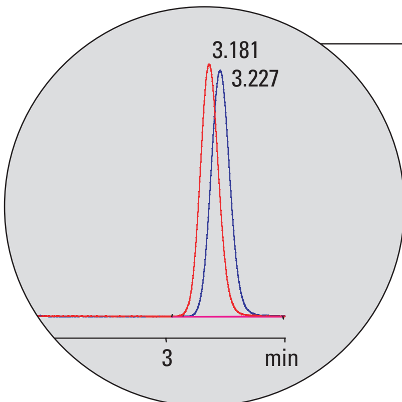
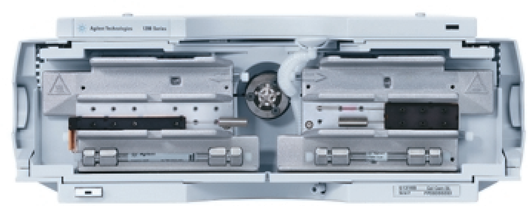
Agilent 1200 Series column compartment SL with temperature control up to 100 °C and post-column cooling for lowest baseline noise

Technical Note





Introduction

As part of the Agilent 1200 Series LC system a new design of column compartment was introduced, which facilitates operation over an enhanced temperature range and includes a post-column cooling device. This Technical Note describes the design of the new column compartment and the influence of the new design on dispersion and detector noise.

Equipment and materials

The instrument used was an Agilent 1200 Series Rapid Resolution LC system, equipped 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

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Low-volume Heater

The Agilent 1200 Series column compartment SL provides for column temperatures from 10 degrees below ambient up to 100 °C for flow rates from 50 µL up to 5 mL/min. A pre-column heater reduces the risk of additional dispersion at low flow rates. To decrease the noise level generated in the detector by high flow rates and elevated column temperatures, post-column cooling devices reduce the temperature of column effluent to same level of the detector optics. These additional heat exchangers can be installed at any position in the column compartment (figure 1). The pre-column heater has an internal delay volume of 1.6 µL and the post-column cooler has an internal delay volume of 1.5 µL. These low delay volumes are important to avoid peak dispersion as much as possible, especially at low flow rates. In the following example isocratic conditions were chosen to show the influence of these different delay volumes on a separation. The standard 3-µL heater was tested in comparison with the new low-delay volume, pre-column heater. Figure 2 shows the influence of the low-delay volume heater on dispersion.

Chromatographic conditions

Sample:	Isocratic standard sample
Column:	2.1 x 100 mm column, ZORBAX SB C-18, 1.8 µm
Mobile phase:	Water/acetonitrile = 30/70
Flow rate:	0.5 mL/min
Detection:	254 nm, 20 Hz

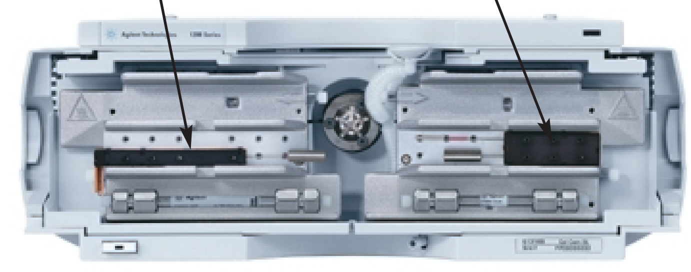

Figure 1
New additional heaters and coolers for lowest peak dispersion at low flow rates.

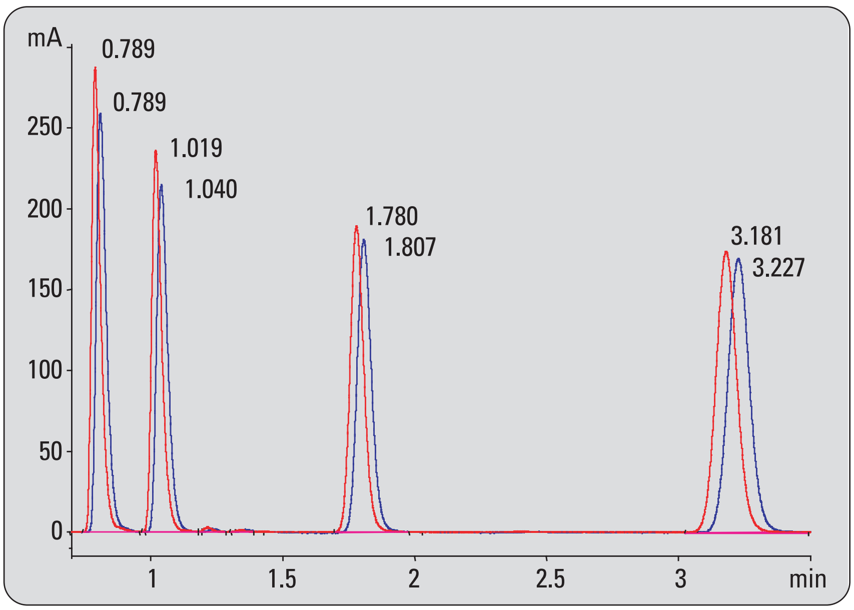

Figure 2
Influence on dispersion of the new low-delay volume, pre-column heater with 1.6 µL compared with the standard 3-µL heater at 0.2 mL/min.

