Performance Characteristics of the Agilent 1200 Series Variable Wavelength Detectors

Faster results, improved sensitivity and absolute data security

Technical Note

Introduction

The Agilent 1200 Series Variable Wavelength Detectors (VWD) are designed for highest optical performance, compliance with GLP regulations and easy maintenance. Two versions are available: the Agilent 1200 Series VWD, and the Agilent 1200 Series VWD SL Plus with high sampling rates for rapid resolution and ultra-fast HPLC. These detectors offer the following features and benefits:

- Higher sampling rates up to 160 Hz for rapid resolution and ultra-fast HPLC (Agilent 1200 Series VWD SL Plus) or up to 20 Hz (Agilent 1200 Series VWD)
- Data recovery card (DRC) provides for unique "data-never-lost insurance" (1200 Series VWD SL Plus)
- Deuterium lamp for highest intensity and lowest detection limit over a wavelength range of 190 to 600 nm
- Optional flow-cell cartridges such as standard (10 mm path length/14 µL volume), high pressure (10 mm/14 µL), micro (3 mm/2 µL) and semi-micro (6 mm/5 µL) are available and can be deployed according to application needs
- Easy front access to flow cell and lamp for fast replacement
- Flow cell and lamp with RFID tag for safe identification
- Lamp information, including part number, serial number, production date, number of ignitions, total burn time
- Cell information, including part number, serial number, production date, nominal path length, volume, maximum pressure
- Built-in electronic temperature control (ETC) for improved baseline stability
- Built-in holmium oxide filter for fast verification of wavelength accuracy

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Optical design

The optical system of the Agilent 1200 Series VWD and VWD SL Plus is shown in figure 1. Its radiation source is a deuterium-arc discharge lamp for the ultraviolet (UV) wavelength range from 190 to 600 nm. The light beam from the deuterium lamp passes through a lens, filter assembly (in open, cut-off or holmium oxide position), entrance slit (1 mm standard), spherical mirror (M1), grating, a second spherical mirror (M2), a beam splitter, and finally through the flow cell to the sample diode. The beam through the flow cell is absorbed depending on the solutions in the cell, in which UV absorption takes place, and the sample photodiode converts the intensity to an electrical signal. Part of the light is directed to the reference photodiode by the beam splitter to obtain a reference signal for compensation of intensity fluctuation of the light source. A slit in front of the reference photodiode cuts out light of the sample bandwidth. Band width is typically 6.5 nm. Wavelength selection is made by rotating the grating, which is driven directly by a stepper motor. This configuration allows fast change of the wavelength. The cut-off filter is moved into the light path above 370 nm to reduce higher order light.

Data sampling rates

Recently there is a major trend to reduce analysis times in order to increase sample throughput. Analysis times as low as 0.6 min and peak widths of 0.4 s are now achievable with modern LC equipment such as the Agilent 1200 Series Rapid Resolution LC system. This has placed high demands on the data sampling rate. Detectors must be fast enough to provide sufficient data points for these small peaks. Table 1 shows the data sampling rate settings of the Agilent 1200 Series VWD and VWD SL Plus.

For rapid resolution and ultra-fast LC application it is recommended to use the 1200 Series VWD SL Plus. For more conventional applications the 1200 Series VWD offers a sufficiently high data sampling rate.

In this publication the performance of the 1200 Series VWD and VWD SL Plus is evaluated and compared to the performance of an earlier 1200 Series VWD model. Performance characteristics evaluated include:

- Drift and noise
- Limit of detection (LOD) for anthracene
- Detection of impurities at levels below 0.03 % of the main compound
- Sensitivity improvements between ultra-fast LC and conventional LC
- Linearity of caffeine
- Influence of data rate on resolution, peak width and peak capacity
- Influence of different cells on resolution, noise and signal-to-noise ratio

Equipment

An Agilent 1200 Series RRLC system was used for the evaluation, comprising the following modules with firmware revisions A.06.01 or higher:

- Agilent 1200 Series Binary Pump SL with Agilent 1200 Series Micro Degasser
- Agilent 1200 Series High Performance Autosampler SL Plus
- Agilent 1200 Series Thermostatted Column Compartment SL
- Agilent 1200 Series VWD and Agilent 1200 Series SL Plus
- Agilent 1200 Series VWD (earlier model)
- Agilent ZORBAX RRHT 1.8 µm columns

