# High-Precision Temperature Control for Supercritical Fluid Chromatography Using the Agilent 1260 Infinity II Multicolumn Thermostat

## Technical Overview

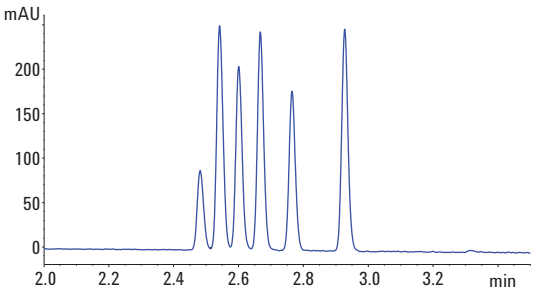

### Author

Edgar Naegele
Agilent Technologies, Inc.
Waldbronn, Germany



### Abstract

This Technical Overview demonstrates the use of the Agilent 1260 Infinity II Multicolumn Thermostat in the Agilent InfinityLab Supercritical Fluid Chromatography (SFC) Solution. It describes how the post column temperature influences the noise at the diode array detector, and how this temperature could be optimized. With the obtained settings, the noise at different column temperatures was measured, and showed stability over a wide column temperature range. The high retention time stability of some compounds with a temperature-sensitive retention time is shown.


Agilent Technologies

## Introduction

Due to the high sensitivity of modern diode array detectors used in LC and SFC systems, the minimization of detector noise is crucial to reach maximum performance. Beyond the electronic noise, typical detector noise in any liquid chromatography detection system may be caused by refractive index effects. These effects are primarily caused by incomplete mixing of the solvents used, and temperature differences between the column and detector cell. The latter can be compensated by adjusting the temperature of the column effluent to the temperature of the detector cell in SFC. Therefore, a highly accurate and stable temperature control is needed. In addition, a highly accurate and stable temperature is needed for the column itself. A stable column temperature is necessary because there are some compounds whose retention times shift considerably with small changes in column temperature. The Agilent 1260 Infinity II Multicolumn Thermostat provides control of both temperatures, the precolumn temperature that determines the column temperature, and the post column temperature that adjusts the column effluent to the detector cell temperature.

This Technical Overview demonstrates and discusses both effects. An optimization process for the minimization of detector noise is given, and the resulting noise over a broad range of column temperatures is displayed. The influence of the column temperature itself is demonstrated by using compounds whose retention is sensitive to temperature changes.

## Experimental

### Instrumentation

The Agilent 1260 Infinity II SFC System comprised the following modules.

- Agilent 1260 Infinity II SFC Control Module (G4301A)
- Agilent 1260 Infinity II SFC Binary Pump (G4782A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1260 Infinity II Diode Array Detector with high-pressure SFC flow cell (G7115A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)

### Instrumental setup

To minimize detector noise, it was necessary to optimize the post column temperature as close as possible to the detector cell temperature. For that purpose, the column effluent was guided through a heat exchanger (3 µL internal volume), which was set to the detector cell temperature. The precolumn temperature adjustment was done by another heat exchanger of the same internal volume. For that purpose, two independent heat-exchanging blocks of the Agilent 1260 Infinity II Multicolumn Thermostat were used (see Results and Discussion).

### Columns

- Agilent ZORBAX RxSil, 4.6 × 150 mm, 5 µm (for noise measurements)
- Agilent ZORBAX RxSil, 3.0 × 100 mm, 1.8 µm (for temperature stability measurements)

### Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07 SR3

### Sample

The sample was a mixture of six compounds: sulfadimethoxine, sulfachloropyrazine, sulfamethazine, sulfamethizole, sulfamerazine, and sulfadiazine (each 10 mg in 25 mL methanol with equivalent volumes in the mixture).

### Chemicals

All solvents were bought from Merck, Germany. Chemicals were bought from Sigma-Aldrich (Germany).

## SFC method for noise measurements

| Parameter | Description |
|---|---|
| Solvent A | $CO_2$ |
| Modifier B | Methanol |
| SFC flow | 2.5 mL/min |
| Isocratic elution | 20 % modifier |
| Stop time | 60 minutes |
| Backpressure regulator (BPR) | 60 °C, 140 bar |
| Column temperature | 20 to 60 °C (step 5 degrees) |
| Precolumn heat exchanger | 3 µL |
| Post column temperature | 42 °C |
| Post column heat exchanger | 3 µL |
| Injection volume | 0.0 µL (blank run) |
| Feed solvent | Methanol |
| Overfeed volume | 4 µL |
| Feed speed | 400 µL/min |
| Diode array detector | 254 nm/4 nm, Ref.: 360 nm/60 nm,<br>16 nm slit, data rate 5 Hz,<br>standard high-pressure flow cell |

