Performance Characteristics of the Agilent 1290 Infinity II Multicolumn Thermostat

Technical Overview

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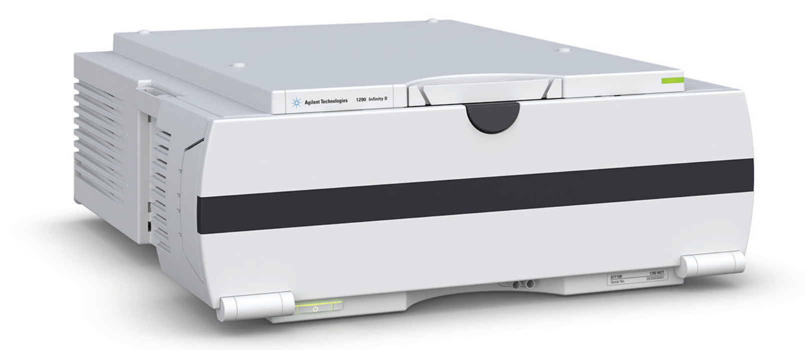
Abstract

This Technical Overview demonstrates the superior usability of the Agilent 1290 Infinity II Multicolumn Thermostat (MCT). Precise column thermostatting over a broad temperature range provides infinite flexibility for optimized speed and selectivity of LC separations. Excellent retention time precision was found at three different temperatures using sulfonamides, the retention times of which are highly sensitive to temperature changes. In addition, high retention time precision was found at extreme conditions using 100 °C and high flow rates. As a result, the 1290 Infinity II MCT achieves excellent temperature precision for highly reproducible retention times.



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Introduction

The Agilent 1290 Infinity II Multicolumn Thermostat (MCT) offers many advantages with respect to performance and usability. The advanced column capacity for up to eight columns with individual precolumn solvent heating in a single MCT is displayed in Figure 1. The door provides superior usability with flexible flap positions at 90° or 180°, and it can be removed completely for highest accessibility. The MCT facilitates precise column thermostatting for maximum application flexibility. This is made possible by Peltier cooling and heating with two independent temperature zones from 20 °C below ambient up to 110 °C. Ultrahigh pressure valves enable a wide range of applications such as column selection from eight columns in a single MCT, sample preparation for analyte enrichment or matrix removal, alternating column regeneration, and many more.

Revolutionary Agilent A-Line Quick-Connect UHPLC column fittings for dead-volume-free fluidic connections enable efficient, fast, and convenient column exchange (Figure 2). In addition, easy-to-install precolumn Quick-Connect heat exchangers are available for precolumn solvent heating. For optimized internal volume contribution, chose the Quick-Connect heat exchanger in accordance to the LC flow applied (1.6 µL for standard flow, 3.0 µL for high flow, and 1 µL for ultralow dispersion).

The 1290 Infinity II MCT combines perfectly with all 1290 Infinity II modules, and also with other Agilent 1260 and 1290 Infinity modules.

This Technical Overview shows temperature precision for reproducible retention times (RT) at three different temperatures using highly temperature-sensitive sulfonamides. The retention times of these compounds are very sensitive even to small temperature changes[1]. High performance and ultrahigh performance liquid chromatography (HPLC and UHPLC) conditions are applied, and the retention time precision is analyzed.

Figure 1. Advanced column capacity for up to eight columns in a single MCT.

Figure 2. Agilent A-Line Quick Connect Fittings.

Experimental

Equipment

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

- Agilent 1290 Infinity II High-speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with Quick-Connect Heat Exchangers for standard flow and high flow
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10-mm Max-Light cartridge cell

Columns

- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 858700-902)
- Agilent ZORBAX RRHD SB-C18, 3 × 50 mm, 1.8 µm (p/n 857700-302)

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.

Agilent RRLC Checkout sample (p/n 5188-6529), containing acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, benzophenone, and acetanilide.

Table 1. Chromatographic conditions for 4.6 × 150 mm, 5 µm column.

