# Performance Comparison of the Agilent 1290 Infinity II Multicolumn Thermostat with the Agilent 1290 Infinity Thermostatted Column Compartment

## Technical Overview


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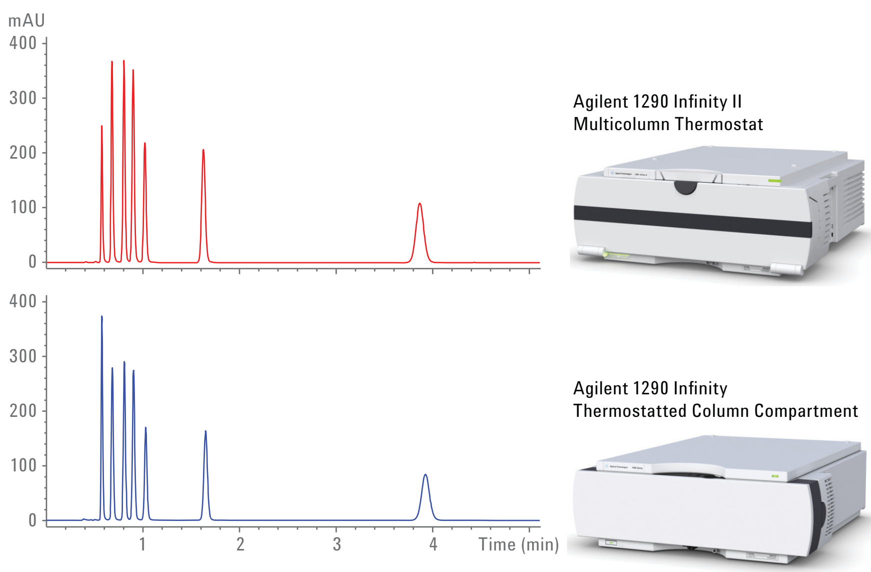
### Abstract

The Agilent 1290 Infinity II Multicolumn Thermostat offers many advantages regarding usability and performance. Flexible open positions of the front door provide for superior usability, and Agilent Quick-Connect heat exchangers and fittings enable fast and easy column exchange. In terms of performance, the 1290 Infinity II Multicolumn Thermostat achieves comparable or better results than the earlier Agilent 1290 Infinity Thermostatted Column Compartment. No significant shifts in retention times of the analyzed compounds were observed.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## Introduction

The Agilent 1290 Infinity II Multicolumn Thermostat features advanced usability and efficiency. In contrast to earlier column compartments, the 1290 Infinity II Multicolumn Thermostat has a front door with flexible opening positions of 90° (desk function) and 180° (Figure 1). For highest accessibility, the door can be removed completely.

Agilent A-Line Quick-Connect and Quick-Turn fittings represent a new generation of fittings, and facilitate faster and easier column exchange with a pressure rating up to 1,300 bar. The 1290 Infinity II Multicolumn Thermostat is based on still-air column thermostatting. Radial temperature gradients from the column center to the column wall are minimized, reducing unnecessary peak broadening, and thereby delivering optimal performance.

Many usability features of the 1290 Infinity Thermostatted Column Compartment were upgraded, maintaining excellent performance[1]. The changes made to the column compartment require robustness tests to prove that existing methods result in the same retention times and resolution as before. This Technical Overview compares the performance of the 1290 Infinity II Multicolumn Thermostat with the earlier Agilent 1290 Infinity Thermostatted Column Compartment with respect to retention time shifts using a mixture of six sulfonamides at different temperatures and flow rates. The retention times of these substances are extremely sensitive to temperature changes, revealing even the smallest temperature deviations. Differences in retention times were calculated for isocratic and gradient analyses.

Figure 1. Flexible open positions of the front door; 90° (desk function, top) or 180° (fully open, bottom).

