An Advanced Heat Exchanger for the Agilent 1290 Infinity Thermostatted Column Compartment

Technical Overview

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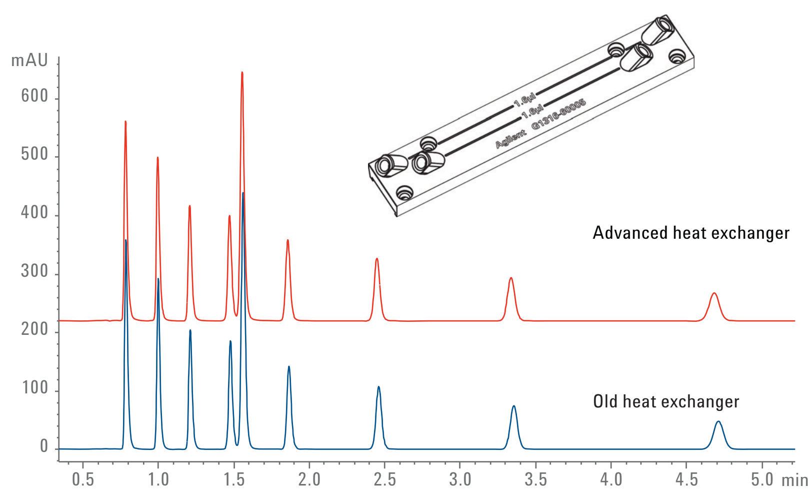
Abstract

Agilent designed an advanced heat exchanger with improved usability for the Agilent 1290 Infinity Thermostatted Column Compartment. The innovative heat exchanger is easy to install with just three screws, and has a removable capillary from the heat exchanger to the column. This Technical Overview demonstrates that the heat exchanger shows no significant differences compared to the previous version with respect to retention times, resolution, and heating and cooling performance.



Introduction

Temperature control in HPLC and UHPLC is mandatory for highly precise retention times. Another demand is that the delay volume of the heat exchanger should be as low as possible to avoid additional peak dispersion. Therefore, Agilent previously introduced heat exchangers with internal volumes of only 1.6 µL. An advanced heat exchanger has been introduced with the same internal volume (p/n G1316-80022), but with improved usability, robustness, and convenience.

If changes are made in a column compartment, tests are needed to prove that existing methods result in the same retention times and resolution as before. Advanced and original heat exchangers were compared at different temperatures using sulfonamides. The retention times of these compounds are very sensitive to temperature changes. Differences in retention times and resolution were calculated for gradient analysis. In addition, the differences at isocratic conditions were evaluated, as well as heating and cooling performance.

Experimental

Instruments

An Agilent 1290 Infinity Binary LC was used with:

- Agilent 1290 Infinity Diode Array Detector with 10-mm cell (G4212A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) equipped with classic L-shaped low dispersion heat exchanger (G1316-80003) (existing design) or low dispersion heat exchanger double (G1316-60005) (as contained in replacement kit G1316-80022)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Binary Pump (G4220A)

Compounds

Test compounds for gradient analysis were sulfathiazole, sulfamerazine, sulfamethazine, sulfamethizole, sulfachloropyrazine, and sulfadimethoxine, purchased from Sigma-Aldrich, Corp., Germany.

The RRLC Checkout Sample (p/n 5188-6529) for isocratic analysis contained nine compounds, 100 ng/µL each, dissolved in water:ACN (65:35):

1. acetanilide
2. acetophenone
3. propiophenone
4. butyrophenone (200 ng/mL)
5. benzophenone
6. valerophenone
7. hexanophenone
8. heptanophenone
9. octanophenone

