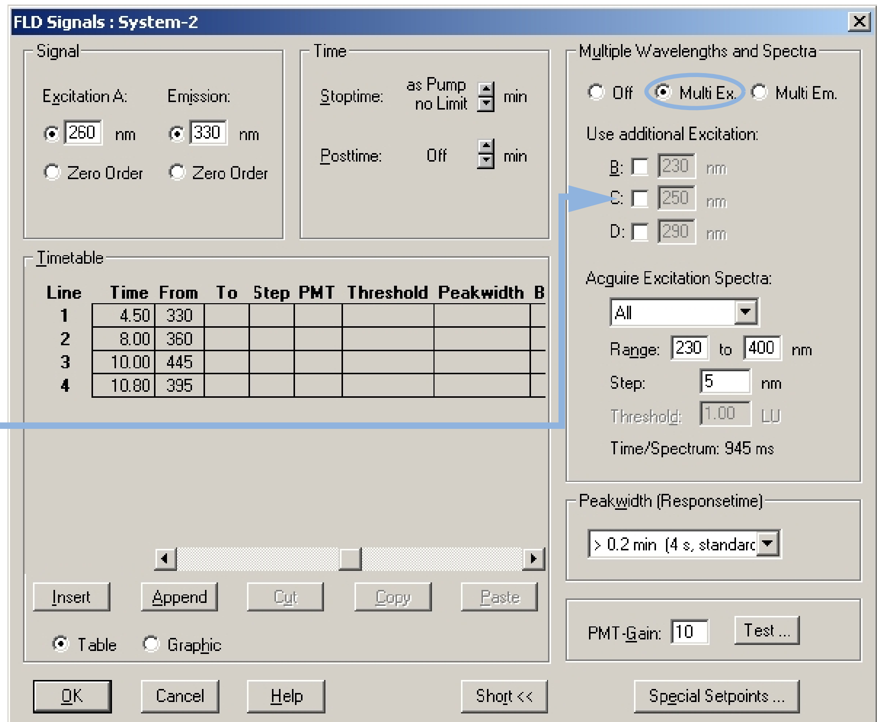


Figure 37 Detector Settings for Excitation Scan

5 Wait until the baseline stabilizes. Start the run.

6 Load the signal.

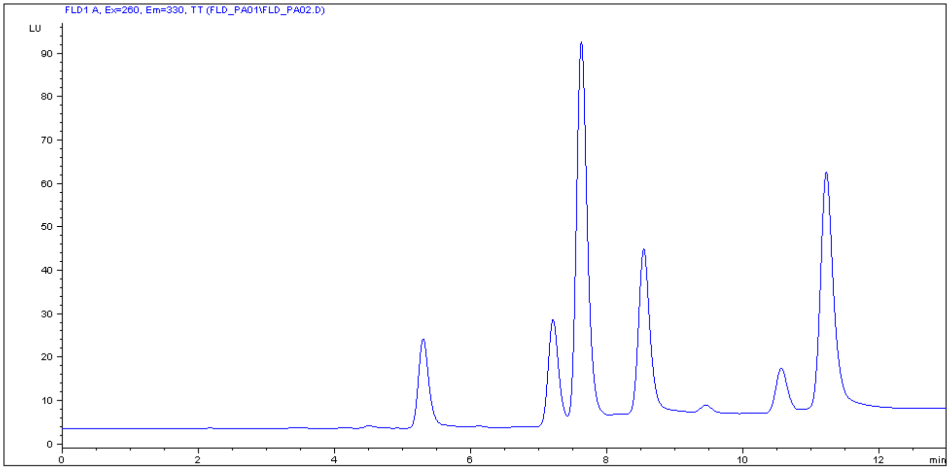


Figure 38 Chromatogram - Excitation Scan at Reference Wavelength 260/330 nm

7 Use the isoabsorbance plot and evaluate the optimal excitation wavelengths (in this example just in the time range of 13 minutes).

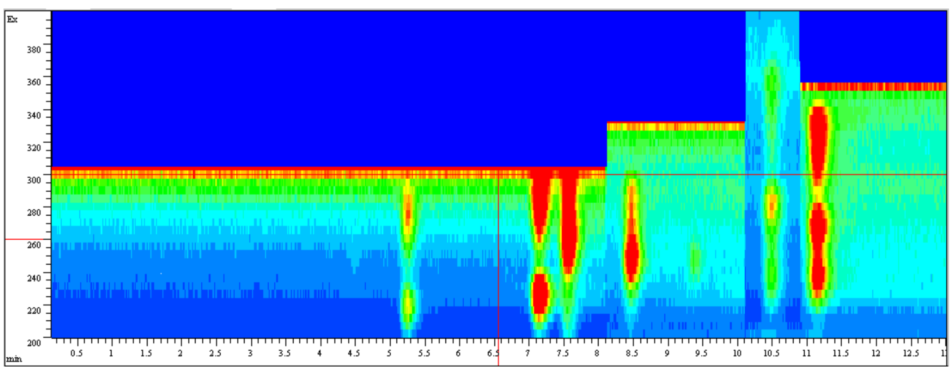


Figure 39 Isoabsorbance Plot - Excitation

The table below shows the complete information about emission (from Figure 36 on page 87) and excitation maxima.

Table 16

Peak #	Time	Emission Wavelength	Excitation Wavelength
1	5.3 min	330 nm	220 / 280 nm
2	7.3 min	330 nm	225 / 285 nm
3	7.7 min	310 nm	265 nm
4	8.5 min	360 nm	245 nm
5	10.7 min	445 nm	280 nm
6	11.3 min	385 nm	270 / 330 nm

Evaluating The System Background

The example below uses water.

1 Pump solvent through your system.

2 Set the fluorescence scan range under FLD special setpoints according to your needs.

NOTE The scan time will increase when the range is enlarged. With the default values, the scan takes about 2 minutes.

3 Set PMT gain to 16.

The wavelength range and step number defines the duration. Using the maximum range, the scan would take approximately 10 minutes.

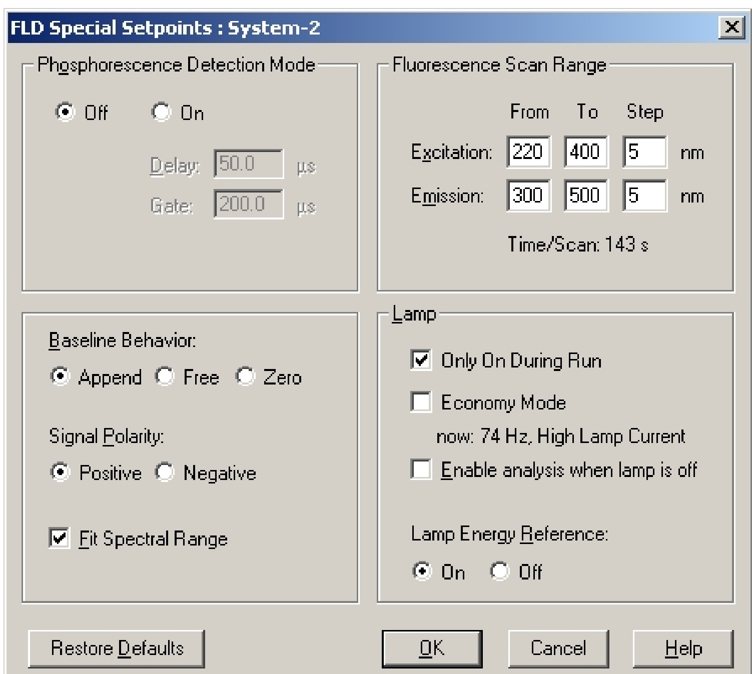


Figure 40 FLD Special Settings

4 Define a data file name and take a fluorescence scan. After the scan is completed, the isoabsorbance scan results appear, see Figure 41 on page 92.

NOTE A low background will improve the signal-to-noise, see also "Reducing Stray Light" on page 114.

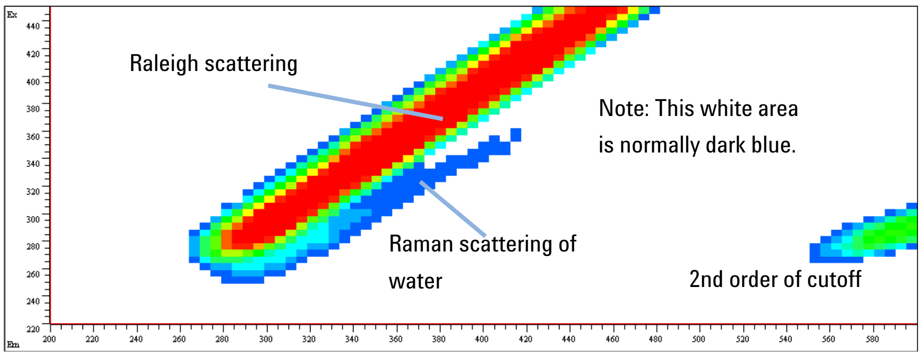


Figure 41 Fluorescence Scan of Water

Solvent Information

Observe the following recommendations on the use of solvents.

- Follow recommendations for avoiding the growth of algae, see pump manuals.
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.4 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials like flow cells, valve materials etc. and recommendations in subsequent sections.

Solvent information for parts of the 1260 Infinity Bio-inert LC system

For the Agilent 1260 Infinity Bio-inert LC system, Agilent Technologies uses highest quality materials (see “Bio-inert Materials” on page 28) in the flow path (also referred to as wetted parts), which are widely accepted by life scientists, as they are known for optimum inertness to biological samples, and ensure best compatibility to common samples and solvents over a wide pH range. Explicitly, the complete flow path is free from stainless steel and free from other alloys containing metals such as iron, nickel, cobalt, chromium, molybdenum or copper, which can interfere with biological samples. The flow downstream of the sample introduction contains no metals whatsoever.

However, there are no materials that combine suitability for versatile HPLC instrumentation (valves, capillaries, springs, pump heads, flow cells etc.) with complete compatibility with all possible chemicals and application conditions. This section recommends the preferred solvents. Chemicals that are known to cause issues should be avoided, or exposure should be minimized, for example, for short-term cleaning procedures. After potentially aggressive chemicals have been used, the system should be flushed with compatible standard HPLC solvents.

PEEK

PEEK (Polyether-Ether Ketones) combines excellent properties with regard to biocompatibility, chemical resistance, mechanical and thermal stability and is therefore the material of choice for biochemical instrumentation. It is stable in the specified pH range, and inert to many common solvents. There is still a number of known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulphuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogenes or aequous halogene solutions, phenol and derivatives (cresols, salicylic acid etc.).

When used above room temperature, PEEK is sensitive to bases and various organic solvents, which can cause it to swell. As normal PEEK capillaries are very sensitive to high pressure, especially under such conditions, Agilent uses stainless-steel cladded PEEK capillaries to keep the flow path free of steel and to ensure pressure stability to at least 600 bar. If in doubt, consult the available literature about the chemical compatibility of PEEK.

Titanium

Titanium is highly resistant to oxidizing acids (for example, nitric, perchloric and hypochlorous acid) over a wide range of concentrations and temperatures. This is due to a thin oxide layer on the surface, which is stabilized by oxidizing compounds. Reducing acids (for example, hydrochloric, sulfuric and phosphoric acid) can cause slight corrosion, which increases with acid concentration and temperature. For example, the corrosion rate with 3 % HCl (about pH 0.1) at room temperature is about 13 µm/year. At room temperature, titanium is resistant to concentrations of about 5 % sulfuric acid (about pH 0.3). The addition of nitric acid to hydrochloric or sulfuric acids significantly reduces corrosion rates. Titanium is subject to corrosion in anhydrous methanol, which can be avoided by adding a small amount of water (about 3 %). Slight corrosion is possible with ammonia > 10 %.

Fused silica

Fused silica is inert against all common solvents and acids except hydrofluoric acid. It is corroded by strong bases and should not be used above pH 12 at room temperature. The corrosion of flow cell windows can negatively affect measurement results. For a pH greater than 12, the use of flow cells with sapphire windows is recommended.

Gold

Gold is inert to all common HPLC solvents, acids and bases within the specified pH range. It can be corroded by complexing cyanides and concentrated acids like aqua regia (a mixture of concentrated hydrochloric and nitric acid).

Zirconium Oxide

Zirconium Oxide (ZrO_2) is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Platinum/Iridium

Platinum/Iridium is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

PTFE

PTFE (polytetrafluorethen) is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Sapphire, Ruby and Al_2O_3-based ceramics

Sapphire, ruby and ceramics based on Al_2O_3 are inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Data above were collected from external resources and are meant as a reference. Agilent cannot guarantee the completeness and correctness of such information. Information can also not be generalized due to catalytic effects of impurities like metal ions, complexing agents, oxygen etc. Most data available refers to room temperature (typically 20 – 25 °C, 68 – 77 °F). If corrosion is possible, it usually increases at higher temperatures. If in doubt, consult additional resources.

5
Optimizing the Detector

This chapter provides information on how to optimize the detector.

Optimization Overview

1 Setting the right PMT value

For most applications a setting of 10 is adequate (see "Finding the Best Signal Amplification" on page 103). The FLD A/D converter exhibits a large linear range making PMT switching unnecessary for most applications. For example, if at high concentrations a peak is cut off; decrease the PMT setting. Remember that low PMT settings decrease the signal to noise ratio.

The built-in PMT gain test uses the parameters in the detector. When using the PMT gain test, the wavelength setting and lamp energy mode (depending on Multiwavelength-Mode and Lamp-Economy) will affect the PMT gain calculation.

NOTE If you have changed one or more parameter(s), you have to press '**OK**' to write down the new settings into the FLD. Then re-enter '**FLD-Signals**' and start the PMT gain test.

2 Using an appropriate response time

For most applications a setting of 4 seconds is adequate (see "Selecting the Best Response Time" on page 111). Only for high speed analyses (short columns at high flow rates) a lower setting is recommended. Bear in mind that even if the response time is too high fast peaks will appear a little smaller and broader but retention time and peak areas are still correct and reproducible.

3 Finding the optimum wavelength

Most fluorescent active molecules absorb at 230 nm (see "Finding the Best Wavelengths" on page 101). Set the excitation wavelength to 230 nm and on-line scan the emission spectra (multi-emission mode). Then set the determined emission wavelength and perform a multi-excitation scan (multi-excitation mode) to find the best excitation wavelength.

4 Evaluating fluorescence spectra

In contrast to diode array based UV detectors where UV spectra are evaluated by taking a spectrum at the peak maximum and selecting a reference spectrum at the baseline, correct fluorescence spectra are obtained by selecting a peak maximum spectrum and a reference around the inflection points. Selecting reference spectra at the baseline is not useful because the spectrum on the baseline is very noisy (no light!).

5 Switching lamp ON only for analysis

Unless maximum sensitivity is needed, the lamp lifetime can significantly be increased by switching it on just for analysis. In contrast to other LC detectors the fluorescence detector equilibrates within seconds after the lamp is switched ON.

NOTE

For highest reproducibility and linearity change the lamp setting to always ON (default is on only during run).

One hour of initial warm-up of the instrument is recommended.

6 Do not overpressurize the detector flow cell

Be aware to not exceed a 20 bar pressure drop after the flow cell when hooking up additional devices like other detectors or a fraction collector. It's better to place a UV detector before the fluorescence detector.

NOTE

When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD = 20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

Design Features Help Optimization

The module has several features you can use to optimize detection:

PMTGAIN	Amplification factor
LAMP	Flash frequency
RESPONSETIME	Data reduction interval

Check Performance Before You Start

Before you start you should check that your detector is performing according to the specifications published by Agilent Technologies.

Your normal LC grade solvents may give good results most of the time but our experience shows that baseline noise can be higher with LC grade solvents than with fluorescence grade solvents.

Flush your solvent delivery system for at least 15 minutes before checking sensitivity. If your pump has multiple channels, you should also flush the channels not in use.

Finding the Best Wavelengths

The most important parameters to be optimized in fluorescence detection are the excitation and emission wavelengths. Generally, it is assumed that the best excitation wavelength can be taken from the excitation spectrum acquired on a spectrofluorimeter. It is also assumed that once the optimal excitation wavelength has been found for one particular instrument type this wavelength can also be applied to other instruments.

Both assumptions are wrong.

The optimum wavelength for the excitation depends on the absorption of the compounds but also on the instrument characteristics, for example the lamp type and the gratings. As most organic molecules absorb best in the ultra-violet range the module was designed to give an optimum signal-to-noise ratio in the 210 nm to 360 nm range of the spectrum. To achieve greatest sensitivity, the absorbance wavelength of your sample molecule should match the wavelength range for your instrument. In other words, an excitation wavelength in the ultra-violet range. Your module has a broad excitation wavelength range, but for higher sensitivity you should choose a wavelength in the ultra-violet range (near 250 nm).

The design elements that contribute to lower efficiency in the lower ultra-violet range are the xenon flash lamp and the gratings. Flash-type lamps shift the optimum wavelength to lower wavelength ranges with the module to a maximum of 250 nm. The excitation grating is blazed for highest efficiency at 300 nm.

A Real Example

Although an excitation wavelength of 340 nm is quoted in the literature the module scan of orthophthalaldehyde, a derivative of the amino acid alanine, (Figure 42 on page 102) shows a maximum between 220 nm and 240 nm.

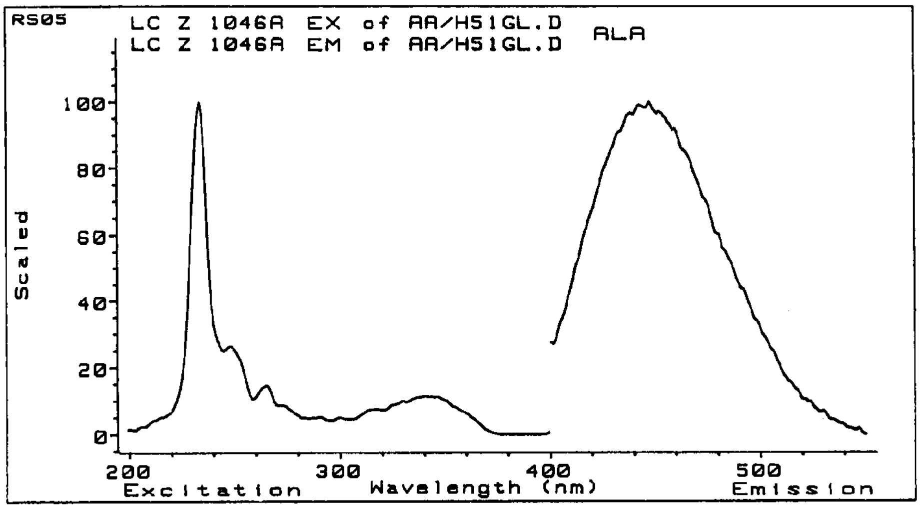


Figure 42 Scan Orthophthalaldehyde Derivative of Alanine

When you are looking for the wavelength by scanning, scan over the whole range. As this example shows a maximum may be found in a completely different wavelength range.