## Experimental

### Instrumentation

The Agilent 1290 Infinity II LC system used for the experiments consisted of the following modules:

- Agilent 1290 Infinity II flexible pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with Quick-Connect standard heat exchangers
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) equipped with low-dispersion heat exchangers
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10-mm Max-Light cartridge cell

### Columns

- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
- Agilent ZORBAX Eclipse Plus C18, 3 × 100 mm, 3.5 µm (p/n 959961-302)
- Agilent ZORBAX Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)

### Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, revision C.01.07 [27]

## Solvents and samples

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfadimethoxine, sulfachloropyrazine, and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich, St. Louis, USA.

Table 1. Chromatographic conditions for isocratic analysis with 3.0 × 100 mm, 3.5-µm column.

| Parameter | Value |
|---|---|
| Mobile phase | A) 80 % water + 0.1 % TFA, 20 % acetonitrile + 0.1 % TFA |
| Flow rate | 1 mL/min |
| Stop time | 10 minutes |
| Injection volume | 1 µL |
| Column temperature | 40 °C |
| Detection | Signal A 254/4 nm, reference 380/100 nm<br>Peak width > 0.013 minutes (0.25 seconds response time)<br>Data rate 20 Hz |

Table 2. Chromatographic conditions for gradient analysis with 2.1 × 100 mm, 1.8-µm column.

| Parameter | Value |
|---|---|
| Mobile phase | A) Water + 0.1 % TFA<br>B) Acetonitrile + 0.1 % TFA |
| Flow rate | 0.3 mL/min |
| Gradient | 0 minutes, 10 %B<br>10 minutes, 40 %B |
| Stop time | 11 minutes |
| Post time | 5 minutes |
| Injection volume | 1 µL |
| Column temperature | 25, 40, and 60 °C |
| Detection | Signal A 254/4 nm, reference 360/100 nm<br>Peak width > 0.025 minutes (0.5 seconds response time)<br>Data rate 10 Hz |

Table 3. Chromatographic conditions for gradient analysis with 3.0 × 100 mm, 3.5-µm column.

| Parameter | Value |
|---|---|
| Mobile phase | A) Water + 0.1 % TFA<br>B) Acetonitrile + 0.1 % TFA |
| Flow rate | 1 mL/min |
| Gradient | 0 minutes, 10 %B<br>10 minutes, 40 %B |
| Stop time | 11 minutes |
| Post time | 5 minutes |
| Injection volume | 1 µL |
| Column temperature | 21, 23, 25, 27, 29, 31, 40, and 60 °C |
| Detection | Signal A 254/4 nm, reference 360/100 nm<br>Peak width > 0.025 minutes (0.5 seconds response time)<br>Data rate 10 Hz |

## Results and Discussion

Six sulfonamides were separated at 21 to 31 °C with temperature intervals of only 2 °C to demonstrate the highly temperature-dependent retention times of the compounds. Gradient conditions and a 1 mL/min flow rate at 21, 23, 25, 27, 29, and 31 °C were chosen for the separation of six sulfonamides. Figure 2 shows the retention time shifts for the 2-degree intervals. All peaks show shorter retention time with increasing temperature, but temperature changes from 31 to 29 °C leads to the coelution of two peaks (A and B) that were separated at 31 °C. Peak B elutes earlier than the other peaks with increasing temperature, resulting in shorter retention time, and reversing the position in the elution order with peak A.

The following analyses were carried out to compare the temperature performance of the Agilent 1290 Infinity II Multicolumn Thermostat with the Agilent 1290 Infinity Thermostatted Column Compartment with respect to retention time shifts:

- Both modules were compared for differences in performance using isocratic conditions
- Both modules 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 using gradient conditions.

The heat exchangers of the 1290 Infinity II Multicolumn Thermostat and of the 1290 Infinity Thermostatted Column Compartment have an internal volume of 1.6 µL. In addition, the same capillaries were used during the analyses with both modules. Therefore, no influence should be expected regarding delay volume, and thus, temperature transfer and hold should be the only factors influencing the retention times of the peaks.