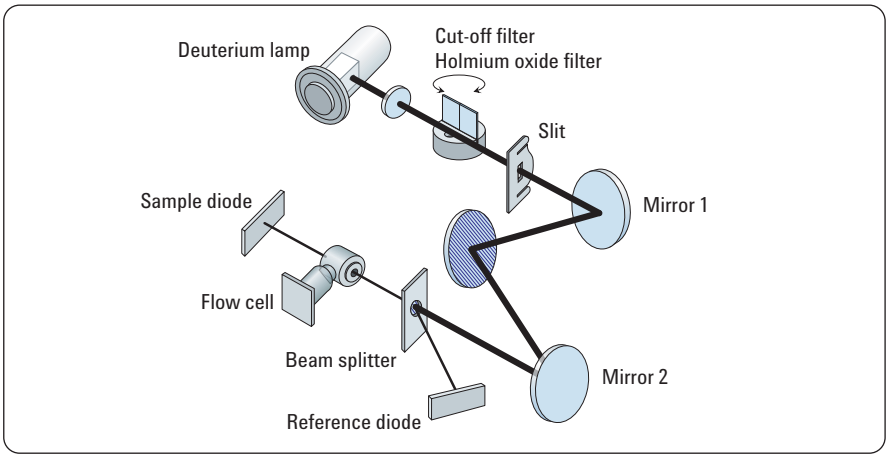

Figure 1
Optical system of 1200 Series VWD and VWD SL Plus.

1200 Series VWD			1200 Series VWD SL Plus		
Peak width	Response time	Sampling rate	Peak width	Response time	Sampling rate
< 0.005 min	< 0.12 s	20 Hz	< 0.0012 min	< 0.03	160 Hz
> 0.005 min	0.12 s	20 Hz	> 0.0012 min	0.03 s	160 Hz
> 0.01 min	0.25 s	20 Hz	> 0.0025 min	0.06 s	160 Hz
> 0.025 min	0.5 s	20 Hz	> 0.005 min	0.12 s	80 Hz
> 0.05 min	1 s	10 Hz	> 0.01 min	0.25 s	40 Hz
> 0.1 min	2 s	5 Hz	> 0.025 min	0.5 s	20 Hz
> 0.2 min	4 s	2.5 Hz	> 0.05 min	1 s	10 Hz
> 0.4 min	8 s	1.25 Hz	> 0.1 min	2 s	5 Hz
			> 0.2 min	4 s	2.5 Hz
			> 0.4 min	8 s	1.25 Hz

Table 1
Peak widths, response times and sampling rates of the 1200 Series VWD and VWD SL Plus.

Noise and drift

Noise and drift are key parameters when evaluating the performance of detectors. Figure 2 shows the noise and drift behavior of the 1200 Series VWD and VWD SL Plus compared to that of the earlier 1200 Series VWD model. The baseline noise of the 1200 Series VWD and VWD SL Plus is typically a factor of three to five times better than the earlier model. The experimental data presented here shows an improvement in baseline noise by a factor of 3.4.

The drift behavior also depends strongly on how sensitive the detector reacts to changes in ambient temperature. The data presented here shows a 2.4-fold improvement in drift for the 1200 Series VWD and VWD SL Plus. Both detectors were within Agilent's specifications.

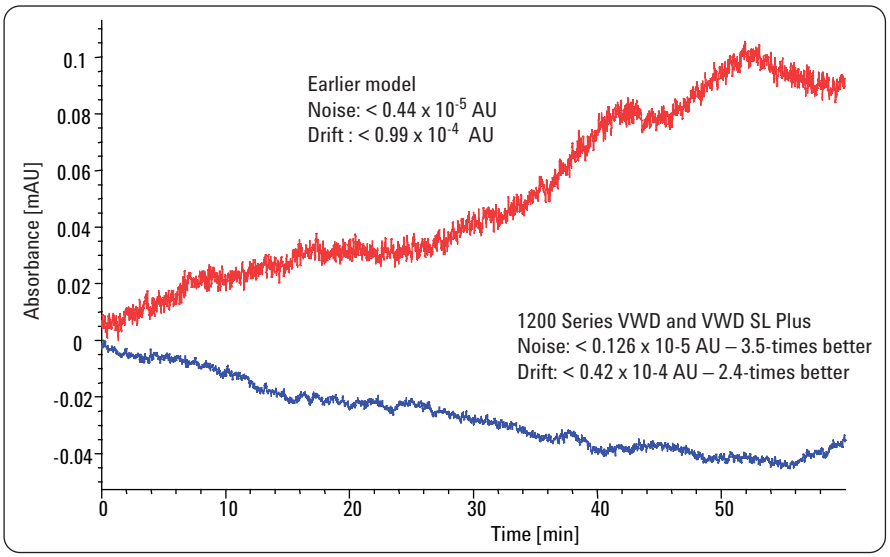

Figure 2
Noise and drift of VWD vs. earlier model.

