Performance characteristics of the Agilent 1290 Infinity Diode Array Detector

Low noise, low refractive index, high speed and data security

Technical Overview

Introduction

The Agilent 1290 Infinity Diode Array Detector (DAD) has a new optical design, which uses a cartridge cell with optofluidic waveguide technology that offers high sensitivity with low dispersion. It provides a wide linear range and a very stable baseline for standard or ultrafast LC applications. The Agilent Max-Light cartridge cell dramatically increases the light transmission by using the principle of total internal reflection along a noncoated fused silica capillary, achieving a new level of sensitivity without sacrificing resolution through cell volume dispersion effects. This design minimizes baseline perturbations caused by refractive index variations within the cell, either generated by gradient analysis, temperature variations or solvent composition inhomogeneities. The stable baseline results in more reliable integration of peak areas.

In this Technical Note, the design is discussed along with the following performance data:

- ASTM drift and noise for the 10 and 60 mm path length cell
- Linearity over a wide range
- Limit of detection for anthracene for the 10 and 60 mm path length cell
- Spectral performance

Agilent Technologies

The new design

The new design of the Agilent 1290 Infinity Diode Array Detector (Figure 1) offers:

Lowest noise and highest linearity—with 10 mm Agilent Max-Light cartridge cell

- Ultra sensitivity—with unique 60 mm Agilent Max-Light high sensitivity cartridge cell
- More reliable and robust peak integration process due to less baseline drift
- Multiple wavelength and full spectral detection at a highest sampling rate (160 Hz)—keeps pace with the analysis speed of ultra-fast LC
- Programmable slit (1 to 8 nm)—provides optimum incident light conditions for rapid optimization of sensitivity, linearity and spectral resolution
- RFID tags for flow cell and lamps ensure data traceability and usage tracking
- Engineered for simplicity and ease of use—cartridge design allows fast, easy exchange of flow cell. Non-coated fused silica fibre cell optics for robust performance and handling

The Agilent 1290 Infinity DAD incorporates electronic temperature control to further enhance the resistance to temperature effects. Although the dispersion volume of the Agilent Max-Light Cartridge Cell (Figure 2) is very small ($V\sigma$ = 1 µL), the path length is a standard 10 mm. However, for even higher sensitivity the alternative Agilent Max-Light high sensitivity cell is available with a path length of 60 mm ($V\sigma$ = 4 µL). Cells are easily exchanged by sliding them in or out of the cell holder and they are auto-aligned in the optical bench. The DAD light source is a deuterium lamp and the operating wavelength range covered is 190 to 640 nm. This is detected by a diode array comprising 1024 diodes. The entrance to the spectrograph is through a programmable optical slit which can give a spectral resolution of 1 to 8 nm. This is generally operated in the middle of the range but can be closed down for optimization to 1 nm for high spectral resolution (rarely required in liquid phase UV spectra). It can also be opened up to 8 nm for maximum light transmission and minimum noise in the signal. The chromatographic signals are extracted from the diode array data within the firmware of the module. Up to 8 individual signals can be defined, each comprising a signal wavelength (nm), a diode bunching bandwidth, and if required, a reference wavelength and bandwidth. Signals can be output up to 160 Hz (160 data points/second) for accurate recording of the fastest (narrowest) chromatographic peaks. At the same time the module can also output full-range spectra to the data system at the same rate of 160 Hz. For regulated laboratories it is important that all the method parameters are recorded. The Agilent 1290 Infinity DAD not only records the instrument setpoints but also has radio-frequency identification tags (RFID) incorporated into the lamp and flowcell cartridge so that the identity and variables of these important components are also recorded by the system.

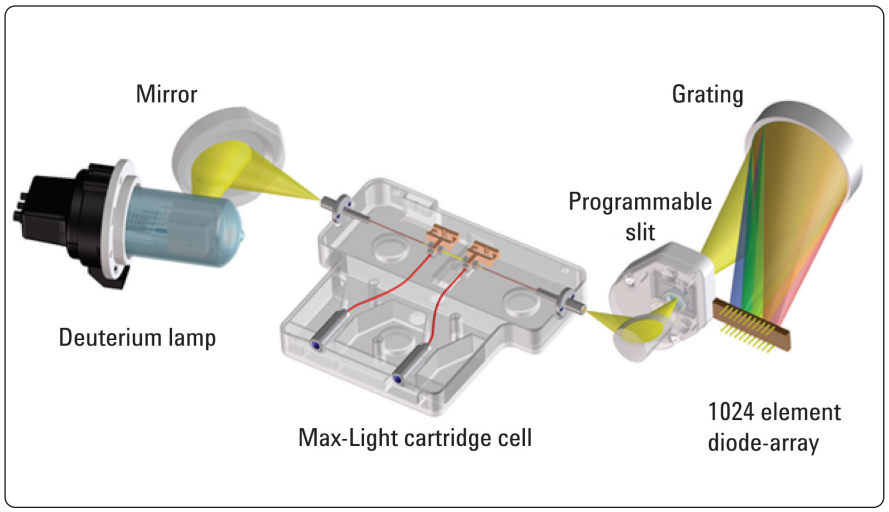


Figure 1
Design of the Agilent 1290 Infinity DAD.