## SFC method for temperature stability measurements

| Parameter | Description |
|---|---|
| Solvent A | $CO_2$ |
| Modifier B | Methanol |
| SFC flow | 1.5 mL/min |
| Gradient | 0 minutes – 5 %B<br>4 minutes – 40 %B |
| Backpressure regulator (BPR) | 60 °C, 140 bar |
| Column temperature | 20 to 80 °C (step 10 degrees) |
| Precolumn heat exchanger | 3 µL |
| Post column temperature | 42 °C |
| Post column heat exchanger | 3 µL |
| Injection volume | 10.0 µL |
| Feed solvent | Methanol |
| Overfeed volume | 4 µL |
| Feed speed | 400 µL/min |
| Diode array detector | 270 nm/4 nm, Ref.: 360 nm/60 nm,<br>16 nm slit, data rate 10 Hz,<br>standard high-pressure flow cell |

## Results and Discussion

To identify the optimum temperature, the detector noise was measured over the post column temperature range of 35 to 45 °C (step size 1 degree), which is the typical range of the detector cell temperature. This was done with the SFC method described in the Experimental section (column temperature 40 °C). The line through the measured data points of detector noise showed a minimum at 60 µAU peak-to-peak noise between 38 and 44 °C for the post column temperature (Figure 1). The direct measurement of the temperature at the surface of the detector cell by a thermocouple confirmed 42 °C as cell temperature. The post column temperature setting at 42 °C was kept for all further experiments.

A set of nine runs with different column temperatures between 20 and 60 °C (step 5 degrees) was done. Each run for the determination of the detector noise was 1 hour, and was done in duplicate. The noise was determined every 10 minutes over the run time, and reported by Agilent ChemStation as peak-to-peak noise. Finally, all values of peak-to-peak noise obtained from one run were averaged and displayed in a chart showing column temperature against peak-to-peak noise (Figure 2). The chart shows that the peak-to-peak detector noise was constant over the entire column temperature range, and typically was approximately 65 µAU.

A minimum of detector noise is especially important when high sensitivity is required, for instance, when determining lower-level impurities at or below 0.1 %, which is required for impurity profiling. This is demonstrated by the separation of a main compound from its impurity at a level of 0.1 %, where the peak of the main compound was still in the linear range of the detector (Figure 3).

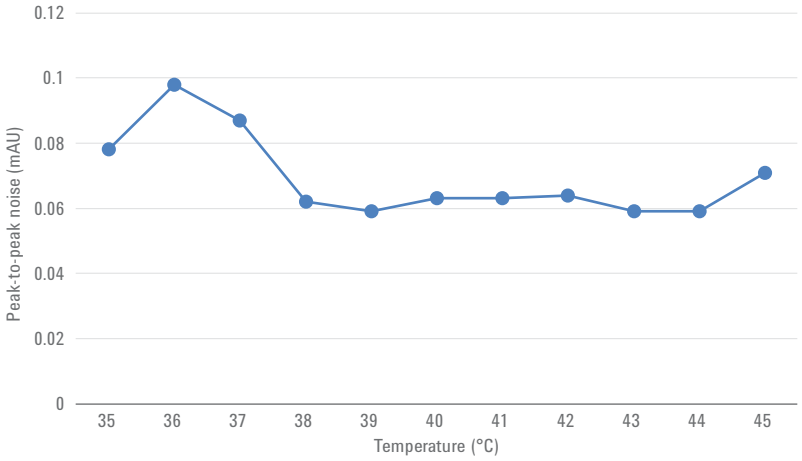



Figure 1. Determination of the post column temperature setting for minimization of detector noise (see *SFC method for noise measurement*).

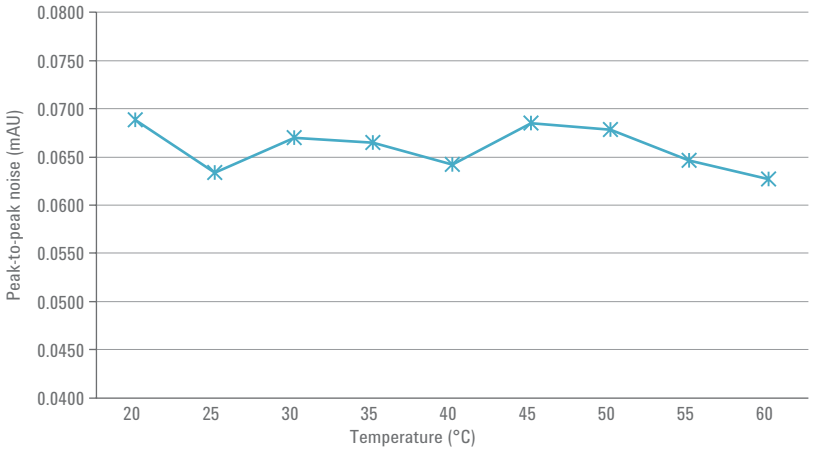



Figure 2. Dependence of peak-to-peak noise on column temperature, showing constant detector noise over a wide range of column temperatures (see *SFC method for noise measurement*).

