Strategies for column and mobile phase selection with the Agilent 1260 Infinity Analytical SFC System

Application Note

Drug Development

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Abstract

Stationary and mobile phase screening is an important aspect of method development in analytical supercritical fluid chromatography (SFC). In this Application Note we describe the automated screening of columns and modifiers using the Agilent 1260 Infinity Analytical SFC System column screening kit. Six different stationary phases and two modifiers were screened to identify the optimum combination. For the selected sample, the Agilent ZORBAX Rx-SIL column and methanol as the modifier gave the best results with baseline separation of all compounds.

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Introduction

Supercritical fluid chromatography (SFC) is well established as an analytical technique for chiral separations and is gaining increasing attention for achiral analytes especially in drug discovery and development. With the increasing costs of organic solvents and environmental awareness to minimize toxic waste production, SFC is also considered as a green alternative to normal phase or reversed phase chromatography for small to medium-sized molecules. In addition, SFC is a complementary separation technique to HPLC for high-throughput and high-resolution analysis of complex samples and impurities. Typical analysis times and column re-equilibration in SFC are decreased by a factor of 3 to 5 compared to standard HPLC. Because there is not yet a standard phase for chiral or achiral SFC applications, a column screening strategy for four to six stationary phases is commonly deployed prior to the actual separation. This helps determine the optimum separation conditions. It also provides an excellent handle for fine-tuning selectivity in a much wider regime than with reversed-phase chromatography.

Selectivity in SFC can be easily changed, optimized, and exploited to fulfill separation requirements by parameters such as:

- Stationary phase
- Modifier type
- Modifier concentration
- Column temperature

In this Application Note we demonstrate a rapid, easy-to-use automated approach to sequentially determine the most appropriate stationary and mobile phases for an isocratic separation of an achiral compound mixture using the Agilent 1260 Infinity SFC System. The system was equipped with the SFC column screening kit for sequential column screening. The 8-position/9-port valve in this kit makes it possible to install up to six columns (maximum column length 250 mm) in the two column compartments. In addition, it is possible to switch between mobile phases automatically by either using the solvent selection upgrade built into the SFC binary pump or an external solvent selection valve.

Experimental

Equipment

An Agilent 1260 Infinity Analytical SFC System (G4309A) was used, including:

- Agilent 1260 Infinity Standard Degasser
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1260 Infinity Diode Array Detector VL with high pressure SFC flow cell
- Agilent 1290 Infinity Thermostatted Column Compartments (G1316C)
- SFC Fusion A5 Module

In addition, the Agilent SFC column screening kit (G4307A) was used, which contains:

- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Valve drive for Agilent 1260 Infinity Thermostatted Column Compartment (G1316C)
- 8-position/9-port valve head
- Manifold and required capillaries

Optional

- Solvent selection upgrade for the Agilent 1260 Infinity SFC Binary Pump or Agilent 1260 Infinity 12 pos/13 port solvent selection valve

Columns

The following columns from Agilent and another vendor were used for column screening (4.6 mm × 150 mm, 5 µm):

- Column 1 Agilent ZORBAX Rx-SIL
- Column 2 Agilent ZORBAX CN
- Column 3 Pyridine
- Column 4 Cyano
- Column 5 Diol
- Column 6 Silica

Chemicals

Solutions of caffeine (Caf), theobromine (Thb), theophylline (Thp) and uracil (Ura) were prepared in methanol at approximately 500 µg/mL.

Results and discussion

Column screening strategy

Column screening was performed using fixed initial chromatographic conditions, modifier concentrations, and column temperature. A sequence to screen the stationary phases using methanol and isopropanol was set up using an isocratic method. The method is summarized in Table 1.

The results of column screening for isopropanol and methanol as the cosolvent are shown in Figures 1 and 2.

Baseline separation of the test mixture could not be achieved with 20% isopropanol as the modifier in the mobile phase and either stationary phase in isocratic mode.

Parameter	Condition
Columns (4.6 mm × 150 mm, 5 µm)	Column 1 Agilent ZORBAX Rx-SIL Column 2 Agilent ZORBAX CN Column 3 Pyridine Column 4 Cyano Column 5 Diol Column 6 Silica
Supercritical Fluid	CO_2 (beverage grade)
Modifier	20% methanol or isopropanol
Separation mode	isocratic
Outlet Pressure	130 bar
Flow Rate	3.0 mL/min
Column inlet temperature	40 °C
Column outlet temperature	37.5 °C
Injection Volume	5 µL
Detection	254/BW nm, Ref WL/BW nm

Table 1
Column screening conditions.