Chromatographic conditions

Column:	Restriction capillary	Wavelength:	254 nm
Mobile phase:	Water	Response time:	2 s
Flow rate:	1 mL/min	Peak width:	> 0.1 min
		Column temp.:	36 °C

Limit of detection of anthracene

Variable wavelength detectors are frequently used for quality control applications. One requirement for ensuring best product quality is the ability to detect all impurities present in a final product. This can be done reliably, if the detector exhibits low noise behavior. Figure 3 shows the evaluation of the limit of detection for the 1200 Series VWD and VWD SL Plus. 5 pg of anthracene were injected in 3 µL of acetonitrile. The limit of detection was 0.228 pg whereby the calculation was based on the 5 pg injection.

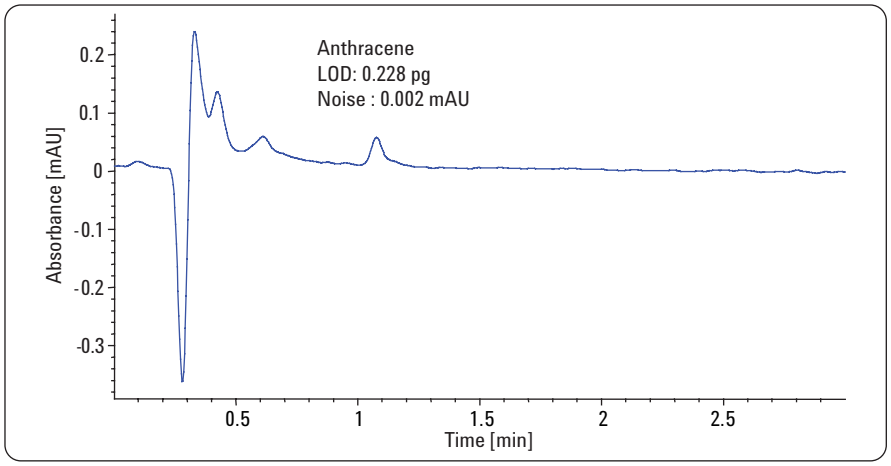

Figure 3
Measurement of the limit of detection of anthracene.

Chromatographic conditions

Sample:	Anthracene, 1.6668 pg/µL, 5.004 pg/3 µL
Column:	ZORBAX SB C18, 2.1 x 50 mm, 1.8 µm
Mobile phase:	Water/Acetonitrile, 30/70
Flow rate:	0.5 mL/min
Detection:	Wavelength 251nm
Peak width:	> 0.025 min (20 Hz)
Cell volume:	14 µL
Path length:	10 mm
Injection vol.:	3 µL
Column temp.:	36 °C

Short 0,12 mm ID capillaries were used to connect injection valve, column and detector. The 1.6 µL volume heat exchanger was used in the detector.

Figure 4 shows a comparison of the 1200 Series VWD and VWD SL Plus with an earlier model for the evaluation of signal-to-noise ratio. Anthracene was used as sample whereby the same amount was injected and the same peak width settings used for both detectors.. The results show that the 1200 Series VWD and VWD SL Plus showed better noise behavior and the signal-to-noise ratio was improved by a factor of 4.6.

Detection of impurities

Figure 5 shows a comparison of the 1200 Series VWD and VWD SL Plus with an earlier model for the analysis of impurities at levels less than 0.03 percent of the main compound. The improved noise behavior of the 1200 Series VWD and VWD SL Plus ensured better identification and quantitative results. The increase in signal-to-noise ratio was by a factor of about 3.

Performance comparison between conventional and ultra-fast LC

Another way to increase performance and sample throughput simultaneously is to use columns with 1.8 µm particles instead of 5 µm particle. Figure 6 shows a comparison between a conventional LC application and an ultra-fast LC application.

A 150 x 4.6 mm, 5 µm column was used for the conventional LC application and the run time was 10 min. For the ultra-fast LC application a 50 x 4.6 mm, 1.8 µm column was used and the

