SFC Method Development Using the Agilent ChemStation Method Scouting Wizard

Technical Overview

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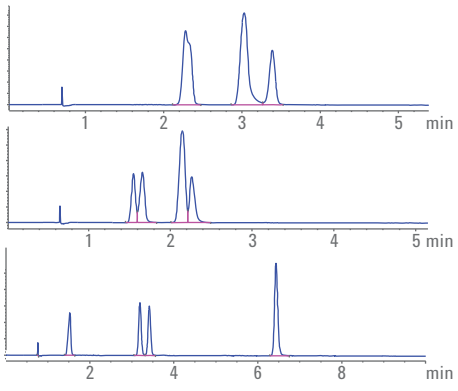
Abstract

This Technical Note demonstrates the enhancement of the Agilent 1260 Infinity SFC System to a method development system. The standard system is enhanced with a second thermostatted column compartment and a 12-solvent selection valve clustered with the SFC pump. For easy method generation, the Agilent ChemStation Method Scouting Wizard is used. The performance of the system is shown by the development of an optimized method with three columns, three solvents, and four initial gradients for the separation of a four-compound mix. Specially designed method development reports are used for data analysis.



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Introduction

Modern supercritical fluid chromatography (SFC) is a versatile tool in today's analytical laboratory for solving separation problems that are difficult to handle by classical HPLC. SFC and HPLC are often used as orthogonal separation techniques. Therefore, it is essential to have enhancements available for SFC systems offering fast and easy-to-use method development capabilities. Such modifications are available for the Agilent 1260 Infinity Analytical SFC Solution, making method development an easy task. The modification, with a second thermostatted column compartment and a 12-solvent selection valve with the SFC pump, offers the capability to use multiple columns and multiple mobile phases. With a software-guided setup, any combination of column, solvent, and gradient can be examined in a fast and error-free way. The data obtained can be displayed by specially designed reports to identify the most efficient combination for the separation.

Experimental

The Agilent 1260 Infinity Analytical SFC Solution (G4309A) comprises:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High-Performance Degasser
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1260 Infinity DAD with high-pressure SFC flow cell
- Agilent 1290 Infinity Thermostatted Column Compartments (TCC) (G1316C)

Additions needed to run the SFC system for automated method development:

- Valve drive upgrade kit for pre-existing G1316C in G4309A (G1353B)
- Agilent 1290 Infinity Thermostatted Column Compartments (TCC) (G1316C)
- Two Agilent 1200 Infinity Series Quick-Change 8-position/9-port valves (G4230A)
- Agilent 1290 Infinity Valve Drive (G1170) with Agilent 1200 Infinity Series Quick-Change 12-position/13-port valve (G4235A)
- Capillary kit for method development (p/n 5067-1595)

Instrument setup

The 1260 Infinity SFC Binary Pump was used with an Agilent 1290 Infinity Valve Drive as a cluster using a 12-position/13-port valve for solvent selection in the OpenLAB CDS ChemStation Edition instrument setup. The solvents were defined in the OpenLAB pump setup menu. The two thermostatted column compartments were also included as a cluster in the OpenLAB instrument setup, and each was equipped with an 8-position/9-port valve for column selection. With the method development capillary kit, up to eight columns could be used. The columns were introduced into the OpenLAB column database and configured in the ChemStation TCC menu.

Columns

- Agilent ZORBAX Rx-SIL, 4.6 × 150 mm, 5 µm (p/n 883975-901)
- Agilent ZORBAX SB-CN, 4.6 × 150 mm, 5 µm (p/n 883975-905)
- Agilent ZORBAX NH2, 4.6 × 150 mm, 5 µm (p/n 883952-708)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.05 with Agilent ChemStation Method Scouting Wizard, Version A02.03.

Standards

Agilent Hybrid SFC/LC Checkout Standard in methanol (p/n 5190-0584) comprising:

- Compound 1: caffeine
- Compound 2: theophylline
- Compound 3: theobromine
- Compound 4: thymine

250 µg/mL each

All solvents were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

Results and Discussion

For the separation of the compounds in the standard sample, a column-scouting experiment was done for all columns with a combination of different gradients and solvents. The Method Scouting Wizard (Figure 1) was used for the setup of all possible combinations out of three columns, four gradients, and three solvents. Every run was done in triplicate. The experiments needed a total run time of approximately 22 hours, including an overhead time for column re-equilibration and solvent exchanges of approximately 4 hours. Bubble chart reports were created for visual data evaluation. These reports show the maximum run time determined by the latest eluting compound by bubble position, and the maximum number of recognized peaks by bubble area. The second and third replicates were used to decide which method would be employed for further optimization.