Table 4. Chromatographic conditions for gradient analysis with 4.6 × 50 mm, 2.7-µm column.

| Parameter | Value |
|---|---|
| Mobile phase | A) Water + 0.1 % TFA<br>B) Acetonitrile + 0.1 % TFA |
| Flow rate | 2 mL/min |
| Gradient | 0 minutes, 10 %B<br>5 minutes, 40 %B |
| Stop time | 5.5 minutes |
| Post time | 2 minutes |
| Injection volume | 1 µL |
| Column temperature | 25, 40, and 60 °C |
| Detection | Signal A 254/4 nm, reference 360/100 nm<br>Peak width > 0.025 minutes (0.5 seconds response time)<br>Data rate 10 Hz |

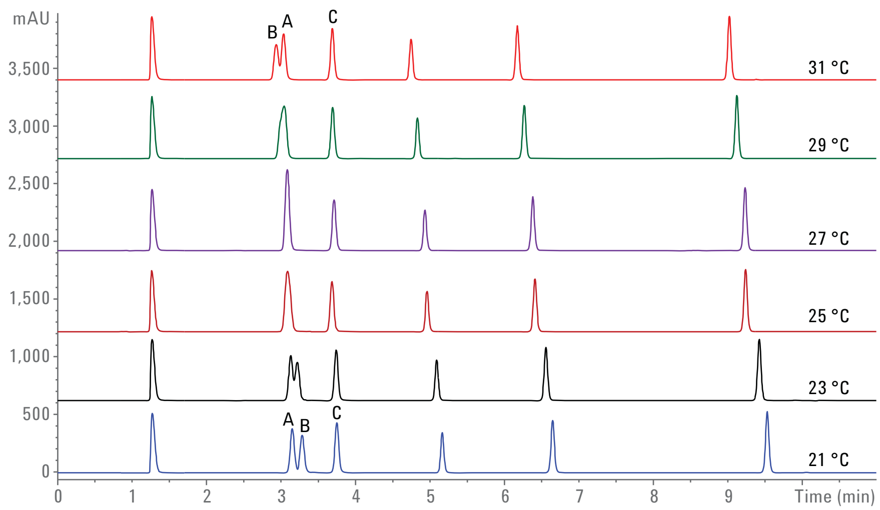


Figure 2. Influence of small temperature changes on the retention times of sulfonamides.

## Isocratic conditions

The separation of the six sulfonamides was performed under isocratic conditions, with a flow rate of 1 mL/min at 40 °C. Figure 3 shows the overlay of the two chromatograms of the 1290 Infinity II Multicolumn Thermostat (MCT, red signal) and the 1290 Infinity Thermostatted Column Compartment (TCC, blue signal). The difference is clearly very small. The deviation of retention times for the last peak was less than 2.1 %, already representing the largest deviation of all peaks. Generally, the tolerable deviation range is 5 %, also within the Agilent 1290 Infinity Intelligent System Emulation Technology (ISET). Therefore, with about 2 % deviation, the retention time shift was acceptable. Using the 1290 Infinity II Multicolumn Thermostat, the peaks eluted slightly earlier.

## Gradient conditions at 0.3, 1, and 2 mL/min flow rates and 25, 40, and 60 °C

Three different temperatures were applied at 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 1290 Infinity II Multicolumn Thermostat from the 1290 Infinity Thermostatted Column Compartment was less than 3.6 % for all temperatures.

