Excellent Reproducibility Using Temperature Gradient Chromatography with the Agilent 1200 Series Rapid Resolution Liquid Chromatography System

Application Brief

Authors

Linda Côté
Agilent Technologies, Inc.
Montréal, Quebéc, CA

Michael Zumwalt
Agilent Technologies, Inc.
9780 S Meridian Blvd., MS 1-1E
Englewood, CO 80112-5910
USA

Andrzej Wnorowski
Analysis and Air Quality Division
Environment Canada
Ottawa, Ontario, CA

Abstract

An Agilent 1200 Series LC system, featuring the G1316B Thermostatted Column Compartment, is used to generate reproducible chromatography with a programmable temperature gradient. Using a mixture of four sulfa drugs for this study, retention time and peak area reproducibility, with methanol as the organic solvent and a 2.5 °C/min column temperature gradient, are less than 0.054 and 0.173 %RSD, respectively. With acetonitrile as the organic solvent and at a temperature gradient of 5.0 °C/min, retention time and peak area reproducibility are less than 0.070 and 0.269 %RSD, respectively. The use of column temperature programming decreases the dependence on organic solvents for chromatography and therefore promotes green chemistry.

Introduction

Over the past 10 years, operating LC columns at higher constant temperatures has been investigated for the rapid analyses of large biomolecules [1], since run times can be drastically shortened when the analytes' thermal diffusivity is increased and the viscosity of the mobile phase is lowered. However, it hasn't been until the past few years that the use of above-normal ambient operating temperatures was appreciated [2] and has found appealing applications [3].

On the other hand, using temperature-programming has been a long-overlooked technique in liquid chromatography and only very recently has raised interest for a potentially wide range of "green chemistry" applications. Many published works [4–6] have confirmed that the application of temperature-programmed liquid chromatography (TPLC) results in improved chromatographic efficiency.

Use of temperature affects many parameters during LC separation. As a result of elevating the temperature, hydrogen bonding weakens and analyte bonding with the stationary phase elongates and weakens, resulting in increased diffusivity and improved peak shape and reduced width. Furthermore, mobile phase viscosity decreases, reducing column backpressure and providing for higher flow rates. As a consequence of changing the polarity of the heated mobile phase and the analytes' interaction with the stationary phase, retention, selectivity, and resolution improve, the analysis is faster, and better results can be obtained.

Because solvent strength is a function of temperature, the effect of varying the temperature during an analysis can be analogous to changing the solubility as a function of the aqueous to organic mobile phase solvent ratio. The effect of varying temperature can be so significant that the need for continued use of organic solvents can be reduced to the point that they are not even used, which is an attractive offering for the promotion of "green," or environment friendly, chemistry.

A specific example of using temperature gradients for chromatographic separations without the use of organic solvents is the "green chromatography" methods being developed by a team at the Analysis and Air Quality Division (AAQD) of Environment Canada in Ottawa. AAQD is developing tandem liquid chromatography methods for the analysis of polar and semipolar environmental pollutants found in air and vehicle emissions. The principle of these methods, which use water as a sole mobile phase, is that the chromatographic separation driving force is not traditional organic solvents gradient but rather the effect of increased elution strength achieved by careful solvent and column temperature programming (ramping), in a similar manner as in gas chromatography separation. TPLC offers faster and more efficient separation; therefore, it affords an equivalent and reliable alternative to traditional organic solvent gradients in addition to using "green solvents." However, an essential condition to achieve reproducible and highly precise separation under TPLC is high instrumental reproducibility in controlling the chromatographic separation temperature.

And yet, the purpose of this work is not to explore liquid chromatography without the use of an organic solvent, but to show that temperature programming of the LC column, using the standard capability of the Agilent G1316B Thermostatted Column Compartment to change the temperature over time, results in extremely consistent retention times and peak area intensities in UV absorbance. In addition, the solvent is preheated before entering the column by passing through the 6-µL preheater chamber, which is also a source of heat for the column compartment. Since interest in using higher temperatures is growing, it is important to show that this standard feature of the Agilent system can be used as a reliable and reproducible technique.