SFC conditions

Parameter	Value
Solvent A	CO_2
Modifier B	Methanol, ethanol, isopropanol
SFC flow	3, 4, and 5 mL/min
Gradient 1	5 % B - 0 minutes, 10 % B - 10 minutes
Gradient 2	5 % B - 0 minutes, 15 % B - 10 minutes
Gradient 3	5 % B - 0 minutes, 20 % B - 10 minutes
Gradient 4	5 % B - 0 minutes, 25 % B - 10 minutes
Stop time	10 minutes
Post time	2 minutes
Backpressure regulator (BPR) temperature	60 °C
Backpressure regulator pressure	100, 120, 140, 160 bar
Column temperature	30, 40, 50, 60 °C
Injection volume	5 µL, 3x loop overfill
Needle wash in vial with methanol	
DAD	254 nm, bandwidth 4 nm, ref. 360 nm, bandwidth 100 nm
Data rate	20 Hz

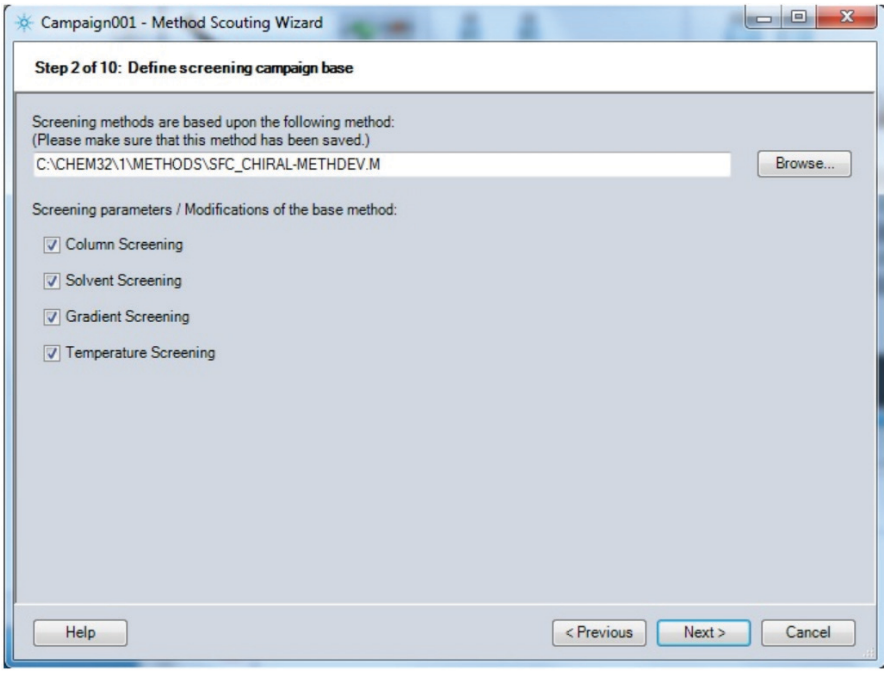

Figure 1. Agilent ChemStation Method Scouting Wizard allows easy setup of column screening, solvent screening, gradient screening, and temperature screening in separate windows, and combines the individual runs into a sequence also comprising automated solvent exchanges and equilibrations.

The first combination comprised the Rx-SIL column with methanol and Gradients 1 to 4 (Figure 2). The bubble chart showed a maximum retention time for Gradient 1 of approximately 3.78 minutes. However, and unfortunately, the peaks of Compounds 1 and 2 were poorly resolved and were completely unresolved for steeper gradients. This can be seen by the very small area of the bubbles in the chart for Gradients 2 to 4, which had the lowest number of recognized peaks.

The second combination comprised the SB-CN column with methanol and Gradients 1 to 4 (Figure 3). The maximum retention times are similar for all gradients, and resolution improves slightly, though still insufficient in all cases.