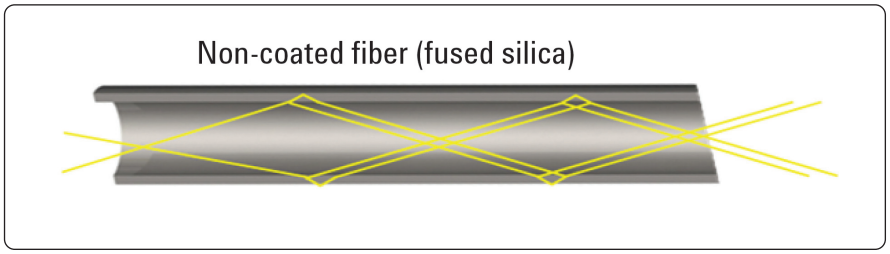


Figure 2
Max-Light Cartridge Cell - Optofluidic waveguides.

Equipment and material

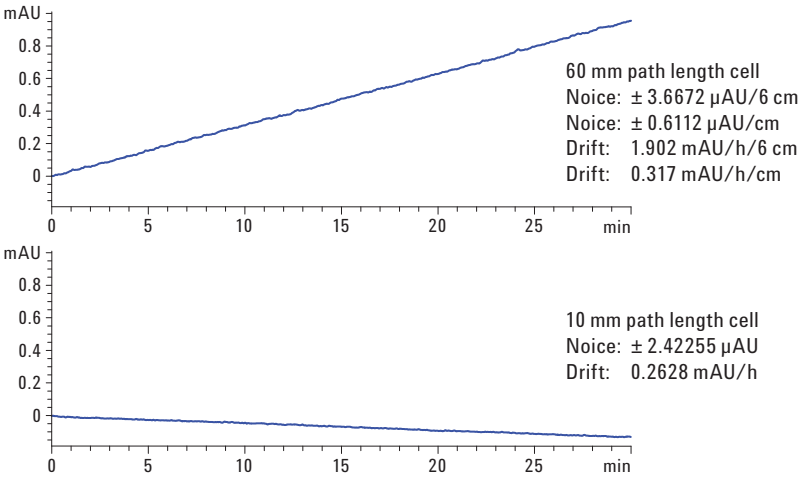
The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Agilent 1290 Infinity Binary Pump with vacuum degasser
- Agilent 1290 Infininty Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity Diode Array Detector for 160-Hz operation

Performance of diode array detection

Baseline noise ASTM and drift for the 10 mm and 60 mm path length cell

The ASTM noise and drift was evaluated using a restriction capillary as the column and water as the mobile phase. The detector was set to a 4-sec response time. The resulting ASTM noise for the 10 mm path length cell was found to be Noise = ±2.4 µAU and the drift was Drift = –0.26 mAU/h. The resulting ASTM noise for the 60 mm path length cell was found to be Noise = ±0.6 µAU/cm and the drift was Drift = 0.32 mAU/h/cm. In Figure 3 an example chromatogram is shown for the noise and drift behavior of both cells.



Conditions for ASTM on the Agilent 1290 Infinity LC system

Column:	Restriction capillary, backpressure 173 bar
DAD:	>0.20 min (4.0 sec response time) (1.25 Hz), 4 nm slit width, Sample wl = 254/4 nm, Ref wl= 360/100 nm
Column temperature:	36 °C
Flow rate :	0.5 mL/min

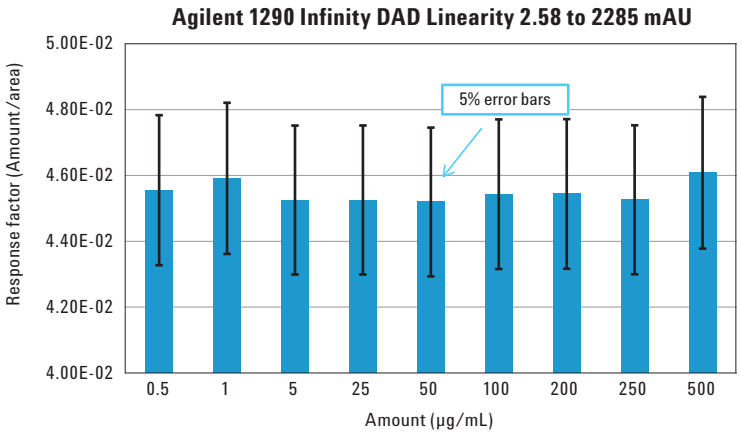
Figure 3
Determination of ASTM noise and drift on Agilent 1290 Infinity DAD.