Parameter	Value
Mobile phase	Solvent A) Water + 0.1 % TFA Solvent B) Acetonitrile + 0.1 % TFA
Flow rate	1.2 mL/min
Gradient	0 minutes, 10 %B 12 minutes, 40 %B 13 minutes, 95 %B
Stop time	15 minutes
Post time	15 minutes
Injection volume	2 µL
Column temperatures	30, 40, and 60 °C
VWD	Signal A 254/4 nm, reference 380/100 nm, peak width > 0.013 minutes (0.25 seconds response time), data rate 20 Hz

Table 2. Chromatographic conditions for 2.1 × 100 mm, 1.8 µm column.

Parameter	Value
Mobile phase	Solvent A) Water + 0.1 % TFA Solvent B) Acetonitrile + 0.1 % TFA
Flow rate	0.3 mL/min
Gradient	0 minutes, 10 %B 10 minutes, 40 %B 11 minutes, 95 %B
Stop time	12 minutes
Posttime	10 minutes
Injection volume	0.2 µL
Column temperatures	30, 40, and 60 °C
VWD	Signal A 254/4 nm, reference 380/100 nm, peak width > 0.013 minutes (0.25 seconds response time), data rate 20 Hz

Table 3. Chromatographic conditions for 3 × 50 mm, 1.8 µm column.

Parameter	Value
Mobile phase	Solvent A) Water Solvent B) Acetonitrile
Flow rate	3.8 mL/min
Gradient	0 minutes, 35 %B 0.38 minutes, 95 %B 0.45 minutes, 95 %B 0.46 minutes, 35 %B
Stop time	0.5 minutes
Post time	0.5 minutes
Injection volume	1 µL
Column temperatures	100 °C
VWD	Signal A 254/4 nm, reference 380/100 nm, peak width > 0.0031 minutes (0.063 seconds response time), data rate 80 Hz

Results and Discussion

Six temperature-sensitive sulfonamides were separated on a commonly-used HPLC column, an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm column, at 30, 40, and 60 °C, and the retention time precision was determined. Figure 3 shows the overlays of six consecutive runs that were also used for retention time precision calculation. The precision is represented by the relative standard deviation (RSD) in percent. At 60 °C, two sulfonamides (peaks 3 and 4) coelute. Table 4 shows the retention time precision values with the HPLC chromatographic conditions with the 4.6 × 150 mm, 5 µm column at 30, 40, and 60 °C.

Excellent retention time precision was achieved for HPLC conditions at all three temperatures with RSDs below 0.062 % for all six sulfonamides.