Instrumental conditions

Gradient analysis	
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959764-902)
Mobile phase	Water + 0.1 % TFA, acetonitrile + 0.1 % TFA
Gradient	From 10 % to 40 % acetonitrile in 10 minutes
Flow rate	0.3 mL/min
Column temperature	25, 40, 60 °C
Detector	254/10 nm, ref 380/100 nm, 20 Hz, slit 4 nm
Injection volume	1 µL, sample cooled to 10 °C
Stop time	10 minutes
Post time	3 minutes
Column	Agilent ZORBAX Eclipse Plus C18, 3 × 100 mm, 3.5 µm (p/n 959961-302)
Mobile phase	Water + 0.1 % TFA, acetonitrile + 0.1 % TFA
Gradient	From 10 % to 40 % acetonitrile in 8 minutes
Flow rate	1 mL/min
Column temperature	21, 23, 25, 27, 40, and 60 °C
Detector	254/10 nm, ref 380/100 nm, 20 Hz, slit 4 nm
Injection volume	1 µL, sample cooled to 10 °C
Stop time	8 minutes
Post time	2 minutes
Column	Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)
Mobile phase	Water + 0.1 %TFA, acetonitrile + 0.1 % TFA
Gradient	From 10 % to 40 % acetonitrile in 5 minutes
Flow rate	2 mL/min
Column temperature	25, 40, 60 °C
Detector	254/10 nm, ref 380/100 nm, 20 Hz, slit 4 nm
Injection volume	1 µL, sample cooled to 10 °C
Stop time	5 minutes
Post time	1 minutes
Isocratic analysis	
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959764-902)
Mobile phase	Water:acetonitrile, 30:70
Flow rate	0.3 mL/min
Column temperature	40 °C
Detector	254/10 nm, ref 380/100 nm, 20 Hz, slit 4 nm
Injection volume	0.5 µL, sample cooled to 10 °C
Stop time	10 minutes

Software

Agilent OpenLAB CDS ChemStation Rev.C.01.05

Results and Discussion

Design of the advanced heat exchanger

The advanced low dispersion heat exchanger consists of a metal block and is installed between the fins of the Agilent 1290 Infinity Thermostatted Column Compartment by tightening three screws (Figure 1). In contrast to the current L-shaped low dispersion heat exchanger, the column inlet capillary and the capillary from injector to column heater can now be replaced if blocked or damaged. Up to four columns (maximum 100 mm in length) can be placed in one 1290 Infinity Thermostatted Column Compartment, as with the existing heat exchanger. Different configurations are possible, and are shown in Figure 1.

Performance comparison

The following tests were performed to compare the temperature behavior and delay volume of the existing and the advanced heat exchanger:

- Both heat exchangers were tested for differences in performance using isocratic conditions.
- The influence of small temperature changes from 21 to 23 to 25 to 27 °C on retention times and resolution was tested at 1 mL/min and gradient conditions.
- Both heat exchangers were tested for differences in retention time and resolution at 0.3, 1, and 2 mL/min flow rates at 25, 40, and 60 °C and gradient conditions.
- Temperature equilibration time was monitored over 10 runs for each temperature change.

Differences under isocratic conditions

Both heat exchangers have an internal volume of 1.6 µL, but they differ in that the capillary from the heater to the column is now removable, in case the fitting is damaged or the capillary is blocked.

To demonstrate that no or only minimal performance differences exist, both heat exchangers were tested under isocratic conditions and at a flow rate of 0.3 mL/min. In Figure 2, the chromatograms of the RR sample are overlaid.

Obviously, the difference is very small. The deviation of retention times for peak 5 is 0.3 %. This means that the peak eluted slightly earlier using the advanced heat exchanger. The deviation of the resolution was –3.4 % for peak 5. This indicates that resolution was minimally diminished using the advanced heater. The deviation of the theoretical plate number for the last peak was –1.4 %, with k' = 5.76.