The structures of the four sulfa drugs are shown in Figure 1.

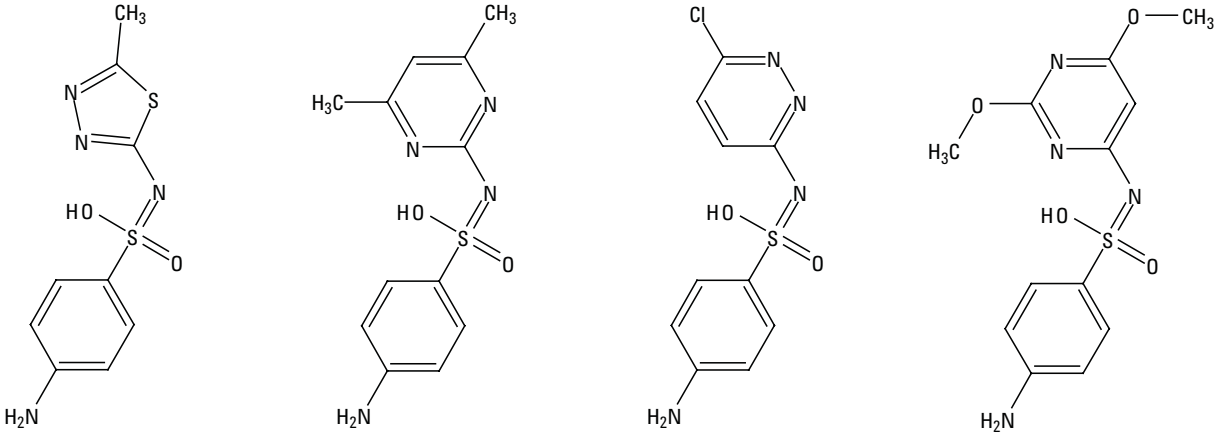

Figure 1. Four sulfonamide compounds contained as mixture analyzed in this work.

Experimental

Sample Preparation

The Electrospray LC Demo Sample (Agilent p/n: 59987-20033) containing the four sulfonamide compounds shown in Figure 1, each at a concentration of 100 ng/µL, is used in this work. No dilutions are made.

Method Details

This work comprises two parts. The first part uses methanol as the organic solvent while the second part uses acetonitrile. Separate column temperature gradients are also used.

LC Conditions

Agilent 1200 Series Rapid Resolution LC system:

G1312B	Binary pump SL
G1379B	Micro-vacuum degasser
G1367C	High performance autosampler SL
G1316B	Thermostatted column compartment SL (column connected to 6-µL heat exchanger)
G1315C	Diode-array detector SL with 2 µL/3 mm flow cell

Column:	Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm (p/n 880975-902)
Column temp:	Gradient
-	Part 1, MeOH, 2.5 °C/min - see Figure 2
-	Part 2, ACN, 5 °C/min - see Figure 5
Mobile phase A:	Water
Mobile phase B:	Part 1, 0.1 % formic acid in MeOH Part 2, 0.1% formic acid in ACN
Flow rate:	1.0 mL/min
Injection volume:	5 µL
Isocratic:	Part 1, 30% B, MeOH Part 2, 40% B, ACN
Stop time:	Part 1, 25 min Part 2, 15 min

UV Detection

Diode-array:	Signal 254 nm, 8 nm (Bw); Reference 450 nm, 100 nm (Bw)