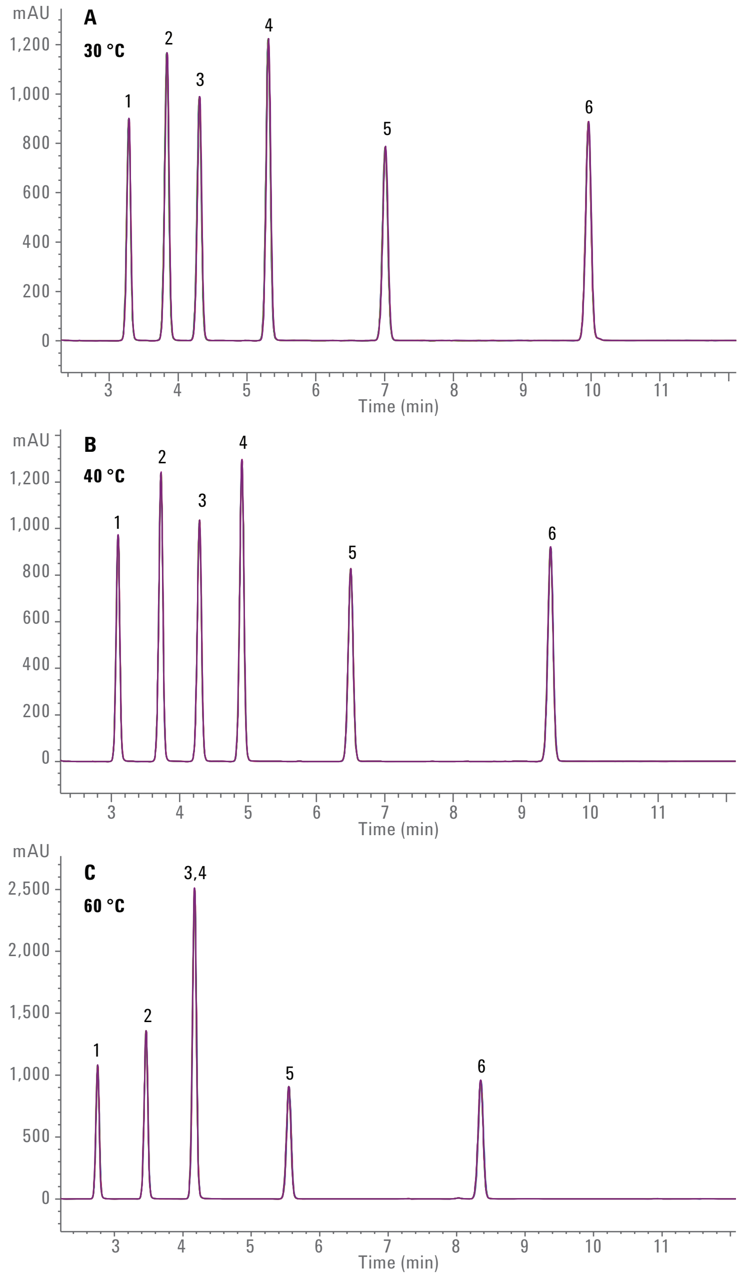

Figure 3. Overlay of six consecutive runs for the HPLC separation of six sulfonamides at 30, 40, and 60 °C. Excellent retention time precision was achieved.

Table 4. Retention time precision using HPLC conditions.

Sulfonamide	Peak	RSD (%) at 30 °C	RSD (%) at 40 °C	RSD (%) at 60 °C
Sulfadiazine	1	0.061	0.036	0.054
Sulfamerazine	2	0.061	0.037	0.04
Sulfamethazine	3	0.061	0.027	0.044
Sulfamethizole	4	0.053	0.03	0.044
Sulfadimethoxine	5	0.036	0.022	0.051
Sulfachloropyrazine	6	0.027	0.019	0.03

The same sample, containing six sulfonamides, was separated using an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column with UHPLC conditions. Figure 4 shows the overlays of six consecutive runs that have also been used for RT precision calculation. Again, at 60 °C, two sulfonamides (peaks 3 and 4) elute very close together. However, although they are not baseline separated, two peaks are observed, which is a clear advantage of the UHPLC separation conditions. Table 5 shows the precision values with the UHPLC chromatographic conditions with the 2.1 ×100 mm, 1.8 µm column at 30, 40, and 60 °C. Again, excellent RT precision was found for UHPLC conditions at all three temperatures with RSDs below 0.086 % for all six sulfonamides.