A comparison of peak width and resolution between peak 1 and 2 clearly shows that the low-delay volume heater creates less peak dispersion at all flow rates, (table 1).

Flow rate	Evaluated Peak	New heater at 50 °C	3 µL heater at 50 °C
0. 5mL/min	Rs of peak 2	3.29	2.86
0.2 mL/min	Rs of peak 2	3.51	3.14

Table 1
Influence of different heaters on resolution.

Post-column cooling of the column effluent

The following example demonstrates the need for a cooling device after the column. At high flow rates and at high column temperatures it is recommended to cool the column effluent down to the temperature of the detector compartment (figure 3). Reducing the noise level improves the limit of detection and quantification.

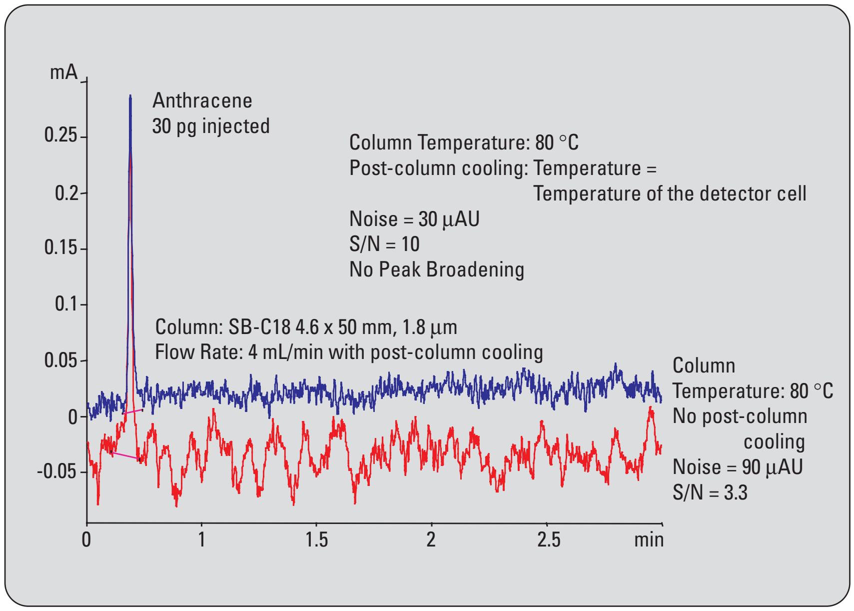

Figure 3
Maintaining sensitivity with post-column cooling.

Chromatographic conditions

Sample:	Anthracene
Column:	4.6 x 50 mm column, ZORBAX SB C-18, 1.8 µm
Mobile phase:	Water/acetonitrile = 40/60
Column temp.:	80 °C for the column
Flow rate:	4 mL/min
Detection:	251/4 nm, ref. 360/80 nm, 20 Hz

At high flow rates and high column temperature the signal-to-noise (S/N) ratio can be significantly improved by active cooling of the column effluent. The new low-delay volume cooler with 1.5 µL-volume can be used as cooling device. The column, for example, is installed at the left side of the column compartment. This side is heated to 80 °C. The column effluent is led through the cooler, which is installed at the right side of the column compartment. At this side the temperature is set to 34 °C, which is equivalent to the temperature of the detector optics. To avoid diffusion insulation material is placed in the column compartment door between the two heating and cooling zones. Cooling of the column effluent in this example helps to keep the S/N ratio up to 10. Without cooling, the S/N ratio drops down to 3.3. The 1.5-µL delay volume of the cooling device after the column does not influence the peak width negatively. At 4-mL/min flow rate this delay volume has no effect.

Conclusion

The design of the Agilent 1200 Series column compartment SL offers the possibility to operate a wide temperature range. Column temperatures from 10 degrees below ambient up to 100 °C can be obtained. To reduce the noise generated in the detector at elevated temperatures and high flow rates a post-column cooling device is used. An additional low-delay volume heater minimizes peak dispersion at low flow rates.

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