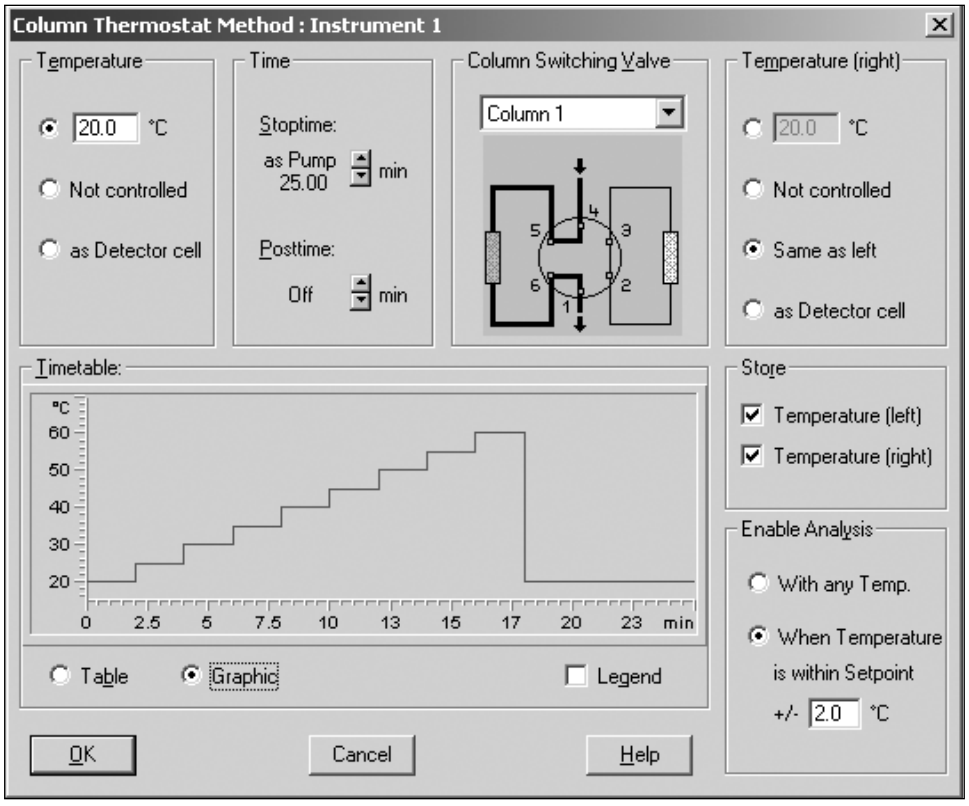

Figure 2. Column temperature gradient of 2.5 °C/min using methanol organic solvent.