NOTE When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD = 20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

Finding the Best Signal Amplification

Increasing the PMTGAIN increases the signal and the noise. Up to a certain factor the increase in signal is higher than the increase in noise.

The step from gain to gain is equal to a factor of 2 (which is the same as on the HP 1046A FLD).

In Figure 43 on page 103 the PMTGAIN was gradually raised from 4 up to 11 (the peak is from the Agilent Technologies isocratic sample which was diluted 1000 times). With increasing PMTGAIN there was an improvement in signal-to-noise up to 10. Above 10 the noise increased proportionately to the signal with no improvement in signal-to-noise.

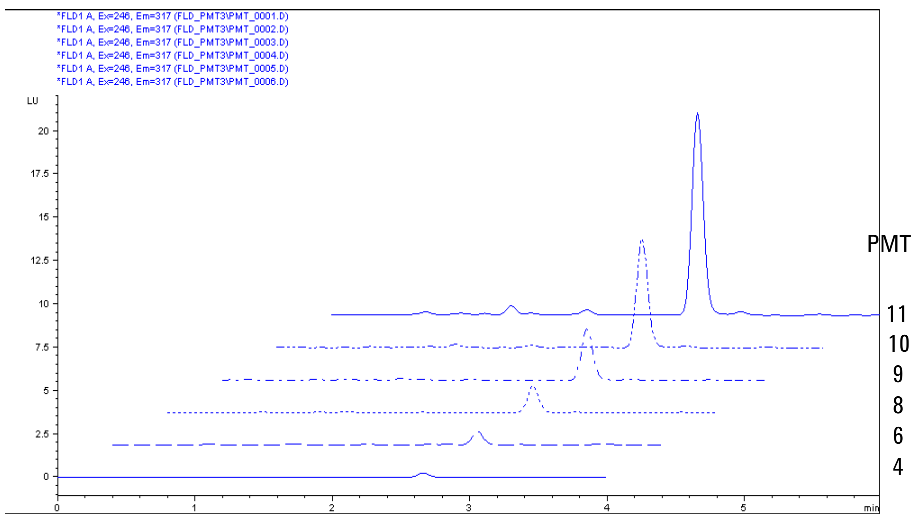


Figure 43 Finding Best PMTGAIN for Biphenyl

The reason for this is the fact, that quantification of baselines (especially at low background levels) is not sufficient for statistically working filter methods. For the best gain, check your solvent under flow conditions with the auto-gain function. Do not use higher values than proposed by the system, if not necessary, because of excessive high fluorescence signals.

Use the PMT test to automatically determine the setting.

FLD Scaling Range and Operating Conditions

When using different FLD

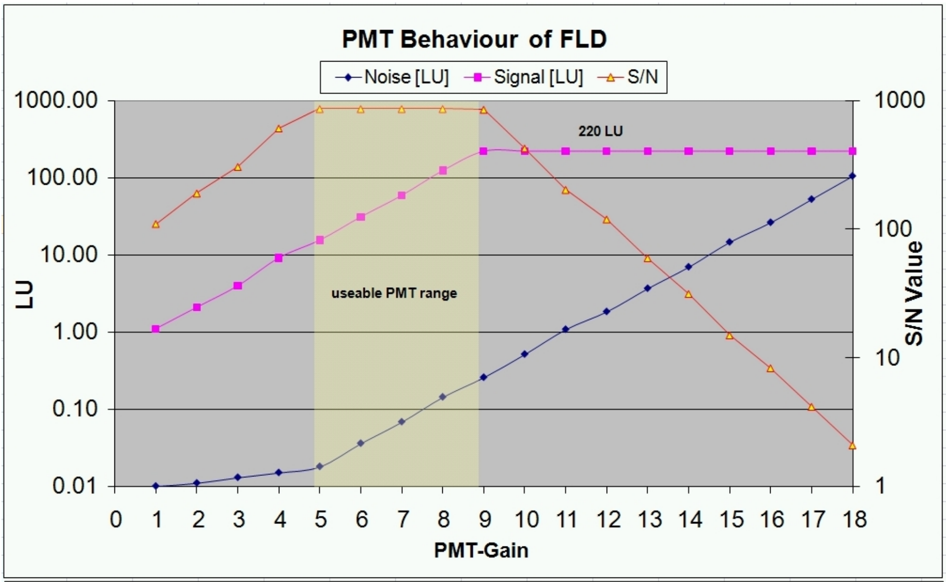
- The signal height of individual G1321 FLD modules may exceed the recommended signal range 0 – 100 LU. Under certain circumstances this could lead to clipped peaks.
- Different G1321 FLD modules show different signal heights with identical methods. This is not a problem in general but could be inconvenient when operating more than one G1321 FLD in the lab.

Both scaling issues can be resolved. Refer to "Optimize the PMT-Gain-Level" on page 105.

Optimize the PMT-Gain-Level

Start the PMT-Gain-Test with your operating conditions (used method parameter, EX-/EM-wavelength, solvent, flow rate, ...). The resulting PMT value will give you the best signal to noise performance with the maximum usable signal range for this method and this specific instrument. For another FLD this PMT level may vary (based on the individual PMT-Gain-Test).

The figure below demonstrates the impact of changing the PMT Gain.



Signal here is the Peak-Height of the highest concentration to measure. **Noise** ist the peak-to-peak baseline noise.
The highest LU level in this example is about 220 LU --> in this case the limit of the detector (may vary!).
---> Highest PMT-Level to not exceed highest concentration level here is 9.
But between PMT Level 5 and 9 the signal and noise increases with the same factor ! --> So S/N is constant.
Below PMT-Level 5 noise of electronic and other purpose get dominant.
---> PMT-Level 5 is the optimal PMT-Level, because (a) S/N cannot be increased and (b) maximum headroom for higher concentrations

IMPORTANT: the LU numbers and the PMT border levels (here 5 and 9) may vary by application (ex/em wavelength, solvent, ...) and by instrument!

Figure 44 PMT Gain Behavior

In this example the maximum output is around 220 LU and further increase of the PMT (above 9) results in a signal overload (clipping) and drop of signal to noise value.

1 Set the PMT-Gain Level

   Now check with your highest concentration amount, that your highest peak does not clip or overflow.

   - If this check is ok, you finished the PMT-Gain-Level optimization. Continue with "Set your Luminescence Units in LU".
   - If the check shows that the highest concentration doesn't fit to the selected range (e.g. by clipping), you may decrease the sensitivity of your FLD by gradually decreasing the PMT-Level by 1 to get roughly half the signal height by each step. Be aware that by that step you will lose sensitivity at low signal levels (LOD).

2 Set your Luminescence Units in LU

   If you are not satisfied with the LU output level of the detector or if you want to align the output of multiple instruments with different output levels you can scale each instrument output.

   The recommended setting of the G1321 FLD is around 100 LU for the highest peak height to get optimum signal to noise and signal range. Lower LU values normally do not influence the performance of the instrument if PMT-Gain Test was executed fine.

   For analog out less than 100 LU is optimum to get best analog signal performance with the default attenuation of 100 LU/ 1 V. Adapt your LU setting such that your maximum signal level under default attenuation is between 50 to 80 LU (analog output equivalent to 500 mV to 800 mV).

   After correct PMT Setting you can scale any instrument to your favorable LU level. We recommend not exceeding around 100 LU. The parameter of choice is called 'Scale factor' and is applicable by the local controller, the Instant Pilot (B.02.07 or later).

In case older revisions are used, the 'Scale factor' can be entered using the command line of

- Agilent ChemStation:
  `PRINT SENDMODULE$(LFLD,"DMUL x.xx")`
- Instant Pilot: Service Mode – FLD, then type
  `DMUL x.xx` and press **SEND**.
- LAN/RS-232 Firmware Update Tool: via Send Instruction menu:
  `DMUL x.xx`
- Agilent LabAdvisor Software: via Instruction menu:
  `DMUL x.xx`

This setting is resident to the instrument even for firmware updates and is independent of the software environment.

The level of LU is no measure of instrument sensitivity! At the lowest concentration limit (limit of detection), the signal to noise (e.g. by Raman S/N Test) is the only measure that can accurately be used to compare chromatograms and results and to confirm the performance of the instrument.

For low background and highest sensitivity keep the flow cell clean and use always fresh water to prevent biological background from native fluorescence by algae and bacteria.

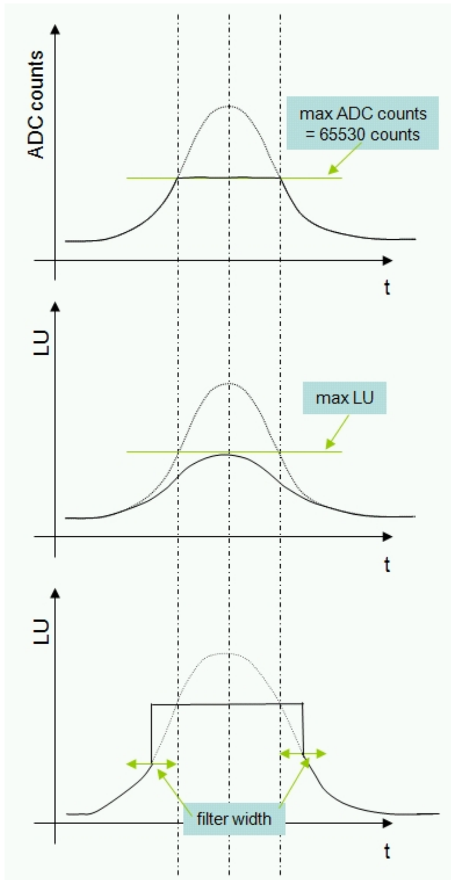
Visualization of ADC Limits

A new firmware (A.06.11) for the Fluorescence Detector G1321A/B has been released that includes a new feature, the "Visualization of ADC Limits".

Up to firmware A.06.10, an "ADC overflow" was not visible in the chromatogram under certain method conditions.

Overflow could be concealed by smoothing of a filter and thus not visible for the user. In the Agilent ChemStation, the "ADC overflow" event was only shown in the logbook.

This problem did only occur if the Peakwidth (Responsetime) parameter has been set similar or larger than the real width of the chromatographic peak.



Raw ADC counts
The measured light intensity is limited by the max range of the ADC-converter.

A filter smoothes the peak making it not clearly visible that the max intensity is reached. Also peak area and peak height are distorted which leads to poor linearity performance.
Note that "max LU" is not a fix number but depends on the intensity of the reference channel!

New implementation (with firmware A.06.11 or above)
While any sample value within the filter width is in state "ADC overflow" the max possible LU is displayed in chromatogram.
Note that "max LU" is slightly dependent on lamp drift and lamp noise but strongly dependent on the excitation wavelength.

As a result, the "ADC overflow" is visible as a real flat peak in the chromatogram showing the user, that the setting of the detector parameter (PMT gain or the concentration of the solution) is set to high.

NOTE

The transfer of methods 1:1 from one FLD to another may result into the above "ADC overflow" problem. For details see "FLD Scaling Range and Operating Conditions" on page 104"FLD Scaling Range and Operating Conditions".

Changing the Xenon Flash Lamp Frequency

Modes

The lamp flash frequency can be changed into the following modes:

Table 17 Flash Lamp Modes

Positioning	296 Hz (Standard), 560 V	63 mJ (18.8 W)
	74 Hz (Economy), 560 V	63 mJ (4.7 W)
Rotation (Multi Ex/Em)	74 Hz (Standard), 950 V	180 mJ (13.3 W)
	74 Hz (Economy), 560 V	63 mJ (4.7 W)

Best sensitivity can be expected with "**no economy**", see Figure 45 on page 109.

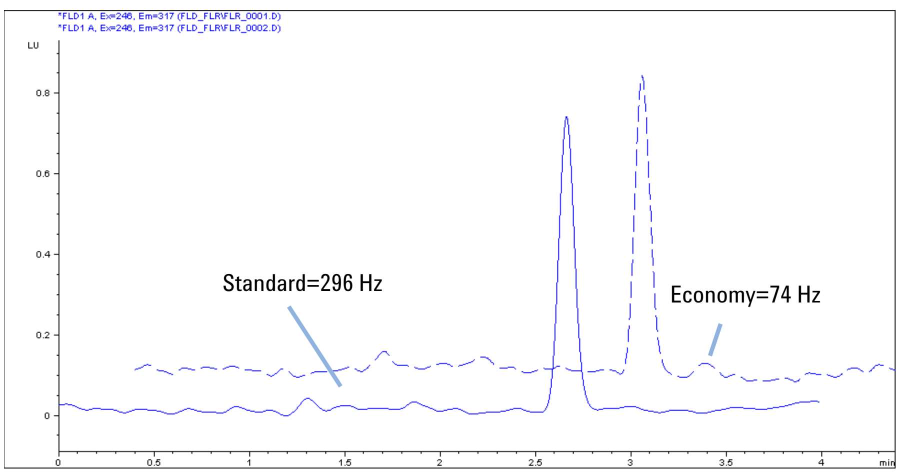


Figure 45 Xenon Flash Lamp Frequency

Lamp Life Savings

There are three ways to save lamp life:

- switch to "**lamp on during run**" without loss of sensitivity.
- switch to "**economy**" mode with a certain loss of sensitivity.
- a combination of the above.

Selecting the Best Response Time

Selecting the Best Response Time

Data reduction using the RESPONSETIME function will increase your signal-to-noise ratio.

For example, see Figure 46 on page 111.

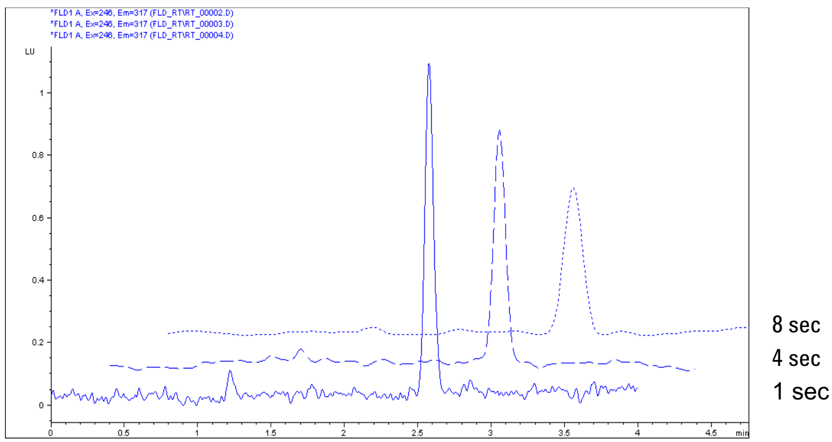


Figure 46 Finding Best Response Time

LC fluorescence detectors typically work with response times of 2 or 4 s. The default of the module is 4 seconds. It is important to know that comparing sensitivity requires using the same response time. A response time of 4 s (default) is equivalent to a time constant of 1.8 s and appropriate for standard chromatographic conditions.

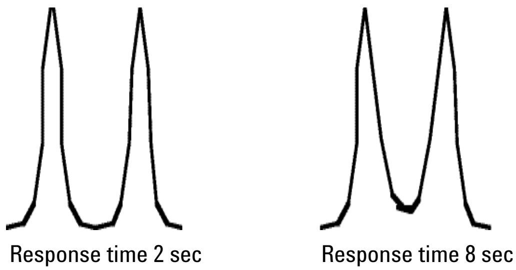


Figure 47 Separation of Peaks using Responsetime

Peakwidth Settings

NOTE Do not use peak width shorter than necessary.

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.

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 the table below.

Table 18 Peakwidth Setting

Peak Width		Data Rate		
At half height [min]	**Response [sec]**	**Hz**	**ms**	
< 0.003	0.03	74.07	13.5	G1321B
> 0.003	0.06	37.04	27.0	
> 0.005	0.12	37.04	27.0	
> 0.01	0.25	37.04	27.0	
> 0.025	0.5	18.52	54.0	G1321A/B
> 0.05	1.0	9.26	108.0	
> 0.1	2.0	4.63	216.0	
> 0.2	4.0	2.31	432.0	
> 0.4	8.0	1.16	864.0	

Reducing Stray Light

Cut-off filters are used to remove stray light and 2^{nd} order or higher stray light by allowing complete transmission above the cut-off and little or no transmission below the cut-off point. They are used between excitation and emission gratings, to prevent any stray excitation light from reaching the photomultiplier tube, when it is measuring emission.

When the emission and excitation wavelengths are close together, the distortion due to scattering severely limits the sensitivity. When the emission wavelength is twice the excitation wavelength the 2^{nd} order light is the limiting factor. To explain the effect of such higher order light, assume the detector is on, but no sample is eluting through the flow cell.

The lamp sends 1 million photons into the flow cell at, for example 280 nm. Scattering on the surface of the flow cell and scattering from the molecules of solvent allow 0.1 % of this light to leave the cell through the window at right angles to the incident light. Without a cut-off filter, these remaining 1000 photons will reach the emission grating. 90 % will be reflected totally without dispersion onto the photomultiplier. The other 10 % disperses at 280 nm (1^{st} order) and at 560 nm (2^{nd} order). To remove this stray light, you need a cut-off filter around 280 nm.

Because of a known set of applications a 295 nm cut-off filter is built-in for undisturbed application up to 560 nm without compromises (see Figure 48 on page 115).

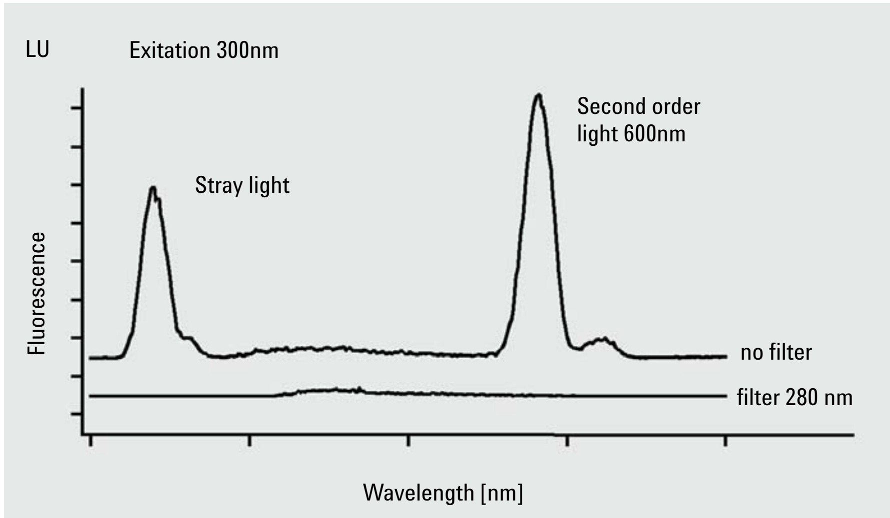


Figure 48 Reducing Stray Light

6
Troubleshooting and Diagnostics

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

Overview of the Module's Indicators and Test Functions

Status Indicators

The module is provided with two status indicators which indicate the operational state (prerun, run, and error states) of the module. The status indicators provide a quick visual check of the operation of the module.

