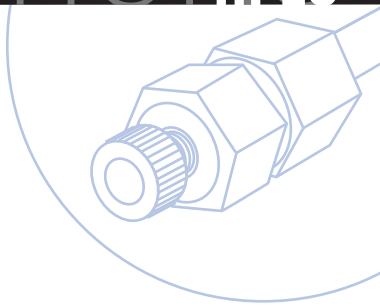

Achiral Stationary Phase Selectivity under Supercritical Fluid Chromatography (SFC) Conditions

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Supercritical fluid chromatography (SFC) is gaining wide acceptance in the pharmaceutical industry in support of drug discovery and medicinal chemistry compound separation and purification. Until recently SFC has found the greatest success in small preparative scale chiral separations in drug discovery laboratories. However, there is significant potential for extending SFC to achiral column screening and method development.

Introduction

Analyses and purifications of reaction mixtures are among the most demanding tasks for organic and analytical chemists in the pharmaceutical industry. This is especially true during the hit-to-lead and lead optimization stages of drug discovery. Today's complex synthetic routes can generate multiple component sample mixtures with a wide range of polarities and functionalities. High Performance Liquid Chromatography (HPLC) has traditionally been the chromatographic method of choice for the separation of complex sample mixtures containing compounds with a range of different functional groups and polarities. HPLC has been the workhorse of the pharmaceutical industry to fulfill these separation needs; however, Supercritical Fluid Chromatography (SFC) is a promising alternative, orthogonal technique that possesses several advantages over HPLC. These advantages include: improved resolution, faster separation, and higher sample throughput. SFC also offers a distinct "green" technology advantage by reducing the usage of toxic solvents.

In SFC the mobile phase consists of carbon dioxide (CO_2) above or near its critical state (temperature >31.1 °C and pressure >73.8 bar) in combination with an organic modifier such as methanol. This relatively non-polar mobile phase can be used with stationary phases typically used in normal or reversed phase HPLC, and the low viscosity and high diffusivity of supercritical CO_2 relative to the solvents typically used in HPLC translates into much faster separations.

SFC first gained popularity in medicinal chemistry laboratories for chiral applications. Due to SFC's intrinsic properties of low viscosity and high diffusivity, chiral separations are easily achieved on the same normal phase columns first introduced for HPLC applications as well as on specialized chiral columns.

Today several medicinal chemistry and analytical laboratories have investigated extending the utility of SFC to achiral separations. Column selection for achiral SFC applications has been under investigation as separation scientists, familiar with the nearly universal applicability of C18 columns for reversed phase HPLC, are searching for a similar universal column for achiral SFC. This technical note will demonstrate that achiral HPLC columns used under SFC conditions can offer quite different selectivities. Therefore, method development for achiral SFC separations must, like chiral separations, involve column screening for the determination of optimal selectivity that will allow analytical scientists to obtain the chromatographic resolution required to meet their needs.

This is particularly important when the ultimate goal is isolation of the compound of interest by preparative chromatography. The column screening approach allows the chromatographer to determine the column that provides the selectivity and chromatographic resolution required to isolate the desired compound from the other components of the sample and, if possible, elutes the desired product early in the chromatographic run so that sample overloading can be used to increase the purification throughput and process efficiency.

Experimental Conditions
Thar SFC Investigator system (Thar Instruments, Pittsburgh, PA, USA) equipped with Variable Wavelength Detector (Gilson UV/VIS-151, Gilson, Middleton, WI, USA) and SuperChrom version 2.6 (Thar Instruments). The mobile phase consisted of industrial carbon dioxide (99.995 % pure, Air Liquide, Houston, Texas, USA) and HPLC grade methanol (Honeywell Burdick and Jackson, Muskegon, Michigan, USA). Four different achiral HPLC columns from Phenomenex (Torrance, CA, USA) were utilized in this study.