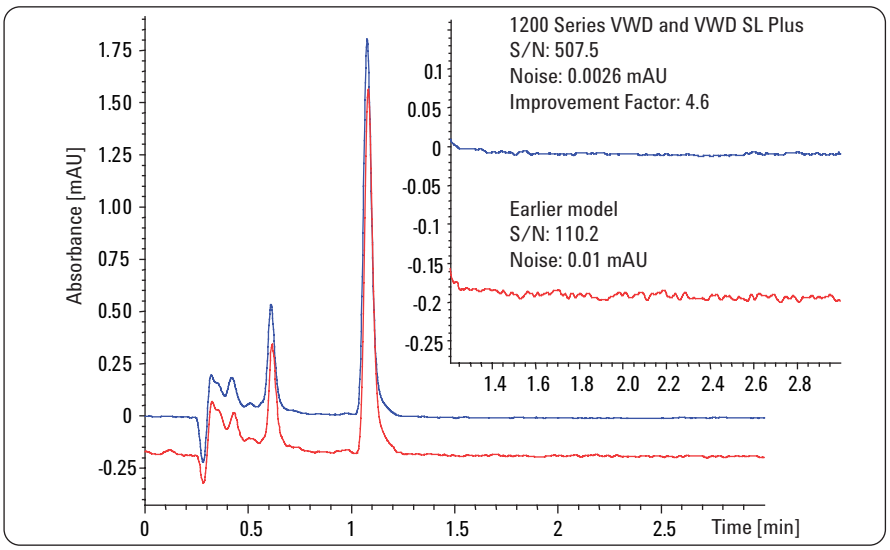

Figure 4
Comparison of signal-to-noise ratio using anthracene as sample.

Chromatographic conditions
Sample: 16.668 pg/µL, 50.04 pg/3 µL
Column: ZORBAX SB C18, 2.1 x 50 mm, 1.8 µm
Mobile phase: Water/Acetonitrile, 30/70
Flow rate: 0.5 mL/min
Detection: (Older model) 251 nm, peak width > 0.025 min (14 Hz), 14 µL flow cell volume, 10 mm path length
(VWD/VWD SL Plus) 251 nm, peak width > 0.025 min (20 Hz), 14 µL flow cell volume, 10 mm path length
Injection vol.: 3 µL
Column temp.: 36 °C
Short 0.12 mm ID capillaries were used to connect injection valve, column and detector. The 1.6 µL volume heat exchanger was used in the detector.

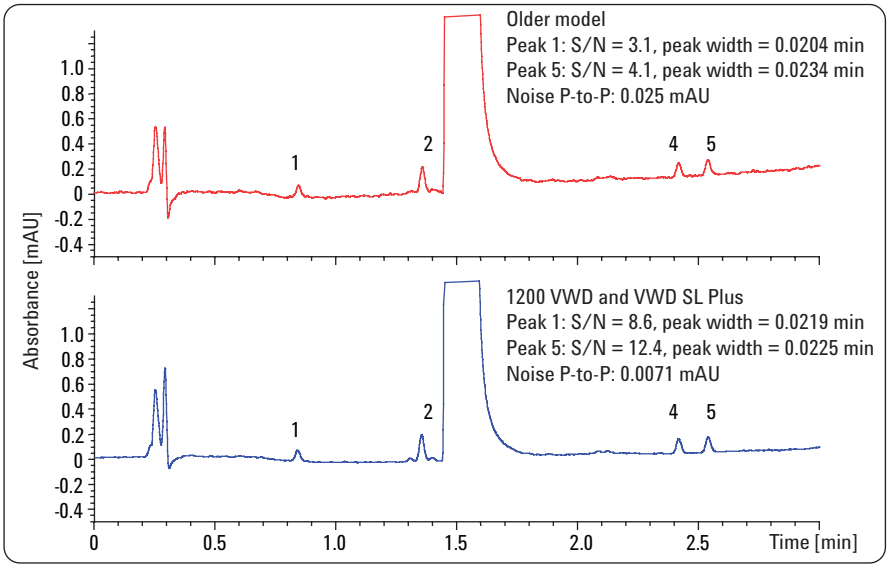

Figure 5
Analysis of impurities at levels < 0.03 % of main component.

Chromatographic conditions
Sample: Tramadol, 2.112 mg/mL, containing four impurities (peaks 1, 2, 3 and 4)
Column: ZORBAX SB C-18, 4.6 x 50 mm, 1.8 µm, for 600 bar operation
Mobile phase: Solvent A: Water + 0.2 % TFA, Solvent B: Acetonitrile + 0.16 % TFA
Gradient: 17 to 45 % B in 2.8 min, hold for 0.2 min
Stop time: 3 min
Post time: 1 min
Flow rate: 2.2 mL/min
Injection vol.: 3 µL, 10 s wash for exterior of needle
Column temp.: 30 °C
Detection: (VWD/VWD SL Plus) 270 nm, peak width = 0.025 min (20 Hz), 10 mm path length
(older model) 270 nm, peak width = 0.025 min (14 Hz), 10 mm path length

run time was decreased to 0.6 min. For this shortened run time the data sampling rate of the 1200 Series VWD was increased from > 0.05 min (peak width) to > 0.0025 min. Increasing the data rate causes an increase in baseline noise and an increase by a factor of 2 was observed in this example. Increasing the data rate also influences the signal-to-noise ratio, which was measured to be about 1.5 to 2 times better for the ultra-fast LC application. Table 2 summarizes the results of both applications.