Error Messages

In the event of an electronic, mechanical or hydraulic failure, the module 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 chapter Error Information).

Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components (see Tests and Calibrations).

Wavelength Recalibration

Wavelength recalibration is recommended after repair of internal components to ensure correct operation of the detector. The detector uses specific properties of the excitation and emission light characteristics (see "Wavelength Calibration Procedure" on page 160).

Status Indicators

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

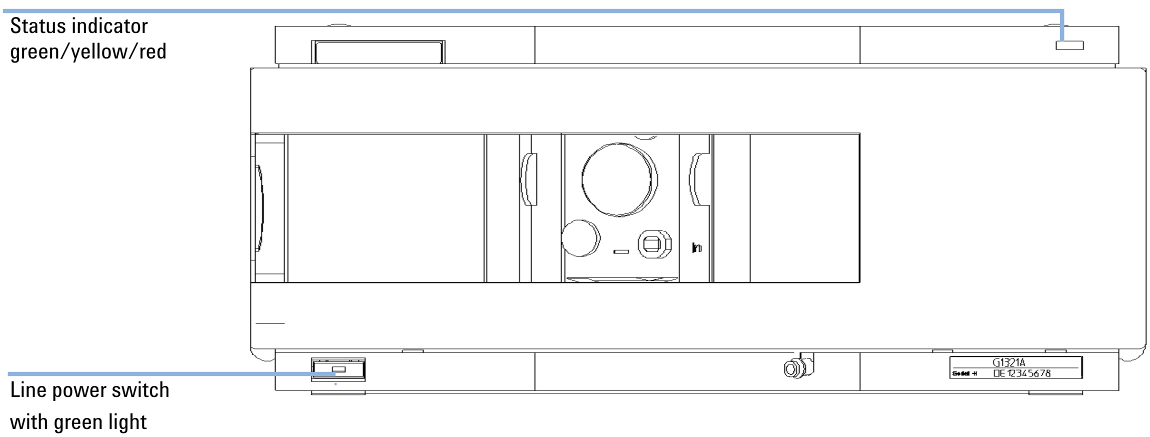


Figure 49 Location of Status Indicators

Power Supply Indicator

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

Module Status Indicator

The module status indicator indicates one of six possible module conditions:

- When the status indicator is *OFF* (and power switch light is on), the module is in a *prerun* condition, and is ready to begin an analysis.
- A *green* status indicator, indicates the module is performing an analysis (*run* mode).
- A *yellow* indicator indicates a *not-ready* condition. The module 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 module has detected an internal problem which affects correct operation of the module. Usually, an error condition requires attention (e.g. leak, defective internal components). An error condition always interrupts the analysis.

  If the error occurs during analysis, it is propagated within the LC system, i.e. a red LED may indicate a problem of a different module. Use the status display of your user interface for finding the root cause/module of the error.
- A *blinking* indicator indicates that the module is in resident mode (e.g. during update of main firmware).
- A *fast blinking* indicator indicates that the module is in a low-level error mode. In such a case try to re-boot the module or try a cold-start (see “Special Settings” on page 224. Then try a firmware update (see “Replacing Module Firmware” on page 180). If this does not help, a main board replacement is required.

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 19 Test Functions avaible vs. User Interface

Test	ChemStation	Instant Pilot G4208A	Lab Advisor
D/A Converter	No	No	Yes
Test Chromatogram	Yes (C)	No	Yes
Wavelength Calibration	Yes	Yes (M)	Yes
Lamp Intensity	Yes	No	Yes
Dark Current	Yes	No	Yes

C	via command
M	section Maintenance
D	section Diagnose

Agilent Lab Advisor Software

The Agilent Lab Advisor software is a standalone product that can be used with or without data system. Agilent Lab Advisor software helps to manage the lab for high quality chromatographic results and can monitor in real time a single Agilent LC or all the Agilent GCs and LCs configured on the lab intranet.

Agilent Lab Advisor software provides diagnostic capabilities for all Agilent 1200 Infinity Series modules. This includes diagnostic capabilities, calibration procedures and maintenance routines for all the maintenance routines.

The Agilent Lab Advisor software also allows users to monitor the status of their LC instruments. The Early Maintenance Feedback (EMF) feature helps to carry out preventive maintenance. In addition, users can generate a status report for each individual LC instrument. The tests and diagnostic features as provided by the Agilent Lab Advisor software may differ from the descriptions in this manual. For details refer to the Agilent Lab Advisor software help files.

The Instrument Utilities is a basic version of the Lab Advisor with limited functionality required for installation, use and maintenance. No advanced repair, troubleshooting and monitoring functionality is included.

7 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What Are Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs which requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the module is switched on, and an entry is written into the module logbook.

If an error occurs outside a method run, other modules will not be informed about this error. If it occurs within a method run, all connected modules will get a notification, all LEDs get red and the run will be stopped. Depending on the module type, this stop is implemented differently. For example, for a pump the flow will be stopped for safety reasons. For a detector, the lamp will stay on in order to avoid equilibration time. Depending on the error type, the next run can only be started, if the error has been resolved, for example liquid from a leak has been dried. Errors for presumably single time events can be recovered by switching on the system in the user interface.

Special handling is done in case of a leak. As a leak is a potential safety issue and may have occurred at a different module from where it has been observed, a leak always causes a shutdown of all modules, even outside a method run.

In all cases, error propagation is done via the CAN bus or via an APG remote cable (see documentation for the APG interface).

General Error Messages

General error messages are generic to all Agilent series HPLC modules and may show up on other modules as well.

Timeout

Error ID: 0062

The timeout threshold was exceeded.

Probable cause	Suggested actions
1 The analysis was completed successfully, and the timeout function switched off the module as requested.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.
2 A not-ready condition was present during a sequence or multiple-injection run for a period longer than the timeout threshold.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

Error ID: 0063

An external instrument has generated a shutdown signal on the remote line.

The module continually monitors the remote input connectors for status signals. A LOW signal input on pin 4 of the remote connector generates the error message.

Probable cause	Suggested actions
1 Leak detected in another module with a CAN connection to the system.	Fix the leak in the external instrument before restarting the module.
2 Leak detected in an external instrument with a remote connection to the system.	Fix the leak in the external instrument before restarting the module.
3 Shut-down in an external instrument with a remote connection to the system.	Check external instruments for a shut-down condition.
4 The degasser failed to generate sufficient vacuum for solvent degassing.	Check the vacuum degasser for an error condition. Refer to the *Service Manual* for the degasser or the 1260 pump that has the degasser built-in.

Remote Timeout

Error ID: 0070

A not-ready condition is still present on the remote input. When an analysis is started, the system expects all not-ready conditions (for example, a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

Probable cause	Suggested actions
1 Not-ready condition in one of the instruments connected to the remote line.	Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
2 Defective remote cable.	Exchange the remote cable.
3 Defective components in the instrument showing the not-ready condition.	Check the instrument for defects (refer to the instrument's documentation).

Lost CAN Partner

Error ID: 0071

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

Probable cause	Suggested actions
1 CAN cable disconnected.	• Ensure all the CAN cables are connected correctly. • Ensure all CAN cables are installed correctly.
2 Defective CAN cable.	Exchange the CAN cable.
3 Defective main board in another module.	Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

Leak

Error ID: 0064

A leak was detected in the module.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak-sensor circuit on the main board.

Probable cause	Suggested actions
1 Loose fittings.	Ensure all fittings are tight.
2 Broken capillary.	Exchange defective capillaries.

Leak Sensor Open

Error ID: 0083

The leak sensor in the module has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak-sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Leak sensor not connected to the main board.	Please contact your Agilent service representative.
2 Defective leak sensor.	Please contact your Agilent service representative.
3 Leak sensor incorrectly routed, being pinched by a metal component.	Please contact your Agilent service representative.

Leak Sensor Short

Error ID: 0082

The leak sensor in the module has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective leak sensor.	Please contact your Agilent service representative.

Compensation Sensor Open

Error ID: 0081

The ambient-compensation sensor (NTC) on the main board in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Compensation Sensor Short

Error ID: 0080

The ambient-compensation sensor (NTC) on the main board in the module has failed (short circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor falls below the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Fan Failed

Error ID: 0068

The cooling fan in the module has failed.

The hall sensor on the fan shaft is used by the main board to monitor the fan speed. If the fan speed falls below a certain limit for a certain length of time, the error message is generated.

Depending on the module, assemblies (e.g. the lamp in the detector) are turned off to assure that the module does not overheat inside.

Probable cause	Suggested actions
1 Fan cable disconnected.	Please contact your Agilent service representative.
2 Defective fan.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Detector Error Messages

These errors are detector specific.

Lamp Cover Open

Error ID: 6622, 6731

The lamp cover in the optical compartment has been removed. The lamp cannot be turned on while this message is on.

Probable cause	Suggested actions
1 Lamp cover removed.	Please contact your Agilent service representative.

FLF Board not found

Error ID: 6620, 6730

The FLF board could not be found by the main board (FLM). This message comes together with some other message generated on the FLF board (e.g. Leak, ...).

Probable cause	Suggested actions
1 FLF board not connected to the FLM board.	Please contact your Agilent service representative.
2 Defective FLF board.	Please contact your Agilent service representative.
3 Defective FLM board.	Please contact your Agilent service representative.

ADC Not Calibrated

Error ID: 6621, 6732

The analog-to-digital converter located on the FLF board cannot calibrate.

Probable cause	Suggested actions
1 Defective ADC or other FLF electronics.	Please contact your Agilent service representative.

A/D Overflow

Error ID: 6618, 6619

This message is not implemented in firmware revision A.03.66 and below.

It indicates an overload situation of the A/D converter (sample signal). The user-interface will show a not-ready condition for the FLD and an info event is written into the logbook. If the message comes up during a run, it includes the time of occurrence and when it disappears.

1200 FLD 1 A/D overflow (RT is 0.32 min) 16:33:24 02/11/99

1200 FLD 1 A/D overflow finished (RT is 0.67 min)16:33:46 02/11/99

If this condition is present prior to a run, the not-ready will prevent the system to start the run/sequence.

With firmware revision A.06.11 and above, the A/D overflow leads into a flat peak in the chromatogram. For details see "Visualization of ADC Limits" on page 107.

Probable cause	Suggested actions
1 PMT setting to high.	Reduce PMT gain.
2 Wavelength setting wrong.	Change wavelength setting.

Flash Lamp Current Overflow

Error ID: 6704

The lamp current of the xenon flash lamp is monitored constantly. If the current gets too high, an error is generated and the lamp is turned OFF.

Probable cause	Suggested actions
1 Shortage of trigger pack assembly or defective FLL board.	Please contact your Agilent service representative.
2 Shortage of flash lamp assembly.	Please contact your Agilent service representative.

Flash Trigger Lost

Error ID: 6722

This message is displayed when the flash trigger is no longer generated.

Probable cause	Suggested actions
1 Firmware problem.	Reboot the detector (power cycle).
2 Multi Mode Off	Please contact your Agilent service representative.
3 Defective encoder.	Please contact your Agilent service representative.

Wavelength Calibration Failed

Error ID: 6703

This message may show up during a wavelength calibration.

If the expected deviation is larger than the specified wavelength accuracy, the message **"Wavelength Calibration Failed"** is displayed and the instrument stays in a **Not Ready** condition.

Probable cause	Suggested actions
1 Flash lamp not ignited or position not correct.	Please contact your Agilent service representative.
2 Cell position not correct.	Check the cell position.
3 Solvent in the cell not clean or air bubble in the cell.	Flush the flow cell.
4 monochromator assembly position not correct (after replacement).	Please contact your Agilent service representative.

Wavelength Calibration Lost

Error ID: 6691

After exchanging the monochromator assemblies, the calibration factors should be reset to defaults values (a new FLM board comes with default values). In this case **"Wavelength Calibration Lost"** is displayed and the instrument stays in a **Not Ready** condition.

Probable cause	Suggested actions
1 Reset of monochromator settings after exchange.	Perform a wavelength calibration.
2 Replacement of FLM board.	Perform a wavelength calibration.

Flow Cell Removed

Error ID: 6616, 6702, 6760

The detector has an automatic cell recognition system. When the flow cell is removed, the lamp is turned off and a **NOT READY** condition exists. If the flow cell is removed during an analysis, a **SHUT DOWN** is generated.

Probable cause	Suggested actions
1 Flow cell has been removed during analysis.	Insert flow cell and turn on the lamp.

No Peaks - No Light Detected

Error ID: 6721

If no peaks are shown in the chromatogram, the user-interface shows the module still in **"Ready"**. There is no feedback mechanism that checks whether the lamp is ON with revision A/B/C Front End Board (FLF). With revision D boards "No Light Detected (6721)" is displayed if the lamp shuts off..

Probable cause	Suggested actions
1 Lamp is off.	Perform a "Lamp Intensity Test" (see "Lamp Intensity Test" on page 140). If no profile available (very low counts).
2 Defective FLL board / Trigger pack.	Please contact your Agilent service representative.
3 Defective Xenon flash lamp.	Please contact your Agilent service representative.
4 Wrong position of monochromator.	Perform a "Wavelength Accuracy Test" (see "Wavelength Accuracy Test" on page 154) to check the wavelength calibration.
5 Defective FLF board.	Please contact your Agilent service representative.

Motor Errors

NOTE Monochromator motor errors may show up during the *initialization* or during *operation* of the detector.There are individual messages for either the excitation or the emission side.If an error occurs, do a lamp ignition. This will clear the error and a re-initialization of the motors is performed.

If motor errors are displayed, please contact your Agilent service representative.

8
Test Functions

This chapter describes the detector’s built in test functions.

Introduction

All tests are described based on the Agilent Lab Advisor Software B.01.04.

Other user interfaces may not provide any test or just a few.

Table 20 Interfaces and available test functions

Interface	Comment	Available Function
Agilent Instrument Utilities	Maintenance tests are available	• Intensity • WL Calibration
Agilent Lab Advisor	All tests are available	• Intensity • ASTM Drift and Noise • Dark Current • D/A Converter • WL Accuracy • WL Calibration • Test Chromatogram (Tools) • Spectra Scan (Tools) • Module Infos (Tools) • Diagnostic (Tools)
Agilent ChemStation	Some tests may be available Adding of temperature	• Some of LabAdvisor tests
Agilent Instant Pilot	Some tests are available	• Intensity • WL Calibration • Spectra Scan (Tools) • Module Infos (Tools) • Diagnostic

For details on the use of the interface refer to the interface documentation.

Diagram of Light Path

The light path is shown in Figure 50 on page 139.

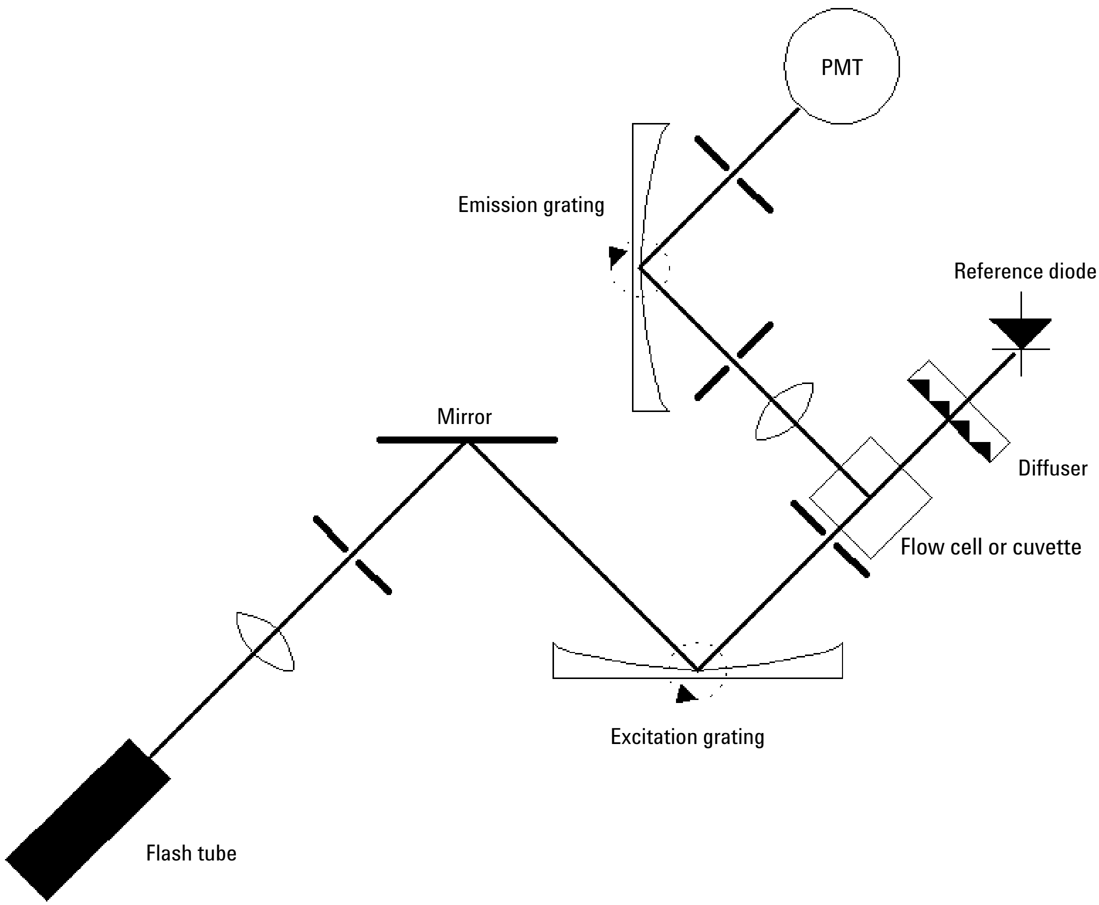


Figure 50 Schematic of the Light Path

Lamp Intensity Test

The intensity test scans an intensity spectrum via the reference diode (200 - 1200 nm in 1 nm steps) and stores it in a diagnosis buffer. The scan is displayed in a graphic window. There is no further evaluation of the test.

Results of this test are stored as lamp history (date code, intensity).