Columns:
Luna® 5 µm NH_2
Luna® 5 µm Silica(2)
Synergi™ 4 µm Polar-RP®
Luna® 5 µm HILIC

The following conditions were the same for all columns:

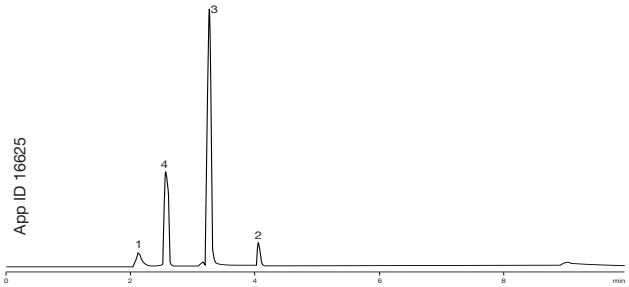
Dimensions:	250 x 4.6 mm
Mobile Phase:	A: Methanol
	B: Carbon Dioxide (CO_2)
Gradient:	A/B (5:95) to (50:50) in 5.5 min, hold 2 min
Flow Rate:	3.0 mL/min
Temperature:	40 °C
Pressure:	200 bar (~2900 psi)
Detection:	UV @ 254 nm
Injection Volume:	1 µL

Two different sample mixtures were injected onto each column under the conditions noted above.

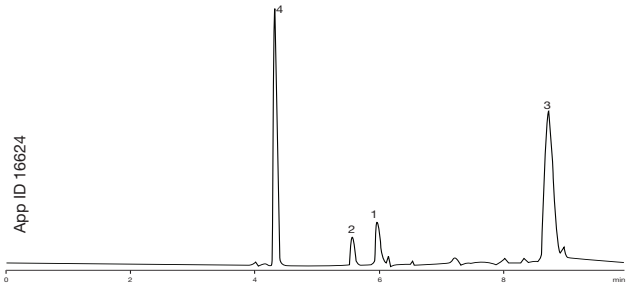
Sample 1:
1. Uracil
2. Hydrocortisone
3. Sulfanilamide
4. Ethyl-2-methyl-3-indoleacetate

Sample 2:
1. Benzoic Acid
2. Caffeine
3. Acetaminophen

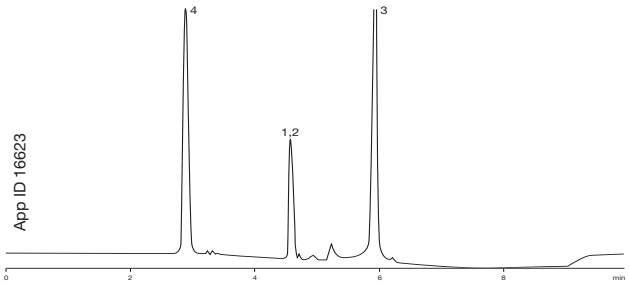
a) Synergi Polar-RP



b) Luna NH_2



c) Luna HILIC



d) Luna Silica(2)

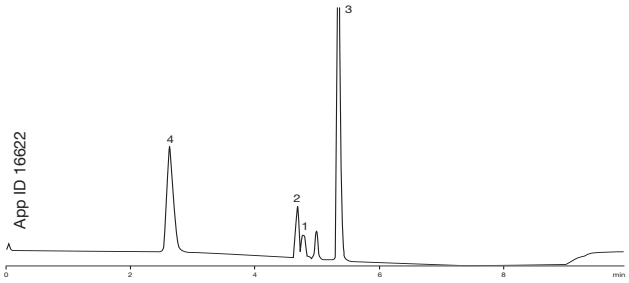
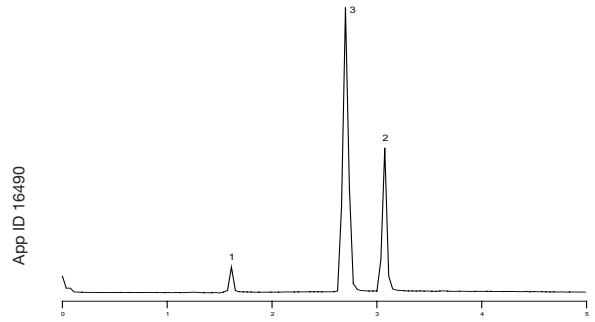
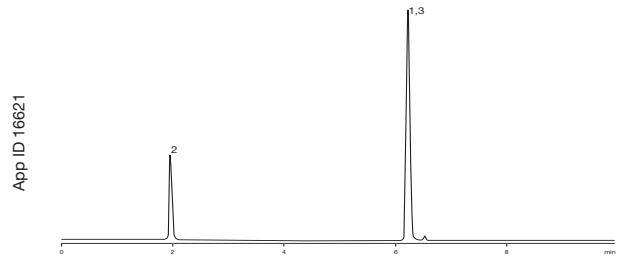


Figure 1: Test mixture recommended by a mutual customer in Europe (Uracil, Hydrocortisone, Sulfanilamide, and Ethyl-2-methyl-3-indoleacetate). Synergi Polar-RP (a), Luna NH_2 (b), Luna HILIC (c), and Luna Silica(2) (d).