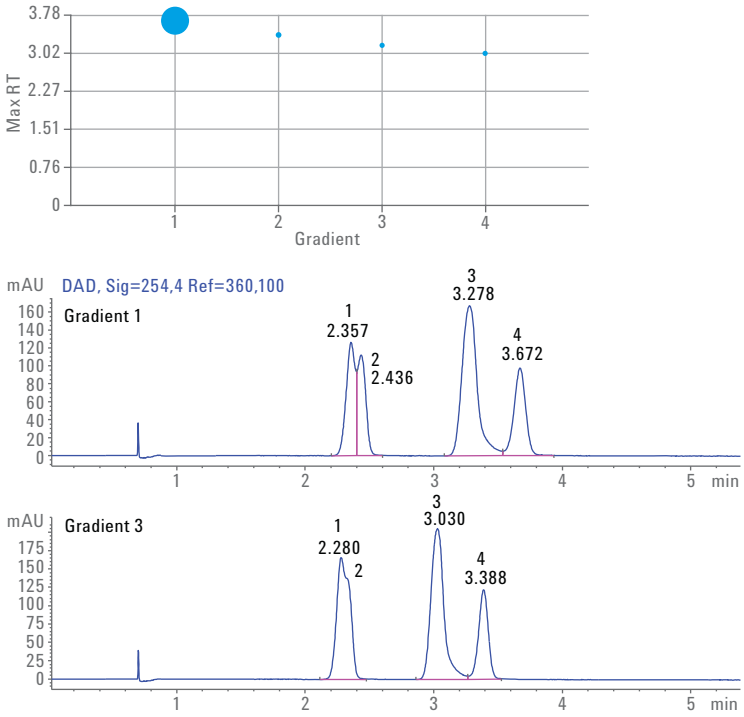

Figure 2. Separation of the four-compound standard mix on an Agilent ZORBAX Rx-SIL column with different Gradients 1 to 4 using methanol as modifier. Gradient 1 showed insufficient resolution of Compounds 1 and 2 and with Gradients 2 to 4 these compounds coeluted.

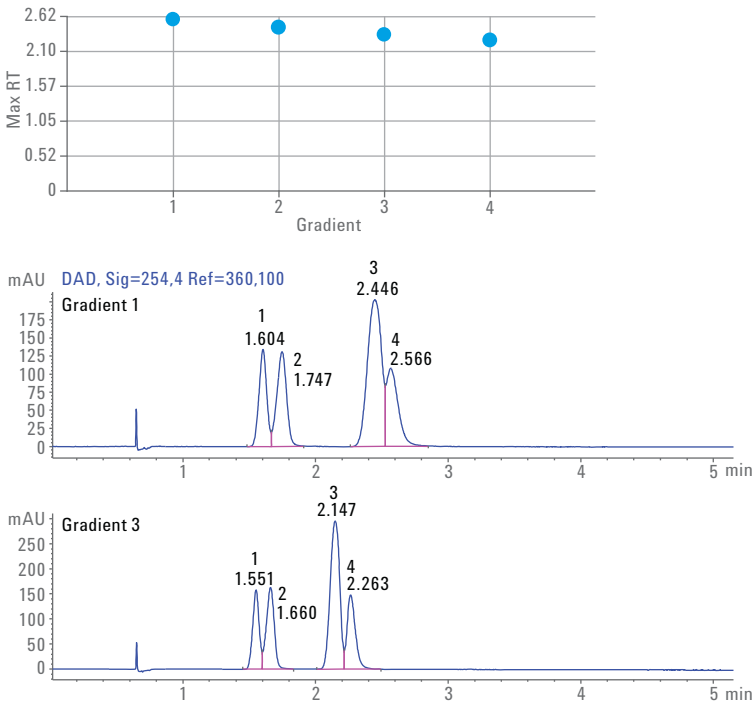

Figure 3. Separation of the four-compound standard mix on an Agilent ZORBAX SB-CN column with Gradients 1 to 4 using methanol as modifier. Gradient 1 showed insufficient resolution of compounds, especially 3 and 4, and with Gradients 2 to 4 these compounds could not achieve sufficient resolution.

The third combination comprised the NH2 column with methanol as eluent and Gradients 1 to 4 (Figure 4). For the first gradient, the resolution of all peaks was sufficient, but the retention time of Compound 4 was very close to the end of the run time. The steeper Gradients 2 to 4 showed sufficient resolution for all compounds in combination with earlier retention of Compound 4. The other possible combinations of all three columns and all four gradients with ethanol and isopropanol as modifiers showed insufficient resolution or peak shapes, or both, and were no longer considered for optimization of the separation (data not shown). Therefore, the third gradient was used, in combination with the NH2 column and methanol as modifier, for further gradient optimization.