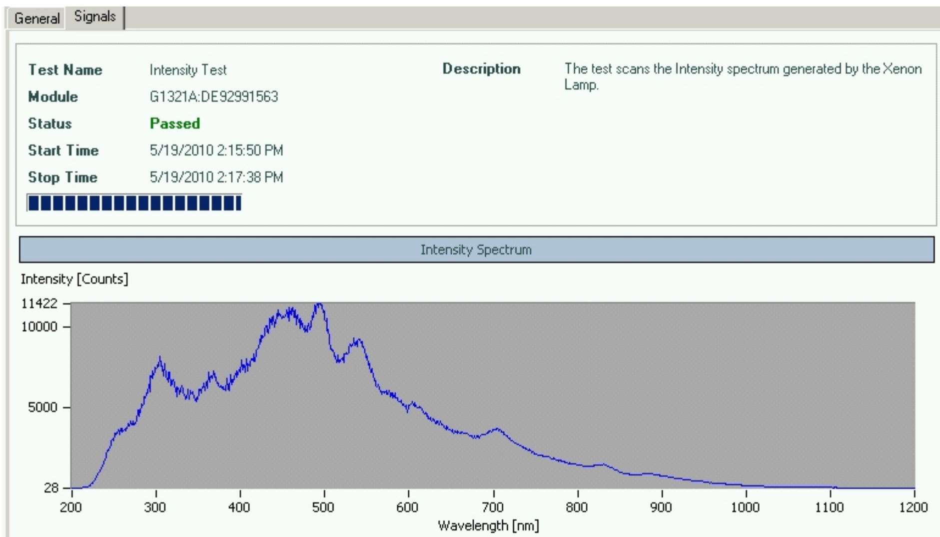


Figure 51 Lamp Intensity Test (Agilent LabAdvisor)

NOTE The profile can vary from instrument to instrument. It is dependig on the age of the lamp and the content of the flow cell (use fresh water).

UV degradation, especially below 250 nm is significantly higher compared to visible wavelength range. Generally the "**LAMP ON during run**" setting or using "**economy mode**" will increase lamp life by a magnitude.

Lamp Intensity History

Results of the lamp intensity test (if the last one is older than one week) are stored as lamp history (date code, intensity of four different wavelengths, 250 nm, 350 nm, 450 and 600 nm) in a buffer. The data/plot can be retrieved via the diagnostics and provides intensity data over a length of time.

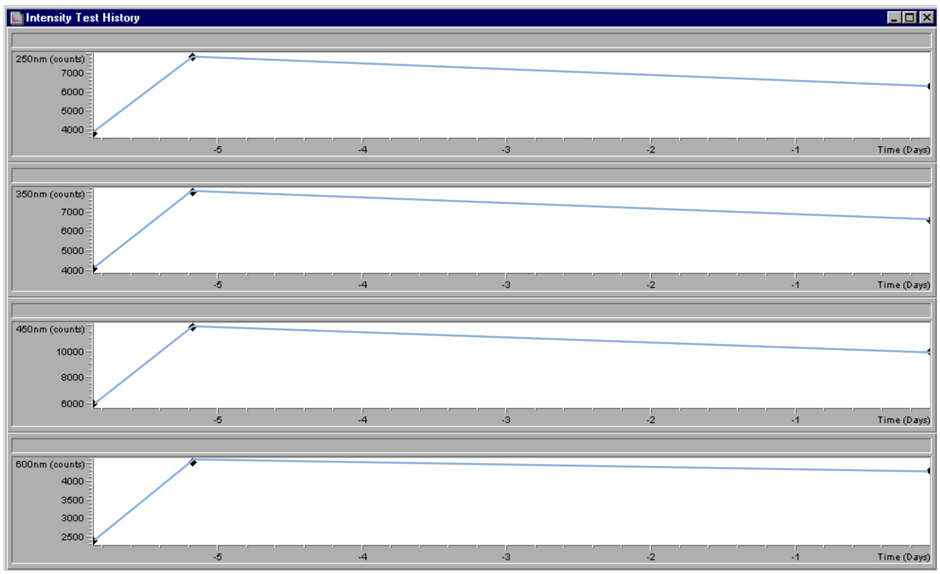


Figure 52 Lamp Intensity History (Agilent ChemStation Diagnostic)

NOTE In the Agilent LabAdvisor the Lamp Intensity History can be found in the **Module Info** section.

Raman ASTM Signal-to-Noise Test

These tests verify the Raman ASTM signal-to-noise for

- single wavelength (at signal) (EX=350 nm, EM=397 nm) or
- single wavelength (at background) (Ex=350 nm, Em=397 nm, dark value 450 nm) or
- dual wavelength (EX=350 nm, EM_A=397 nm, EM_B=450 nm)

NOTE The specification single wavelength at signal can be measured with the Agilent LabAdvisor. All other have to be set up manually as described for "Dual Wavelength Verification" on page 147 with the information from Table 23 on page 143 and Table 24 on page 143.

Table 21 Raman Signal-to-Noise Test Conditions

Duration	approximately 23 minutes
Report Style (Agilent ChemStation)	Performance+Noise
Noise Determination	5 to 20 minutes
Solvent	LC grade water, degassed
Flow rate	0.5 - 1 ml/min
Specification (single wavelength at signal)	>500 (according to settings in Table 22 on page 143)
Specification (single wavelength at background)	>3000 (according to settings in Table 23 on page 143)
Specification (dual wavelength)	>300 (according to settings in Table 24 on page 143)

Table 22 Settings for Single Wavelength Specifications (at signal)

Time	EX	EM	PMT	Baseline
0	350	397	12	Free
20.30	350	450	12	Free

Table 23 Settings for Single Wavelength Specifications (at background)

Time	EX	EM	PMT	Baseline
0	350	450	14	Free
20.30	350	397	14	Free

Table 24 Settings for Dual Wavelength Specifications (Multi-EM Scan)

Time	EX	EM_A	EM_B	Spectra	From	To	Step	PMT	Baseline	Fit Spectra
00.00	350	397	450	None	280	450	10	12	Free	OFF
20.30	350	450	450	None	280	450	10	12	Free	OFF

Formula for the Raman ASTM S/N value (see Figure 53 on page 144 for details):

$$\text{RamanASTM} = \frac{\text{Intensity (Raman)} - \text{Intensity (Dark)}}{\text{ASTMNoise (Raman or Dark)}}$$

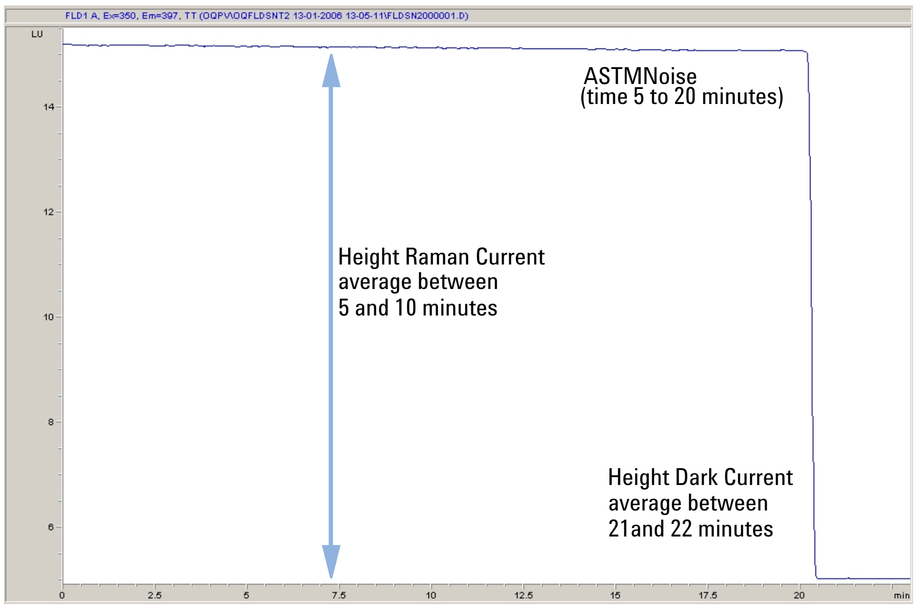


Figure 53 Raman ASTM signal/noise calculation

Procedure using an Agilent LabAdvisor

1 Set up the HPLC system and the LabAdvisor.

2 Flush the flow cell with clean bi-distilled water.

3 Start the test in the LabAdvisor.

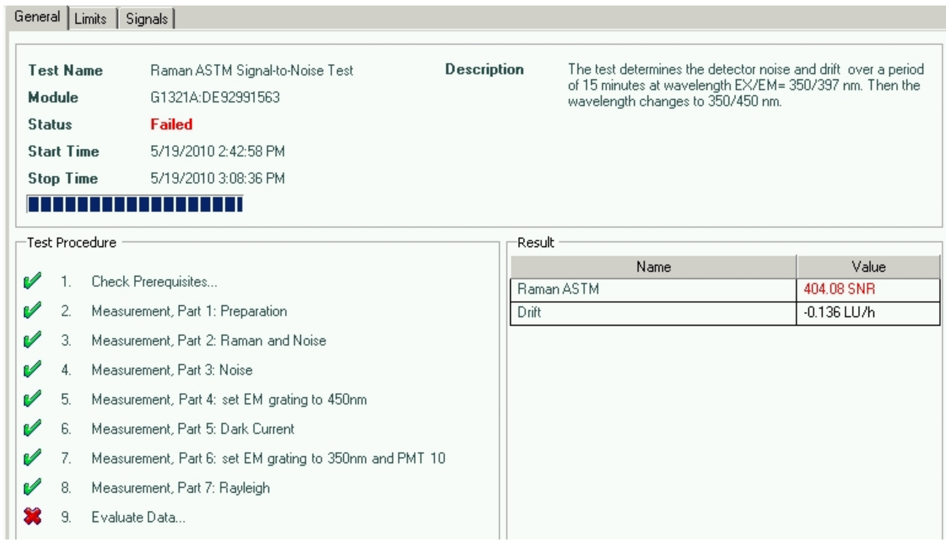


Figure 54 Raman ASTM Signal-to-Noise Test (Agilent LabAdvisor)

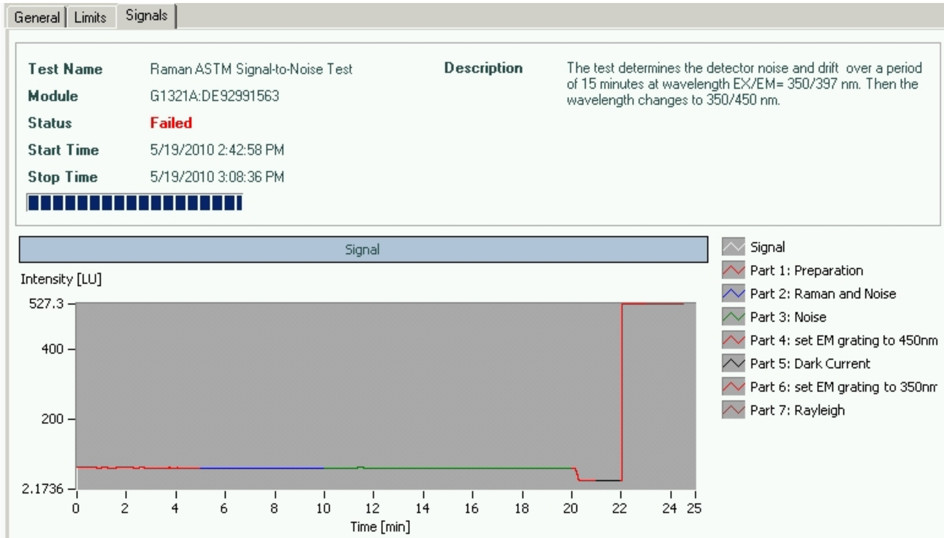


Figure 55 Raman ASTM Signal-to-Noise Test (Agilent LabAdvisor)

In case of failing this test (as shown above) see “Interpretation of the Results” on page 148.

Procedure using an Agilent ChemStation

Procedure using an Agilent ChemStation

The Agilent ChemStation should be used for the verification of the Dual Wavelength Check only.

1 Set up the HPLC system and the Agilent LabAdvisor.
2 Flush the flow cell with clean bi-distilled water.
3 Turn on the FLD lamp.
4 Select "**Verification (OQ/PV)**".

NOTE Assure that the FLD signal is signal 1 (in case you use an additional detector in the system). Otherwise wrong calculations may be done due to wrong signals.

Dual Wavelength Verification

For specfications and settings see Table 21 on page 142 and Table 24 on page 143.

1 Open **Method and Run Control**.
2 Open method OQFLDSNT.M.
3 Modify the time table according to Table 24 on page 143.
4 Save the method as OQFLDSNT2.M.
5 Open sequence OQFLDSNT.S.
6 Modify the sequence to use a different store location and and to call up method OQFLDSNT2.M.
7 Save the sequence as OQFLDSNT2.S
8 Select customize sequence and select OQFLDSNT2.S.
9 Set the limits to 300.
10 When the run is completed a report is displayed and the status line shows the "**Raman signal/noise ratio =** " value should be >300.

Interpretation of the Results

If the test shows low Raman values, check for:

- ✔ correctly positioned flow cell,
- ✔ clean flow cell (flush with clean bi-distilled water),
- ✔ no air bubble(s) (check via fluorescence scan or visual check of cell/cuvette),
- ✔ solvent inlet filter (may create air bubbles in flow cell).

Using the Built-in Test Chromatogram

This function is available from the Agilent ChemStation, LabAdvisor and Instant Pilot.

The built-in Test Chromatogram can be used to check the signal path from the detector to the data system and the data analysis or via the analog output to the integrator or data system. The chromatogram is continuously repeated until a stop is executed either by means of a stop time or manually.

NOTE The peak height is always the same but the area and the retention time depend on the set peakwidth, see example below.

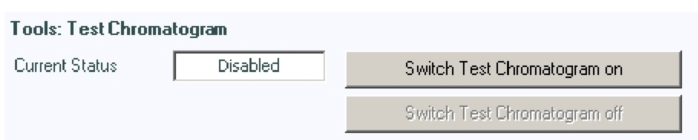
Procedure Using the Agilent LabAdvisor

This procedure works for all Agilent 1200 Infinity detectors (DAD, MWD, VWD, FLD and RID). The example figure is from the RID detector.

1 Assure that the default LC method is loaded via the control software.

2 Start the Agilent Lab Advisor software (B.01.03 SP4 or later) and open the detector's **Tools** selection.

3 Open the test chromatogram screen



4 Turn the **Test Chromatogram** on.

5 Change to the detector's **Module Service Center** and add the detector signal to the Signal Plot window.

6 To start a test chromatogram enter in the command line: STRT

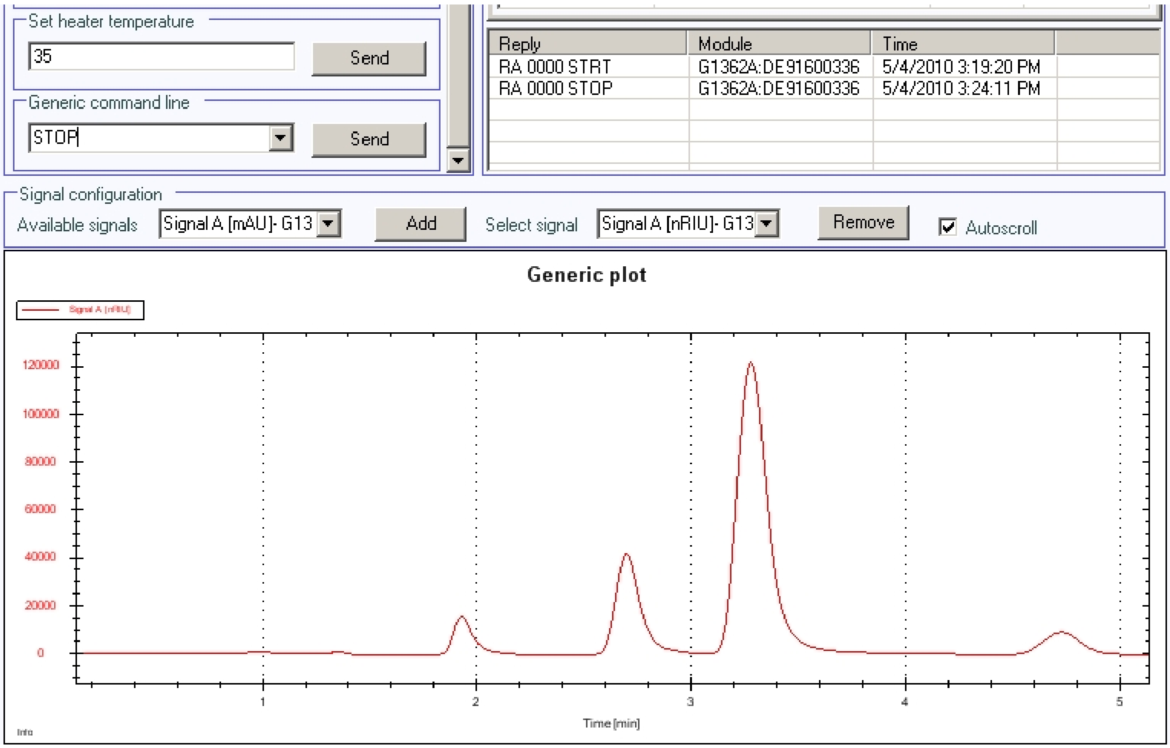


Figure 56 Test Chromatogram with Agilent Lab Advisor

7 To stop the test chromatogram enter in the command line: STOP

NOTE The test chromatogram is switched off automatically at the end of a run.

Wavelength Verification and Calibration

The wavelength calibration is based on a Glycogen solution, which acts as a strong elastic light scatterer (refer to ASTM Test Method E388-72-1993 "*Spectral Bandwidth and Wavelength Accuracy of Fluorescence Spectrometers*"). The Glycogen solution is introduced into the flow cell and then the built-in wavelength calibration functionality is used.

The algorithm is based on evaluating different grating orders and calculating the wavelength scales of both, excitation and emission monochromator, by applying the fundamental grating equation.

NOTE A complete wavelength calibration is not always required. In most cases a quick wavelength accuracy verification is sufficient enough, see Table 25 on page 151.

Table 25 Reasons for doing a Verification or Calibration

	Verification	WL calibration
interest	X	
GLP compliance	X	
cell change	X	(X)
lamp change	X	(X)
monochromator change		X
main board change		X
optical unit change		X

(X) only required, if deviation is too large.

NOTE Prior to a wavelength calibration, a wavelength accuracy verification should be performed, see "Wavelength Accuracy Test" on page 154. If the deviation is more than ±3 nm, the wavelength calibration should be done as described in "Wavelength Calibration Procedure" on page 160.

NOTE

The duration of the wavelength calibration is about 15 minutes plus setup time for the calibration sample and system. Depending on the maximum intensity found during this scan, the PMT gain will be changed automatically and requires an additional 1 minute per scan.

Table 26 on page 153 shows the steps performed during the wavelength calibration.

The excitation grating and the emission grating are calibrated using Rayleigh stray light from the flow cell or cuvette measured with the photomultiplier tube.