Linearity for different caffeine concentrations

The linearity was tested using certified caffeine standards from 0.5 µg/mL to 500 µg/mL. For this concentration range, very good linearity was obtained. The coefficient of correlation was 0.99996. The response factors were all within the 5% error range over an absorbance range of 2.58 to 2285 mAU (Figure 4).

Influence of data rate

To obtain optimum results the data rate must be selected appropriately. If the data rate is too low all gained resolution is lost in the detector. If data rate is too high, the noise level may not be appropriate for the peak width obtained in the chromatogram and sensitivity is lost because the signal-to-noise ratio is decreasing. Therefore, the data rate should be selected so that 15 to 30 data points can be acquired over the fastest peak.



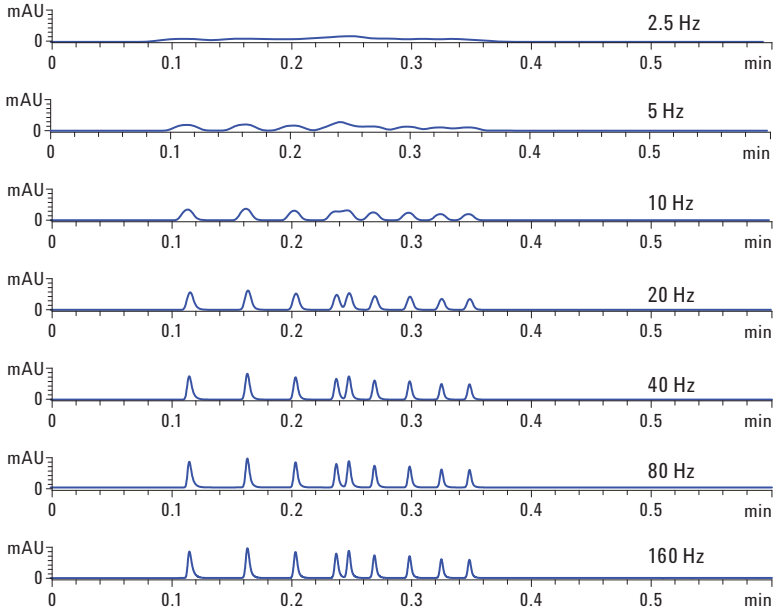
Chromatographic Method

Column:	Agilent ZORBAX RRHD SB C18, 50 mm × 2.1 mm, 1.8 µm
	Sample Caffeine with 0.5,1,5,25,50,100,200,250,500 µg/mL
Injection volume:	4 µl
Column temperature:	30 °C
Mobile Phases:	water (A) and acetonitrile (B)
Flow:	0.5 mL/min, 92% water, 8% acetonitrile isocratic
Stop time:	2.5 min
DAD:	273/10 nm, Ref 380/80, 20 Hz

Figure 4
Linearity of DAD using response factors.

In Figure 5 an example of an ultrafast application is shown with a peak width for the first peak of 184 msec. In this example, it is demonstrated that a data rate of 160 Hz is needed to get the most out of a chromatogram in terms of resolution, peak width and peak height. In Table 1, the results are combined.

The chromatographic conditions combine an ultrafast gradient with high flow rate and automatic delay volume reduction (ADVR) for the auto sampler.



Chromatographic method	
Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 50 mm × 2.1 mm, 1.8 µm
Sample:	Set of 9 compounds, 100 ng/uL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/mL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone (p/n 5188-6529)
Injection volume:	1µl with Automatic Delay Volume Reduction (ADVR)
Column temperature:	60 °C
Mobile phases:	water(A) and Acetonitrile (B)
Gradient:	at 0 min 35% B, at 0.3 min 95% B
Flow:	1.5 mL/min
Stop time:	0.6 min
DAD:	2.5 up to 160 Hz, 245/10 nm, Ref 360/80