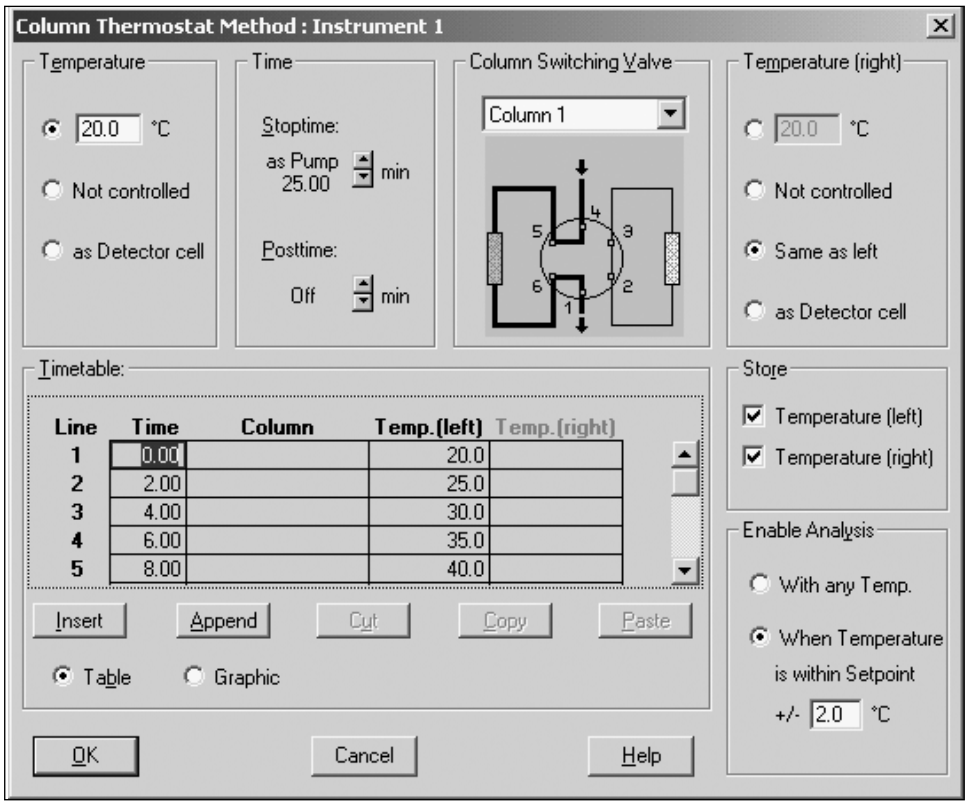

Figure 3. Column temperature gradient table.

Results and Discussion

Part 1

In the first part of this work, methanol, with 0.1% formic acid, is used as the organic solvent. The temperature gradient of the G1316B is ramped at 2.5 °C/min and is shown in Figures 2 and 3. The reproducibility of injecting 10 × 5 µL of the sulfonamide mix is shown as an overlay of the chromatographic runs in Figure 4. The percent relative standard deviations of the retention times and peak areas for each compound are shown in Tables 1 and 2. Reproducibility of only the last two eluting peaks is considered because in this case the first two compounds at RT = 6.7 min., sulfamethizole and sulfachloropyridazine, are not resolved. Time did not allow for the further development of chromatographic resolution. However, in Part 2, using acetonitrile, all four peaks are resolved and results for each one are provided.

As shown in Table 1, the retention time reproducibility for sulfamethazine (RT = 9.25) is only 0.053% and only 0.034% for sulfadimethoxine (RT = 20.46 min). These excellent results are complemented by the results for the corresponding peaks areas, which are shown in Table 2, and include 0.17 %RSD for both compounds.

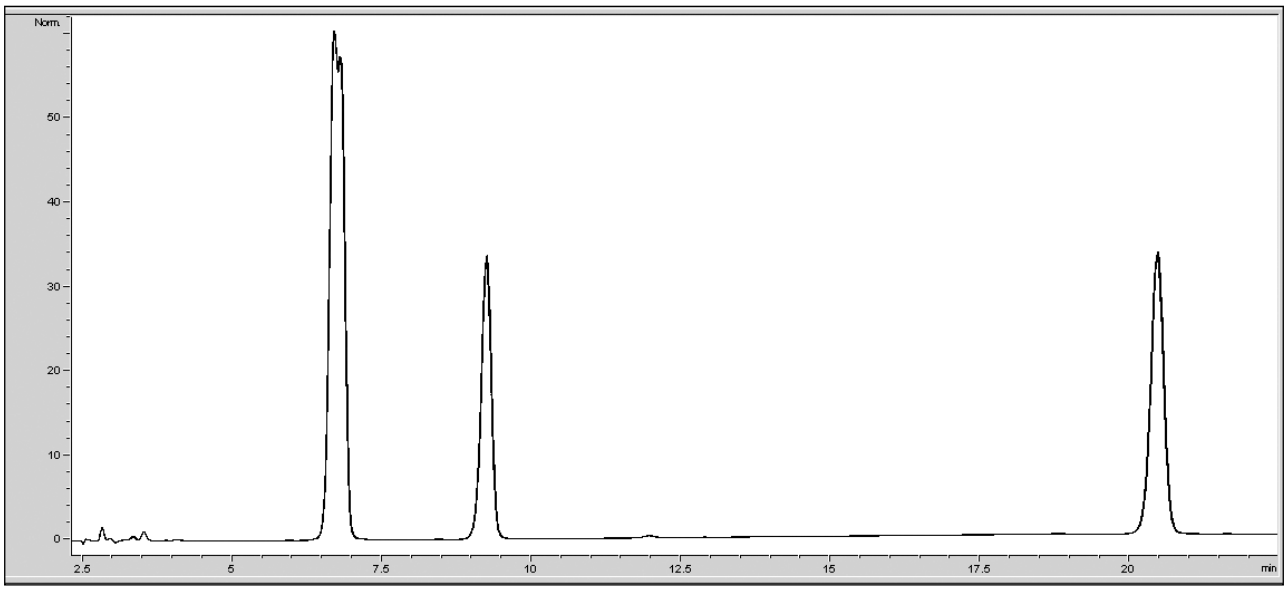

Figure 4. Chromatographic reproducibility displayed in overlay of 10 injections.

