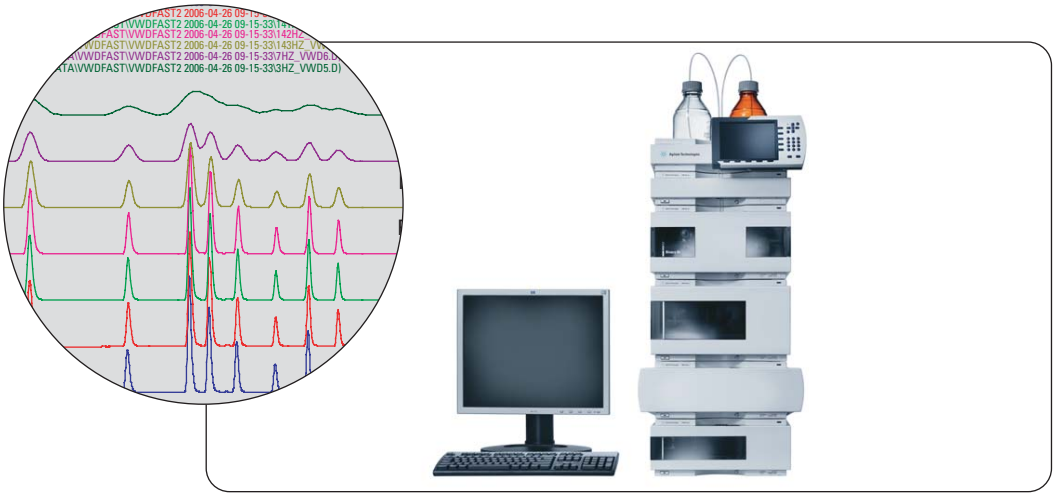
Performance of the Agilent 1200 Series variable wavelength detector SL using different detector cells and different data rates up to 55 Hz

Technical Note



Introduction

In 2006, the introduction of the Agilent 1200 Series Rapid Resolution LC system included the development of a faster variable wavelength detector, the Agilent 1200 Series variable wavelength detector SL (VWD SL) The data acquisition rate was increased to 55 Hz which ensures that for fast and ultra fast applications enough data points for optimum resolution, precision and sensitivity are provided. In this Technical Note the performance of the Agilent 1200 Series VWD SL is evaluated showing the influence of data rate on resolution, sensitivity, linearity and noise behavior at different data rates and using different detector cells.

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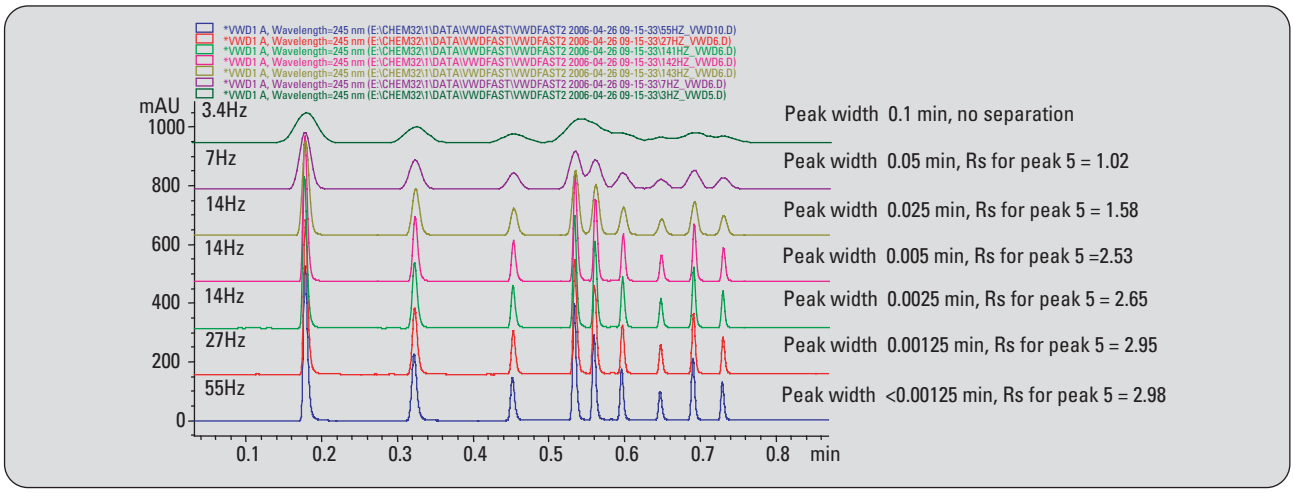

Figure 1
Influence of data rate on peak width.