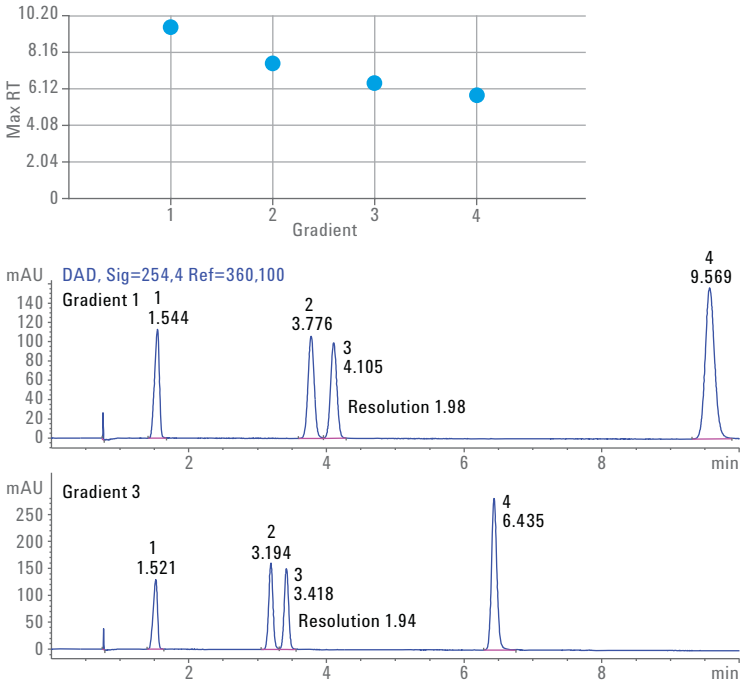

Figure 4. Separation of the four-compound standard mix on an Agilent ZORBAX NH2 column with different Gradients 1 to 4 using methanol as modifier. Gradient 1 showed good resolution of Compounds 2 and 3 but high retention near the end of the run for Compound 4. Gradients 2 to 4 led to an earlier elution of Compound 4 while maintaining resolution of Compounds 2 and 3.

So far, the experiments were done at a column temperature of 40 °C and a backpressure of 120 bar. Since temperature can influence selectivity and retention time behaviors for SFC separations, the next parameter to optimize was temperature while using the NH2 column, Gradient 3, and methanol as modifier (Figure 5). The resolution obtained for a column temperature of 30 °C was slightly better than at 40 °C. However, at higher temperatures Compounds 2 and 3 lost resolution. The retention time was shifted only slightly.

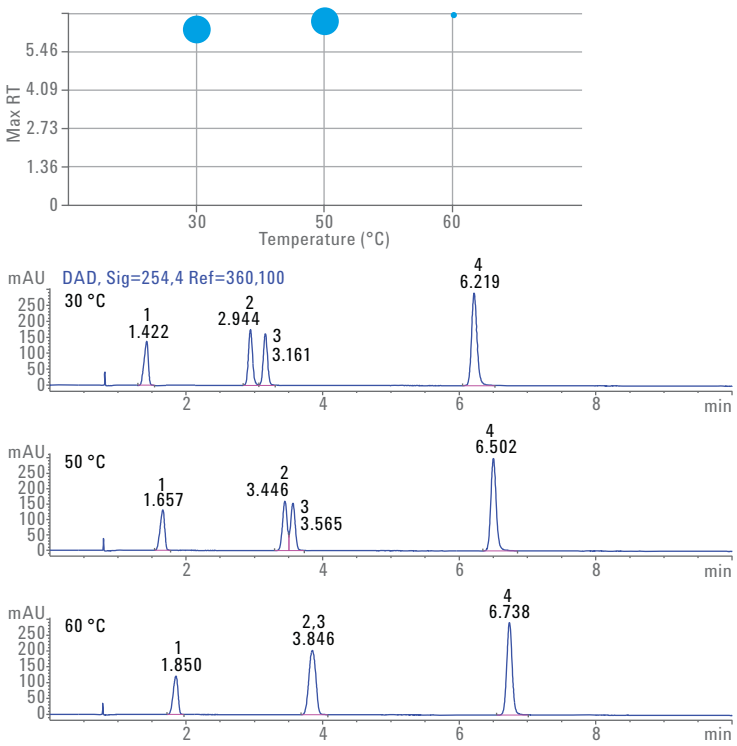

Figure 5. Separation of the four-compound standard mix on an Agilent ZORBAX NH2 column with Gradient 3 using methanol as modifier and different temperatures. A column temperature of 30 °C showed good resolution of Compounds 2 and 3. At higher temperatures of 50 and 60 °C, they lost their resolution.

