Using the Agilent 1290 Infinity II Multicolumn Thermostat with Extreme Temperatures and Flow Rates

Technical Overview

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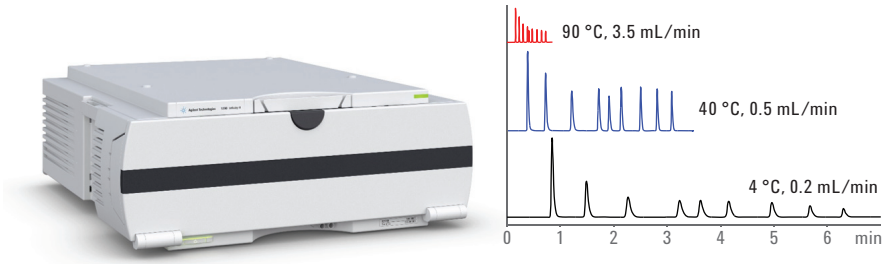
Abstract

This Technical Overview describes the performance of the Agilent 1290 Infinity II Multicolumn Thermostat when using extreme temperatures and flow rates. The analysis of nine compounds under standard conditions was optimized towards high and low settings of temperature and solvent flow. Excellent retention time precision was found at column temperatures of 4 and 90 °C, with flow rates as low as 0.2 up to 3.5 mL/min. Optimized for high throughput, a baseline separation of the nine compounds was achieved in less than 0.8 minutes. These findings point out the superior usability of the 1290 Infinity II Multicolumn Thermostat over a wide range of temperature and flow settings, covering the most demanding applications with focus on throughput or selectivity.



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Introduction

The Agilent 1290 Infinity II Multicolumn Thermostat provides extended heating and cooling capabilities from 4 °C (20 degrees below ambient) up to 110 °C for up to eight columns (Figure 1). The column compartment can be divided into two independent temperature zones that are controlled separately. Agilent Quick-Connect heat exchangers are available in three different sizes (1.0, 1.6, and 3.0 µL), featuring internal volumes optimized for low dispersion, standard flow, and high throughput applications, respectively (Figure 2). Each of the eight columns that can be mounted in the 1290 Infinity II Multicolumn Thermostat has a distinct heat exchanger, enabling an optimum combination of columns and heat exchangers. Together with the integrated 8-column selection valve and the two independent temperature zones, the design of the 1290 Infinity II Multicolumn Thermostat enables a system setup suitable for diverse applications from method development to routine analyses.

With commonly used temperature and solvent flow settings of 30 to 60 °C and 0.3 to 1.2 mL/min, respectively, the 1290 Infinity II Multicolumn Thermostat has already proven excellent performance[1]. To demonstrate the performance under nonstandard conditions, we applied some rather unusual separation methods using high or low solvent flows, with high or low temperature settings. Such parameters might be used when separating thermally labile or chiral substances, or when high-throughput analyses of hardly separable compounds are demanded. A mixture of standard compounds comprising eight phenones and acetanilide was used, providing a sample that is stable under all conditions applied in this Technical Overview. Temperature stability and precision of the 1290 Infinity II Multicolumn Thermostat were measured by means of retention time relative standard deviations (RSDs) of each compound, calculated out of a series of six consecutive runs.

Figure 1. The Agilent 1290 Infinity II Multicolumn Thermostat holds up to eight columns, each with a distinct heat exchanger, in two independent temperature zones.

Figure 2. Agilent Quick-Connect heat exchangers and column holder clips.

Experimental

Instrumentation

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

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Vialsampler (G7129B) with integrated sample cooler (option #100)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with divider assembly, 8-column selection valve (G4239C) and Agilent Quick-Connect heat exchangers for standard flow (option #062), high flow (option #063), and ultralow dispersion (option #064)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with 10-mm Max-Light cartridge cell

Columns

The following two columns were used for low/standard flow and high flow applications, respectively:

- Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
- Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm (p/n 835975-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR1 [106]

Solvents and sample

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). The Agilent RRLC Checkout Sample (p/n 5188-6529) used for analysis consisted of eight phenones (acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, and benzophenone) and acetanilide, each at a concentration of 100 µg/mL, dissolved in water/acetonitrile (65/35 by volume).