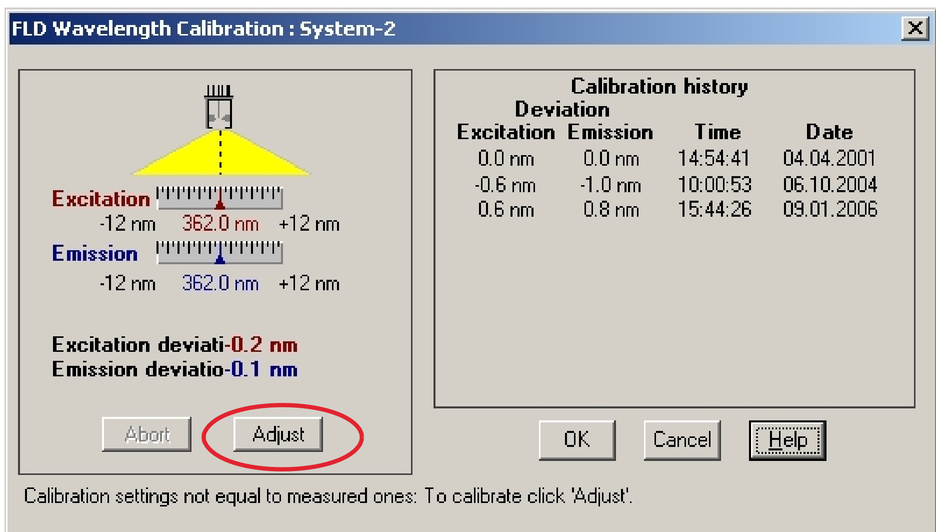


Figure 57 Wavelength Calibration (Agilent ChemStation Diagnostic)

Table 26 Wavelength Calibration Steps

Step	Description	Duration
1	Preparation	max 30 s
2	Excitation rotation scan, full circle	60 s
3	Excitation rotation scan, high resolution	44 s
4	Excitation position scan, low resolution	55 s variable
5	Excitation position scan, high resolution	260 s variable
6.n	Emission rotation scan, full circle (# of scans depends on the required PMT gain, 1 minute per scan)	61 s variable
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
7	Emission rotation scan, high resolution, part I	44 s
8	Emission rotation scan, high resolution, part II	44 s
9	Emission position scan, low resolution	50 s variable
10	Emission position scan, high resolution	250 s variable

NOTE

Variable times means that they could be a little bit longer.

When the lamp is off, the calibration process will stop within the first two steps with " Wavelength Calibration Failed" , see "Wavelength Calibration Failed" on page 134.

Wavelength Accuracy Test

Using the Agilent LabAdvisor

1. Set up the HPLC system and the Agilent LabAdvisor.
2. Flush the flow cell with clean bi-distilled water.
3. Turn on the FLD lamp.
4. Run the Wavelength Accuracy Test.
5. The FLD will change into the multi-excitation mode with emission wavelength at 397 nm and scan in the range of the expected maximum of 350 nm ±20 nm.

   As result, the maxima should be found at 350 nm ±3 nm, see Figure 58 on page 154.

   The FLD will change into the multi-emission mode with excitation wavelength at 350 nm and scan in the range of the expected maximum of 397 nm ± 20 nm.

   As result, the maxima should be found at 397 nm ± 3 nm, see Figure 58 on page 154.

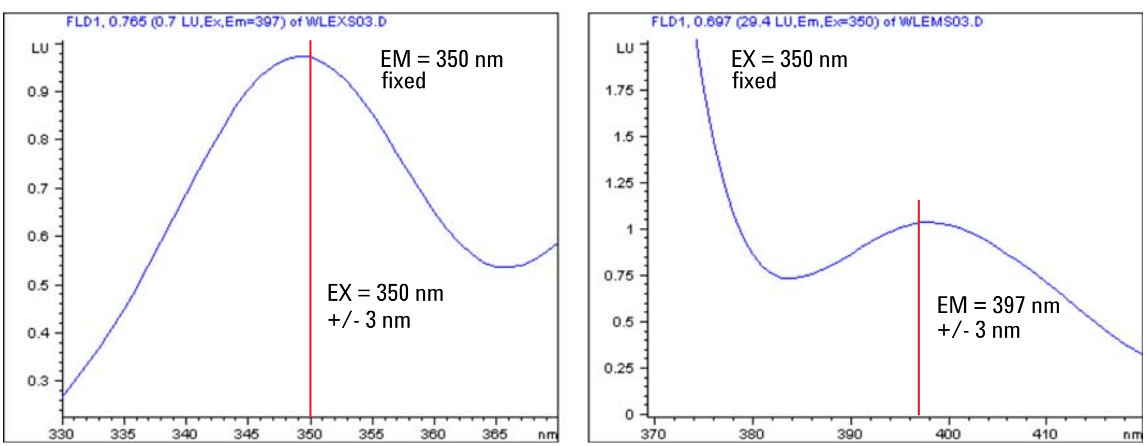


Figure 58 Excitation and Emission Spectrum (expected results)

NOTE If the limits are not met, check for "Interpretation of the Results" on page 148 or perform "Wavelength Calibration Procedure" on page 160.

The below figures show an example of a failed test. The excitation scan did not find a maximum (just an up-slope).

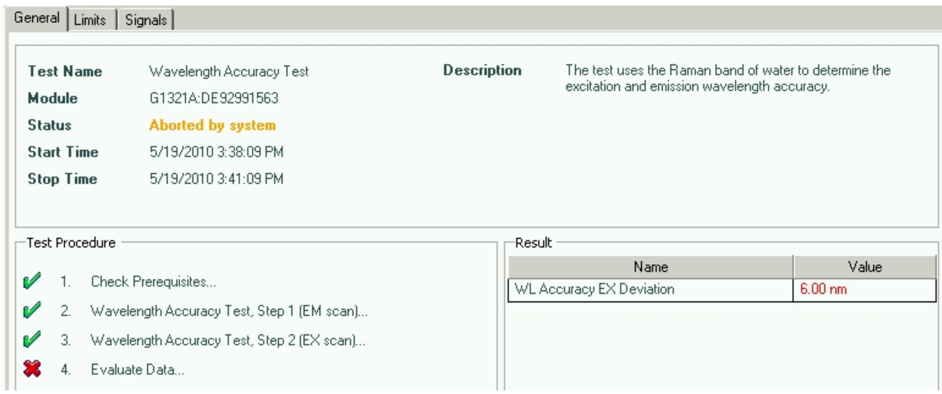


Figure 59 Wavelength Accuracy Test (Agilent LabAdvisor)

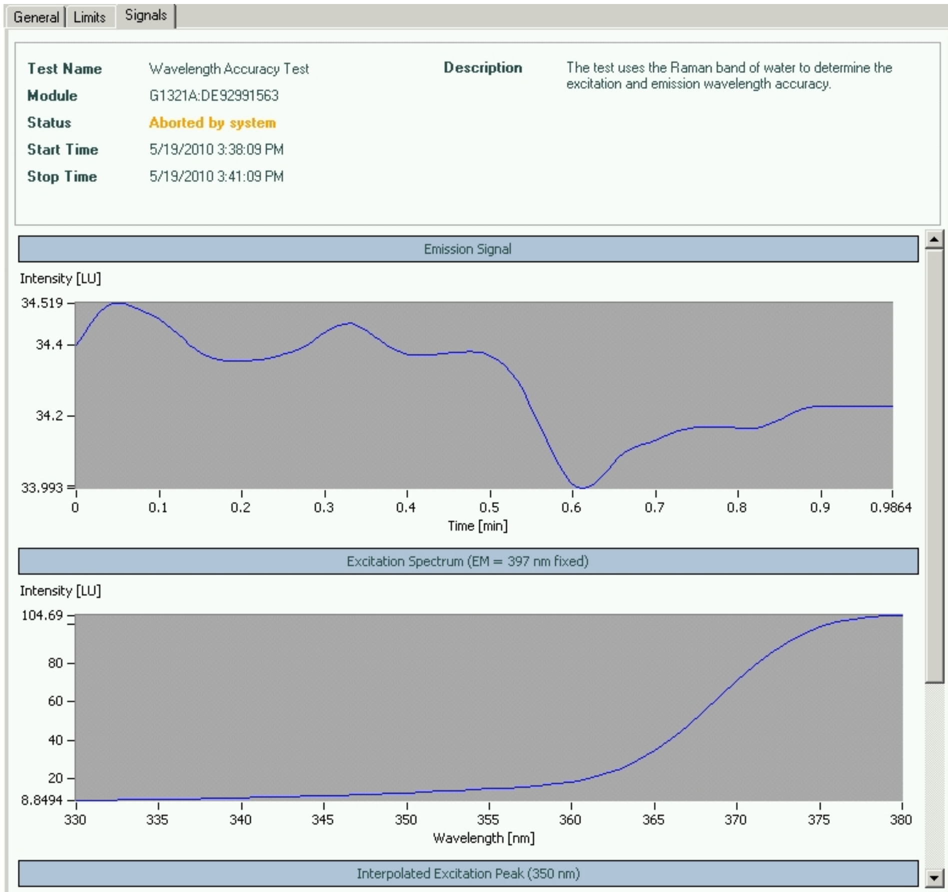


Figure 60 Wavelength Accuracy Test (Agilent LabAdvisor)

Using the Agilent ChemStation (Manually)

1 Create the methods WLEMTEST and WLEXTEST as listed Table 27 on page 157.

Table 27 Method Settings

Setting	Check of EM WL 397 nm WLEMTEST	Check of EX WL 350 nm WLEXTEST
Peak Width	>0.2 min (4 s, standard)	>0.2 min (4 s, standard)
Fit Spectral Range	OFF	OFF
PMT Gain	12	12
Flash Lamp	ON	ON
Spectrum Range	EM 367 - 417 nm step 1 nm	EX 330 - 380 nm step 1 nm
Store Spectra	All w/o signal	All w/o signal
EX Wavelength	350 nm, ON	350 nm, OFF
EM Wavelength	397 nm, OFF	397 nm, ON
Multi WL Settings	Multi EM	Multi EX

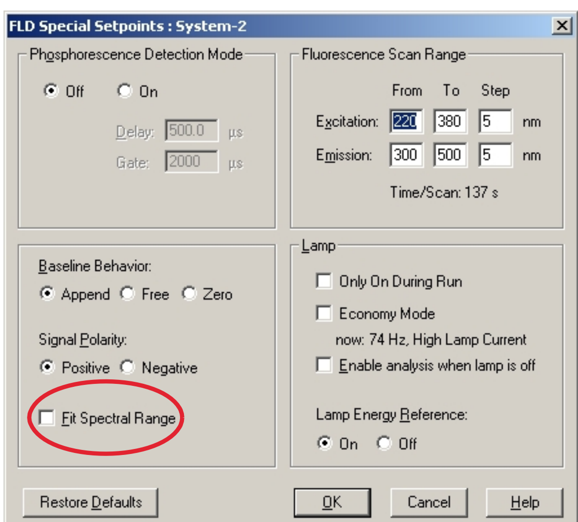


Figure 61 Special Setpoints Settings

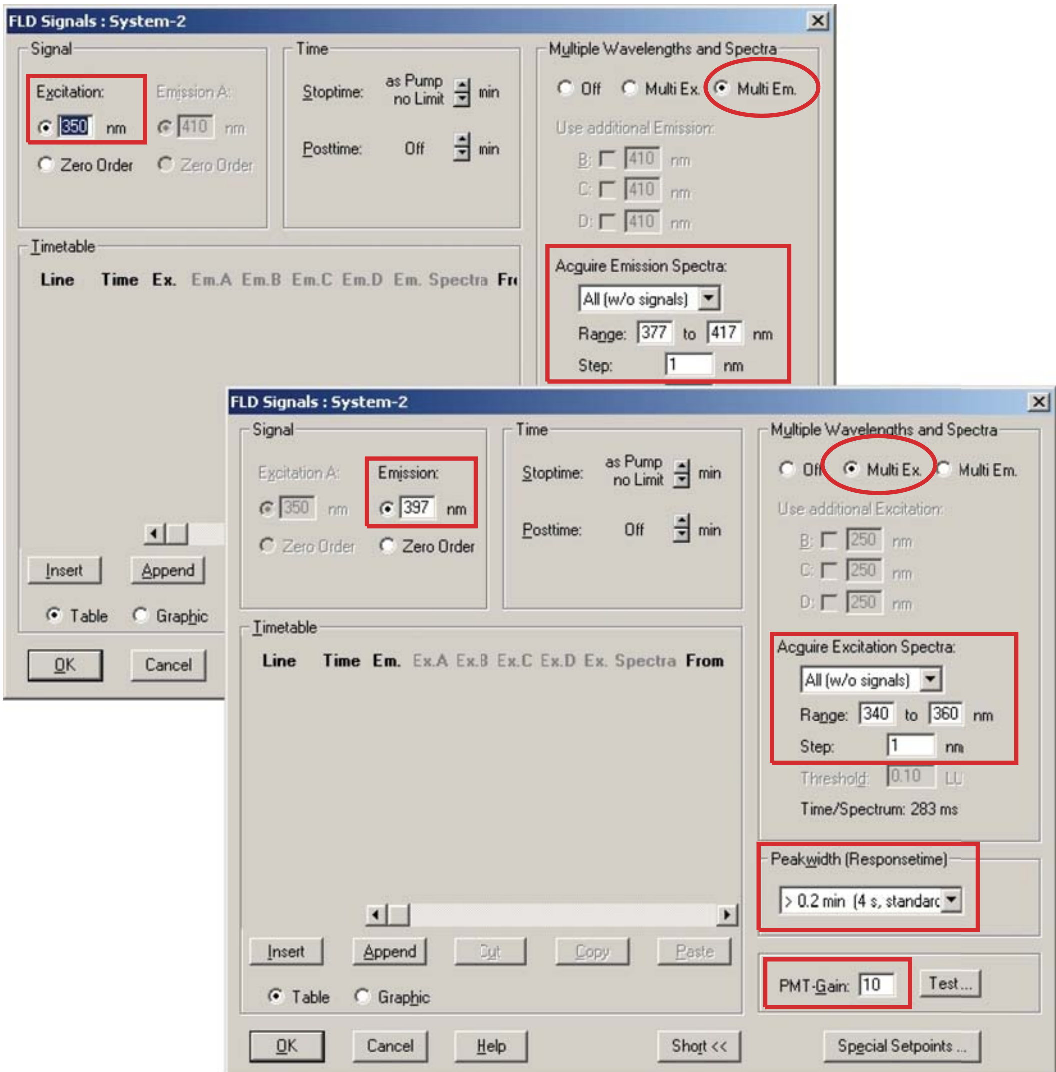


Figure 62 Settings for EM / EX Scan

2 Load the method **WLEXTEST**. The FLD will change into the multi-emission mode and scan in the range of the expected maximum of 397 nm ±20 nm.

3 Start the pump and flush the cell with water for a few minutes to assure a clean flow cell. Flow rate should be 0.5 to 1 ml/min and the baseline stable.

NOTE You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp ON.

4 Open the Online Spectra plot and observe the maximum as shown in Figure 58 on page 154 (left).

5 Load the method **WLEMTEST**. The FLD will change into the multi-excitation mode and scan in the range of the expected maximum of 350 nm ±20 nm.

6 Open the Online Spectra plot and observe the maximum as shown in Figure 58 on page 154 (right).

Wavelength Calibration Procedure

When If application requires, or see Table 26 on page 153.

Tools required

Description
Laboratory balance

Parts required

p/n	Description
5063-6597	Calibration Sample, Glycogen
9301-1446	Syringe
9301-0407	Needle
5061-3364	Filter regen Cellulose 30/45 luer 100/pk
0100-1516	Fitting male PEEK, 2/pk

1 Preparation of the Glycogen Calibration Sample.
   a To prepare 10 ml of the calibration solution you have to use 10 mg of the Glycogen sample (a tolerance of ±20% is not critical).
   b Fill the prepared amount into a suitable bottle/vial.
   c Fill 10 ml of distilled water into the vial and shake.
   d Wait 5 minutes and shake again. After 10 minutes the solution is ready.
2 Preparation of the Flow Cell.
   a Flush the flow cell with water.
   b Remove the inlet capillary from the flow cell.
   c Take the syringe and fix the needle to the syringe adapter.
   d Suck about 1.0 ml of the calibration sample into the syringe.
   e Keep the syringe in a horizontal position.
   f Remove the needle.

g Add the filter to the syringe and fit the needle to filter.

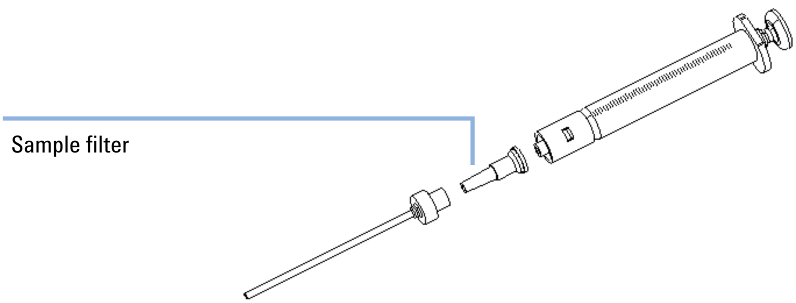


Figure 63 Syringe with Sample Filter

h Lift the needle tip and carefully eject approximately 0.5 ml to remove air out of the syringe and to flush the needle.

i Add the PEEK fitting to the needle tip and fix both at the flow cell inlet.

NOTE Do not inject the calibration sample without the sample filter.

j Slowly inject about 0.2 ml and wait for about 10 seconds to inject another 0.1 ml. This will assure that the cell is filled properly.

3 Wavelength Calibration.

a From the user interface start the FLD wavelength calibration (see Figure 66 on page 163).

- Agilent LabAdvisor: **Calibrations**
- Agilent ChemStation: **Diagnosis > Maintenance > FLD Calibration**
- Instant Pilot (G4208A): **Maintenance > FLD > Calibration**

NOTE If the wavelength calibration process fails, refer to “Wavelength Calibration Failed” on page 134.

b If a deviation is displayed, press **Yes** (LabAdvisor) to adjust to new values or **Adjust** and **OK** (ChemStation, see next page). The history table will be updated.