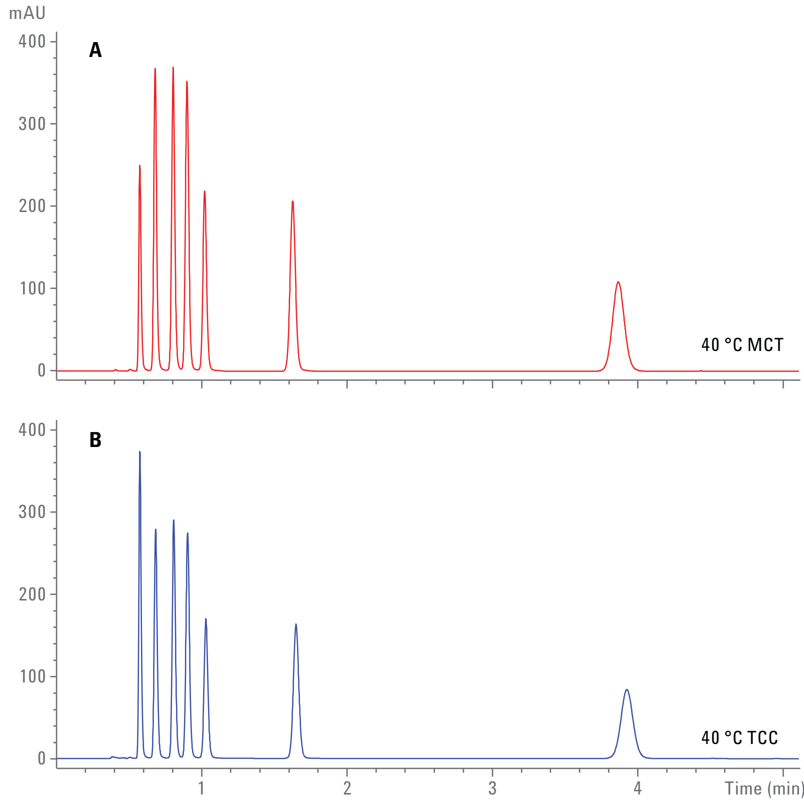


Figure 3. Comparison of chromatograms of an Agilent 1290 Infinity II Multicolumn Thermostat (MCT, red signal) and an Agilent 1290 Infinity Thermostatted Column Compartment (TCC, blue signal) under isocratic conditions at 40 °C and a 1 mL/min flow rate.

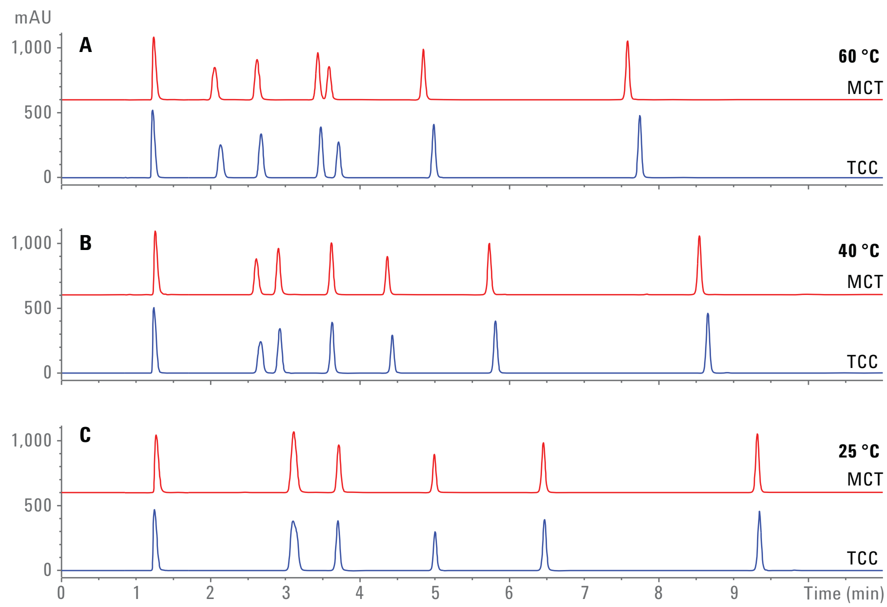


Figure 4. Overlay of chromatograms of an Agilent 1290 Infinity II Multicolumn Thermostat (MCT, red signals) and an Agilent 1290 Infinity Thermostatted Column Compartment (TCC, blue signals) at different temperatures under gradient conditions using a 2.1 × 100 mm, 1.8 µm column and a 0.3 mL/min flow rate.