Chromatographic conditions

Chromatographic conditions for standard flow application with an Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm column	
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.5 mL/min
Gradient	0.0 minutes – 40 %B 2.5 minutes – 95 %B
Stop time	3.5 minutes
Post time	2.0 minutes
Injection volume	0.2 µL
Sample temperature	8 °C
Column temperature	40 °C
Detection	Signal A 240/4 nm, reference 360/80 nm Peak width >0.0063 minutes (0.13 seconds response time), 40 Hz
Chromatographic conditions for low-flow/low-temperature application with an Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm column	
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.2 mL/min
Gradient	0.0 minutes – 50 %B 4.0 minutes – 98 %B
Stop time	7.0 minutes
Post time	6.0 minutes
Injection volume	0.2 µL
Sample temperature	8 °C
Column temperature	4 °C
Detection	Signal A 240/4 nm, reference 360/80 nm Peak width >0.013 minutes (0.25 seconds response time), 20 Hz
Chromatographic conditions for low-flow/high-temperature application with an Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm column	
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.2 mL/min
Gradient	0.0 minutes – 50 %B 5.0 minutes – 98 %B
Stop time	6.0 minutes
Post time	6.0 minutes
Injection volume	0.2 µL
Sample temperature	8 °C
Column temperature	90 °C
Detection	Signal A 240/4 nm, reference 360/80 nm Peak width >0.013 minutes (0.25 seconds response time), 20 Hz

Results and Discussion

An Agilent RRLC checkout sample containing nine standard compounds was analyzed using different solvent flow and temperature settings. The 1290 Infinity II Multicolumn Thermostat has two temperature zones. The zone where the column was installed was always set to the temperature described in the methods section. The other zone was controlled separately and held constantly at 40 °C to demonstrate the simultaneous preparation or regeneration of another column, which can be implemented as a time-saving step in routine analyses.

Chromatographic conditions for high-flow/low-temperature application with an Agilent ZORBAX SB-C18,4.6 × 50 mm, 3.5 µm column	
Mobile phase	A) Water B) Acetonitrile
Flow rate	2.5 mL/min
Gradient	0.0 minutes – 60 %B 2.0 minutes – 98 %B
Stop time	2.0 minutes
Post time	1.0 minutes
Injection volume	1 µL
Sample temperature	8 °C
Column temperature	4 °C
Detection	Signal A 240/4 nm, reference 360/80 nm Peak width >0.0063 minutes (0.13 seconds response time), 40 Hz

Chromatographic conditions for high-flow/high-temperature application with an Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm column	
Mobile phase	A) Water B) Acetonitrile
Flow rate	3.5 mL/min
Gradient	0.0 minutes – 50 %B 0.8 minutes – 98 %B
Stop time	1.0 minutes
Post time	1.0 minutes
Injection volume	1 µL
Sample temperature	8 °C
Column temperatures	90 °C
Detection	Signal A 240/4 nm, reference 360/80 nm Peak width >0.0063 minutes (0.13 seconds response time), 40 Hz

In a scoping experiment, the sample was separated using an HPLC method with common temperature and solvent flow settings of 40 °C and 0.5 mL/min, respectively, and a gradient optimized to achieve baseline separation within 3.5 minutes. Six consecutive runs were evaluated regarding retention time precision and resolution (Figure 3A). Retention time precision was excellent (RSDs < 0.06 %). Resolution was greater than 3.0 for all compounds. This scoping method served as a reference for the evaluation of retention time precision in the following experiments.

The conditions of the scoping method were changed in a manner to demonstrate an analytical challenge where very low solvent flow and temperature settings (0.2 mL/min, 4 °C) are required. Again, the RRLC checkout sample was separated with a gradient adapted to the lower solvent flow and temperature settings. In a third experiment, the temperature of the column was raised to 90 °C while maintaining a solvent flow of 0.2 mL/min. Figure 3 shows three chromatogram overlays of six consecutive runs each of the RRLC mix separated by applying the reference method, the low-flow/low-temperature method, and the low-flow/high-temperature method, respectively. All compounds were separated with high retention time precision. Retention time RSDs of the three different methods are compared in Table 1. The different setting of the 1290 Infinity II Multicolumn Thermostat temperature zone not equipped with a column had no adverse effects on the performance.