Table 1. Retention Time Reproducibility for 10 Injections of Sulfamethazine and Sulfadimethoxine

Sulfamethazine		**Sulfadimethoxine**	
Run number	**RT [minute]**	**Run number**	**RT [minute]**
1	9.25137	1	20.45863
2	9.25954	2	20.47676
3	9.25221	3	20.47479
4	9.24497	4	20.45886
5	9.24951	5	20.46019
6	9.24473	6	20.46480
7	9.24574	7	20.46136
8	9.24535	8	20.46442
9	9.25253	9	20.47504
10	9.24439	10	20.46290
Mean:	9.24903	Mean:	20.46578
S.D.:	0.00494	S.D.:	0.00706
% RSD:	***0.05338***	***% RSD:***	***0.03449***

Table 2. Peak Area Reproducibility for 10 Injections of Sulfamethazine and Sulfadimethoxine

Sulfachloropyridazine		**Sulfadimethoxine**	
Run number	**Area**	**Run number**	**Area**
1	390.40219	1	498.85330
2	390.59979	2	498.96521
3	391.09012	3	499.23676
4	391.04861	4	498.34360
5	390.68967	5	498.52740
6	391.63257	6	499.57095
7	391.85172	7	500.12451
8	392.03073	8	499.44321
9	392.26016	9	500.92892
10	392.01077	10	500.52737
Mean:	391.36163	Mean:	499.45212
S.D.:	0.67612	S.D.:	0.85176
% RSD:	***0.17276***	***% RSD:***	***0.17054***

Part 2

In the second part of this work, acetonitrile with 0.1% formic acid is the organic mobile phase solvent and the temperature gradient of the G1316B is ramped at 5 °C/min and is shown in Figure 5. In this case, all four peaks are chromatographically resolved. The reproducibility of injecting 10 × 5 µL of the sulfonamide mix is shown as an overlay of the chromatographic runs in Figure 6. The %RSDs of the retention times and peak areas for each compound are shown in Tables 3 and 4.

As shown in Table 3, the retention time reproducibility is excellent for all four compounds: sulfamethizole (RT = 3.70 min), 0.259 %RSD; sulfachloropyridazine (RT = 3.86 min), .037 %RSD; sulfamethazine (RT = 4.88), 0.070 %RSD; and sulfadimethoxine (RT = 6.86 min), 0.061 %RSD. As was the case for Part 1, these excellent results are complemented by the results for the corresponding peaks areas, which are shown in Table 4, and include 0.258, 0.232, 0.258, and 0.269 %RSD for the same four compounds, respectively.