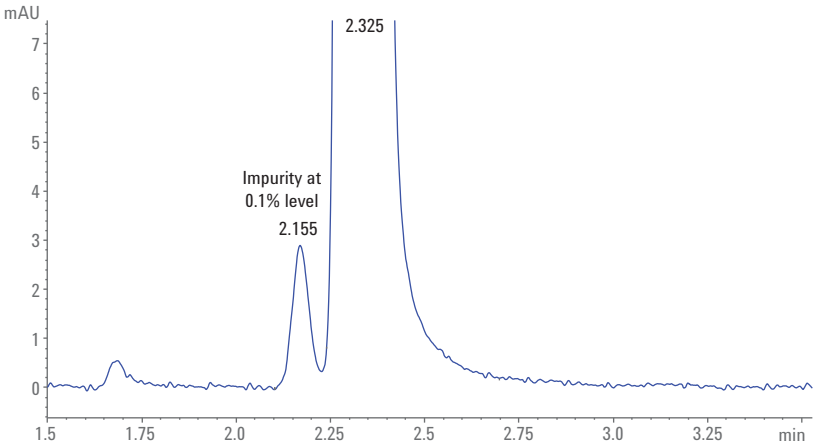



Figure 3. Detection of a low-level impurity at 0.1 % compared to the main peak on the SFC system with minimized noise.

For the evaluation of the stability of the column temperature and the precolumn temperature adjustment, a mixture of six compounds was used. The mixture included compounds with highly temperature-sensitive retention and temperature-dependent coelution. Under the given conditions, the six compounds were clearly separated at a column temperature of 30 °C (Figure 4).