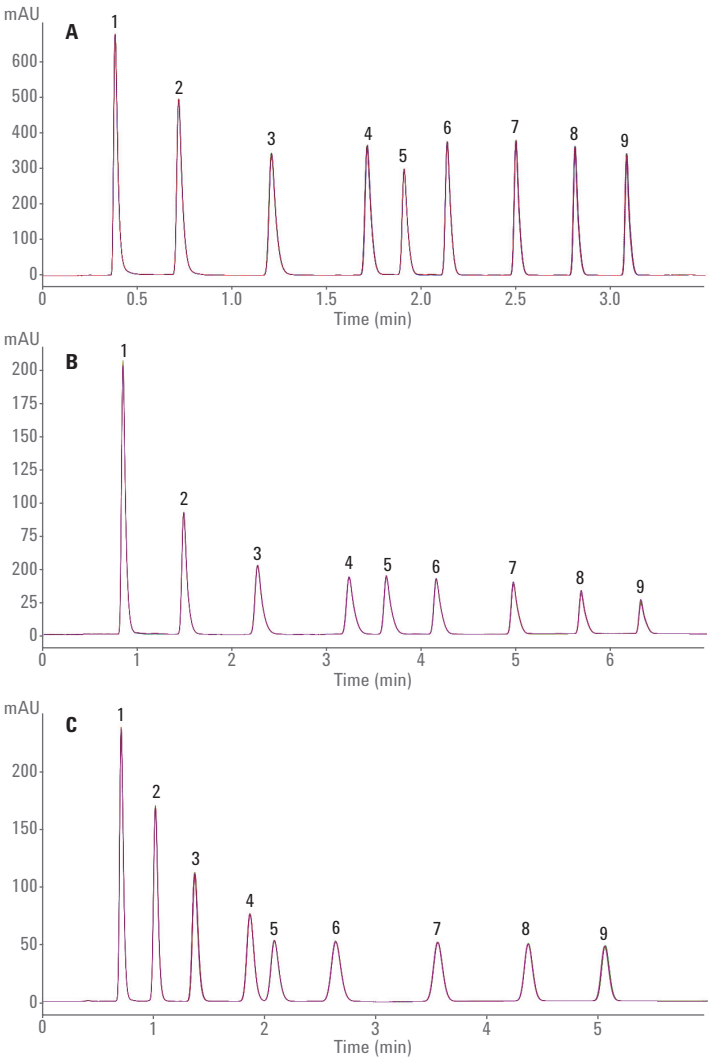

Figure 3 Separation of an RRLC checkout sample on an Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm column with 1.8 µm particle size applying a standard HPLC method (A), a low-flow/low-temperature method (B), and a low-flow/high-temperature method (C). Overlays of six consecutive runs each. For peak numbering, see Table 1.

Table 1. Comparison of retention time precisions applying low solvent flow settings combined with low and high temperature settings.

Peak	Compound	Reference RSD (%)	RSD (%) at 4 °C	RSD (%) at 90 °C
1	Acetophenone	0.053	0.046	0.041
2	Propiophenone	0.050	0.056	0.053
3	Butyrophenone	0.058	0.066	0.058
4	Valerophenone	0.052	0.059	0.070
5	Hexanophenone	0.024	0.053	0.073
6	Heptanophenone	0.032	0.042	0.075
7	Octanophenone	0.040	0.042	0.066
8	Benzophenone	0.045	0.030	0.046
9	Acetanilide	0.045	0.016	0.035

To demonstrate high throughput applications, parameters of the standard HPLC method were changed to yield a maximum solvent flow using an analytical column with 4.6 mm inside diameter, while the gradient was adjusted accordingly. Again, low and high temperatures were set to simulate the demands of special separation methods. With a column temperature of 4 °C, the RRLC checkout sample was separated within 1.8 minutes using a solvent flow of 2.5 mL/min. At 90 °C, the mix was separated in less than 0.8 minutes at a solvent flow of 3.5 mL/min. Figure 4 shows three chromatogram overlays of six consecutive runs each of the RRLC mix separated by applying the reference method, the high-flow/low-temperature method, and the high-flow/high-temperature method, respectively. Retention time precisions yielded with the two high solvent flow methods were excellent (RSD < 0.07 %) and comparable to those of the reference method (Table 2). As in the low-flow experiments, the different setting of the second temperature zone in the 1290 Infinity II Multicolumn Thermostat did not impair the excellent retention time precision.