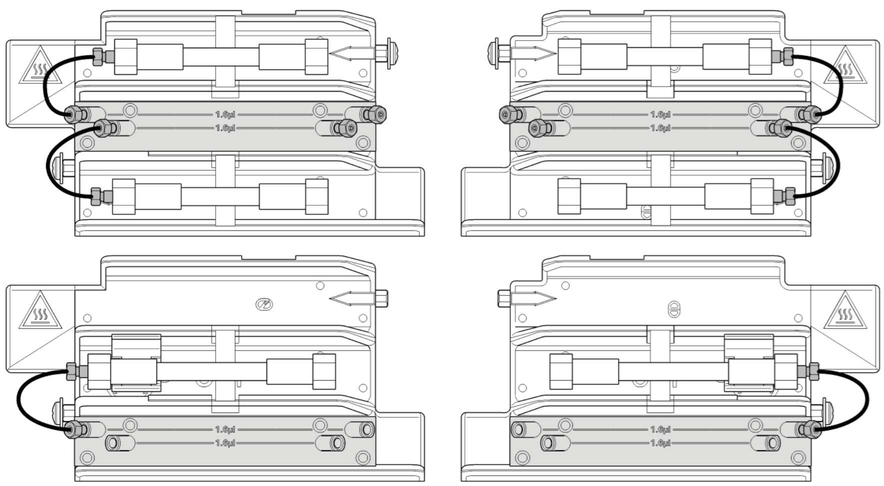


Figure 1. Different configurations of the advanced heat exchanger in the Agilent 1290 Infinity Thermostatted Column Compartment.

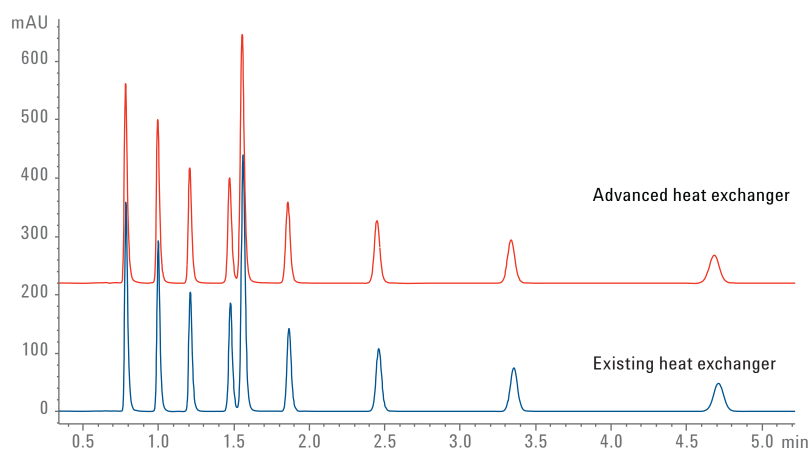


Figure 2. Comparison of chromatograms with Agilent advanced and existing heat exchangers using isocratic conditions at 0.3 mL/min flow rate to analyze the RRLC Checkout Sample.

Differences in retention time with small temperature changes

It was possible to determine to what extent the two types of heat exchangers differed by applying small temperature changes using sulfonamides to indicate the temperature effects. Gradient conditions and a 1 mL/min flow rate at 21, 23, 25, and 27 °C were chosen for the separation of six sulfonamides (Figure 3).

The first two peaks, sulfathiazole and sulfamerazine, were partially separated at 21 and 27 °C; at 23 and 25 °C, both compounds coeluted. Sulfamethazine, peak 3, showed only minimal changes in retention for all applied temperatures. Sulfamethizole, peak 4, sulfachloropyrazine, peak 5, and sulfadimethoxine, peak 6, changed to lower retention times with increasing temperature.

The deviation of retention times of the advanced heat exchanger was smaller than 0.5 % for all temperatures compared to the existing heat exchanger. This corresponded to a temperature deviation of approximately +0.2 °C measured for the last peak. The resolution deviation was smaller than –1.3 % for all peaks, which meant slightly less resolution. These results proved that both heaters behaved very similarly in this temperature range.

Differences in retention time and resolution at 0.3, 1, and 2 mL/min flow rates and 25, 40, and 60 °C

Three different temperatures were applied at a 0.3 mL/min flow rate on a 2.1 × 100 mm, 1.8 µm column (Figure 4). The deviation of retention times of the advanced heat exchanger was smaller than 0.7 % for all temperatures and the deviation of resolution was smaller than –0.4 %.