Equipment

An Agilent 1200 Series Rapid Resolution LC system was used with the following modules:

- Agilent 1200 Series binary pump SL and vacuum degasser for high-speed and high-resolution applications on short and long sub 2-µm particle columns
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with new design for column temperatures up to 100 °C
- Agilent 1200 Series variable wavelength detector SL for 55-Hz operation
- ZORBAX SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particles

Chromatographic conditions

Column:	4.6 x 50 mm ZORBAX SB C-18, 1.8 µm, 600 bar
Test sample:	Set of 9 compounds, 100 ng/µL each, 1. acetanilide, 2. acetophenone, 3: propiophenone, 4. butyrophenone, 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone, 9. octanophenone
Pump:	Solvent A: H_2O, Solvent B: ACN
Gradient:	35 to 95 % B in 0.5 min, hold over 0.5 min, stop time 1.3, post time: 0.5 min
Flow rate:	5 mL/min
Autosampler:	
Injection volume:	2 µL, external needle wash 5 sec
Thermostatted column compartment:	
	Temperature: 70 °C
VWD detector:	245 nm, pre-run balancing, 14 µL detector cell with 10-mm path length
Instrument configuration:	
	Standard delay configuration for the Agilent 1200 Series Rapid Resolution system

Data rate (Hz)	Set peak width (min)	Actual peak width of peak 9 at 5sigma (min)	Resolution for peak 5	Peak capacity
55	< 0.00125	0.011	2.98	46
27	> 0.00125	0.011	2.95	46
14	> 0.0025	0.011	2.65	46
14	> 0.005	0.012	5.53	43
14	> 0.025	0.019	1.58	27
7	> 0.05	0.029	1.02	18
3	> 0.1	0.038	-	14
				no separation for peak 9

Table 3
Influence of data rate on peak width, resolution and peak capacity.

Data rate and resolution

The chromatograms clearly demonstrate that data rate must be increased when using high flow rates and short columns that result in run times less than 1 min. and peak widths of < 0.5 s. Increasing the data rate ensures optimum resolution and high peak capacity (table 1). The data in table 1 clearly shows that only an optimum data rate provides best resolution and peak capacity. Compared to a 7-Hz data rate, the peak width with a data rate of 55 Hz decreases by 164 %. This results in a 192 % gain in resolution, and a 156 % gain in peak capacity.

Data rate and noise

The Agilent 1200 Series VWD SL is able to collect data at a rate of 55 Hz, which is especially important for high throughput applications. For high resolution and high sensitivity analysis it is very important that the noise level of the detector is as low as possible. Table 2 shows the influence of data rate on noise level. For most high resolution applications with run times less than 5 minutes a data rate setting of 20 Hz is sufficient. For mobile phases with formic acid or trifluoro-acetic-acid (TFA) the noise level might increase due to the dependence of the TFA spectrum on the acetonitrile concentration. Even small variations can cause an increase of baseline noise.

Data rate	Noise level (ASTM)
3 Hz	1.708-2.876 µAU
7 Hz	4.147-4.9 µAU
14, (PW>0.0025 min)	29.42-37.16 µAU
28 Hz	47.82-56.76 µAU
55 Hz	74.40-81.11 µAU

Table 2
Influence of data rate on noise level.

Chromatographic conditions

Column:	4.6 x 50 mm ZORBAX SB C-18, 1.8 µm, 600 bar
Test sample:	Blank
Pump:	Solvent A: H2O, Solvent B: ACN = 50/50 Flow rate: 2mL/min
Thermostatted column compartment:	Temperature: 34 °C
VWD detector:	254 nm, pre-run balancing, 14 µL flow cell

Limit of detection

The limit of detection was determined by injecting 10 pg of anthracene in 1µL injection volume. The data rate used was 14 Hz. The limit of detection was calculated based on signal-to-noise ratio (peak-to-peak noise). At a data rate of 14 Hz the limit of detection was 0.62 pg. At 27 Hz with its higher noise level, the limit of detection was 0.77 pg.