A twofold increase in the limit of detection was achieved for the late eluting peaks. The Agilent 1200 Series VWD and VWD SL Plus, which typically deliver a threefold increase in signal-to-noise ratio, were able to take advantage of the columns with 1.8 µm particles and facilitated a 10 to 20-fold increase in speed and a fivefold increase in signal-to-noise ratio.

Detection linearity

The detection linearity of the 1200 Series VWD and VWD SL Plus was determined using caffeine. Eleven different concentrations from 0.1 to 1000 µg/mL were analyzed (table 3). Over the tested concentration range the linearity was within ± 5 %, (figure 7).

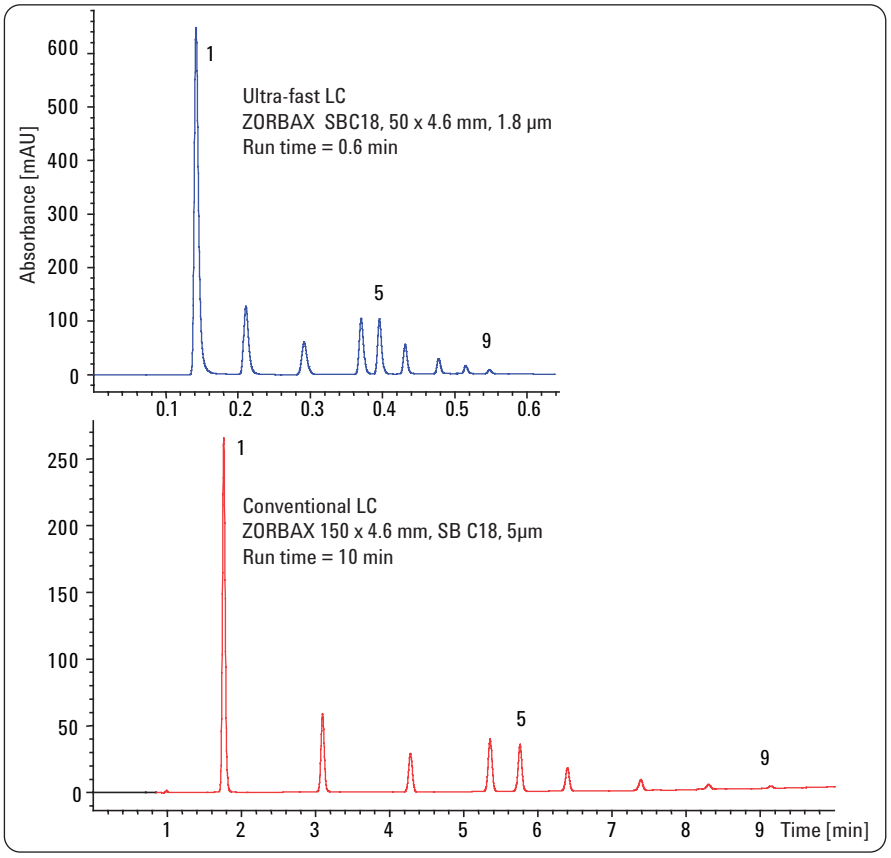

Figure 6
Performance comparison between conventional LC and ultra-fast LC.

Chromatographic conditions for ultra-fast LC

Sample:	Phenone test mix (order no. 5188-6529), diluted 1:10
Column:	ZORBAX SB C18, 50 x 4.6 mm, 1.8 µm
Gradient:	50-100 % ACN in 0.3 min
Flow rate:	5 mL/min
Stop time:	0.6 min
Temperature:	60 °C
Injection vol.:	3 µL
Detection:	Peak width > 0.0025 min, data rate 160 Hz, standard cell path length 10 mm

Chromatographic conditions for conventional LC

Sample:	Phenone test mix (order no. 5188-6529), diluted 1:10
Column:	ZORBAX SB C18, 150 x 4.6 mm, 5µm
Gradient:	35 to 95 % ACN in 10 min
Flow rate:	1.5 mL/min
Stop time:	10 min
Temperature:	50 °C
Injection vol.:	3 µL
Detection:	Peak width > 0.05 min, data rate 10 Hz, standard cell path length 10 mm

Parameter	Conventional LC	Ultra-fast LC
Run time	10 min	0.6 min (17 times faster)
Peak width		
peak 2	0.0492 min	0.00698 min
S/N peak 2	1814.9	2001.0
S/N peak 5	1094.6	1618.7
Resolution		
peak 5	4.24	2.37
S/N peak 9	55.8	124.2

Table 2
Performance comparison between conventional LC and ultra-fast LC

Calibration level	Amount (µg/mL)
1	0.977
2	1.953
3	3.906
4	7.812
5	15.625
6	31.25
7	62.5
8	125
9	250
10	500
11	1000

Table 3
Concentrations used for linearity evaluation

Influence of data sampling rate on resolution, peak width and peak capacity

It is important to set the data sampling rate correctly to generate sufficient data points. About 20 data points per peak are required to obtain correct quantitative results. If the data sampling rate is too low, peaks separated in the column become merged in the detector and lead to incorrect quantification. Setting the data sampling rate too high causes an increase in baseline noise. To select the optimum data sampling rate, the peak width of the smallest peak should be used as a reference and the rate set accordingly. Figure 8 shows an example of an ultra-fast analysis with different data sampling rate settings.