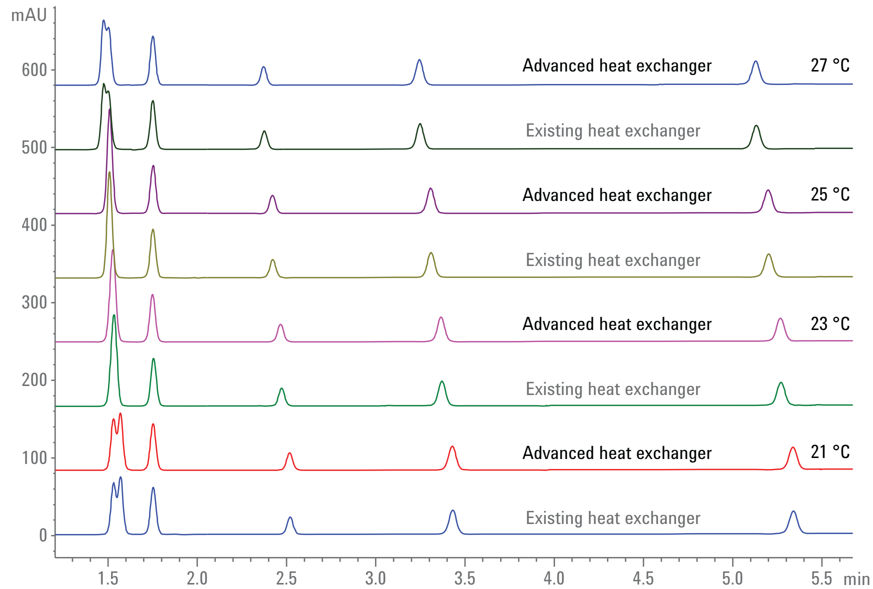


Figure 3. Influence of small temperature changes on the elution of sulfonamides.

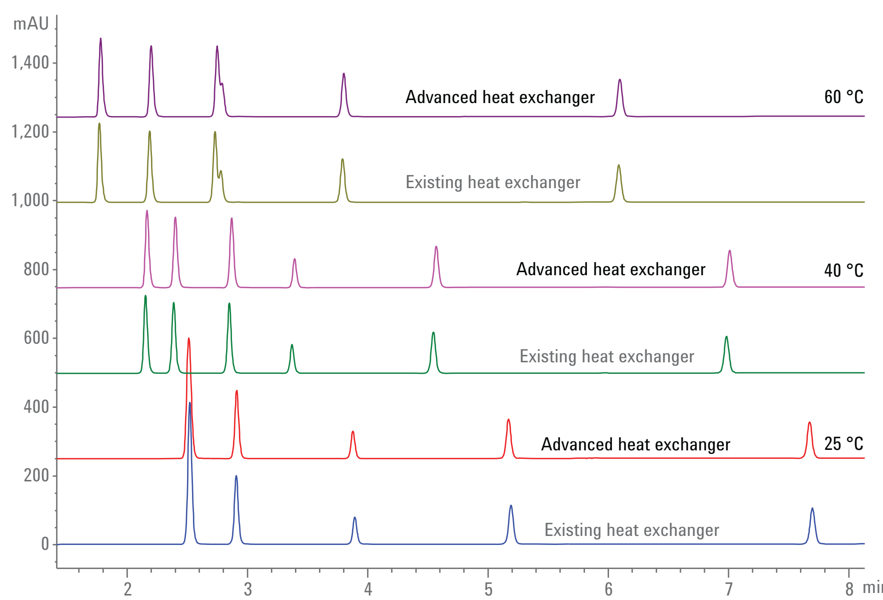


Figure 4. Overlay of chromatograms of existing and advanced Agilent heat exchangers at different temperatures and a 0.3 mL/min flow rate.

The same experiment was done using a 3 × 100 mm, 3.5 µm column at a 1 mL/min flow rate (Figure 5). The deviation of retention times of the advanced heat exchanger was smaller than 0.5 %, and the deviation of resolution was smaller than –1.6 %.

Finally, the experiments were repeated on a 4.6 × 50 mm, 1.8 µm column and a flow rate of 2 mL/min (Figure 6). The deviation of retention times of the advanced exchanger was smaller than 0.9 % and the deviation of resolution smaller than –3.4 %.