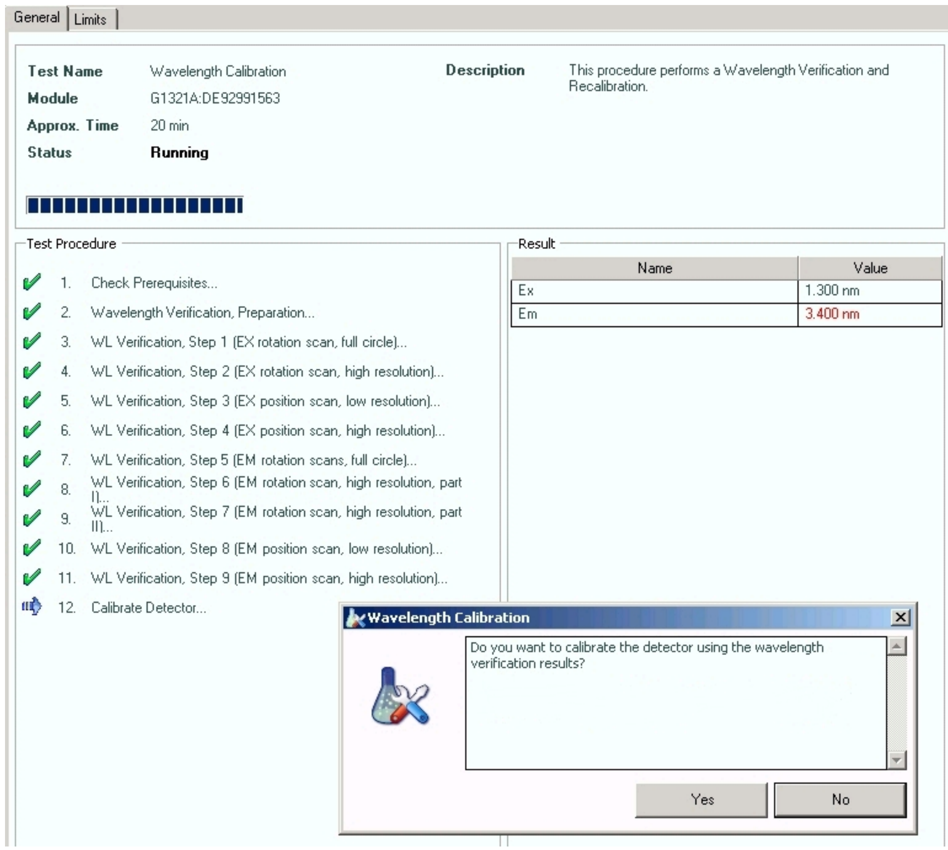


Figure 64 Wavelength Calibration (Agilent LabAdvisor)

WL Calibration History

Date	Deviation of Excitation	Deviation of Emission
02/11/2010 12:54	0.3	-1.6
02/09/2010 12:22	0.0	0.0
02/09/2010 11:48	13.2	12.5
10/20/2009 10:41	-2.2	0.5
07/21/2009 13:41	23.2	-1.1
07/21/2009 12:22	0.1	0.1
07/21/2009 11:31	-19.7	-6.6
08/25/2006 12:05	-0.2	0.2
01/09/2006 16:02	-0.2	-0.1
01/09/2006 15:30	0.6	0.8

Figure 65 Calibration History (Agilent LabAdvisor)

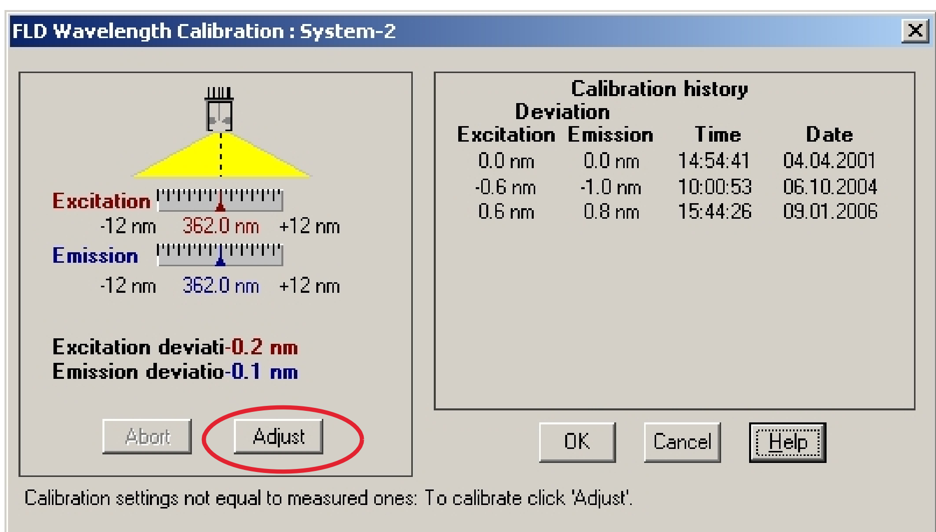


Figure 66 Wavelength Calibration (Agilent ChemStation)

NOTE To look at the history table (ChemStation) start a wavelength calibration and abort immediately. No changes are made to the calibration at this time.

NOTE Rinse the flow cell with pure water at a minimum of 1.5 ml/min to get rid of the Glycogen from the cell and the capillaries. When organic solvent is sequentially applied (without rinsing), a blockage of capillaries may occur.

4 Verification using “Wavelength Accuracy Test” on page 154.

   a Refit the capillary to the flow cell.

   b Follow the procedure “Wavelength Accuracy Test” on page 154.

9 Maintenance

This chapter provides general information on maintenance of the detector.

Introduction to Maintenance

The module is designed for easy maintenance. Maintenance can be done from the front with module in place in the system stack.

NOTE

There are no serviceable parts inside.

Do not open the module.

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

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened.

→ Do not remove the cover of the module.

→ Only certified persons are authorized to carry out repairs inside the module.

WARNING

Personal injury or damage to the product

Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

→ Use your Agilent products only in the manner described in the Agilent product user guides.

CAUTION

Safety standards for external equipment

→ If you connect external equipment to the instrument, make sure that you only use accessory units tested and approved according to the safety standards appropriate for the type of external equipment.

Overview of Maintenance

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

Table 28 Simple Repairs

Procedure	Typical Frequency	Notes
Flow cell exchange	If application requires a different flow cell type or if defective.	Complete Assembly A wavelength calibration check should be performed after replacement. If the flow cell is removed and inserted, then a quick calibration check is performed. If this fails, you must do a wavelength recalibration, see "Wavelength Verification and Calibration" on page 151.
Flow cell flushing	If flow cell is contaminated.	
Leak sensor drying	If leak has occurred.	Check for leaks.
Leak handling System replacement	If broken or corroded.	Check for leaks.

Cleaning the Module

To keep the module case clean, use a soft cloth slightly dampened with water, or a solution of water and mild detergent.

WARNING

Liquid dripping into the electronic compartment of your module can cause shock hazard and damage the module

➔ Do not use an excessively damp cloth during cleaning.

➔ Drain all solvent lines before opening any connections in the flow path.

Exchanging a Flow Cell

BIO inert For bio-inert modules use bio-inert parts only!

When If an application needs a different type of flow cell or the flow cell is defective (leaky).

Tools required

Description

Wrench, 1/4 inch
for capillary connections

Parts required

#	p/n	Description
1	G1321-60005	Flow cell, 8 µL, 20 bar (pH 1 – 9.5)
1	G1321-60015	Flow cell, 4 µL, 20 bar (pH 1 – 9.5)
1	G5615-60005	Bio-inert flow cell, 8 µL, 20 bar (pH 1–12) includes Capillary Kit Flow Cells BIO (p/n G5615-68755)
1	G1321-60007	FLD Cuvette Kit, 8 µL, 20 bar

Preparations Turn off the flow.

CAUTION

Sample degradation and contamination of the instrument

Metal parts in the flow path can interact with the bio-molecules in the sample leading to sample degradation and contamination.

→ For bio-inert applications, always use dedicated bio-inert parts, which can be identified by the bio-inert symbol or other markers described in this manual.

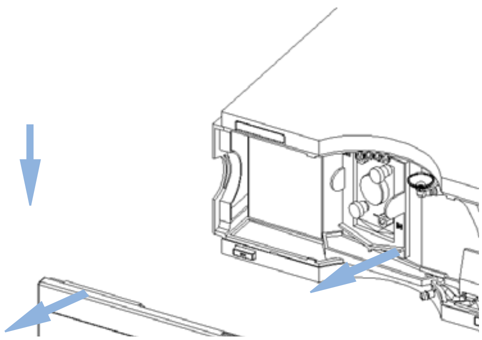
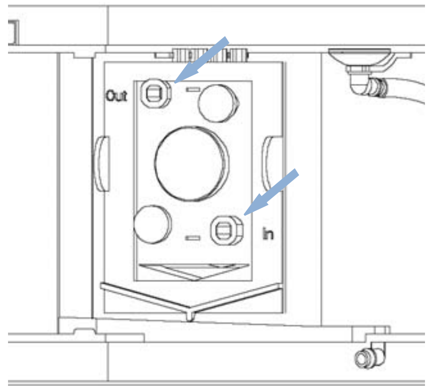
→ Do not mix bio-inert and non-inert modules or parts in a bio-inert system.

NOTE

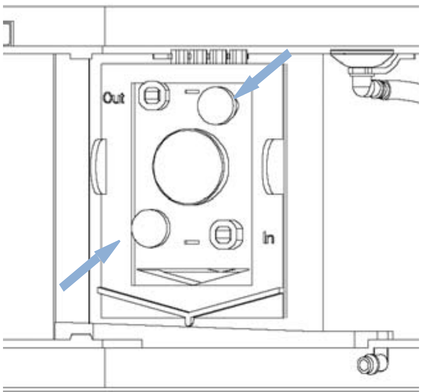
DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance.

NOTE

In case the flow cell is not used for some time (stored), then flush the flow cell with iso-propanol and close the cell with Plug-Screw (0100-1259).

1 Press the release buttons and remove the front cover for access to the flow cell area. 	**2** Disconnect the capillaries from the flow cell. 

3 Unscrew the thumb screws and pull the flow cell out of the compartment.

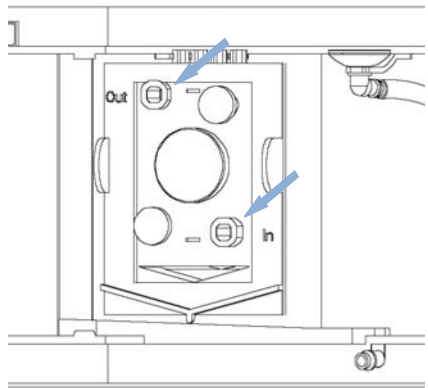


NOTE

The label attached to the flow cell provides information on part number, cell volume and maximum pressure. The cell type will be automatically detected.

There are no parts that can be replaced on the flow cell. If defective (leaky) the flow cell has to be replaced completely.

4 Insert the flow cell and tighten the thumb screws. Reconnect the capillaries to the flow cell. DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance or damage.



NOTE

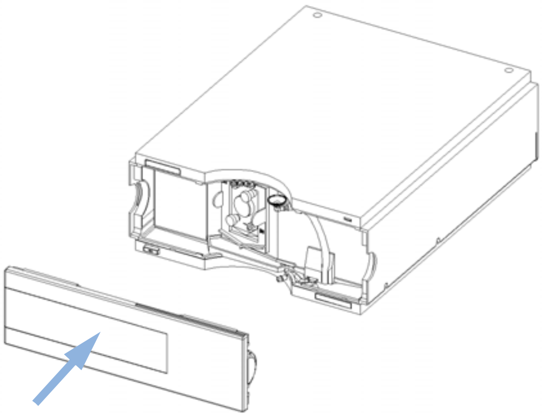
If an additional detector is added to the system, the fluorescence detector should be the last detector in the flow path except for evaporative detectors, like LC-MSD. Otherwise the back pressure generated by the other detector may overload the flow cell and will lead to a defective cell (maximum pressure is 20 bar (2 MPa)).

Always use the outlet capillary set supplied with the accessory kit.

NOTE

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

5 Replace the front cover.



NOTE

Perform a wavelength verification to check the correct positioning of the flow cell, as described in chapter “Wavelength Verification and Calibration” on page 151.

How to use the Cuvette

The cuvette is used for off-line measurements (no flow system required) and is basically a standard flow cell with a few changes:

- wide bore capillary connections for easier injections with a syringe
- identification lever for cell auto-recognition system.

1 Install the cuvette instead of the standard flow cell.
2 Connect the waste tubing to the outlet of the cuvette.
3 Use the syringe (see “Cuvette Kit” on page 185) to inject the compound.
4 Setup the parameters for the Fluorescence Scan (under Special Setpoints).
5 Select “Take Fluorescence Scan” on the user-interface to start the off-line measurement.

Flow Cell Flushing

When	If flow cell is contaminated	
Tools required	**Description**	
	Glass syringe	
	Adapter	
Parts required	**#**	**Description**
	1	Bidistilled water, nitric acid (65 %), tubings to waste

WARNING

Dangerous concentration of nitric acid

The nitric acid flushing procedure is not an infallible remedy for a dirty cell. It is to be used as a last attempt to salvage the cell before cell replacement. Note that the cell is a consumable item.

→ Give proper attention to safety.

NOTE

Aqueous solvents in the flow cell can built up algae. Algae do fluoresce. Therefore do not leave aqueous solvents in the flow cell for longer periods. Add a small percentage of organic solvents (e.g. Acetonitrile or Methanol ~5 %).

1. Flush with bidistilled water.
2. Flush with nitric acid (65 %) using a glass syringe.
3. Leave this solution in the cell for about one hour.
4. Flush with bidistilled water.

NOTE

Do not exceed the pressure limit of 20 bar (0.2 MPa).

Correcting Leaks

When	If a leakage has occurred in the flow cell area or at the capillary connections
Tools required	**Description**
	Tissue
	Wrench, 1/4 inch for capillary connections

1 Remove the front cover.
2 Use tissue to dry the leak sensor area and the leak pan.
3 Observe the capillary connections and the flow cell area for leaks and correct, if required.
4 Replace the front cover.

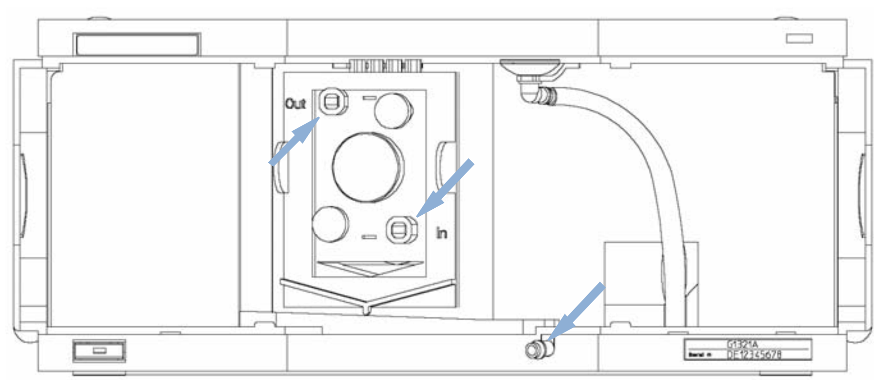


Figure 67 Observing for Leaks

Replacing Leak Handling System Parts

When	If the parts are corroded or broken		
Parts required	**#**	**p/n**	**Description**
	1	5041-8389	Leak funnel
	1	5061-3356	Leak funnel holder
	1	5042-9974	Leak tubing (1.5 m, 120 mm required)

1 Remove the front cover.
2 Pull the leak funnel out of the leak funnel holder.
3 Pull out the leak funnel with the tubing.
4 Insert the leak funnel with the tubing in its position.
5 Insert the leak funnel into the leak funnel holder.
6 Replace the front cover.

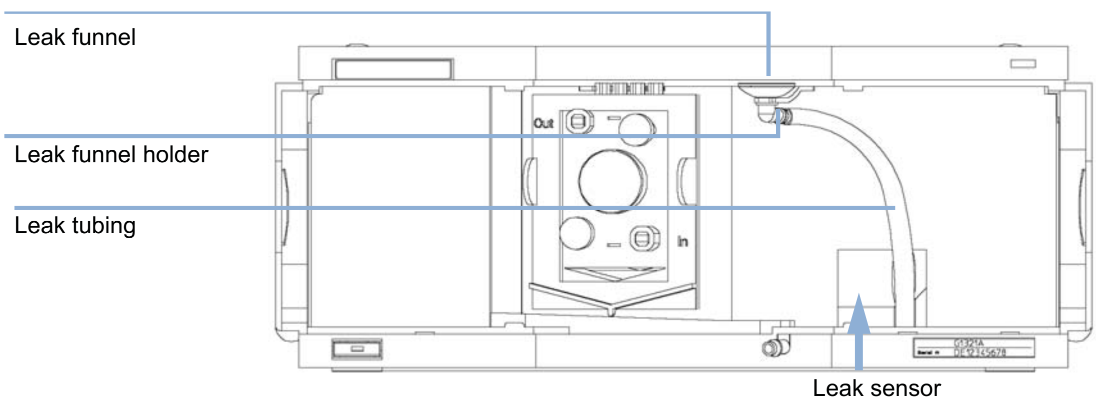


Figure 68 Replacing Leak Handling System Parts

Replacing the Interface Board

When	For all repairs inside the detector or for installation of the board		
Parts required	**#**	**p/n**	**Description**
	1	G1351-68701	Interface board (BCD) with external contacts and BCD outputs
	1	G1369B or G1369-60002	Interface board (LAN)
OR	1	G1369C or G1369-60012	Interface board (LAN)

1 To replace the interface board unscrew the two screws, remove the board, slide in the new interface board and fix it with the board's screws.

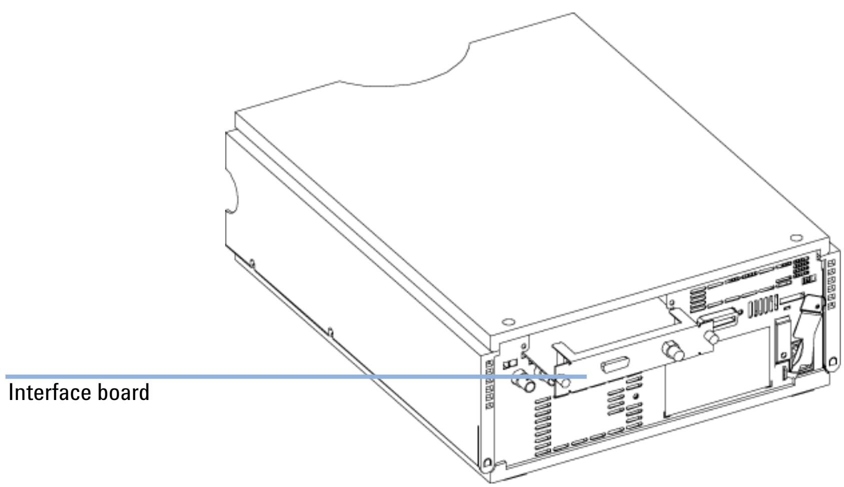


Figure 69 Location of the Interface Board

Replacing Module Firmware

When	The installation of newer firmware might be necessary • if a newer version solves problems of older versions or • to keep all systems on the same (validated) revision. The installation of older firmware might be necessary • to keep all systems on the same (validated) revision or • if a new module with newer firmware is added to a system or • if third party control software requires a special version.
Tools required	**Description**
	LAN/RS-232 Firmware Update Tool
OR	Agilent Lab Advisor software
OR	Instant Pilot G4208A (only if supported by module)

Parts required	**#**	**Description**
	1	Firmware, tools and documentation from Agilent web site

Preparations Read update documentation provided with the Firmware Update Tool.

