Agilent Max-Light Cartridge Cell Information for G4212A and G4212B DAD

This document should give you some guidance on how to use the Agilent Max-Light Cartridge Cells for the Agilent 1290/1260 Infinity Diode Array Detector.

Agilent Technologies

Information about the Max-Light Cartridge Cells

NOTE The flow independent information can be used for the Max-Light Cartridge Test Cell as well.

Specifications

Table 1 Specifications for Max-Light Cartridge Flow Cells

Cartridge Cells	G4212-60008 (10 mm, V(σ) = 1.0 µL), for details see 1200 Infinity Series DAD Manual) G5615-60018 (10 mm, V(σ)= 1.0 µL) Bio-inert, for details see page 7 G4212-60007 (60 mm, V(σ)= 4.0 µL), for details see page 6 G5615-60017 (60 mm, V(σ) = 4.0 µL) Bio-inert, for details see page 7 G4212-60032 (3.7 mm, V(σ) = 0.9 µL) HDR, for details see page 8 G4212-60038 (10 mm, V(σ) = 0.6 µL) ULD, for details see page 8 G4212-60011 (Test Cell)
Maximum pressure	60 bar (870 psi)
pH range	1.0-12.5 (solvent dependent)

For the definition of V(σ) see "Description of the dispersion volume V(σ) of" on page 2.

Description of the dispersion volume V(σ) of

Dispersion in HPLC systems is not only the simple cumulative effect of subcomponent volumes like fittings, tubing, flow cells, valves, etc. The geometric shape of the cavities as well as the characteristics of flow through those components have to be considered. Laminar mixing, turbulent mixing, non-ideal flow patterns as well as basic dynamic parameters like flow rate, viscosity and diffusion affect the Newtonian flow and contribute to extra column dispersion and related loss of resolution and/or sensitivity.

Rather than describing the extra column contribution of the flow cell by simply specifying the geometric cell volume, Agilent provides an dispersion effective cell volume V(σ), which is derived as follows:

V(σ) has been experimentally determined by the injection of 50 nl Thiourea in a flow of 10%H2O/90%ACN at a flow rate of 0.5 mL/min. The corresponding chromatographic peak has been evaluated by measuring the tangent width, which is then multiplied by the flow rate and divided by a factor of 4 to achieve the V(σ) value.

It should be pointed out that the sample injection has not been done with a standard auto sampler and system capillaries. To minimize the system related extra column contributions the sample has been injected by a special micro injection device (fast switching valve with 50 nL micro groove) with a 50 µm ID capillary connected to the cell fitting. The remaining system contribution to the dispersion has been checked and determined by replacing the UV cell under test by a nano dispersion cell with 13 nL volume.

Recommendations

For G4212-60007 and G4212-60008

The use of Peek-FS capillaries is not recommended. In combination with the SST zero dead volume fitting (e.g. at the inlet) the capillary could break and the glass particles could block/damage the flow cell.

Inline Pressure Relief Valve Kit (G4212-68001)

When several detectors are installed in a system the connecting capillary and fittings between the detectors must be carefully chosen to keep chromatographic influence on peak shape small. On the other hand narrow bore connection capillaries generate a significant pressure drop dependent on flow rate and solvent properties.

The pressure relief valve is designed to protect the flow cell of a Agilent 1200 Infinity Diode Array Detector (G4212A/B).

Agilent strongly recommends installing the pressure relief valve at the outlet of the detector as soon as a second detector is installed like in LC/MS applications.

Application Information

For the analysis and characterization of proteins and large biomolecules for SEC, AEX and RP applications add 100 mM salt into mobile phase or 10% organic to prevent secondary interaction

For cation exchange chromatography the usage of an Agilent Diode Array Detector G1315C/D with the respective bio-inert flow cell is highly recommended to avoid unspecific interaction of the protein with the flow cell

For applications with mobile phases of a pH above 12.5 use an Agilent Diode Array Detector G1315C/D and the respective bio-inert flow cell.