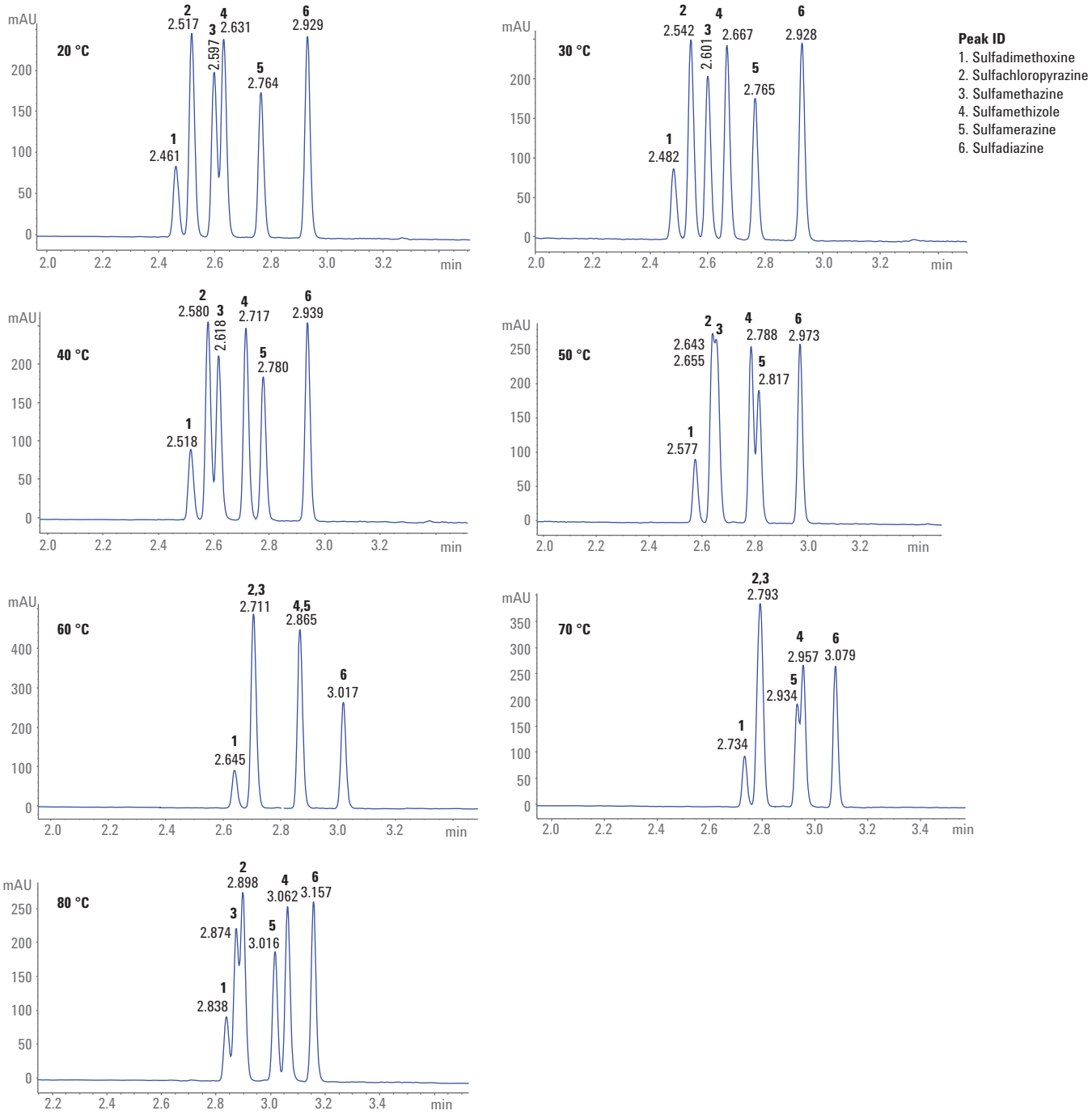



Figure 4. Temperature-dependent elution behavior of six compounds. The best separation was obtained at 30 °C. All other temperatures show temperature-dependent retention-time shifts up to complete coelution and reversal of the elution order (see *SFC method for temperature stability measurements*).

At a lower column temperature (20 °C), peak 4 moved to a shorter retention time, and started to coelute with peak 3. At a higher column temperature (40 °C), peaks 2 and 4 moved markedly to longer retention times. At a column temperature of 50 °C, peaks 2 and 3 coeluted completely. The same situation occurred at a column temperature of 60 °C for peaks 4 and 5. At higher column temperatures (70 °C), peaks 4 and 5 started to separate again but in reversed order. At the highest column temperature in the experiment (80 °C), peaks 4 and 5 again showed good separation. Peaks 2 and 3 also started to separate again at that temperature, but with reversed order and partial coelution with peak 1. To gain a better overview of the temperature-dependent movements, retention times were plotted against temperature (Figure 5). This chart shows clearly that peaks 4 and 5 overlapped completely at 60 °C with reversed retention above and below this temperature. Compounds 2 and 3 coeluted over a broader temperature range between 50 and 70 °C with reversed retention above and below this range. At 80 °C, the peaks of compounds 1, 2, and 3 eluted closely to each other, and were no longer separated.

The precision of the precolumn heating temperature and temperature stability are reflected in the retention time RSD values, which were calculated for all compounds at each applied column temperature for 10 runs. To get a comprehensive overview, all data points were plotted (Figure 6). The chart shows that the retention time RSDs for all six compounds were typically distributed within a small range of approximately 0.003 to 0.01 % for each column temperature. As an example for the frequently used column temperature of 40 °C, the RSDs were between 0.014 and 0.022 %. Table 1 summarizes the measured values for all retention times, and the calculated RSD values.

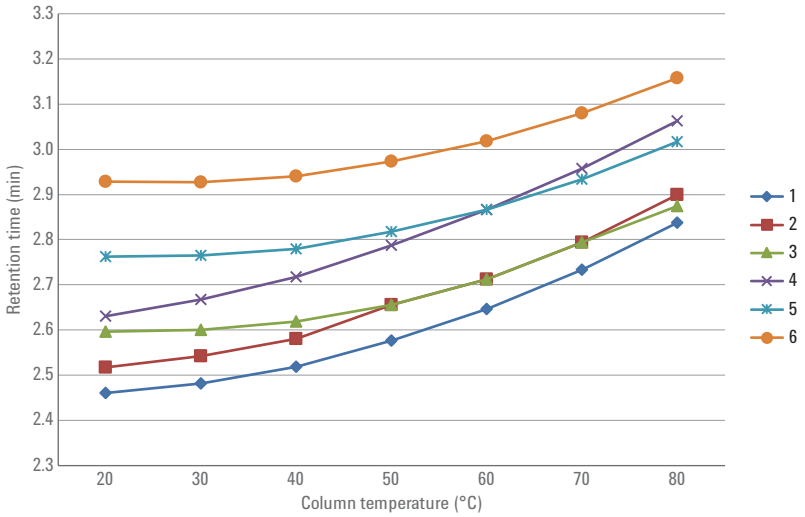



Figure 5. Dependence of retention time on column temperature, showing retention time changes of the six compounds in the mixture.