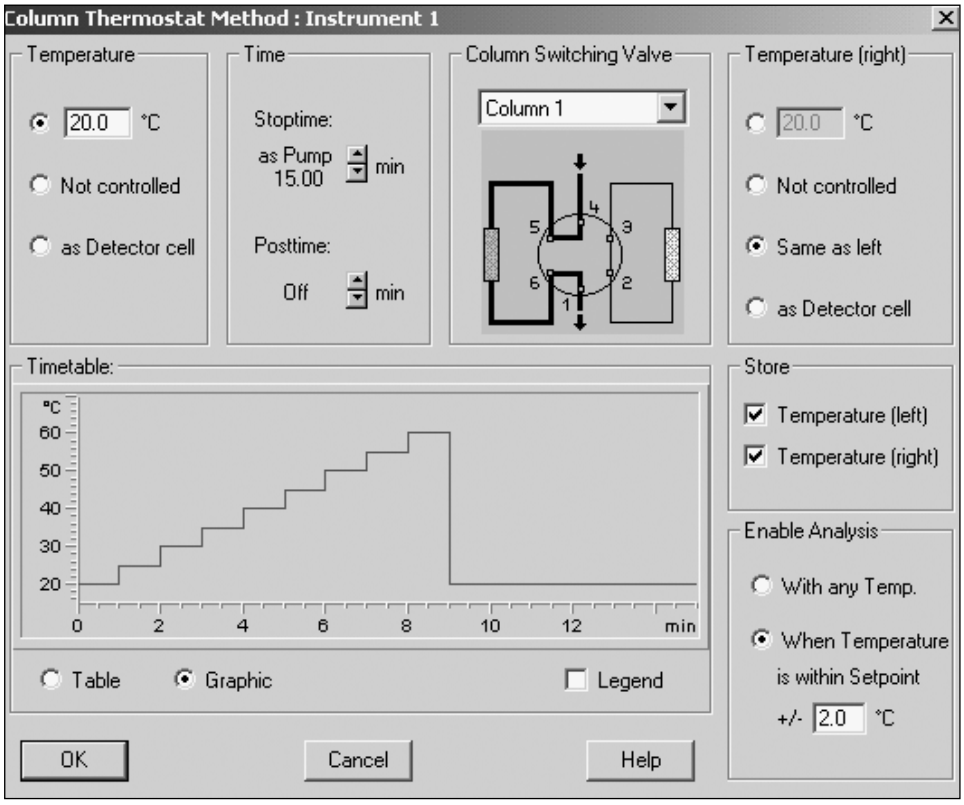

Figure 5. Column temperature gradient of 5 °C/min using acetonitrile organic solvent.

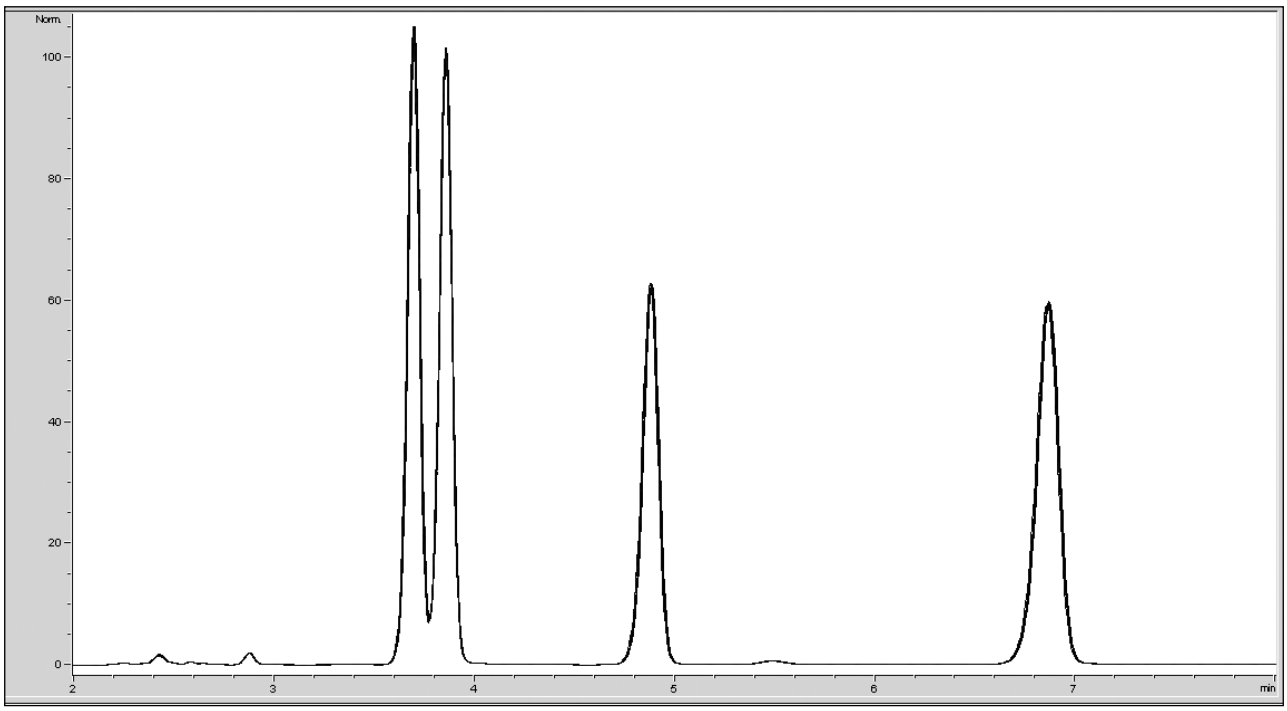

Figure 6. Chromatographic reproducibility displayed in overlay of 10 injections.