To upgrade/downgrade the module's firmware carry out the following steps:

1. Download the required module firmware, the latest LAN/RS-232 FW Update Tool and the documentation from the Agilent web.
   - http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761
2. For loading the firmware into the module follow the instructions in the documentation.

Module Specific Information

There is no specific information for this module.

Tests and Calibrations

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

- "Lamp Intensity Test" on page 140.
- "Wavelength Verification and Calibration" on page 151

10
Parts for Maintenance

This chapter provides information on parts for maintenance.

Overview of Maintenance Parts

p/n	Description	
	G1321-60005	Flow cell, 8 µL, 20 bar (pH 1 – 9.5)
OR	G1321-60015	Flow cell, 4 µL, 20 bar (pH 1 – 9.5) requires a 0.12 mm i.d. capillary (e.g. p/n G1316-87318, 300 mm long), part of Capillary kit for 0.12 mm id (p/n G1316-68716)
OR	G5615-60005	Bio-inert flow cell, 8 µL, 20 bar (pH 1–12) includes Capillary Kit Flow Cells BIO (p/n G5615-68755)
	G5615-68755	Capillary Kit Flow Cells BIO includes Peek Capillary i.d. 0.18 mm, 1.5 m lg and PEEK Fittings 10/PK (p/n 5063-6591)
	G1321-60007	FLD Cuvette Kit, 8 µL, 20 bar
	9301-0407	Needle
	9301-1446	Syringe
	5067-4691	Front Panel DAD/VWD/FLD (1260/1290)
	5041-8388	Leak funnel
	5041-8389	Leak funnel
	5041-8387	Tube clip
	5062-2463	Corrugated tubing, PP, 6.5 mm id, 5 m
	5062-2462	PTFE Tubing flexible i.d. 0.8 mm, o.d. 1.6 mm, 2 m, re-order 5 m (flow cell to waste)
	5181-1516	CAN cable, Agilent module to module, 0.5 m
	5181-1519	CAN cable, Agilent module to module, 1 m
	G1369B or G1369-60002	Interface board (LAN)
	5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
	5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)
	01046-60105	Agilent module to general purpose (Analog)
	G1351-68701	Interface board (BCD) with external contacts and BCD outputs

Parts for wavelength calibration, see “Standard Accessory Kit” on page 186.

Cuvette Kit

p/n	Description
G1321-60007	FLD Cuvette Kit, 8 µL, 20 bar includes:
5062-2462	PTFE Tubing flexible i.d. 0.8 mm, o.d. 1.6 mm, 2 m, re-order 5 m (flow cell to waste)
79814-22406	SST Fitting
0100-0043	SST front ferrule
0100-0044	SST back ferrule
0100-1516	Fitting male PEEK, 2/pk
9301-0407	Needle
9301-1446	Syringe

Accessory Kit

Standard Accessory Kit

Accessory kit (G1321-68755) contains some accessories and tools needed for the installation and repair/calibration of the detector.

Item	p/n	Description
1	5062-2462	PTFE Tubing flexible i.d. 0.8 mm, o.d. 1.6 mm, 2 m, re-order 5 m (flow cell to waste)
2	0100-1516	Fitting male PEEK, 2/pk
3	G1315-87311	Capillary column – detector 380 mm lg, 0.17 i.d., (includes SST ferrule front, SST ferrule back and SST fitting).
4	0100-0043	SST front ferrule
5	0100-0044	SST back ferrule
6	79814-22406	SST Fitting

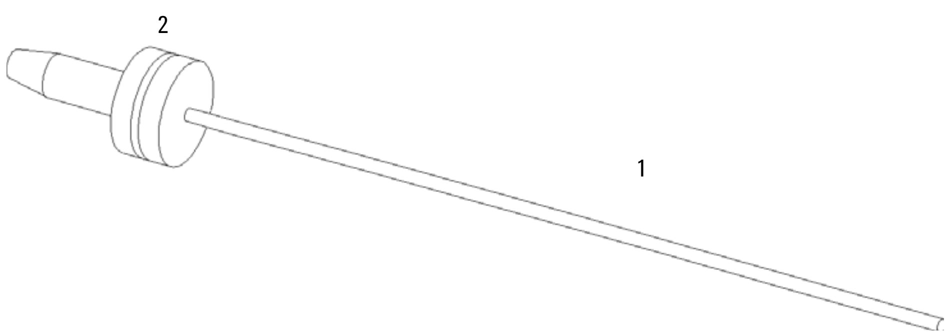


Figure 70 Waste Tubing Parts

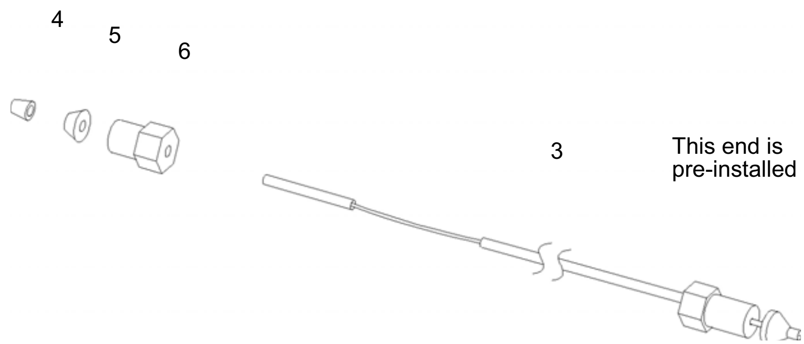


Figure 71 Inlet Capillary (Column-Detector) Parts

Capillary Kit Flow Cells BIO

Capillary Kit Flow Cells BIO includes Peek Capillary i.d. (G5615-68755) includes:

p/n	Description
0890-1763	Peek Capillary i.d. 0.18 mm, 1.5 m lg
5063-6591	PEEK Fittings 10/PK

11
Identifying Cables

This chapter provides information on cables used with the 1290 series of HPLC modules.

Cable Overview

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

Analog cables

p/n	Description
35900-60750	Agilent module to 3394/6 integrators
35900-60750	Agilent 35900A A/D converter
01046-60105	Analog cable (BNC to general purpose, spade lugs)

Remote cables

p/n	Description
03394-60600	Agilent module to 3396A Series I integrators
	3396 Series II / 3395A integrator, see details in section "Remote Cables" on page 194
03396-61010	Agilent module to 3396 Series III / 3395B integrators
5061-3378	Remote Cable
01046-60201	Agilent module to general purpose

BCD cables

p/n	Description
03396-60560	Agilent module to 3396 integrators
G1351-81600	Agilent module to general purpose

CAN cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

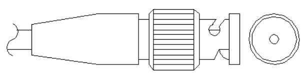
LAN cables

p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

RS-232 cables

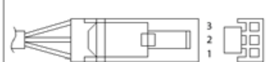
p/n	Description
G1530-60600	RS-232 cable, 2 m
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It's also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

Analog Cables

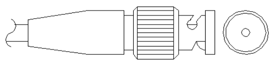


One end of these cables provides a BNC connector to be connected to Agilent modules. The other end depends on the instrument to which connection is being made.

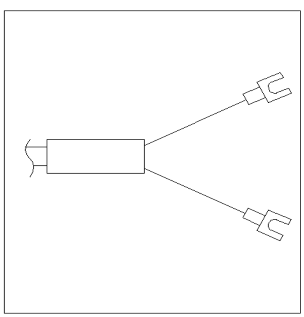
Agilent Module to 3394/6 Integrators

p/n 35900-60750	Pin 3394/6	Pin Agilent module	Signal Name
	1		Not connected
	2	Shield	Analog -
	3	Center	Analog +

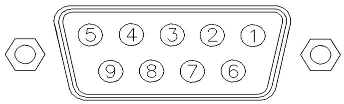
Agilent Module to BNC Connector

p/n 8120-1840	Pin BNC	Pin Agilent module	Signal Name
	Shield	Shield	Analog -
	Center	Center	Analog +

Agilent Module to General Purpose

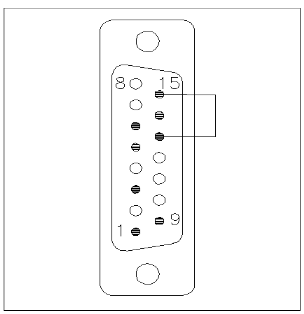
p/n 01046-60105	Pin	Pin Agilent module	Signal Name
	1		Not connected
	2	Black	Analog -
	3	Red	Analog +

Remote Cables



One end of these cables provides a Agilent Technologies APG (Analytical Products Group) remote connector to be connected to Agilent modules. The other end depends on the instrument to be connected to.

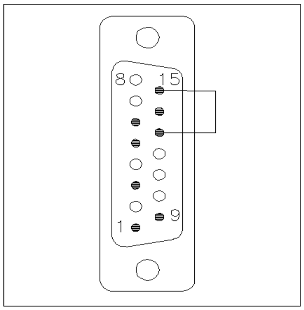
Agilent Module to 3396A Integrators

p/n 03394-60600	Pin 3396A	Pin Agilent module	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

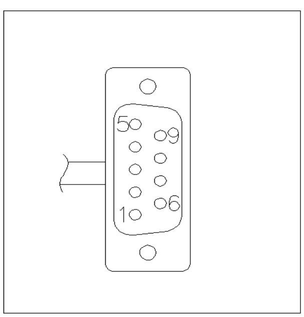
Agilent Module to 3396 Series II / 3395A Integrators

Use the cable Agilent module to 3396A Series I integrators (03394-60600) and cut pin #5 on the integrator side. Otherwise the integrator prints START; not ready.

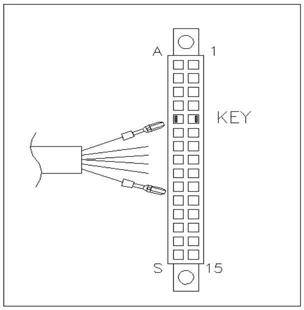
Agilent Module to 3396 Series III / 3395B Integrators

p/n 03396-61010	Pin 33XX	Pin Agilent module	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	14	7 - Red	Ready	High
	4	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

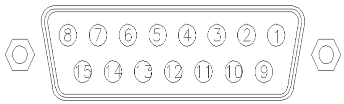
Agilent Module to Agilent 35900 A/D Converters

p/n 5061-3378	Pin 35900 A/D	Pin Agilent module	Signal Name	Active (TTL)
	1 - White	1 - White	Digital ground	
	2 - Brown	2 - Brown	Prepare run	Low
	3 - Gray	3 - Gray	Start	Low
	4 - Blue	4 - Blue	Shut down	Low
	5 - Pink	5 - Pink	Not connected	
	6 - Yellow	6 - Yellow	Power on	High
	7 - Red	7 - Red	Ready	High
	8 - Green	8 - Green	Stop	Low
	9 - Black	9 - Black	Start request	Low

Agilent Module to General Purpose

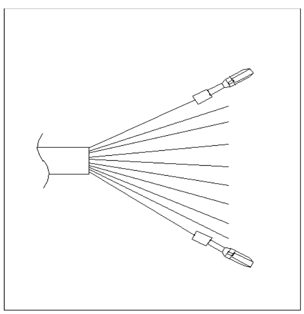
p/n 01046-60201	Wire Color	Pin Agilent module	Signal Name	Active (TTL)
	White	1	Digital ground	
	Brown	2	Prepare run	Low
	Gray	3	Start	Low
	Blue	4	Shut down	Low
	Pink	5	Not connected	
	Yellow	6	Power on	High
	Red	7	Ready	High
	Green	8	Stop	Low
	Black	9	Start request	Low

BCD Cables

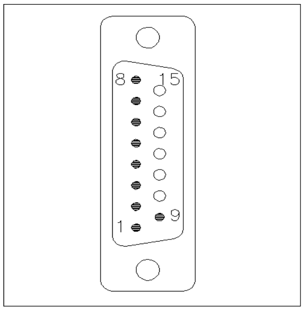


One end of these cables provides a 15-pin BCD connector to be connected to the Agilent modules. The other end depends on the instrument to be connected to

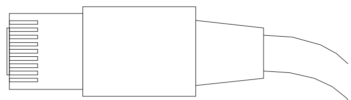
Agilent Module to General Purpose

p/n G1351-81600	Wire Color	Pin Agilent module	Signal Name	BCD Digit
	Green	1	BCD 5	20
	Violet	2	BCD 7	80
	Blue	3	BCD 6	40
	Yellow	4	BCD 4	10
	Black	5	BCD 0	1
	Orange	6	BCD 3	8
	Red	7	BCD 2	4
	Brown	8	BCD 1	2
	Gray	9	Digital ground	Gray
	Gray/pink	10	BCD 11	800
	Red/blue	11	BCD 10	400
	White/green	12	BCD 9	200
	Brown/green	13	BCD 8	100
	not connected	14		
	not connected	15	+ 5 V	Low

Agilent Module to 3396 Integrators

p/n 03396-60560	Pin 3396	Pin Agilent module	Signal Name	BCD Digit
	1	1	BCD 5	20
	2	2	BCD 7	80
	3	3	BCD 6	40
	4	4	BCD 4	10
	5	5	BCD0	1
	6	6	BCD 3	8
	7	7	BCD 2	4
	8	8	BCD 1	2
	9	9	Digital ground	
	NC	15	+ 5 V	Low

CAN/LAN Cables



Both ends of this cable provide a modular plug to be connected to Agilent modules CAN or LAN connectors.

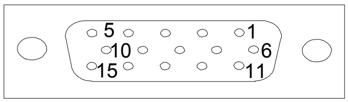
CAN Cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

LAN Cables

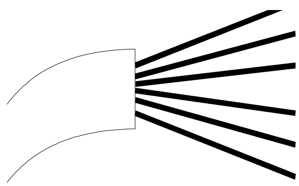
p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

External Contact Cable



One end of this cable provides a 15-pin plug to be connected to Agilent modules interface board. The other end is for general purpose.

Agilent Module Interface Board to general purposes

p/n G1103-61611	Color	Pin Agilent module	Signal Name
	White	1	EXT 1
	Brown	2	EXT 1
	Green	3	EXT 2
	Yellow	4	EXT 2
	Grey	5	EXT 3
	Pink	6	EXT 3
	Blue	7	EXT 4
	Red	8	EXT 4
	Black	9	Not connected
	Violet	10	Not connected
	Grey/pink	11	Not connected
	Red/blue	12	Not connected
	White/green	13	Not connected
	Brown/green	14	Not connected
	White/yellow	15	Not connected

Agilent Module to PC

p/n	Description
G1530-60600	RS-232 cable, 2 m
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It's also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

12
Hardware Information

This chapter describes the detector in more detail on hardware and electronics.

Firmware Description

The firmware of the instrument consists of two independent sections:

- a non-instrument specific section, called *resident system*
- an instrument specific section, called *main system*

Resident System

This resident section of the firmware is identical for all Agilent 1100/1200/1220/1260/1290 series modules. Its properties are:

- the complete communication capabilities (CAN, LAN and RS-232C)
- memory management
- ability to update the firmware of the 'main system'

Main System

Its properties are:

- the complete communication capabilities (CAN, LAN and RS-232C)
- memory management
- ability to update the firmware of the 'resident system'

In addition the main system comprises the instrument functions that are divided into common functions like

- run synchronization through APG remote,
- error handling,
- diagnostic functions,
- or module specific functions like
    - internal events such as lamp control, filter movements,
    - raw data collection and conversion to absorbance.

Firmware Updates

Firmware updates can be done using your user interface:

- PC and Firmware Update Tool with local files on the hard disk
- Instant Pilot (G4208A) with files from a USB Flash Disk
- Agilent Lab Advisor software B.01.03 and above

The file naming conventions are:

PPPP_RVVV_XXX.dlb, where

PPPP is the product number, for example, 1315AB for the G1315A/B DAD,

R the firmware revision, for example, A for G1315B or B for the G1315C DAD,

VVV is the revision number, for example 102 is revision 1.02,

XXX is the build number of the firmware.

For instructions on firmware updates refer to section *Replacing Firmware* in chapter *"Maintenance"* or use the documentation provided with the *Firmware Update Tools*.

NOTE Update of main system can be done in the resident system only. Update of the resident system can be done in the main system only.

Main and resident firmware must be from the same set.

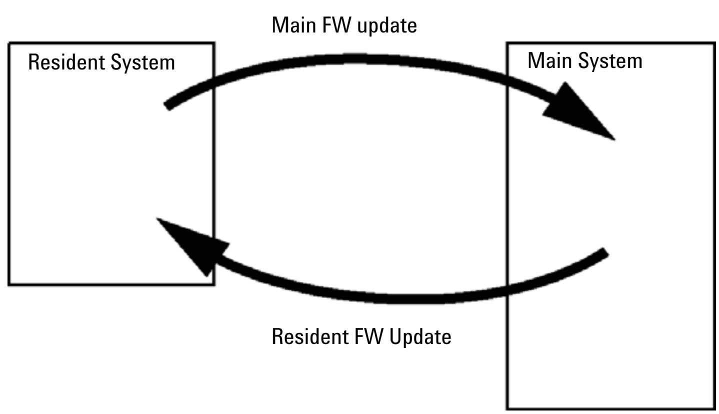


Figure 72 Firmware Update Mechanism

NOTE

Some modules are limited in downgrading due to their main board version or their initial firmware revision. For example, a G1315C DAD SL cannot be downgraded below firmware revision B.01.02 or to a A.xx.xx.

Some modules can be re-branded (e.g. G1314C to G1314B) to allow operation in specific control software environments. In this case the feature set of the target type are use and the feature set of the original are lost. After re-branding (e.g. from G1314B to G1314C), the original feature set is available again.

All these specific informations are described in the documentation provided with the firmware update tools.

The firmware update tools, firmware and documentation are available from the Agilent web.

- http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761

Optional Interface Boards

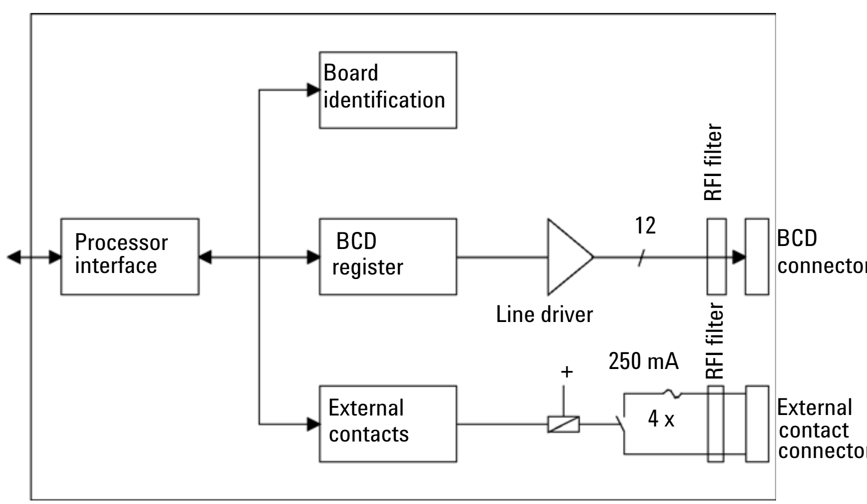
BCD / External Contact Board

The Agilent 1200 Infinity Series modules have one optional board slot that allows to add an interface board to the modules. Some modules do not have this interface slot. Refer to "Interfaces" on page 214 for details.