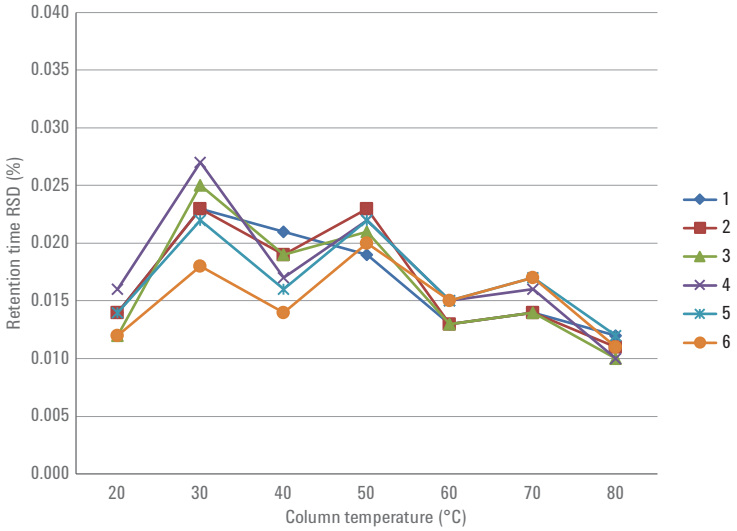



Figure 6. Retention time RSDs obtained for the set of the six compounds at different column temperatures (10 runs at each temperature level).

Table 1. Average retention time and retention time RSD values for all six compounds at all measured temperature levels (10 runs at each temperature level, see *SFC method for temperature stability measurements*).

| | 20 °C | | 30 °C | | 40 °C | | 50 °C | | 60 °C | | 70 °C | | 80 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound | RT | RSD (%) | RT | RSD (%) | RT | RSD (%) | RT | RSD (%) | RT | RSD (%) | RT | RSD (%) | RT | RSD (%) |
| 1 | 2.460 | 0.014 | 2.482 | 0.023 | 2.518 | 0.021 | 2.577 | 0.019 | 2.646 | 0.013 | 2.734 | 0.014 | 2.838 | 0.012 |
| 2 | 2.517 | 0.014 | 2.542 | 0.023 | 2.581 | 0.019 | 2.655 | 0.023 | 2.712 | 0.013 | 2.794 | 0.014 | 2.899 | 0.011 |
| 3 | 2.596 | 0.012 | 2.600 | 0.025 | 2.619 | 0.019 | 2.656 | 0.021 | 2.712 | 0.013 | 2.794 | 0.014 | 2.875 | 0.010 |
| 4 | 2.630 | 0.016 | 2.667 | 0.027 | 2.718 | 0.017 | 2.788 | 0.022 | 2.866 | 0.015 | 2.957 | 0.016 | 3.063 | 0.010 |
| 5 | 2.763 | 0.014 | 2.765 | 0.022 | 2.780 | 0.016 | 2.818 | 0.022 | 2.866 | 0.015 | 2.934 | 0.017 | 3.017 | 0.012 |
| 6 | 2.928 | 0.012 | 2.927 | 0.018 | 2.940 | 0.014 | 2.974 | 0.020 | 3.018 | 0.015 | 3.080 | 0.017 | 3.158 | 0.011 |

## Conclusion

This Technical Overview describes a routine to determine the post column temperature adjustment to minimize the detector noise caused by temperature differences between column effluent and detector cell temperature. With the optimized post column temperature applied to the column effluent, the detector noise was determined over a broad temperature range from 20 to 60 °C as peak-to-peak noise of approximately 65 µAU. This low level of noise is excellent, and enables the sensitive measurements of low-level impurities at or even below 0.1 %. The stability of the column temperature, achieved by precolumn heating, was determined by the analysis of a set of compounds with high temperature sensitivity. The typical RSD values of the retention time over a range of 20 to 80 °C was between 0.010 to 0.025 %. These excellent peak-to-peak noise and retention time RSD values can be achieved using the Agilent 1260 Infinity II Multicolumn Thermostat for column heating and post column temperature adjustment.

www.agilent.com/chem


Published in the USA, October 15, 2017
5991-7625EN

Agilent Technologies