Table 3. Retention Time Reproducibility for 10 Injections of the Sulfonamide Mixture

Sulfamethizole		Sulfachloropyridazine		Sulfamethazine		Sulfadimethoxine	
Run number	**RT [minute]**	**Run number**	**RT [minute]**	**Run number**	**RT [minute]**	**Run number**	**RT [minute]**
1	3.69646	1	3.85627	1	4.88303	1	6.86720
2	3.70082	2	3.85946	2	4.88593	2	6.87043
3	3.69392	3	3.85611	3	4.87673	3	6.86049
4	3.69758	4	3.85719	4	4.88322	4	6.87079
5	3.69479	5	3.85584	5	4.87763	5	6.86407
6	3.69896	6	3.85911	6	4.88234	6	6.86591
7	3.69932	7	3.85840	7	4.88560	7	6.87134
8	3.69850	8	3.85852	8	4.88409	8	6.86998
9	3.69915	9	3.85909	9	4.88310	9	6.86504
10	3.69490	10	3.85639	10	4.87736	10	6.85989
Mean:	3.69744	Mean:	3.85764	Mean:	4.88190	Mean:	6.86651
S.D.:	0.00231	S.D.:	0.00142	S.D.:	0.00342	S.D.:	0.00419
% RSD:	***0.06257***	***% RSD:***	***0.03682***	***% RSD:***	***0.06999***	***% RSD:***	***0.06103***

Table 4. Peak Area Reproducibility for 10 Injections of the Sulfonamide Mixture

Sulfamethizole		Sulfachloropyridazine		Sulfamethazine		Sulfadimethoxine	
Run number	**Area**	**Run number**	**Area**	**Run number**	**Area**	**Run number**	**Area**
1	459.36658	1	456.68210	1	353.19196	1	463.56354
2	459.13544	2	456.83420	2	353.38800	2	462.94131
3	459.98608	3	457.92313	3	354.35135	3	464.18039
4	460.21707	4	458.12100	4	354.24716	4	464.30734
5	459.91040	5	457.37457	5	354.29434	5	464.32480
6	461.23935	6	458.68213	6	355.17117	6	465.06543
7	460.87387	7	458.27908	7	354.31567	7	465.21423
8	461.90726	8	459.02490	8	355.17407	8	466.11273
9	462.11920	9	459.47849	9	355.80191	9	465.98492
10	462.54395	10	459.83719	10	355.86966	10	467.02435
Mean:	460.72992	Mean:	458.22368	Mean:	354.58053	Mean:	464.87191
S.D.:	1.19146	S.D.:	1.06260	S.D.:	0.91413	S.D.:	1.25173
% RSD:	***0.25860***	***% RSD:***	***0.23190***	***% RSD:***	***0.25781***	***% RSD:***	***0.26926***

Conclusions

The performance of a standard capability with the 1200 Series G1316B Thermostatted Column Compartment is evaluated for retention time and peak area reproducibility of the chromatographic elution for four sulfonamide compounds, given two different organic mobile phases (MeOH and ACN, both with 0.1% formic acid) and two different temperature gradients (2.5 and 5.0 °C/min, respectively). Excellent reproducibility is seen among the two chromatographically resolved peaks of the MeOH, 2.5 °C/min run, with retention times of less than 0.053 %RSD and peak areas of less than 0.170 %RSD. Using ACN and 5.0 °C/min, excellent reproducibility is again seen, with retention times of less than 0.070 %RSD and peak areas of less than 0.269 % RSD.

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