The backpressure used in SFC can have some influence on selectivity and retention time. For the experimental verification of backpressure influence, the temperature was kept at 40 °C (Figure 6). A backpressure of 100 bar showed a slightly better resolution of Compounds 2 and 3, comparable to the resolution obtained at 120 bar. At higher backpressures of 140 and 160 bar, Compounds 2 and 3 lost resolution.

Consequently, the final statistical evaluation of the separation was done on the NH2 column with Gradient 3 and methanol as modifier, at a column temperature of 30 °C, and at system backpressure of 100 bar (Table 1). The RSD values of the retention times were typically better than 0.3 %, and the area RSDs were typically better than 1.6 %. The resolution obtained for the critical Compound 3 was above 2.0.

Conclusions

This Technical Overview demonstrates the enhancement of the Agilent 1260 Infinity Analytical SFC Solution to an SFC method-development system by the addition of a second thermostatted column compartment and a 12-solvent selection valve used with the SFC pump. Together with the Agilent ChemStation Method Scouting Wizard for fast and automated method development, this makes SFC method development an easy task. The example given here shows the column and solvent scouting method with some predefined gradients and fine tuning by means of column temperature and system backpressure. For the final method, a statistical evaluation was done showing excellent RSD values of retention time and area precision.

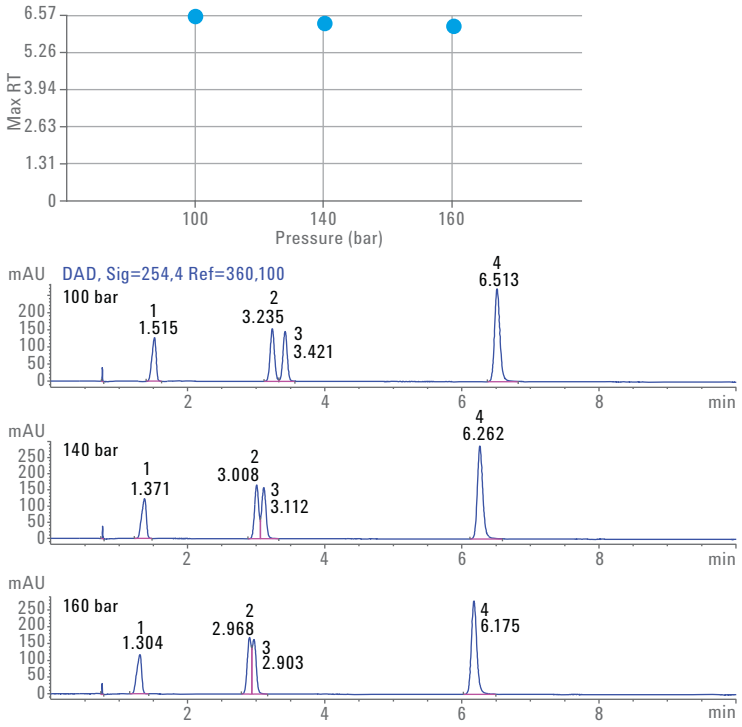

Figure 6. Separation of the four-compound standard mix on an Agilent ZORBAX NH2 column with Gradient 3 using methanol as modifier at 40 °C and different backpressures. The backpressure of 100 bar showed good resolution of Compounds 2 and 3. At higher backpressures of 140 and 160 bar, they lost resolution.

Table 1. Separation of a four-compound standard mix with the final method: Agilent ZORBAX NH2 column, Gradient 3, methanol, 30 °C, 100 bar. The averages (av), standard deviations (std) and relative standard deviations (rsd (%)) were calculated for retention time (RT), peak area and resolution from 10 runs.

	Compound 1			Compound 2		
	RT	Area	Resolution	RT	Area	Resolution
av	1.444	587.91	10.13	3.008	733.31	13.98
std	0.010	8.298	0.195	0.009	14.247	0.171
rsd	0.68	1.41	1.93	0.29	1.94	1.22
	Compound 3			Compound 4		
	RT	Area	Resolution	RT	Area	Resolution
av	3.235	686.41	2.06	6.327	1619.53	24.25
std	0.009	11.167	0.015	0.011	23.735	0.177
rsd	0.27	1.63	0.72	0.17	1.47	0.73

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Published in the USA, August 1, 2014
5991-5029EN

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