Performance optimization using the Agilent 1260 Infinity Analytical SFC system

Technical Overview

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Abstract

Supercritical Fluid Chromatography (SFC) has gained a wide interest and acceptance in many small molecule applications especially for the separation of chiral compounds. This is due to unique selectivity, high separation speed, efficiency, and low operating costs. As there is no universal stationary phase available for SFC separations, screening of different columns is required in order to achieve optimum separation. In this Application Note the influence of backpressure, column temperature and modifier concentration are investigated in order to fine-tune the selectivity of a separation in SFC.

Introduction

Selectivity in SFC can be easily altered, and exploited to fulfill separation requirements through a variety of parameters. In a previous note column screening and mobile phase selection in SFC was described.[1]

This publication describes how to optimize a typical SFC separation by changing:

- System backpressure
- Modifier concentration
- Column temperature

Predicting the influence of these parameters on a chromatographic separation is difficult and very often depends on the stationary phase and the investigated analytes. We have demonstrated in two different, and independent experiments how to obtain and optimize the separation of a 4-compound mixture in a limited number of experiments.

Experimental

Equipment

The Agilent 1260 Infinity Analytical SFC System (G4309A) was used, equipped with a column screening kit (G4307A) and a 12-position/13-port solvent selection valve.

Columns

The following columns were used for column screening (4.6 mm × 150 mm, 5 µm):

- Agilent ZORBAX Rx-SIL
- Silica column from other vendor

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Chemicals

Solutions of Caffeine (Caf), Theobromine (Thb), Theophylline (Thp) and Uracil (Ura) were prepared in methanol at approximately 500 µg/mL.

Results and Discussion

1. System backpressure

The following experiment demonstrates the influence of the system backpressure on retention and resolution. When the system backpressure is increased, the density of the supercritical fluid is changed. In the experiment shown, the backpressure regulator setting was varied between 110 to 190 bar. The flow rate was kept constant at 3 mL/min, with the organic modifier concentration at 20% while the column temperature was 40 °C.

The chromatograms in Figure 1 shows that higher pressure results in decreased retention times. Backpressure also affects resolution for peak pair 1/2, the resolution decreased from 1.6 at 110 bar to 1.0 at 190 bar and for peaks 3/4 from 4.1 to 2.5. Note that the resolution of peaks 2/3, in contrast, increased from 1.5 to 2.2.

2. Organic modifier concentration

Different organic solvents have been used as modifiers in SFC such as methanol, ethanol, 2-propanol, and acetonitrile. Methanol which is the most widely used co-solvent was used in this study. The impact on retention times and the resolution with methanol concentrations between 5% and 25% concentrations is shown in Figure 2. The flow rate was kept at 3 mL/min. The column temperature was 40 °C, while the column outlet temperature and the system backpressure remained at 38 °C and 130 bar. The analysis time decreased with the increase of modifier. This is best seen in peak pair 3 and 4, where baseline separation was achieved only below 10% modifier. Below a modifier concentration of 5% the separation could not be further improved.

