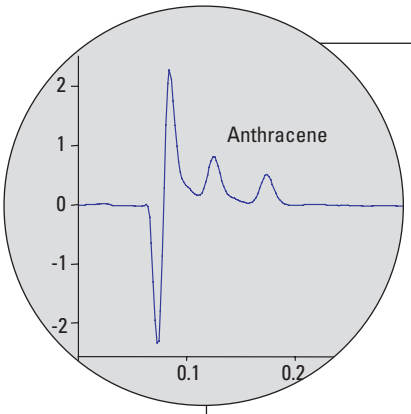
Performance of the Agilent 1200 Series diode-array detector SL using different detector cells and different data rates up to 80 Hz

Technical Note



Introduction

The introduction in 2006 of the Agilent 1200 Series Rapid Resolution LC system also included new design elements in the 80 Hz diode-array detector (DAD) that was first introduced in 2004. Two new detector cells are the most significant innovations. This Technical Note demonstrates the performance of these new cells as well as the overall performance of the Agilent 1200 Series DAD SL.

Equipment and materials

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 diode-array detector SL for 80-Hz operation, including new data protection tool
- ZORBAX SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particles

Data rate and resolution

A data rate of 80 Hz is needed for peak widths of less than 0.3 s such as those shown in figure 1 (see also literature reference 1.) 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 0.5 min and peak widths of 0.3 s. Increasing the data rate ensures optimum resolution and high peak capacity, (table 1). The data in table 1 shows clearly that only an optimum data rate provides best resolution and peak capacity. Compared to a 20-Hz data rate, which is the maximum signal rate available on most standard diode-array detectors, the peak width with a data rate of 80 Hz decreases by 30 %. This results in a 30 % gain in resolution, a 40 % in peak capacity and a 70 % gain in apparent column efficiency.

Data rate and noise

The Agilent 1200 Series DAD SL is able to collect data at a rate of 80 Hz, which is especially important for high throughput applications. For high resolution and high sensitivity analysis it 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 application 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. The following example shows to what extent the data rate can influence the limit of detection for a specific application. In this case 10 pg of anthracene were injected and the limit of detection was evaluated for data rates of 10 Hz and 80 Hz.

The limit of detection was calculated based on signal-to-noise ratio (peak-to-peak noise). At a data rate of 10 Hz the limit of detection was 0.6 pg. At 80 Hz, with its higher noise level, the limit of detection was 2.4 pg. Although the noise level increased by a factor of 10, the limit of detection decreased only by a factor of 4. This is due to the increased peak height obtained at 80 Hz.

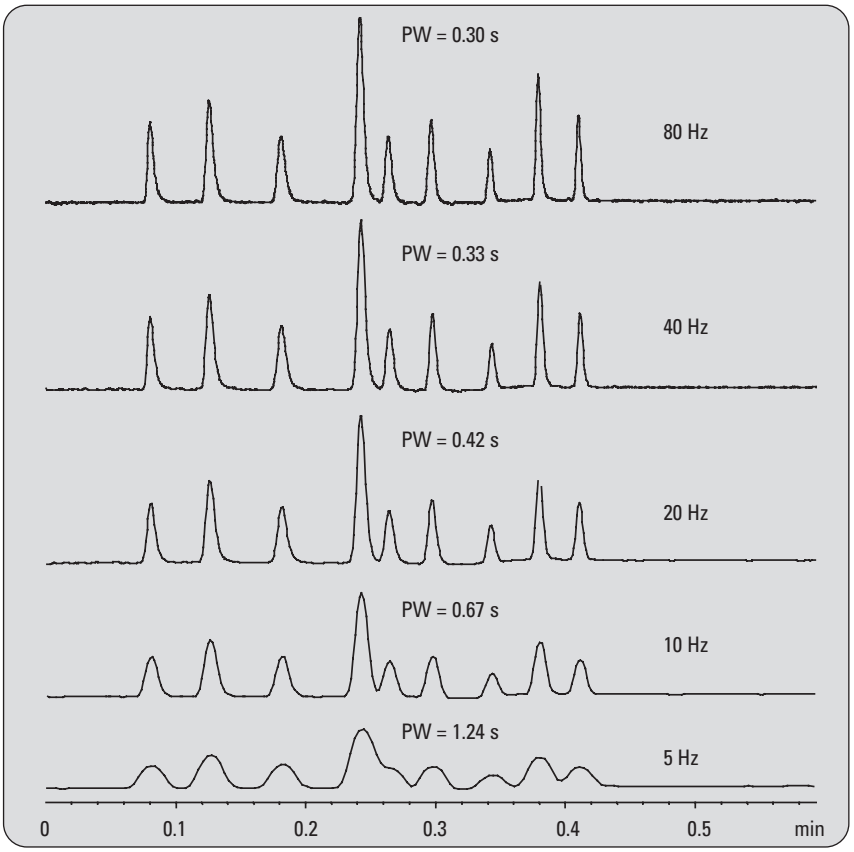


Figure 1
Influence of data rate on peak width.

Chromatographic conditions

Sample: Set of 9 compounds, 100 ng/uL each, dissolved in water/ ACN (65/35)
1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone (200 mg/µL)
5. Benzophenone
6. Valerophenone
7. Hexanophenone
8. Heptanophenone
9. Octanophenone

Column: ZORBAX SB-C18, 4.6 x 30 mm, 1.8 µm
Gradient: 50-100 % ACN in 0.3 min
Temp.: 50 °C
Flow Rate: 5 mL/min
Injection volume: 3 µL
Flow cell: 5 µL

Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

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

Data rate	Noise level (ASTM)
80 Hz	< 0.038 mAU
40 Hz	< 0.024 mAU
20 Hz	< 0.015 mAU
10 Hz	< 0.0091 mAU
5 Hz	< 0.0047 mAU
2.5 Hz	< 0.0023 mAU

Table 2
Influence of data rate on noise level.

Chromatographic conditions

Column: 100 x 2.1 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Solvent: Water/ACN = 50/50
Flow rate: 1 mL/min
Temp.: 50 °C
Detection: Signal 254/10 nm, reference 380/80 nm
13 µL flow cell
Slit width 8 nm

Chromatographic conditions

Column:	30 x 2.1 mm, ZORBAX SB C-18, 1.8 µm
Test sample:	Anthracene, 10 pg/µL
Solvent:	Water/Acetonitrile = 25/75
Flow rate:	1.3 mL/min
Run time:	3 min
Injection vol.:	1 µL
Temperature:	50 °C
Detection:	Signal 215/4 nm, reference 360/80nm Flow cell 13-µL Data rates 10 and 80 Hz Slit width 4 nm

Detector cells

In addition to the 13-µl flow cell, two new flow cells have been designed for the Agilent 1200 Series DAD SL.

- 5-µl flow cell with a path length of 6 mm, which is best suited for low dispersion using 4.6 or 3 mm ID columns
- 2-µl flow cell with a path length of 3 mm, which is best suited for lowest dispersion using 2.1-mm ID columns

Figure 3 and table 3 show the differences when using these cells.

Chromatographic conditions

Sample:	Set of 9 compounds, 100 ng/uL each, dissolved in water/ ACN (65/35) 1. Acetanilide 2. Acetophenone 3. Propiophenone 4. Butyrophenone 5. Benzophenone 6. Valerophenone 7. Hexanophenone 8. Heptanophenone 9. Octanophenone
Column:	100 x 2.1mm, ZORBAX SB C-18, 1.8 µm for 600 bar operation
Solvent:	A = Water, B = Acetonitrile
Gradient:	35 to 95 %B in 5 min, hold at 95 % for 1 min
Stop time:	8 min
Post time:	5 min
Flow rate:	0.6 mL/min
Injection vol.:	3 µL, wash exterior of needle for 10 s
Temperature:	50 °C
Detection:	Signal 245/10 m, reference 360/80 nm Flow cells 2 µL, 5 µL and 13 µL Data acquisition rate 20 Hz Peak width = > 0.01 min Slit width 8 nm

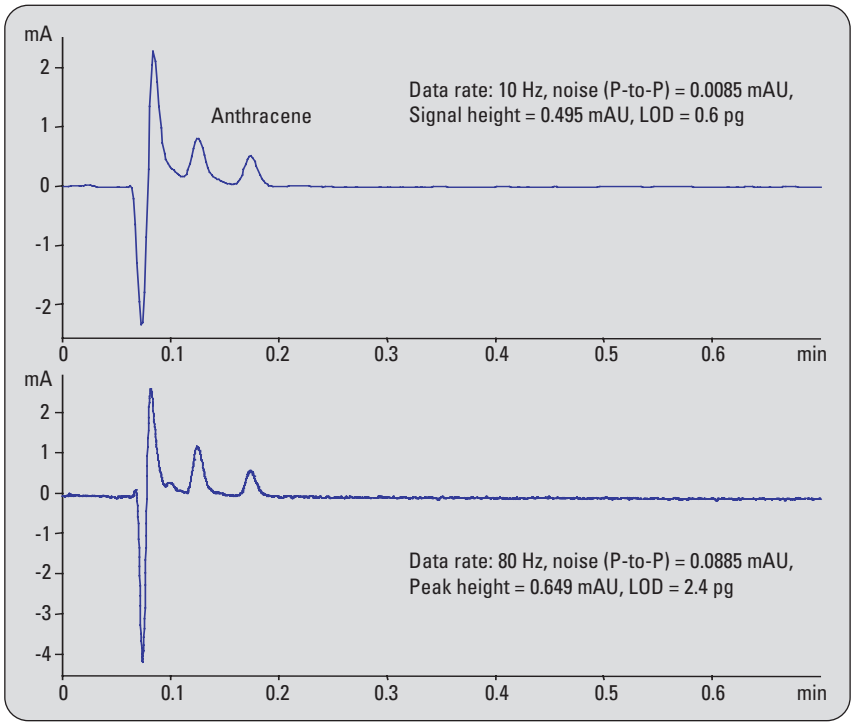


Figure 2
Influence of data rate on limit of detection for the analysis of 10 pg of anthracene.

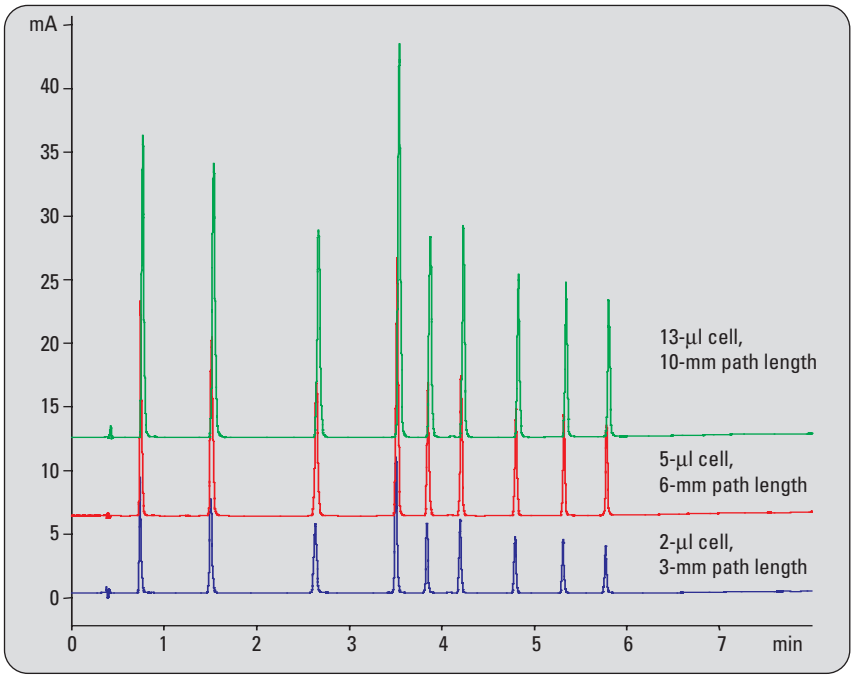


Figure 3
Comparison of different flow cells for the Agilent 1200 Series DAD SL at constant injection volume and concentration.

Cell type	Resolution of Peak 5	Noise peak-to-peak (mAU)	Signal-to-noise for peak 4
13-µL cell, 10-mm path length 3-µL injection volume	6.89	0.0509	5589
5-µL-cell, 6-mm path length 3-µL injection volume	7.66	0.0583	4008
2-µL cell, 3-mm path length 3-µL injection volume	7.85	0.0536	2289

Table 3
Performance of different detector cells at constant injection volume and concentration, column ID was 2.1 mm.

The 2-µL cell gives the best resolution whereas the 13-µL cell with 10-mm path length gives the best signal-to-noise ratio as would be expected. The peak-to-peak noise level is comparable for all cells and even the smallest cell shows very good noise characteristics. For lowest dispersion with **2.1-mm ID columns**, the 2-µL cell offers the best performance. If maximum sensitivity is needed, it is advantageous to use the 13-µl cell with 10-mm path length. For **columns with 3-mm ID**, the 5-µL cell is recommended as a good compromise between sensitivity and resolution. If highest resolution is needed, the 2-µL cell is the best choice. If highest sensitivity is needed, the 13-µL cell should be used. For **4.6-mm ID columns** with lengths of 100 and 150 mm, the 13-µL 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 2-µl cell is the best choice.

Conclusion

With its range of flow cells and selectable data rate up to 80 Hz, the Agilent 1200 Series diode-array 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 10 µAU at 10 Hz, and achieves a limit of detection for anthracene of 0.6 pg (peak-to-peak signal to noise.)

References

1.

"High-speed Agilent 1100 Series diode-array detector SL for optimization of resolution, sensitivity, spectral sensitivity and linearity" *Agilent Application Note, publication number 5989-3070EN*, **2005.**

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Published April 1, 2006
Publication Number 5989-5033EN

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