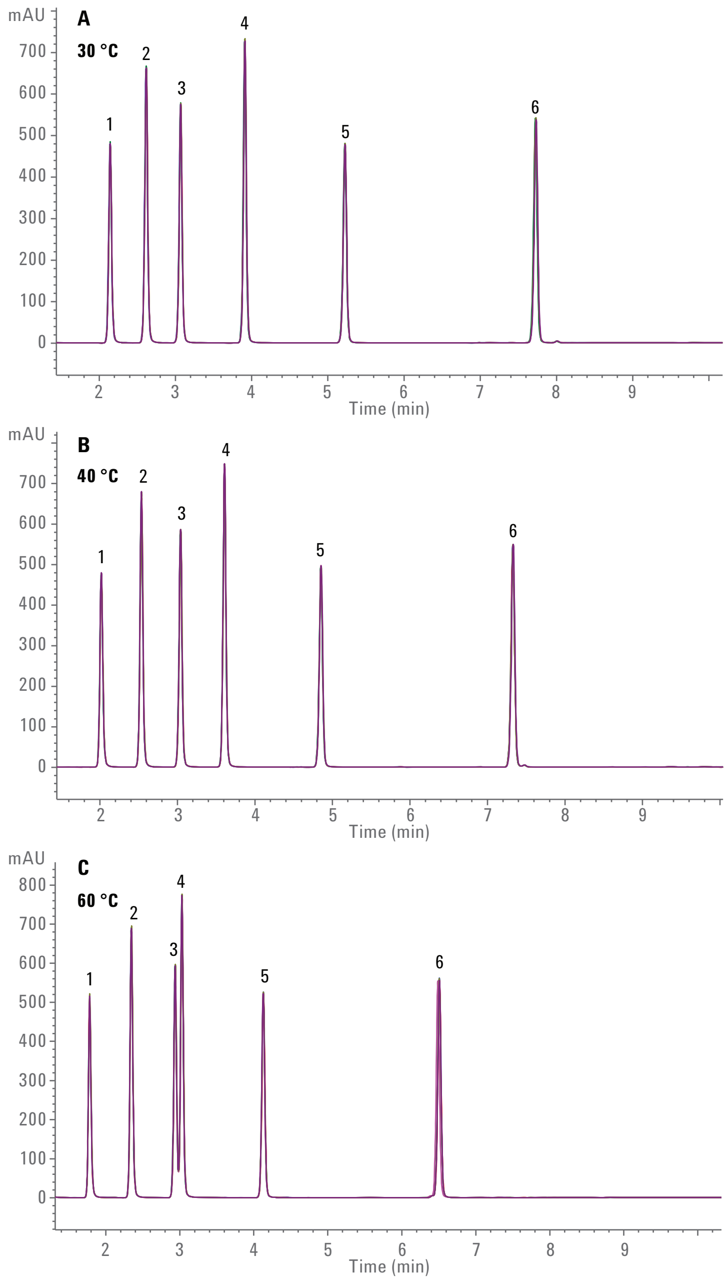

Figure 4. Overlay of six consecutive runs for the UHPLC separation of six sulfonamides at 30, 40, and 60 °C. Excellent retention time precision was achieved.

Table 5. Retention time precision using UHPLC conditions.

Sulfonamide	Peak	RSD (%) at 30 °C	RSD (%) at 40 °C	RSD (%) at 60 °C
Sulfadiazine	1	0.072	0.049	0.083
Sulfamerazine	2	0.059	0.051	0.055
Sulfamethazine	3	0.049	0.044	0.039
Sulfamethizole	4	0.059	0.028	0.085
Sulfadimethoxine	5	0.041	0.034	0.073
Sulfachloropyrazine	6	0.051	0.03	0.053

Although column temperatures above 60 °C are rarely used for standard applications, the next experiment demonstrates the superior performance of the 1290 Infinity II MCT at elevated temperatures. With a high flow rate of 3.8 mL/min, and at 100 °C, fast analyses are possible, resulting in run times below 0.5 minutes. Figure 5 shows an overlay of six consecutive runs of a separation of the RRLC checkout sample, containing nine different peaks using a Quick-Connect standard flow heat exchanger (1.6 µL) and a Quick-Connect high flow heat exchanger (3 µL for flows higher than 2.5 mL/min). Despite the harsh conditions, excellent RT precision was found for all nine peaks with RSDs lower than 0.066 % for the 1.6-µL heat exchanger, and even lower than 0.035 % for the 3-µL heat exchanger, see Table 6.