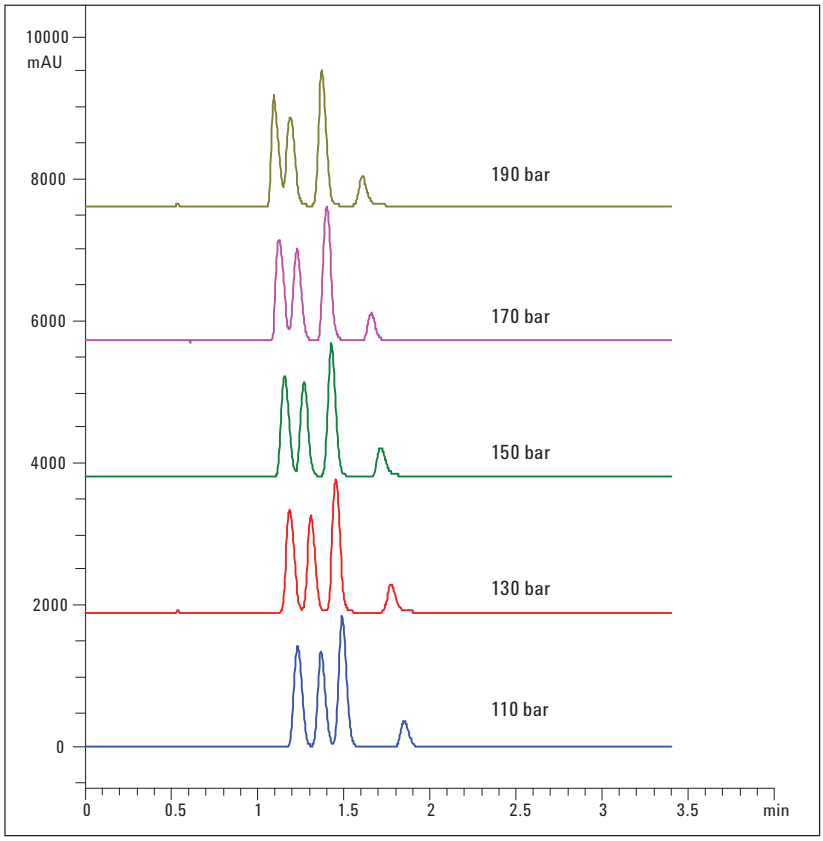

Figure 1
Chromatograms show the influence of the system backpressure on the retention times and on resolution.

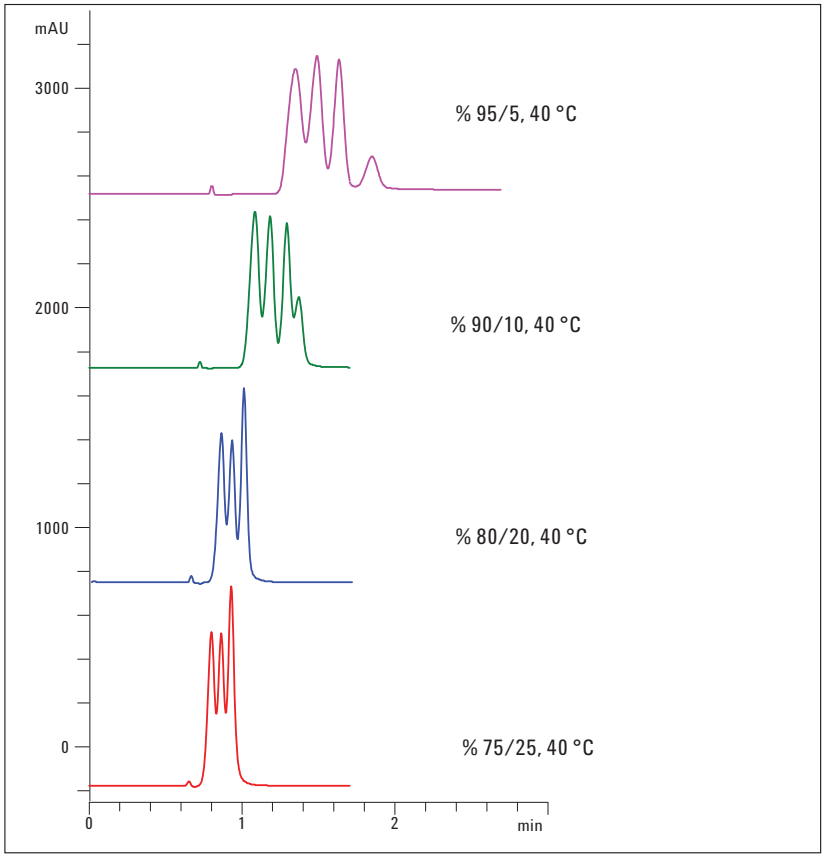

Figure 2
Chromatograms showing the influence of organic modifier concentration retention times and resolution.

3. Influence of column temperature

Based on the previous result, the influence of the column temperature was investigated for the analyte mixture example using a co-solvent concentration of 5% methanol. The column temperature was varied between 10 °C and 40 °C, while all other parameters were similar as in experiment 2.

The influence of the column temperature is best seen in peak pairs 1/2 and 2/3 (Figure 3). The resolution increased from 0.8 and 1.0 respectively at 40 °C to 1.2 and 1.4 respectively at 10 °C. Resolution of peak 3/4 (1.5), however, was not affected with the column temperature. In general column temperature had a moderate impact on retention. While in LC, lowering the temperature typically results in increased retention in the SFC example shown, we observed the opposite behaviour. Here, the best results were achieved at a column temperature of 10 °C, this might be different for other analyte mixture/stationary phase combinations.