The same experiment was done using a 3 × 100 mm, 3.5 µm particle column at 1 mL/min flow rate (Figure 5). The deviation of retention times of the 1290 Infinity II Multicolumn Thermostat from the 1290 Infinity Thermostatted Column Compartment was less than 1.9 % for all temperatures.

Finally, the experiments were repeated on a 4.6 × 50 mm, 2.7 µm particle column and a flow rate of 2 mL/min (Figure 6). The deviation of retention times of the 1290 Infinity II Multicolumn Thermostat from the 1290 Infinity Thermostatted Column Compartment was smaller than 0.8 % for all temperatures. Table 5 summarizes the results, with more detailed information for each temperature.

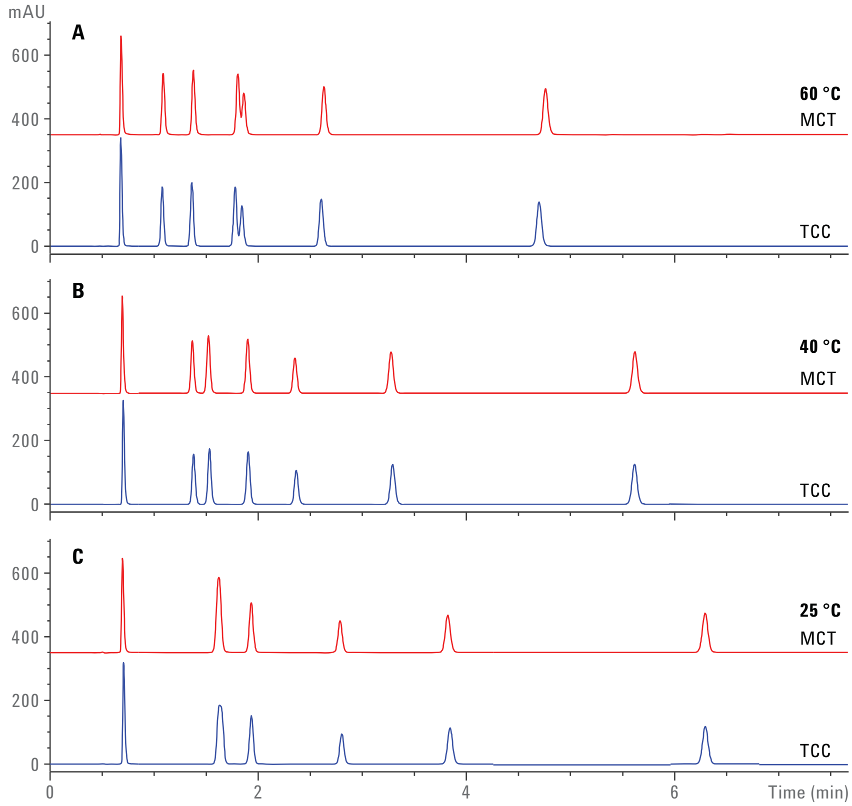


Figure 5. Overlay of chromatograms of an Agilent 1290 Infinity II Multicolumn Thermostat (MCT, red signals) and an Agilent 1290 Infinity Thermostatted Column Compartment (TCC, blue signals) at different temperatures under gradient conditions using a 3 × 100 mm, 3.5 µm column and a 1 mL/min flow rate.