Optional Interface Boards

p/n	Description
G1351-68701	Interface board (BCD) with external contacts and BCD outputs
2110-0004	Fuse for BCD board, 250 mA

The BCD board provides a BCD output for the bottle number of the Agilent 1200 Series autosampler and four external contacts. The external contact closure contacts are relay contacts. The maximum settings are: 30 V (AC/DC); 250 mA (fused).



There are general purpose cables available to connect the BCD output, see "BCD Cables" on page 197 and the external outputs, see "External Contact Cable" on page 200 to external devices.

Table 29 Detailed connector layout (1200)

Pin	Signal name	BCD digit
1	BCD 5	20
2	BCD 7	80
3	BCD 6	40
4	BCD 4	10
5	BCD 0	1
6	BCD 3	8
7	BCD 2	4
8	BCD 1	2
9	Digital ground	
10	BCD 11	800
11	BCD 10	400
12	BCD 9	200
13	BCD 8	100
15	+5V	Low

LAN Communication Interface Board

The Agilent modules have one optional board slot that allows to add an interface board to the modules. Some modules do not have this interface slot. Refer to “Interfaces” on page 214 for details.

p/n	Description	
	G1369B or G1369-60002	Interface board (LAN)
OR	G1369C or G1369-60012	Interface board (LAN)

NOTE One board is required per Agilent 1260 Infinity instrument. It is recommended to add the LAN board to the detector with highest data rate.

NOTE For the configuration of the G1369 LAN Communication Interface card refer to its documentation.

The following cards can be used with the Agilent 1260 Infinity modules.

Table 30 LAN Boards

Type	Vendor	Supported networks
Interface board (LAN) (G1369B or G1369-60002) or Interface board (LAN) (G1369C or G1369-60012)	Agilent Technologies	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) *recommended for re-ordering*
LAN Communication Interface board (G1369A or G1369-60001)	Agilent Technologies	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) (*obsolete*)
J4106A[1]	Hewlett Packard	Ethernet/802.3, RJ-45 (10Base-T)
J4105A[1]	Hewlett Packard	Token Ring/802.5, DB9, RJ-45 (10Base-T)
J4100A[1]	Hewlett Packard	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) + BNC (10Base2)

1 These cards may be no longer orderable. Minimum firmware of these Hewlett Packard JetDirect cards is A.05.05.

Recommended LAN Cables

p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

Electrical Connections

- The CAN bus is a serial bus with high speed data transfer. The two connectors for the CAN bus are used for internal module data transfer and synchronization.
- Two independent analog outputs provide signals for integrators or data handling.
- 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 Agilent 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 module from a computer through a RS-232C connection. This connector is activated and can be configured with the configuration switch.
- The power input socket accepts a line voltage of 100 – 240 VAC ± 10 % with a line frequency of 50 or 60 Hz. Maximum power consumption varies by module. There is no voltage selector on your module because the power supply has wide-ranging capability. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

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

Rear view of the module

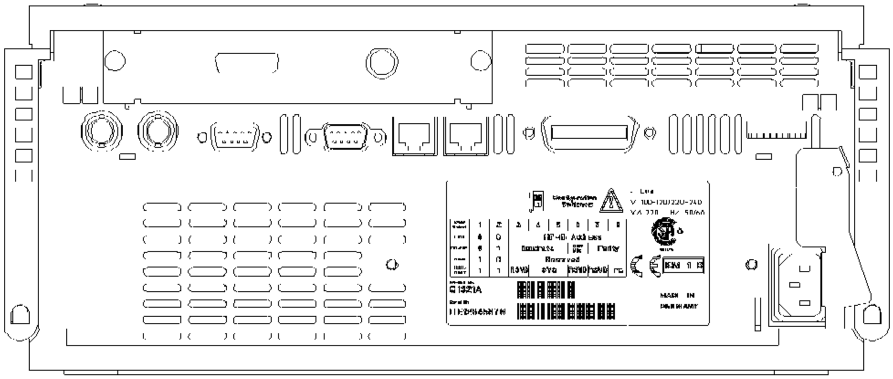


Figure 73 Rear View of Detector – Electrical Connections and Label

NOTE The GPIB interface has been removed with the introduction of the 1260 Infinity modules.

Serial Number Information

Serial Number Information 1260 Infinity

The serial number information on the instrument labels provide the following information:

CCXZZ00000	Format
CC	Country of manufacturing • DE = Germany • JP = Japan • CN = China
X	Alphabetic character A-Z (used by manufacturing)
ZZ	Alpha-numeric code 0-9, A-Z, where each combination unambiguously denotes a module (there can be more than one code for the same module)
00000	Serial number

Serial Number Information 1200 Series and 1290 Infinity

The serial number information on the instrument labels provide the following information:

CCYWWSSSSS	Format
CC	country of manufacturing • DE = Germany • JP = Japan • CN = China
YWW	year and week of last major manufacturing change, e.g. 820 could be week 20 of 1998 or 2008
SSSSS	real serial number

Interfaces

The Agilent 1200 Infinity Series modules provide the following interfaces:

Table 31 Agilent 1200 Infinity Series Interfaces

Module	CAN	LAN/BCD (optional)	LAN (on-board)	RS-232	Analog	APG Remote	Special
Pumps							
G1310B Iso Pump G1311B Quat Pump G1311C Quat Pump VL G1312B Bin Pump G1312C Bin Pump VL 1376A Cap Pump G2226A Nano Pump G5611A Bio-inert Quat Pump	2	Yes	No	Yes	1	Yes	
G4220A/B Bin Pump G4204A Quat Pump	2	No	Yes	Yes	No	Yes	CAN-DC- OUT for CAN slaves
G1361A Prep Pump	2	Yes	No	Yes	No	Yes	CAN-DC- OUT for CAN slaves
Samplers							
G1329B ALS G2260A Prep ALS	2	Yes	No	Yes	No	Yes	THERMOSTAT for G1330B
G1364B FC-PS G1364C FC-AS G1364D FC-µS G1367E HiP ALS G1377A HiP micro ALS G2258A DL ALS G5664A Bio-inert FC-AS G5667A Bio-inert Autosampler	2	Yes	No	Yes	No	Yes	THERMOSTAT for G1330B CAN-DC- OUT for CAN slaves
G4226A ALS	2	Yes	No	Yes	No	Yes	

Table 31 Agilent 1200 Infinity Series Interfaces

Module	CAN	LAN/BCD (optional)	LAN (on-board)	RS-232	Analog	APG Remote	Special
Detectors							
G1314B VWD VL G1314C VWD VL+	2	Yes	No	Yes	1	Yes	
G1314E/F VWD	2	No	Yes	Yes	1	Yes	
G4212A/B DAD	2	No	Yes	Yes	1	Yes	
G1315C DAD VL+ G1365C MWD G1315D DAD VL G1365D MWD VL	2	No	Yes	Yes	2	Yes	
G1321B FLD	2	Yes	No	Yes	2	Yes	
G1362A RID	2	Yes	No	Yes	1	Yes	
G4280A ELSD	No	No	No	Yes	Yes	Yes	EXT Contact AUTOZERO
Others							
G1170A Valve Drive	2	No	No	No	No	No	[1]
G1316A/C TCC	2	No	No	Yes	No	Yes	
G1322A DEG	No	No	No	No	No	Yes	AUX
G1379B DEG	No	No	No	Yes	No	Yes	
G4225A DEG	No	No	No	Yes	No	Yes	
G4227A Flex Cube	2	No	No	No	No	No	CAN-DC- OUT for CAN slaves [1]
G4240A CHIP CUBE	2	Yes	No	Yes	No	Yes	CAN-DC- OUT for CAN slaves THERMOSTAT for G1330A/B (NOT USED)

[1] Requires a HOST module with on-board LAN (e.g. G4212A or G4220A with minimum firmware B.06.40 or C.06.40) or with additional G1369C LAN Card

NOTE The detector (DAD/MWD/FLD/VWD/RID) is the preferred access point for control via LAN. The inter-module communication is done via CAN.

- CAN connectors as interface to other modules
- LAN connector as interface to the control software
- RS-232C as interface to a computer
- REMOTE connector as interface to other Agilent products
- Analog output connector(s) for signal output

Overview Interfaces

CAN

The CAN is inter-module communication interface. It is a 2-wire serial bus system supporting high speed data communication and real-time requirement.

LAN

The modules have either an interface slot for an LAN card (e.g. Agilent G1369B/C LAN Interface) or they have an on-board LAN interface (e.g. detectors G1315C/D DAD and G1365C/D MWD). This interface allows the control of the module/system via a PC with the appropriate control software. Some modules have neither on-board LAN nor an interface slot for a LAN card (e.g. G1170A Valve Drive or G4227A Flex Cube). These are hosted modules and require a Host module with firmware B.06.40 or later or with additional G1369C LAN Card.

NOTE If an Agilent detector (DAD/MWD/FLD/VWD/RID) is in the system, the LAN should be connected to the DAD/MWD/FLD/VWD/RID (due to higher data load). If no Agilent detector is part of the system, the LAN interface should be installed in the pump or autosampler.

RS-232C (Serial)

The RS-232C connector is used to control the module from a computer through RS-232C connection, using the appropriate software. This connector can be configured with the configuration switch module at the rear of the module. Refer to *Communication Settings for RS-232C*.

NOTE There is no configuration possible on main boards with on-board LAN. These are pre-configured for

- 19200 baud,
- 8 data bit with no parity and
- one start bit and one stop bit are always used (not selectable).

The RS-232C is designed as DCE (data communication equipment) with a 9-pin male SUB-D type connector. The pins are defined as:

Table 32 RS-232C Connection Table

Pin	Direction	Function
1	In	DCD
2	In	RxD
3	Out	TxD
4	Out	DTR
5		Ground
6	In	DSR
7	Out	RTS
8	In	CTS
9	In	RI

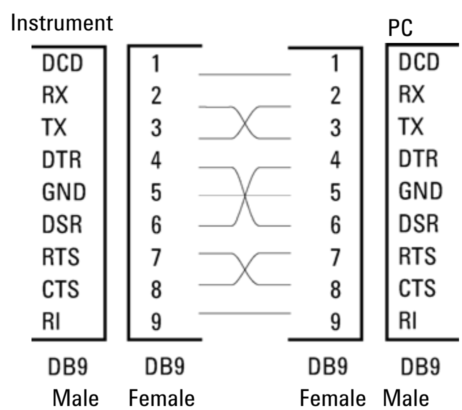


Figure 74 RS-232 Cable

Analog Signal Output

The analog signal output can be distributed to a recording device. For details refer to the description of the module's main board.

APG Remote

The APG Remote connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features as common shut down, prepare, and so on.

Remote control allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

The subminiature D connector is used. The module provides one remote connector which is inputs/outputs (wired- or technique).

To provide maximum safety within a distributed analysis system, one line is dedicated to **SHUT DOWN** the system's critical parts in case any module detects a serious problem. To detect whether all participating modules are switched on or properly powered, one line is defined to summarize the **POWER ON** state of all connected modules. Control of analysis is maintained by signal readiness **READY** for next analysis, followed by **START** of run and optional **STOP** of run triggered on the respective lines. In addition **PREPARE** and **START REQUEST** may be issued. The signal levels are defined as:

- standard TTL levels (0 V is logic true, + 5.0 V is false),
- fan-out is 10 ,
- input load is 2.2 kOhm against + 5.0 V, and
- output are open collector type, inputs/outputs (wired- or technique).

NOTE All common TTL circuits operate with a 5 V power supply. A TTL signal is defined as "low" or L when between 0 V and 0.8 V and "high" or H when between 2.0 V and 5.0 V (with respect to the ground terminal).

Table 33 Remote Signal Distribution

Pin	Signal	Description
1	DGND	Digital ground
2	PREPARE	(L) Request to prepare for analysis (for example, calibration, detector lamp on). Receiver is any module performing pre-analysis activities.
3	START	(L) Request to start run / timetable. Receiver is any module performing run-time controlled activities.
4	SHUT DOWN	(L) System has serious problem (for example, leak: stops pump). Receiver is any module capable to reduce safety risk.
5		Not used
6	POWER ON	(H) All modules connected to system are switched on. Receiver is any module relying on operation of others.
7	READY	(H) System is ready for next analysis. Receiver is any sequence controller.
8	STOP	(L) Request to reach system ready state as soon as possible (for example, stop run, abort or finish and stop injection). Receiver is any module performing run-time controlled activities.
9	START REQUEST	(L) Request to start injection cycle (for example, by start key on any module). Receiver is the autosampler.

Special Interfaces

There is no special interface for this module.

Setting the 8-bit Configuration Switch (without On-board LAN)

The 8-bit configuration switch is located at the rear of the module.

This module does not have its own on-board LAN interface. It can be controlled through the LAN interface of another module, and a CAN connection to that module.

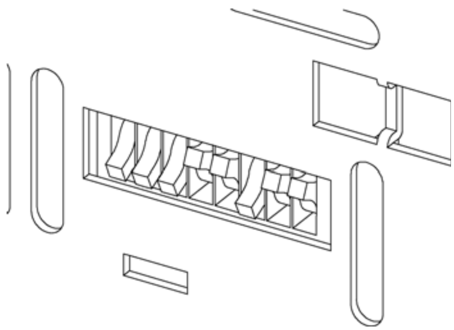


Figure 75 Configuration switch (settings depend on configured mode)

All modules without on-board LAN:

- default should be ALL DIPS DOWN (= best settings)
  - Bootp mode for LAN and
  - 19200 baud, 8 data bit / 1 stop bit with no parity for RS-232
- DIP 1 DOWN and DIP 2 UP allows special RS-232 settings
- for boot/test modes DIPS 1+2 must be UP plus required mode

NOTE For normal operation use the default (best) settings.

Switch settings provide configuration parameters for serial communication protocol and instrument specific initialization procedures.

NOTE With the introduction of the Agilent 1260 Infinity, all GPIB interfaces have been removed. The preferred communication is LAN.

NOTE The following tables represent the configuration switch settings for the modules without on-board LAN only.

Table 34 8-bit Configuration Switch (without on-board LAN)

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baudrate			Data Bits	Parity	
Reserved	1	0	Reserved					
TEST/BOOT	1	1	RSVD	SYS		RSVD	RSVD	FC

NOTE The LAN settings are done on the LAN Interface Card G1369B/C. Refer to the documentation provided with the card.

Communication Settings for RS-232C

The communication protocol used in the column compartment supports only hardware handshake (CTS/RTR).

Switches 1 in down and 2 in up position define that the RS-232C parameters will be changed. Once the change has been completed, the column instrument must be powered up again in order to store the values in the non-volatile memory.

Table 35 Communication Settings for RS-232C Communication (without on-board LAN)

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baudrate			Data Bits	Parity	

Use the following tables for selecting the setting which you want to use for RS-232C communication. The number 0 means that the switch is down and 1 means that the switch is up.

Table 36 Baudrate Settings (without on-board LAN)

Switches			Baud Rate	Switches			Baud Rate
3	4	5		3	4	5	
0	0	0	9600	1	0	0	9600
0	0	1	1200	1	0	1	14400
0	1	0	2400	1	1	0	19200
0	1	1	4800	1	1	1	38400

Table 37 Data Bit Settings (without on-board LAN)

Switch 6	Data Word Size
0	7 Bit Communication
1	8 Bit Communication

Table 38 Parity Settings (without on-board LAN)

Switches		Parity
7	8	
0	0	No Parity
0	1	Odd Parity
1	1	Even Parity

One start bit and one stop bit are always used (not selectable).

Per default, the module will turn into 19200 baud, 8 data bit with no parity.

Special Settings

The special settings are required for specific actions (normally in a service case).

Boot-Resident

Firmware update procedures may require this mode in case of firmware loading errors (main firmware part).

If you use the following switch settings and power the instrument up again, the instrument firmware stays in the resident mode. It is not operable as a module. It only uses basic functions of the operating system for example, for communication. In this mode the main firmware can be loaded (using update utilities).

Table 39 Boot Resident Settings (without on-board LAN)

Mode Select	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8
TEST/BOOT	1	1	0	0	1	0	0	0

Forced Cold Start

A forced cold start can be used to bring the module into a defined mode with default parameter settings.

CAUTION

Loss of data

Forced cold start erases all methods and data stored in the non-volatile memory. Exceptions are calibration settings, diagnosis and repair log books which will not be erased.

→ Save your methods and data before executing a forced cold start.

If you use the following switch settings and power the instrument up again, a forced cold start has been completed.

Table 40 Forced Cold Start Settings (without on-board LAN)

Mode Select	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8
TEST/BOOT	1	1	0	0	1	0	0	1

Early Maintenance Feedback

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 module 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

EMF counters increment with use and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. Some counters can be reset to zero after the required maintenance procedure.

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 maintenance cycle is dependent on the requirements for use. 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 the default **EMF** limits should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by the **EMF 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.

Instrument Layout

The industrial design of the module 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 module 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.

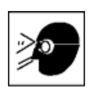
13 Appendix

This chapter provides safetey and other general information.

General Safety Information

Safety Symbols

Table 41 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect risk of harm to the operator and 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.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

→ 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 loss of data, or damage of equipment.

→ Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

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.

Safety Standards

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

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 fuse holders must be avoided.

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 whenever possible. When inevitable, this has to 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, observe appropriate safety procedures (for example, 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.

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.

Lithium 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.

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

UV-Radiation (UV-lamps only)

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 42 UV-Radiation Limits

Exposure/day	Effective Irradiance
8 hours	0.1 µW/cm2
10 minutes	5.0 µW/cm2

Typically the radiation values are much smaller than these limits:

Table 43 UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50-cm distance	average 0.016 µW/cm2
Lamp installed, 50-cm distance	maximum 0.14 µW/cm2

Solvent Information

Flow Cell

To protect optimal functionality of your 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.
- If the flow cell is transported while temperatures are below 5 °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 a small % of organic solvents (e.g. acetonitrile or methanol ~5 %).

Use of Solvents

Observe the following recommendations on the use of solvents.

- Brown glass ware can avoid growth of algae.
- Small particles can permanently block capillaries and valves. Therefore always filter solvents through 0.4 µm filters.
- 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 sulfuric acid and nitric acid, especially at higher temperatures (if your chromatography method allows, replace 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:

    $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

    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,
- Solvents containing strong complexing agents (e.g. EDTA),
- Mixtures of carbon tetrachloride with 2-propanol or THF.

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http://www.agilent.com

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In This Book

This manual contains technical reference information about the Agilent 1260 Infinity Fluorescence Detector G1321B and Agilent 1200 Series Fluorescence Detector G1321A (obsolete).

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

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