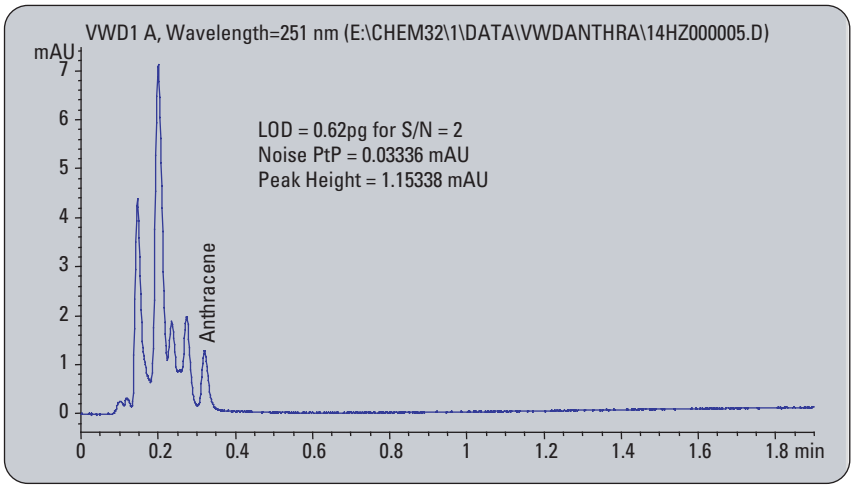

Figure 2
Limit of detection for the analysis of 10 pg of anthracene.

Chromatographic conditions

Column:	2.1 x 50 mm ZORBAX SB C-18, 1.8 µm, 600-bar column directly connected to autosampler and detector
Test sample:	10 pg/µL anthracene
Pump:	Solvent A: H_2O, Solvent B: ACN = 25/75 Flow rate: 1.3 mL/min
Thermostatted column compartment:	Temperature: 50 °C
VWD detector:	251 nm, PW > 0.0025 min, 14 Hz, pre-run balancing, 14 µL standard cell

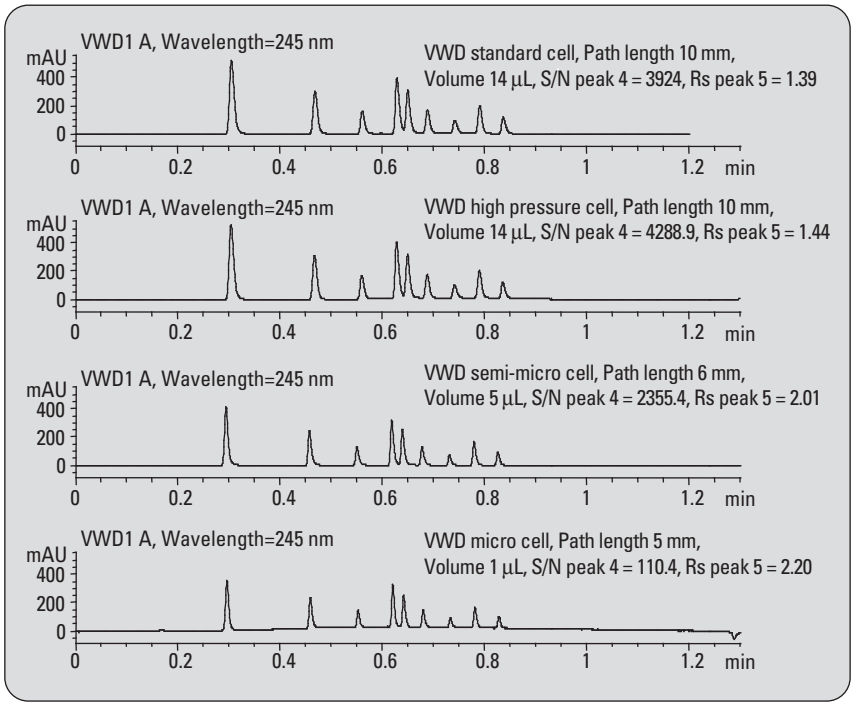

Figure 3
Comparison of different flow cells for the Agilent 1200 Series VWD SL at constant injection volume and concentration.