Installation

1. Unpack the Max-Light Cartridge Cell.
2. Remove the plastic plugs from the Max-Light Cartridge Flow Cell inlet and outlet

3 Remove the black hoods, that secure the cell light inlet and outlet.

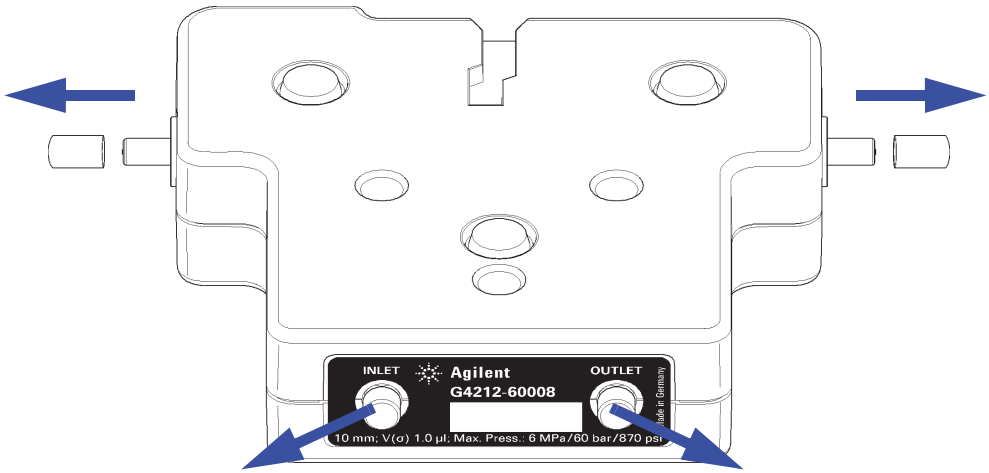

Figure 1 Remove hoods and plugs from Max-Light Cartridge Cell

NOTE Never touch the cell light input and outlet. Contamination on the window will reduce the performance of the Max-Light Cartridge Cell (intensity, wavelength accuracy).

4 Keep the hoods and plugs for later use in case the Max-Light Cartridge Cell is removed from the detector and stored for some time.

NOTE The hoods protect the cell light inlet and outlet against contamination.

5 Place the Max-Light Cartridge Cell in the cell cartridge drawer of the detector and connect the capillaries as described in the detector's *User Manual*.

During Use

1 Do not let buffers stay for long times in the Max-Light Cartridge Flow Cell. Flush the Max-Light Cartridge Flow Cell when finished with the application.

2 In case of a leak occurs due to an overpressure situation, reduce the flow/pressure. If the leak continues afterwards, the Max-Light Cartridge Flow Cell must be replaced (no repair possible).

Storage

1 Flush the Max-Light Cartridge Flow Cell with iso-propanol or methanol and insert the plugs into the cell inlet and outlet.

2 Remove the Max-Light Cartridge Cell from the flow cell cartridge drawer of the detector as described in the detector's *User Manual*.

3 Replace the black hoods, that secure the cell light inlet and outlet.

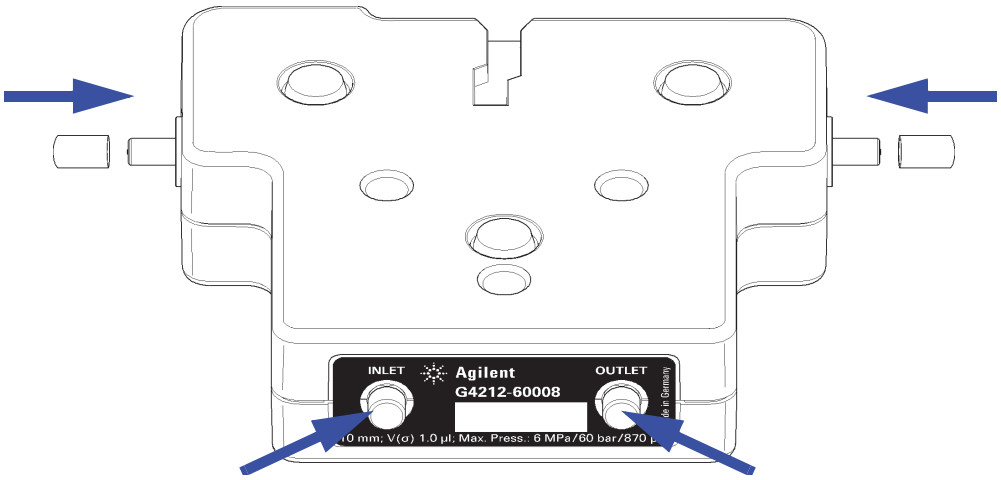

Figure 2 Replace the plugs and hoods

4 Store the Max-Light Cartridge Cell in safe location.

Cleaning

Please refer to detector's *User Manual* for information on cleaning the Max-Light Cartridge Cell.