In table 4 the results are combined and show that a data sampling rate of 160 Hz is optimum for ultra-fast applications.

Influence of different flow cells on resolution, noise and signal-to-noise ratio

A variety of flow cells are available for the 1200 Series VWD and VWD SL Plus, which can be installed using the same quick and simple mounting procedure. The flow cells have integrated radio-frequency identification (RFID) tags that hold specific information about the cell such as part number, cell volume, path length, and so on. An ID tag reader in the detector collects this information and transfers it to the control software. Table 5 shows the cells that are available.

In table 6 recommendations are made what flow cell matches the column used. If more than one selection is appropriate, use the larger flow cell to get the best detection limit. Use the smaller flow cell for best peak resolution.

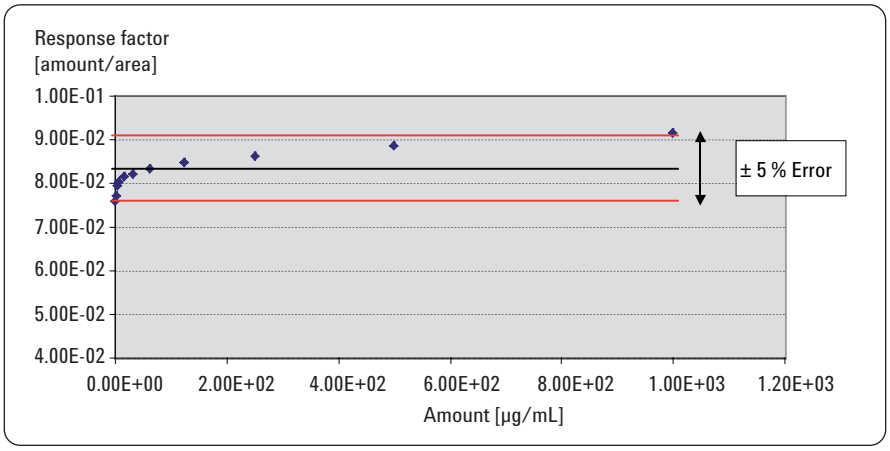

Figure 7
Linearity of 1200 Series VWD and VWD SL Plus from 1 to 1000 µg/mL.

Chromatographic conditions
Sample: Caffeine at 11 concentrations
Column: ZORBAX SB C18, 50 x 4.6 mm, 1.8 µm
Mobile phase: Water/Acetonitrile, 85/15
Flow rate: 1 mL/min
Detection: 272 nm, response 2 s ,peak width > 0.1 min, standard flow cell with 14 µL volume and 10 mm path length
Injection vol.: 10 µL
Column temp.: 36 °C