Linearity

The upper limit of linearity is > 2 AU measured with caffeine in methanol at 265 nm.

Detector cells

In addition to the 14-µL flow cell, 3 more flow cells are available for the Agilent 1200 Series VWD SL.

- VWD standard cell, G1314-60086, path length 10 mm, volume 14µL
- VWD high pressure cell, G1314-60082, path length 10 mm, volume 14 µL
- VWD semi-micro cell, G1314-60083, path length 6 mm, volume 5 µL
- VWD micro cell, G1314-60081, path length 5 mm, volume 1 µL

Figure 3 and table 3 show the differences between these cells. The VWD micro cell offers the best resolution, whereas the 14-µL cells with 10-mm path length have the best signal-to-noise ratio, as would be expected. The peak-to-peak noise level is comparable for 3 cells, whereas the micro cell shows less favorable noise characteristics.

- For lowest dispersion and best resolution with 2.1-mm ID columns, the micro cell offers the best performance. If maximum sensitivity is needed, it is advantageous to use the 14-µL cells with the 10-mm path length.
- For columns with 3-mm ID, the semi-micro cell is recommended as a good compromise between sensitivity and resolution. If highest resolution is needed, the 1-µL cell is the best choice. If highest sensitivity is needed, the 13-µL cell should be used.

Chromatographic conditions

Column:	4.6 x 50 mm ZORBAX SB C-18, 1.8 µm, 600 bar
Test sample:	Set of 9 compounds, 100 ng/µL each, 1. acetanilide, 2. acetophenone, 3: propiophenone, 4. butyrophenone, 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone, 9. octanophenone
Pump:	Solvent A: H_2O, Solvent B: ACN Gradient: 35 to 95 % B in 0.5 min, hold over 0.5 min, Stop time 1.3, Post time: 0.5 min, Flow rate: 3 mL/min
Autosampler:	Injection volume: 2 µL, external needle wash 5 sec
Thermostatted column compartment:	Temperature: 50 °C
VWD detector:	245 nm, pre-run balancing, 0.00125 min PW

Cell type	Resolution of peak 5	Noise peak-to-peak (mAU)	Signal-to-noise for peak 4
VWD standard cell, G1314-60086, path length 10 mm, volume 14 µL volume 5 µL	1.39	0.08474	3924
VWD high pressure cell, G1314-60082, path length 10 mm, volume 14 µL	1.44	0.08472	4289
VWD semi-micro cell, G1314-60083, path length 6 mm	2.01	0.08595	2355
VWD micro cell, G1314-60081, path length 5 mm, volume 1 µL	2.20	2. 5999	110

Table 3
Performance of different detector cells at constant injection volume and concentration.

VWD Detector cell	Resolution	Sensitivity
Standard cell	+	+++
High pressure cell	+	+++
Semi-micro cell	++	++
Micro cell	+++	+

Table 4
Overview for selecting the optimum cell either for sensitivity or resolution.

- For 4.6-mm ID columns with lengths of 100 and 150 mm, the 14-µL cell is always recommended. The flow rates for 4.6-mm ID columns are typically above or equal to 1.5 mL/min and a post-column delay volume of a few micro-liters does not significantly influence the performance. If highest resolution is needed with, for example, short 4.6-mm ID columns, the micro cell is the best choice.

Conclusions

With its range of flow cells and selectable data rate up to 55 Hz, the Agilent 1200 Series variable-wavelength detector SL is suitable for a wide range of applications, from ultra-fast LC to high-resolution LC with optimum sensitivity. It provides lowest noise (ASTM), typically less than 3 µAU at 3 Hz, and achieves a limit of detection for anthracene of 0.62 pg (peak-to-peak signal to noise = 2). Linearity is typical > 2 mAU. Four detector cells are available for different application needs. Table 4 gives an overview about the relation of resolution and sensitivity for the four available cells.

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Published July 1, 2006
Publication Number 5989-5361EN

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