Special Information of 60 mm Cartridge Flow Cell

Application Information

The geometrical volume of the 60 mm cell is 6 times larger than the 10 mm cell. However, the chromatographic relevant dispersion volume, the square roots of variances, accounting for cell specific geometrical volume shape and fluidic flow pattern, have been determined as $V(\sigma) = 4$ µl and $V(\sigma) = 1$ µl in for the 10 mm cell.

Due to the larger dispersion volume, the 60 mm cell is primarily designed for 4.6 mm column applications to achieve highest sensitivity with no additional peak broadening. However, if sensitivity is important the 60 mm cell will also be advantageous in case of smaller columns (3 mm, 2.1 mm) but depending on the chromatographic system and method additional peak broadening might occur.

The upper limit of concentration

Care should be taken in methods where high background absorption of solvents or modifiers are present. When using the 60 mm cell the detector will measure 6 times the background absorption as in case of the 10 mm cell, which will reduce the remaining dynamic absorbance range for sample peaks. Furthermore those UV absorbing modifiers could compromise the sensitivity gain (signal/noise) of 60 mm cell.

The linearity limit of the detector is seen at about 2 AU for both, the 10 mm and the 60 mm Max-Light Cartridge Flow Cell. Using firmware revision B.06.25 and below, the 60 mm Max-Light Cartridge Cell linearity limit would be 333 mAU/cm.

Required Detector Firmware

For use of the 60 mm Max-Light Cartridge Flow Cell a detector firmware B.06.26 (introduced December 2009) or later is required.

NOTE If the 60 mm Max-Light Cartridge Flow Cell is used with detector firmware B.06.25 and below, the detector output (digital and analog) is normalized to 1 cm. This means the peak height would be the same as on the 10 mm Cartridge Flow Cell, the noise is reduced by a factor of 6 and the linearity limit would be 333 mAU/cm.

The firmware can be obtained from the Agilent web

https://www.chem.agilent.com/en-US/Support/Downloads/firmware/Pages/default.aspx

Lab Advisor (Utility) Software

At the time of the introduction of the Agilent Lab Advisor (Utility) Software B.01.03, introduced in 2009 with the Agilent 1290 Infinity LC, the limits for certain tests were not final for the 60 mm Cartridge Flow Cell.

NOTE

At shipment start (March 2010) the final specification for 60 mm Max-Light Cartridge Cell has not been set.

The typical noise specification is +/- 0.6 µAU/cm, measured at 254/360 nm with 4 nm slit and RT=4 s (TC=2 s).

Reference conditions:

- Wavelength: 254 nm/4 nm with Reference Wavelength 360 nm/100 nm, Slitwidth 4 nm, TC 2 s, (or with RT = 2.2 * TC), ASTM
- Max-Light Cartridge Cell (60 mm, σ_V = 4.0 µl) with flow of 0.5 ml/min LC grade water or Max-Light Cartridge Test Cell

As soon as the final specification have been set, the Agilent LabAdvisor (Utility) Software will be updated accordingly. This version B.01.04 was introduced July 2010.

NOTE

When the noise of the 60 mm Max-Light Cartridge Flow Cell is normalized to 1cm, the noise would be close to 1/6 of the 10 mm Max-Light Cartridge Flow Cell.

Elevated noise, especially at the lower spectral end, could occur in case of dirty flow cell or in case of high background absorption of solvents and modifiers or fluctuating residual gas concentrations.

Special Information for Bio-Inert Cartridge Flow Cells

For bio-inert applications use the specified BIO Max-Light Cartridge Flow Cell only, see “Specifications for Max-Light Cartridge Flow Cells" on page 2.

Both bio-inert Max-Light Cartridge Flow Cells include

- a Peek Capillary 1.5 m i.d. 0.18 mm (0890-1763) and
- PEEK Fittings 10/PK (5062-8541)

Recommendations

Assure that

- the capillary ends are right-angled when cutting capillaries
- no pliers or wrenches are used to fix the PEEK fittings at the flow cell
- no metal ferrules are used at the cell unions to prevent contaminations and damage
- the flow cell is bypassed when flush procedures at pH > 12.5 are used.

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Special Information on HDR and ULD Max-Light Cartridge Flow Cells

ASTM Drift and Noise Test, Cell Test, Intensity Test, Quick Noise Test, Self Test and Slit Test will be similar as with the Standard Max-Light Cartridge Flow Cell (10 mm / 60 mm).

NOTE Instrument specifications of the G4212A and G4212B DAD should be measured with the Standard 10 mm flow cell.

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Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn