

More Chromatographic Power for the Agilent 1200 Series LC

How the Agilent 1200 Series VWD SL Plus and ZORBAX ZORBAX RRHT 1.8 μm columns can boost speed and sensitivity, and cut solvent consumption

Technical Note



Introduction

The Agilent 1200 Series LC is a robust and reliable workhorse for conventional HPLC methodologies and is deployed worldwide in chemical, environmental, food and pharmaceutical laboratories for a vast range of applications in routine analysis through new product research and development. Significant performance gains in terms of detection limits, analysis speed and solvent consumption can be attained through simple addition of the new Agilent 1200 Series Variable Wavelength Detector (VWD) SL Plus and Agilent ZORBAX RRHT 1.8 µm columns with sub-2-micron particles. These include:

- 5 times shorter run and cycle times for increased productivity
- Simultaneous 3 times improved signal-to-noise ratio using the new Agilent 1200 Series VWD and fast analysis with sub-2-micron particle columns
- 50 percent less solvent consumption



Overview

Significant hardware improvements in the Agilent 1200 Series VWD SL Plus facilitate lowest baseline noise and drift for highest sensitivity and lowest limits of detection.

- 160 Hz data acquisition rate gives up to 100 percent gains in resolution for ultra-fast LC, making the 1200 Series VWD SL Plus future proof for fastest separations
- Electronic Temperature Control (ETC) for maximum baseline stability and practical sensitivity under fluctuating ambient temperature and humidity conditions
- Latest electronics with built-in LAN ensure highest up-time and a secure investment
- Data Recovery Card (DRC) and radio frequency identification (RFID) technology for flow cells and lamps provide for a new level of data security and traceability
- A wide linear range facilitates reliable, simultaneous quantification of primary compounds, by-products and impurities
- Programmable wavelength switching enables sensitivity and selectivity to be optimized for the elution of each analyte
- Extensive diagnostics, error detection and display through the Agilent 1200 Series Instant Pilot or Agilent ChemStation make the VWD SL Plus easy to use and maintain
- Wavelength accuracy verification helps you to comply with GLP – an automatic holmium oxide filter can be programmed at the beginning of your chromatography to verify the accuracy of the set wavelength
- Early Maintenance Feedback (EMF) continuously tracks instrument usage such as lamp burn-time – user-settable limits and feedback messages inform you of problems before they happen.

In addition to the 1200 Series VWD SL Plus, deploying ZORBAX RRHT 1.8 μ m columns with sub-2-micron particles gives further performance improvements. For example, 50 mm columns

packed with 1.8 µm particles give the same resolution as 150 mm columns packed with 5 µm particles but in considerably shorter analysis times. Table 1 shows that shorter columns with small particles achieve the chromatographic efficiency of longer columns with large particles.

Experimental

Equipment

Initial configuration:

- Agilent 1200 Series LC system, including quaternary pump and standard autosampler
- Agilent 1200 Series VWD ("B" model) with 13 µL flow cell
- Agilent ZORBAX SB-C18 column, 150 x 4.6 mm, 5 μm (PN 883975-902

Configuration for detector upgrade: • Agilent 1200 Series LC system, including quaternary pump and standard autosampler

- Agilent 1200 Series VWD SL Plus
- Agilent ZORBAX SB-C18 column, 150 x 4.6 mm, 5 μm (PN 883975-902)

Third configuration for deployment of sub-2-micron column technology:

- Agilent 1200 Series LC system, including quaternary pump and standard autosampler
- Agilent 1200 Series VWD SL Plus
- Agilent ZORBAX SB-C18 column, 50 x 4.6 mm, 1.8 μm (PN 827975-902)

Chromatographic conditions

- Sample: Tramadole with 4 impurities in the range 0.7 to 1.25 %
- Mobile phase: Water with 0.1 % TFA and Acetonitrile with 0.650 % TFA

Column Length	Column Efficiency [N]			Reduction in
[mm]	5 µm Particles	3.5 µm Particles	1.8 µm Particles	Analysis Time [%]
150	12.500	21.000	35.000	
100	8.500	14.000	23.250	33 %
75	6.000	10.500	17.500	50 %
50	4.200	7.000	12.000	67 %
30	n.a.	4.200	6.500	80 %
15	n.a.	2.100	2.500	90 %



Comparison of columns with different lengths and particle sizes, showing reduction in analysis times (all columns with of 4.6 mm inside diameter).



Figure 1

Chromatogram of analysis using VWD "B" model and column packed with 5 μm particles.

Chromatographic conditions

Results and discussion

Three steps were performed to evaluate the gain in performance through deployment of the new detector and columns with sub-2-micron technology.

In the first step the Agilent 1200 Series LC system was equipped with an earlier "B" model of the VWD and a 150 mm ZORBAX column packed with 5 μ m particles. Figure 1 shows the chromatogram from the analysis of the Tramadol impurity mixture.

In the second step the old VWD "B" model was replaced by the new VWD SL Plus. Figure 2 shows the chromatogram from the analysis of the Tramadol impurity mixture. The lower noise level of the VWD SL Plus as well as a twofold increase in the signal-to-noise ratio can be seen clearly (table 2). Figure 3 shows the noise levels of both detectors. Even at a data acquisition rate of 40 Hz the noise level of the VWD SL Plus is a factor 1.8 lower.

In a final step the 150 mm column packed with 5 µm particles was replaced by a 50 mm column packed with 1.8 µm particles. Figure 4 shows the chromatogram from the analysis of the Tramadol impurity mixture. The signalto-noise ratio increased by a factor of three. The run time was shortened from 15 min to 3 min, representing a fivefold increase in analysis speed. Comparing the chromatograms obtained in all three steps clearly shows that upgrading to the new VWD SL Plus and ZORBAX RRHT 1.8 µm columns with sub-2-micron particles increases chromatographic performance in terms of signal-to-noise ration, analysis speed and solvent consumption. This is summarizes in table 2.



Figure 2

Comparison of chromatograms obtained with the VWD "B" model and the new VWD SL Plus and using a column packed with 5 μm particles.

Chromatographic conditions

 Flow rate:
 1 mL/min

 Gradient:
 0 min, 10 %B; 8 min, 45 %B; 10.5 min, 45 %B; 11min, 10 %B; 15 min, 10 %B

 Column temp.:
 30 °C

 Injection vol.:
 5 μL

 Detection:
 270 nm. response time 0.25 s (equivalent to 40 Hz)





Comparison of the noise levels of the VWD "B" model and the new VWD SL Plus.

VWD SL Plus with 50 x 4.6 mm, 1.8 µm ZORBAX column	VWD SL Plus with 150 x 4.6, 5 μm column	WVD "B" model with 150 x 4.6, 5 µm column
275.8	149.8	63.0
314.2	203.4	84.9
337.7	210.9	89.5
254.0	157.3	71.2
0.03063 mAU	0.05771 mAU	0.1059 mAU
3 min including equilibration time	15 min including equilibration time	15 min including equilibration time
~8 mL	~16 mL	~16 mL
	VWD SL Plus with 50 x 4.6 mm, 1.8 µm ZORBAX column 275.8 314.2 337.7 254.0 0.03063 mAU 3 min including equilibration time ~8 mL	VWD SL Plus with 50 x 4.6 mm, 1.8 µm ZORBAX columnVWD SL Plus with 150 x 4.6, 5 µm column275.8149.8314.2203.4337.7210.9254.0157.30.03063 mAU0.05771 mAU3 min including15 min includingequilibration time ~8 mL~16 mL



Comparison of chromatographic performance.

Conclusion

The chromatographic performance of a standard Agilent 1200 Series LC system can be significantly improved through addition of the new Agilent 1200 Series Variable Wavelength Detector SL Plus and deployment of Agilent ZORBAX RRHT 1.8 μ m columns with sub-2-micron particles. The signal-to-noise ratio can be increased by a factor of three and analysis speed can be increased by a factor of five. A further benefit is reduced solvent consumption, which can be cut by 50 %.





Final chromatogram from the analysis of the Tramadol impurity mixture using the Agilent 1200 Series VWD SL Plus and ZORBAX RRHT 1.8 µm columns with sub-2-micron particles.

Chromatographic conditions

 Flow rate:
 2 mL/min

 Gradient:
 0 min, 20 %B; 2.7 min, 45 %B; 3 min, 45 %B; 3.1 min, 20 %B; 4.2 min, 20 %B

 Column temp.:
 30 °C

 Injection vol.:
 5 μL

 Detection:
 270 nm, response time 0.25 s (equivalent to 40 Hz)

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