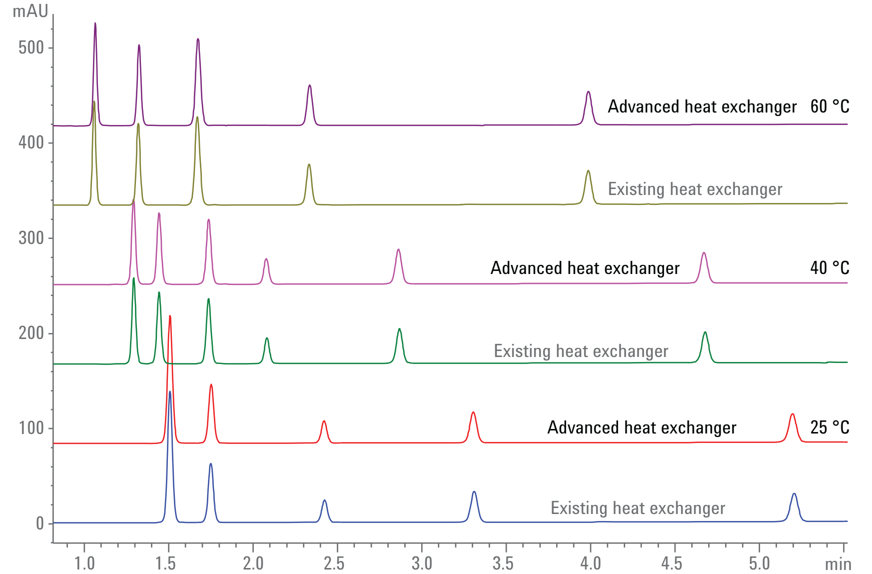


Figure 5. Overlay of chromatograms of Agilent existing and advanced heat exchangers at different temperatures and a 1 mL/min flow rate.

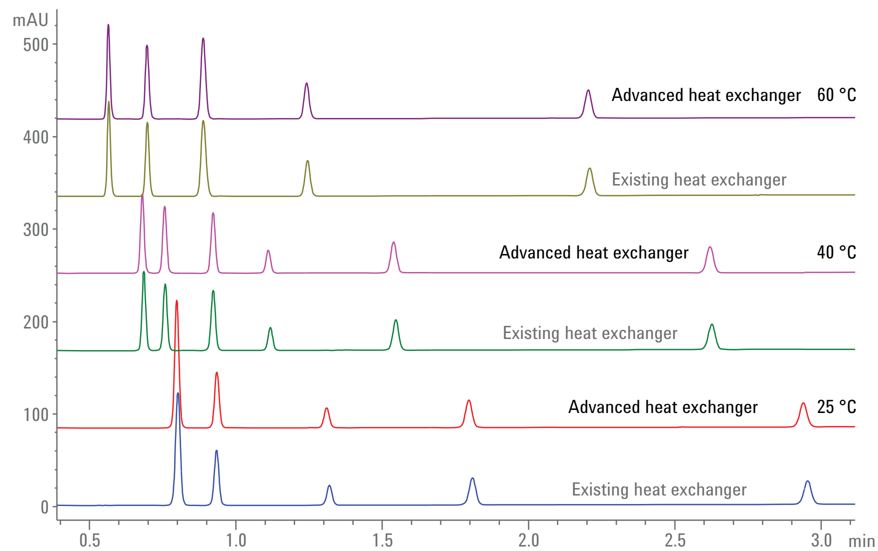


Figure 6. Overlay of chromatograms of Agilent existing and advanced heat exchangers at different temperatures and a 2 mL/min flow rate.

Table 1 summarizes the results, with more detailed information. Only resolution values that are lower with the advanced heat exchanger are considered.

Table 1. Deviation of retention times and resolution at 0.3, 1, and 2 mL/min flow rates and at 25, 40, and 60 °C using the Agilent advanced low dispersion heat exchanger double in comparison with the existing low dispersion heat exchanger.