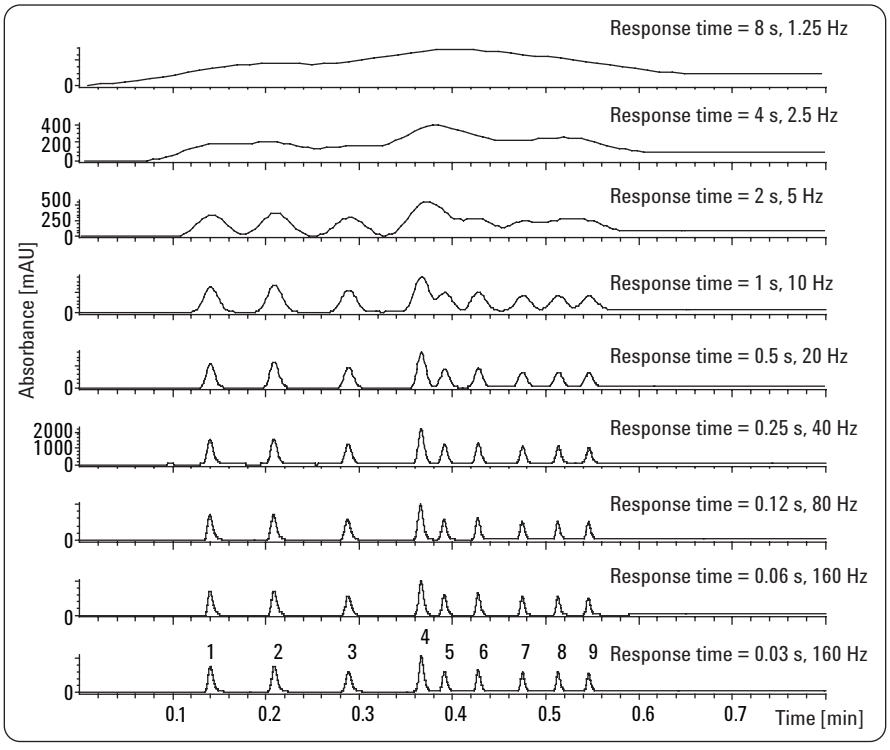

Figure 8
Influence of data sampling rate on resolution and peak width.

Chromatographic conditions
Sample: Phenone test mix (order no. 5188-6529)
Column: ZORBAX SB C18, 50 x 4.6 mm, 1.8µm
Gradient: 50-100 % ACN in 0.3 min
Flow rate: 5 mL/min
Stoptime: 0.8 min
Temperature: 60 °C
Injection vol.: 2 µL
Detection: See figure for response time and data sampling rate, standard flowcell with 14 µL volume and 10 mm path length

For ultra-fast applications with short columns and higher flow rates, the parameter settings shown in table 7 are recommend.

Flow cell path length

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

Absorbance (A) = $\log I_0/I = \varepsilon C d$

where:

- A is defined as the quotient of the intensity of the transmitted light, I_0, divided by the intensity of the incident light, I0 .
- Ɛ is the extinction coefficient, which is a characteristic of a given substance under a precisely-defined set of conditions of wavelength, solvent, temperature and other parameters.
- C is the concentration of the absorbing species (usually in g/L or mg/L).
- d is the path length of the cell used for the measurement.

As a result, flow cells with longer path lengths yield higher signals. Although noise usually increases slightly with increasing path length, there is a gain in signal-to-noise ratio. When increasing the path length, the cell volume usually increases as well. Typically, this causes more peak dispersion. As a rule-of-thumb the flow cell volume should be about one third of the peak volume at half height.

Response time (s)	Resolution peak 5	Peak width peak 2 (min)	Peak width peak 6 (min)
0.03 (160 Hz)	2.61	0.00635 (0.381 s)	0.0049
0.06 (160 Hz)	2.56	0.00656	0.005
0.12 (80 Hz)	2.48	0.00667	0.00521
0.25 (40 Hz)	2.11	0.00764	0.00642
0.5 (20 Hz)	1.45	0.0107	0.00976
1 (10 Hz)	0.89	0.0183	0.0172
2 (5 Hz)	n.a.	0.0343	
4 (2.5 Hz)	n.a.		
8 (1.25 Hz)	n.a.		

Table 4
Influence of response time on resolution and peak width.

	Cell type Standard	Semi-micro	Micro	High pressure
Max. pressure [bar]	40	40	120	400
Path length [mm]	10	6	3	10
Volume [µL]	14	5	2	10

Table 5
Flow cells for 1200 Series VWD and NWD SL Plus.

Column length	Typical peak width	Recommended flow cell			
<= 5 cm	0.025 min	Micro flow cell	Semimicro flow cell	Standard flow cell	
10 cm	0.05 min				
20 cm	0.1 min				
>= 40 cm	0.2 min				
	Typical flow rate	0.05 - 0.2 mL/min	0.2 - 0.4 mL/min	0.4 - 0.8 mL/min	1 - 5 mL/min
	Internal column diameter	1.0 mm	2.1 mm	3.0 mm	4.6 mm

Table 6
Flow cell recommendations.

Column inside diameter	2.1 mm	3.0 mm	4.6 mm
Practical flow rate (ml/min)	0.4-5	1-5	2-5
Flow cell volume, path length	2 µL, 3 mm	5 µL, 6 mm	14 µL, 10 mm*

Table 7
Flow cell recommendations for ultra-fast analysis.
* For ultra fast analysis with step gradients the micro flow cell (2 µL 3 mm) gives the best performance. If longer columns (> 50 mm) for higher resolution are used, then the next larger flow cell is the preferred choice for higher sensitivity.