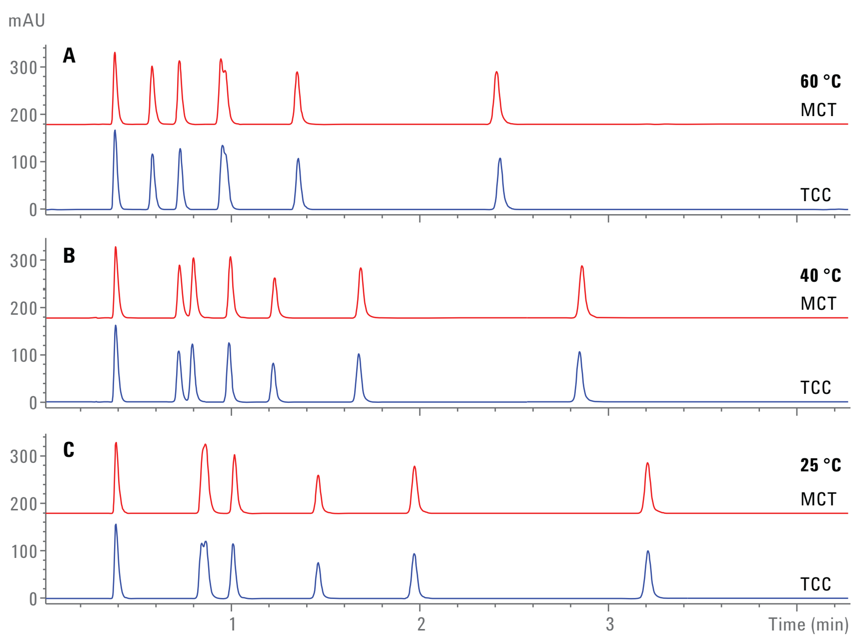


Figure 6. Overlay of chromatograms of an Agilent 1290 Infinity II Multicolumn Thermostat (MCT, red signals) and an Agilent 1290 Infinity Thermostatted Column Compartment (TCC, blue signals) at different temperatures under gradient conditions using a 4.6 × 50 mm, 2.7-µm column and a 2 mL/min flow rate.

## Conclusion

This Technical Overview presents a performance comparison between the Agilent 1290 Infinity II Multicolumn Thermostat and the Agilent 1290 Infinity Thermostatted Column Compartment. Six sulfonamides with retention times that are highly sensitive to temperature changes were analyzed under isocratic and gradient conditions with both modules, and the retention time shifts were examined. No significant shifts in retention time were observed. For the isocratic analysis, a retention time shift of less than 2.1 % was found whereby a shift of less than 5 % is regarded as tolerable (also in accordance to the Agilent 1290 Infinity Intelligent System Emulation Technology). For gradient analysis, three different temperatures were tested for retention time shifts: 25, 40, and 60 °C. None of the observed retention time shifts were outside the tolerance range of 5 %, with a maximum of 3.6 % found at 0.3 mL/min and 60 °C. The 1290 Infinity II Multicolumn Thermostat shows comparable temperature performance regarding retention time compared to the 1290 Infinity Thermostatted Column Compartment even for extremely temperature-sensitive analytes such as the sulfonamides used.

Table 5. Deviation of retention times and resolution at 0.3, 1, and 2 mL/min flow rates and at 25, 40, and 60 °C for the Agilent 1290 Infinity II Multicolumn Thermostat (MCT) from the Agilent 1290 Infinity Thermostatted Column Compartment.

| | Maximum RT deviation (%) | | |
|---|---|---|---|
| Temperature | MCT at 0.3 mL/min | MCT at 1 mL/min | MCT at 2 mL/min |
| 25 °C | 1.69 | 1.27 | 0.79 |
| 40 °C | 2.21 | 1.28 | 0.4 |
| 60 °C | 3.57 | 1.86 | 0.42 |

## References

1. Schneider, S., Performance Characteristics of the Agilent 1290 Infinity II Multicolumn Thermostat, *Agilent Technologies Technical Overview*, publication number 5991-5533EN, **2015**.

2. Muehlebach, A., Advance efficiency with new Agilent 1290 Infinity II Multicolumn Thermostat, *Access Agilent Newsletter* (www.agilent.com/chem/accessagilent), May 2015.

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Published in the USA, August 1, 2015
5991-6076EN

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