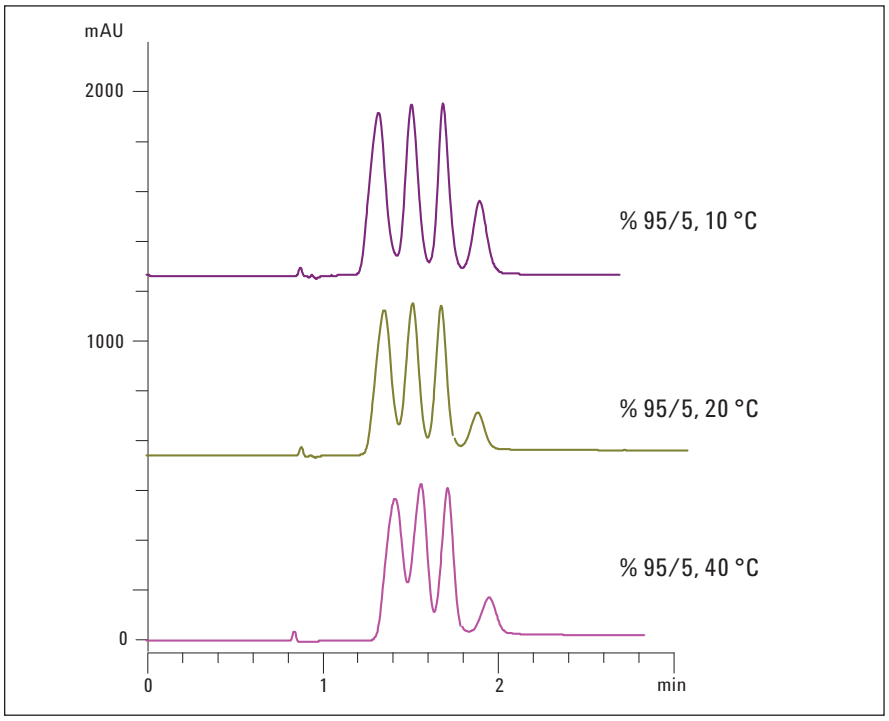

Figure 3
Influence of the column temperature on retention times and resolution.

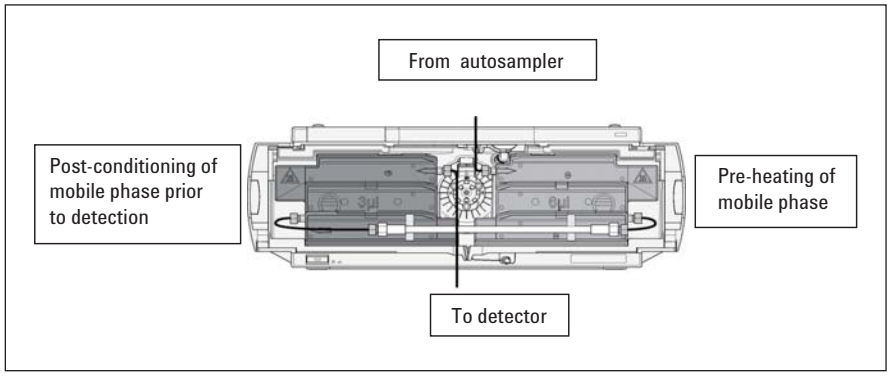

Figure 4
Different zones in the thermostatted column compartment to pre and post condition mobile phase temperatures.

General recommendations

In general, it is very important to precondition the temperature of the mobile phase before it enters the detector flow cell in order to minimize noise (Figure 4). The right heat exchanger in the column compartment is used to pre-heat the mobile phase prior to entering the column. The left heat exchanger is applied to change the temperature of the effluent to an empirically derived optimum prior to entrance into the detector. This is necessary since the refractive index of carbon dioxide changes as much as 50 times more compared to water with small changes in temperature. A temperature change of, for example, 38 °C to 49 °C resulted in an increased baseline noise of over an order of magnitude.

Conclusions

This Technical Overview demonstrates the importance of system backpressure, mobile phase concentration, and column temperature.

A typical strategy for method development for separation in SFC consists of column screening and optimization steps. A logical approach for successful SFC method development should contain a series of experiments in the following order:

1. Stationary phase choice has a major influence on retention and selectivity
2. Co-solvent selection
3. Co-solvent concentration
4. Column temperature
5. System backpressure

These steps can be easily automated in SFC by using the method development capabilities of the Agilent 1260 Infinity LC system, rendering SFC into a robust and sensitive orthogonal separation option.

Reference

1.
"Strategies for column screening and mobile phase selection in Supercritical Fluid Chromatography," Agilent Technologies publication number 5990-7147EN.

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