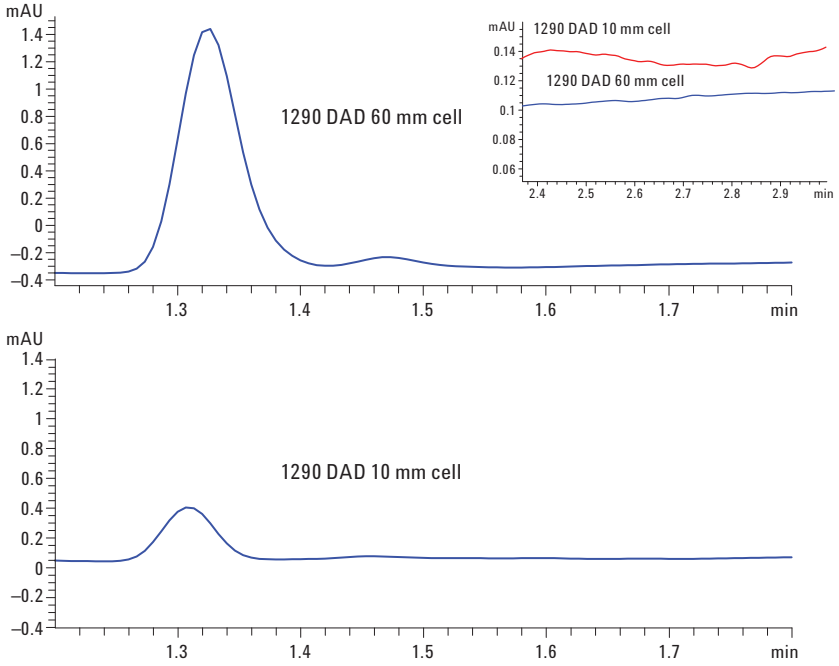
Figure 5
Influence of data rate on resolution and peak width.

Data rate (Hz)	Resolution peak 5	Peak width last peak (min)	Peak height (mAU) of peak 3
160	1.89	0.00307	1171.2
80	1.83	0.00323	1131.1
40	1.57	0.00381	1006.4
20	1.06	0.00565	738.6
10	0.56	0.0102	431.2
5	–	0.0203	217.1
2.5	–	–	–

Table 1
Influence of data rate on resolution, peak width and peak height.

Limit of detection for anthracene

The limit of detection for anthracene was evaluated using the DAD at 2.5 Hz and both available detector cells. The injected concentration was as low as 10.5 pg/µL. Figure 6 shows the overlaid chromatograms of both cells. The results are combined in Table 2. The limit of detection for a signal-to-noise ratio of 2 is 41 fg for the 60-mm path length detector cell.



Chromatographic method

Column:	Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm, 1.8 µm
Sample:	Anthracene 10.5 pg in 1 µL
Injection volume:	1 µL
Column temperature:	36 °C
Mobile phases:	Water and Acetonitrile
Isocratic:	Water/ACN = 35/65
Flow:	0.5 mL/min
Stop time:	3 min
DAD:	2.5 Hz, 251/4 nm, Ref 450/80, 8 mm slit width

Figure 6
LOD of Anthracene comparing the 10-mm and the 60-mm path length cell. Red is the 10-mm path length cell and blue the 60-mm path length cell.

Parameter	DAD 1290 60 mm cell	DAD 1290 10 mm cell
Noise PtoP (mAU)	0.0034908	0.004848
Peak height (mAU)	1.787442	0.357384
Signal/noise (mAU) keine Einheit	512	74
LOD (FG)	41	284
Factor (improvement) vs. 10 mm DAD cell	6.9	

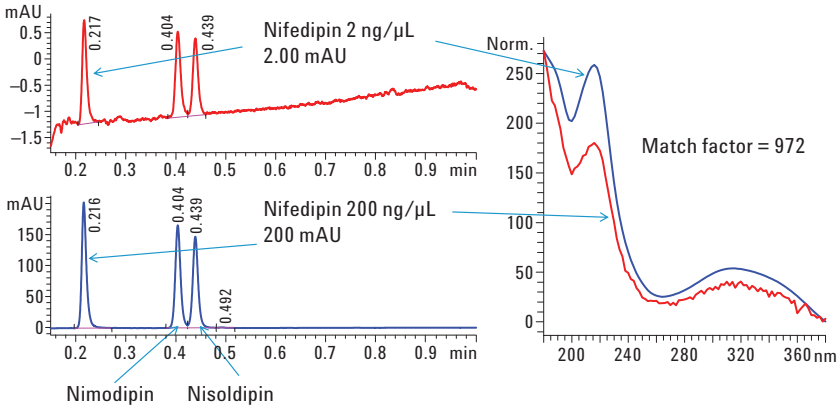
Table 2
Results of LOD measurements, LOD for Anthracene = 41 fg with S/N =2 at 10 Hz for the 60 mm path length cell and 284 fg for the 10 mm path length cell.