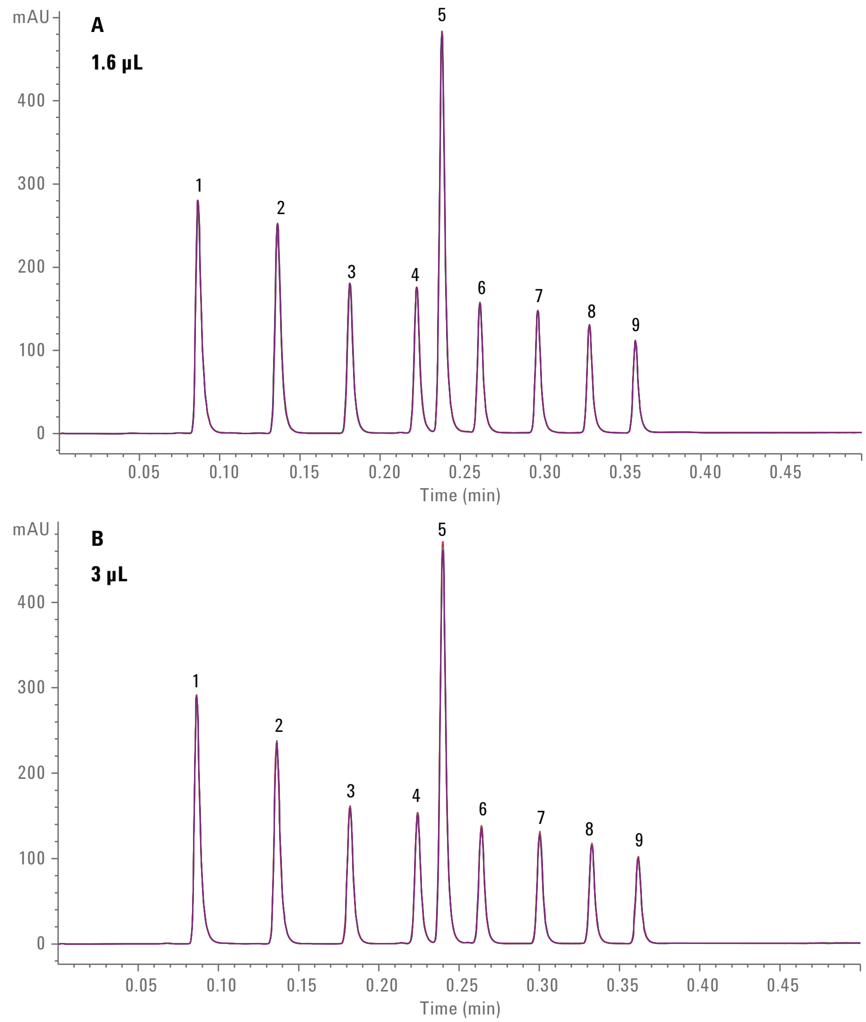

Figure 5. Overlay of six consecutive runs of RRLC checkout sample separation at 100 °C with 3.8 mL/min flow rate using the 1.6- and 3-µL heat exchangers.

Table 6. Retention time precision at 100 °C and 3.8-mL/min flow rate.

Compound	Peak	RSD (%) 1.6-µL heat exchanger	RSD (%) 3-µL heat exchanger
Acetophenone	1	0.065	0.024
Propiophenone	2	0.055	0.034
Butyrophenone	3	0.042	0.016
Valerophenone	4	0.028	0.014
Hexanophenone	5	0.021	0.021
Heptanophenone	6	0.015	0.015
Octanophenone	7	0.013	0.013
Benzophenone	8	0.006	0.013
Acetanilide	9	0.005	0.016

Conclusion

This Technical Overview presents the usability and thermostatting performance of the Agilent 1290 Infinity II Multicolumn Thermostat. HPLC and UHPLC conditions were applied at four temperatures of 30, 40, 60, and 100 °C. For six temperature-sensitive sulfonamides, analyzed under HPLC and UHPLC conditions at 30, 40, and 60 °C, excellent retention time RSDs of below 0.062 % were achieved under HPLC conditions, and below 0.086 % under UHPLC conditions. Extreme conditions, at 100 °C and a high flow rate of 3.8 mL/min for sample separation in less than 0.5 minutes, also revealed high precision values below 0.066 % for the Quick-Connect standard flow heat exchanger, and even lower than 0.035 % for the Quick-Connect high flow heat exchanger. The 1290 Infinity II Multicolumn Thermostat combines superior usability and flexibility with precise column thermostatting over a broad temperature range, resulting in optimized speed and selectivity for LC separation.

Reference

1. An Advanced Heat Exchanger for the Agilent 1290 Infinity Thermostatted Column Compartment, *Agilent Technologies Technical Overview*, publication number 5991-5166EN, **2014**.

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