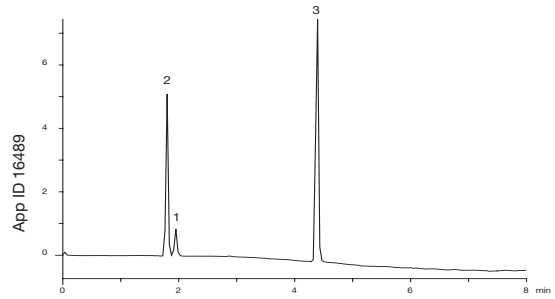
a) Synergi Polar-RP



b) Luna NH_2



c) Luna HILIC



d) Luna Silica(2)

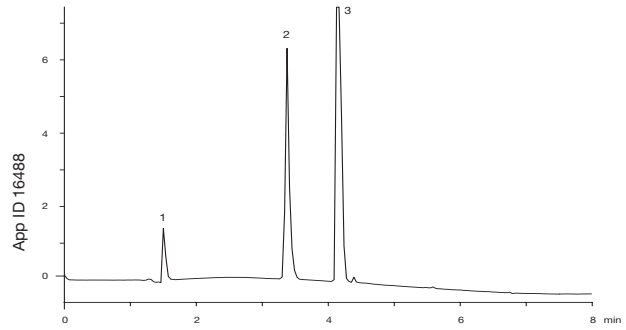


Figure 2: Test mixture 2 (Benzoic acid, Caffeine, and Acetaminophen). Synergi Polar-RP (a), Luna NH_2 (b), Luna HILIC (c), and Luna Silica(2) (d).

Results

The Thar SFC Investigator system was used to screen several different Phenomenex achiral column chemistries using two different sample mixtures. The results show that under the same SFC mobile phase conditions the different column stationary phases can have a dramatic effect on selectivity, and therefore the overall chromatographic resolution and separation.

Each sample was injected onto each of the four columns chosen for this study. These columns were originally developed for very different modes of separation: reversed phase (Synergi Polar-RP), normal phase (Luna Silica(2) and Luna NH_2), and HILIC (Luna HILIC). Significant shifts in retention time as well as reversals in elution order clearly illustrate the very different selectivities provided by these HPLC columns under SFC conditions. These selectivity differences can be further exploited to allow for the selection of a column that best meets the requirements for the chromatographic separation problem at hand.

For analytical chemists, the difference in selectivity demonstrated through the use of orthogonal phases will offer the ability to very effectively develop complementary chromatographic methods. This is very important for a complete and thorough understanding of their unknown sample mixtures.

Conclusion

The analytical SFC applications shown in this technical note demonstrate that the differences in selectivity offered by achiral HPLC columns can be utilized for SFC separations as well.

SFC is a technology that must be considered in preparative chromatography, due to its geometric scalability, reduction in waste organic solvents and unique selectivities. The Phenomenex columns included in this study clearly demonstrate both 1) varied selectivity from one column chemistry to another and 2) robustness and reproducibility of individual columns when employed under SFC conditions. This indicates that the applicability of achiral HPLC columns that are already present in many medicinal chemistry or analytical laboratories can easily be extended beyond the limits for their originally intended application areas to allow for their use in SFC application development.

Ordering Information

Part No.	Description	Dimensions
00G-4450-E0-TN	Luna 5 µm HILIC 200 Å	250 x 4.6 mm
00G-4336-E0-TN	Synergi 4 µm Polar-RP 80 Å	250 x 4.6 mm
00G-4274-E0-TN	Luna 5 µm Silica(2) 100 Å	250 x 4.6 mm
00G-4378-E0-TN	Luna 5 µm NH_2 100 Å	250 x 4.6 mm

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