Spectral conformation of trace level compounds using ultra-fast chromatographic conditions

A library search is executed for the measured trace level spectrum. Match factors are calculated and tabulated in the Library Search Result table. Highest spectral match is achieved for nifedipin with a match factor of 972. The spectral library analysis confirms compound id based on chromatographic retention. This positive spectral conformation significantly enhances confidence in qualitative analytical results. See Figure 7.

Peak purity analysis of trace level compounds under ultra-fast LC conditions

The Agilent 1290 Infinity DAD enables peak purity analysis under ultra-fast LC conditions, even for trace level compounds. In this case, the spectral analysis confirms that the nifedipin peak as identified by chromatographic retention was pure. This positive purity confirmation significantly increases confidence in quantitative chromatographic results. The Agilent 1290 Infinity DAD provides lowest noise over the complete wavelength range for significantly increased spectral quality, see Figure 8



Column:	50 mm × 4.6 mm ZORBAX RRHTSB C-18, 1.8 µm, 600 bar
Sample	Nifedipin, Nimodipin and Nisoldipin each ~200 ng/µL and ~2 ng/µL solution
Injection volume:	1 µL, needle cleaning with methanol for 6 sec
Column temperature:	80 °C
Sample temperature:	10 °C
Mobile Phases:	water + 400 µL TFA/l and Acetonitrile
Gradient:	65% to 70% in 0.85 min
Flow:	4 mL/min
Stop time:	1 min
Post time:	1 min
DAD:	80 Hz, 245/4 nm, Ref 450/80, slit 8 nm, all spectra

Figure 7
Analysis of Nifedipin, Nimodipin and Nisoldipin; 1290 for spectral and purity evaluation.

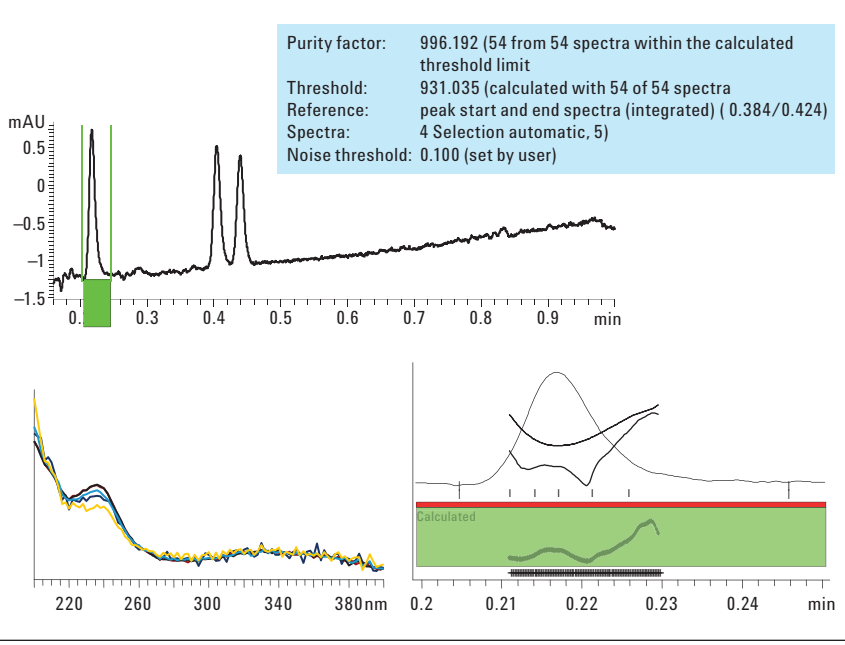


Figure 8
Peak purity analysis for Nifedipin.

Conclusion

The new design of the Agilent 1290 Infinity DAD offers lowest noise <±3 µAU and small drift behavior <0.5 mAU/h for the 10-mm path length cell. The 60-mm path length cell shows noise as low as <1 µAU/cm and drift as low as <0.5 mAU/h/cm The linear range typically goes up to 2300 mAU for the 10-mm path length cell. Data rates up to 160 Hz enable excellent quantitation even for peak widths <200 msec. The limit of detection for Anthracene was as low as 41 fg with a S/N ratio of 2 using the 60-mm path length cells. The new design allows for an easy change of detector cell. RFID tags in the lamp and the flow cell ensure traceability of lamp and cell usage.

www.agilent.com/chem/1290

April 1, 2010
Publication Number 5990-5601EN

Agilent Technologies