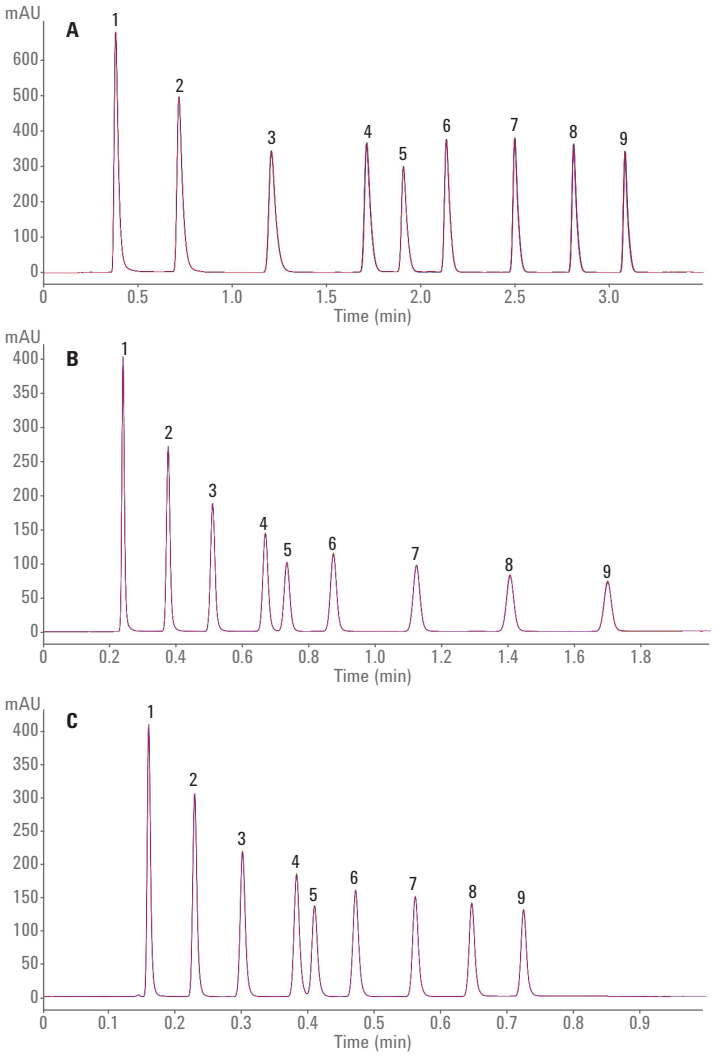

Figure 4. Separation of an RRLC checkout sample on an Agilent ZORBAX SB-C18, 4.6 × 50 mm column with 3.5 µm particle size applying a high-flow/low-temperature method (B), and a high-flow/high-temperature method (C), compared with a standard HPLC method (A) on an Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm column. Overlays of six consecutive runs each. For peak numbering, see Table 2.

Table 2. Comparison of retention time precisions applying high solvent flow settings combined with low and high temperature settings.

Peak	Compound	Reference RSD (%)	RSD (%) at 4 °C	RSD (%) at 90 °C
1	Acetophenone	0.064	0.032	0.069
2	Propiophenone	0.048	0.025	0.040
3	Butyrophenone	0.059	0.015	0.054
4	Valerophenone	0.031	0.026	0.025
5	Hexanophenone	0.027	0.024	0.024
6	Heptanophenone	0.033	0.014	0.014
7	Octanophenone	0.040	0.014	0.007
8	Benzophenone	0.044	0.014	0.014
9	Acetanilide	0.043	0.013	0.015

Conclusion

This Technical Overview describes the performance of the Agilent 1290 Infinity II Multicolumn Thermostat when extreme temperature and flow settings are applied. The separation of a standard mixture of nine different compounds was carried out at temperatures of 4 and 90 °C with a solvent flow from 0.2 up to 3.5 mL/min. A method using common temperature and flow settings of 40 °C and 0.5 mL/min, respectively, served as a reference. Temperature stability and precision were measured by means of retention time RSDs of the different analytes, calculated out of six consecutive runs. Under conditions of the reference method, excellent retention time RSDs of 0.064 % or smaller could be achieved. At 4 °C and 0.2 mL/min, retention time RSDs were as low as 0.069 %. When this low solvent flow was combined with a column temperature of 90 °C, retention time precision did not decline significantly (RSDs < 0.076 %). Using the Agilent Quick-Connect high-flow heat exchanger at a solvent flow of 2.5 mL/min with 4 °C column temperature, retention time precision was excellent (RSDs < 0.033 %). Under extreme temperature conditions of 90 °C, the solvent flow was raised to 3.5 mL/min, yielding RSDs still below 0.070 %. These results demonstrate that the 1290 Infinity II Multicolumn Thermostat delivers excellent performance not only under commonly used conditions, but also with extreme temperature and solvent flow settings. The benefit of this high temperature stability from 4 to 90 °C, combined with a separately controllable second temperature zone, is superior flexibility for routine analyses, method development, and extraordinary analytical challenges.

Reference

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

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