Temperature/ flow rate	Advanced heat exchanger at 0.3 mL/min		Advanced heat exchanger at 1 mL/min		Advanced heat exchanger at 2 mL/min	
	Maximum RT deviation (%)	Maximum negative Rs deviation (%)	Maximum RT deviation (%)	Maximum negative Rs deviation (%)	Maximum RT deviation (%)	Maximum negative Rs deviation (%)
25 °C	0.28	–0.4	0.07	–0.1	0.76	–2.2
40 °C	0.7	–0.0	0.21	–1.6	0.88	–3.35
60 °C	0.63	–0.3	0.5	–1.3	0.36	–0.0
Column dimension	2.1 × 100 mm, 1.8 µm		3 × 100 mm, 3.5 µm		4.6 × 50 mm, 1.8 µm	

Temperature equilibration time monitored over 10 runs for each temperature change

The heating and cooling behavior of the heat exchangers was tested by changing the temperature from 25 to 40 °C and from 40 to 60 °C, and *vice versa*. Ten runs were done at 25 °C, followed by 10 runs at 40 °C, and 10 runs at 60 °C. During the change of temperature, no additional equilibration time was programmed. The next line of the sequence with the higher (lower) temperature started immediately. If, for example, after five runs the peaks overlaid exactly, it was assumed that the temperature was equilibrated. Figure 7 shows an example, applying a 1 mL/min flow rate and a temperature change from 40 to 60 °C using the existing heat exchanger.

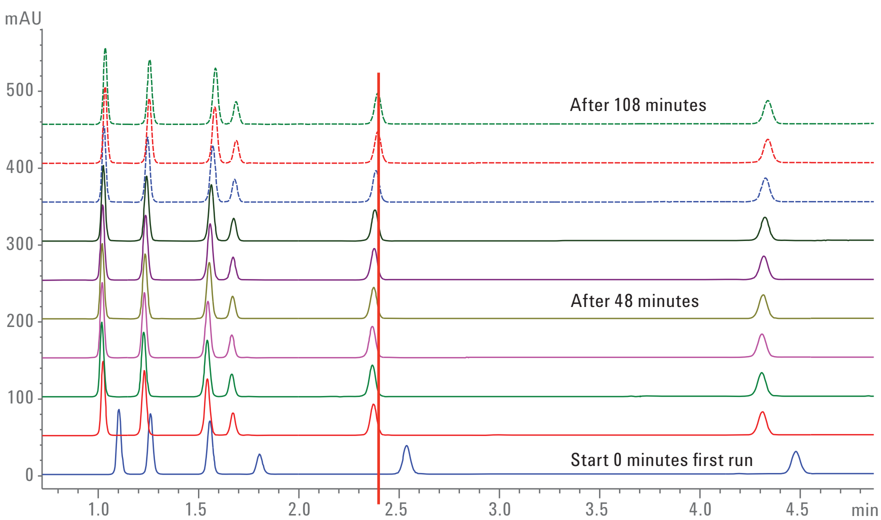


Figure 7. Temperature equilibration time after the change from 40 to 60 °C for the existing heat exchanger.

Table 2 summarizes the results for the different temperature changes at different flow rates. It was found that the advanced heat exchanger, especially at higher flow rates, equilibrated three to four times faster than the previous version. This was mainly due to the new design that uses one massive aluminum block. The cool-down experiments showed no significant difference for both heaters. The cool-down time depended not only on the heat exchanger itself, but also on complete oven environment, which significantly influenced the cool-down time until equilibration was reached.

Table 2. Result of different temperature changes at different flow rates.

Temperature change/flow rate	At 0.3 mL/min		At 1 mL/min		At 2 mL/min	
	Exisiting (min)	New (min)	Exisiting (min)	New (min)	Exisiting (min)	New (min)
25 to 40 °C	39	26	72	24	54	18
40 to 60 °C	39	26	72	24	48	12
Column dimensions	2.1 × 100 mm, 1.8 µm		3 × 100 mm, 3.5 µm		4.6 × 50 mm, 1.8 µm	

Conclusions

Agilent designed an advanced low dispersion heat exchanger for the Agilent 1290 Infinity Thermostatted Column Compartment. Comparison of the advanced and existing low dispersion heat exchanger showed no significant differences in retention time, resolution, or plate number. The deviation of retention time was found to be smaller than 0.9 % from 21 to 60 °C and the resolution differed less than –3.4 %. In addition to more convenient handling and advanced robustness, temperature-heating equilibration times were three times faster using the advanced heat exchanger.

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