Parameter	14 µL volume flow cell, 10 mm path length, 2 mL/min flow rate	14 µL volume flow cell, 10 mm path length, 1 mL/min flow rate	2 µL volume flow cell, 3 mm path length, 1 mL/min flow rate	5 µL volume flow cell, 6 mm path length, 1 mL/min flow rate
Peak width of peak 2	0.0229 min	0.0442 min	0.0400 min	0.0417 min
Peak width of peak 5	0.0214 min	0.0425 min	0.0383 min	0.0396 min
Peak width of peak 9	0.0211 min	0.0425 min	0.0387 min	0.0400 min
Resolution of peak 5	4.16	4.06	4.48	4.33
Signal-to-noise of peak 2	6318.5	4760.5	3389.4	4926.9
Signal-to-noise of peak 9	365.6	264.5	188.6	271.3

Table 8
Influence of different cell dimensions on peak width, resolution and signal-to-noise ratio.

Figure 9 and table 8 show the influence of different flow cells on peak width, signal-to-noise ratio, resolution and noise. The 14 µL volume flow cell was evaluated using two different flow rates. With a flow rate of 2 mL/min and a 3 minute gradient, the performance of columns with 1.8 µm particles was significantly better compared to a flow rate of 1 mL/min and a 6 minute gradient. At a flow rate of 1 mL/min the 2 µL volume flow cell gave smaller peak widths due to less peak dispersion after the column. The performance of the 5 µL volume flow cell was between the 14 and 2 µL volume flow cells. The different path lengths yielded significantly different signal-to-noise ratios. As expected the 2 µL volume flow cell with 3 mm path length showed the lowest signal to noise ratio. The 14 µL volume flow cell with 10 mm path length showed best results at a flow rate of 2 mL/min. The 2 µL volume flow cell yielded the best resolution due to the lowest dispersion volume after the column. In the example presented here at a flow rate of 1 mL/min, the 5 µL volume flow cell might be the best choice, see table 8.

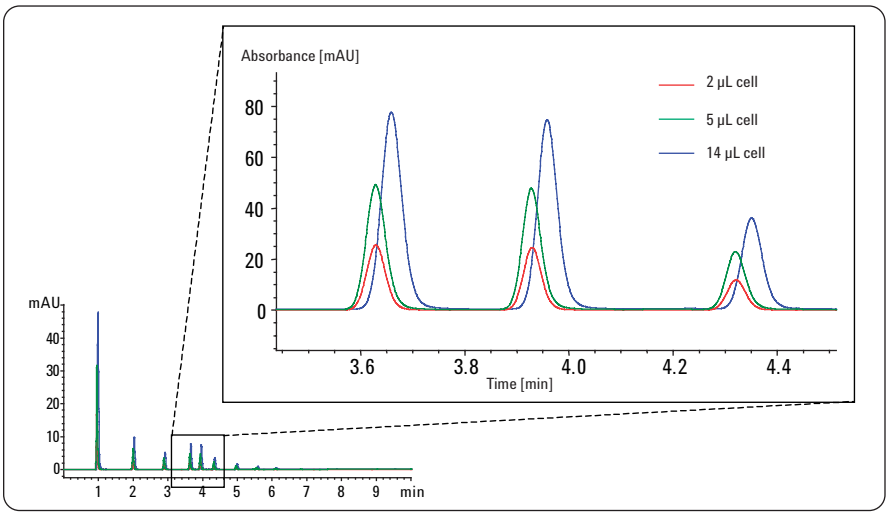

Figure 9
Influence of cell volume and path length on performance.

Chromatographic conditions

Sample:	Phenone test mix (order no. 5188-6529), diluted 1:10
Column:	ZORBAX SB C18, 50 x 4.6 mm, 1.8 µm
Gradient:	35-95 % ACN in 3 min (35-95 % in 6 min)
Flow rate:	2 mL/min (1 mL/min)
Stoptime:	10 min
Temperature:	30 °C
Injection vol.:	3 µL
Detection:	Data sampling rate 20 Hz, peak width > 0.025 min, 14 µL volume flow cell with 10 mm path length, 5 µL cell with 6 mm path length, 2 µL cell with 3 mm path length

Conclusion

The Agilent 1200 Series Variable Wavelength Detector and Agilent 1200 Series Variable Wavelength Detector SL Plus offer data sampling rates up to 160 Hz, which facilitates collection of sufficient data points for ultra-fast LC analysis with peak widths as narrow as 0.381 seconds. Design changes have significantly improved baseline noise and drift characteristics compared with earlier VWD models. Noise is typically a factor of three to five better and drift about three times better than earlier models. The 1200 Series VWD and VWD SL Plus have extended the earlier linearity specification of 2000 mAU up to 2500 mAU.

A wide range of flow cells facilitates optimum support for all applications regardless of whether highest sensitivity or lowest dispersion volume after the column is required. RFID tags for lamp and cells keep track of lamp performance, cell usage and provides for identification of the cell and lamp used in the chromatographic method. The data recovery card available for the 1200 Series VWD SL Plus ensures no loss of data even when the connection between the detector and the control software is interrupted.

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