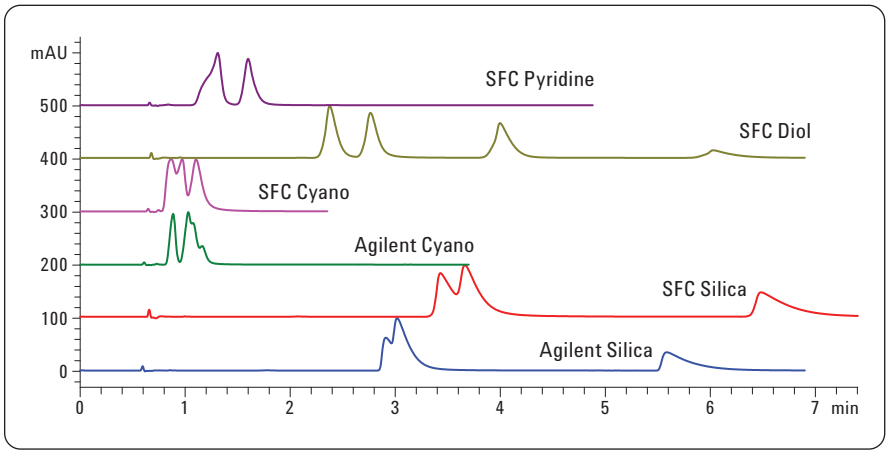

Figure 1
Results of the columns screening with isopropanol as modifier.

In contrast, similar separation conditions substituting methanol for isopropanol resulted in significant improved separation efficiency with all stationary phases. Baseline separation, for all four analytes, however, was achieved only on the Agilent ZORBAX Rx-SIL stationary phase (Figure 2). Separation of all components was achieved in less than 3 min. The results are summarized in Table 2.

In order to obtain further optimization, such as an increase in resolution or peak capacity, the next steps would be to test different modifier concentrations or screen different column temperatures.

Conclusions

This Application Note demonstrates the column and modifier screening as part of a typical method development procedure on the Agilent Infinity 1260 Analytical SFC System. The addition of the SFC column screening kit to the system offers high flexibility and performance for automated selection of the most appropriate stationary phase for analytical SFC and prepreparative SFC method development. The screening experiments, column conditioning, column flushing, and equilibration methods can be easily integrated into ChemStation sequences for an unattended and automated workflow. The SFC system used in the study demonstrated a high degree of robustness with no manual interference, thereby offering an attractive and orthogonal alternative to HPLC.

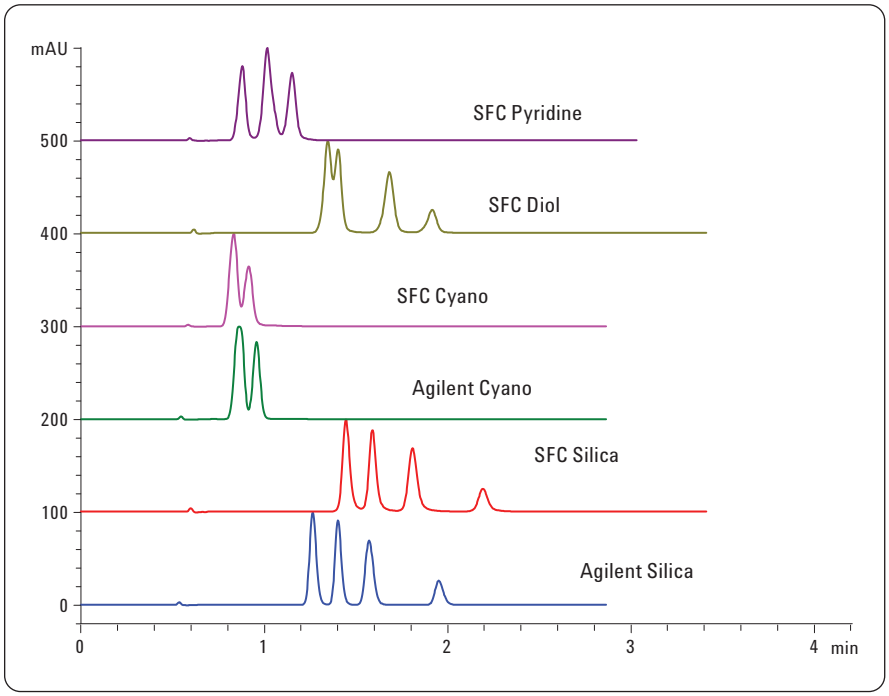

Figure 2
Results of the columns screening experiments with methanol as modifier.

Compound	Retention time (min)	5σ peak width (min)	Resolution
Caffeine	1.26	0.04	N/A
Theobromine	1.4	0.04	2.1
Theophylline	1.57	0.05	2.3
Uracil	1.95	0.05	4.5

Table 2
Summary of results on the Agilent Silica column.

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Published July 12, 2011
Publication Number 5990-7147EN

Agilent Technologies