Using Core Shell Columns *for* Improved Separation of Pharmaceutical Compounds *by* Supercritical Fluid Chromatography

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Abstract

Superficially porous (core shell) particles consist of a non-porous silica core surrounded by a thin shell of porous silica, have gained in popularity as a solid support for chromatographic phases over the last decade. Core shell particles offer reduced resistance to solute mass transfer into the porous structure due to the small diffusion distances which leads to very fast and efficient separations. Since the overall particle size is larger, core shell particles operate with lower pressure than sub-2 micron particles, and do not require specialized instrumentation to deal with the increased pressure. Similar benefits of using superficially porous particles have been demonstrated for supercritical fluid chromatography (SFC), but the selection of available phases is limited to those for reversed phase, or bare silica. In this study, we evaluate a 2.6-micron superficially porous 2-ethylpyridine stationary phase made specifically for SFC applications. We compare its performance to several available superficially porous HILIC (hydrophilic interaction liquid chromatography) or unbonded silica phases, as well as fully porous, sub-2 micron silica and 2-ethylpyridine columns. Using a mixture of 17 small drug-like compounds under similar SFC conditions, we determined that superficially porous 2-ethylpyridine columns outperformed comparable silica phases for overall selectivity.

Introduction

SFC analysis has increased in prevalence in the last ten years for the analysis of pharmaceutical compounds, as it benefits from high peak capacities and efficiencies.[1-3] Run times on SFC systems are typically 3-5 times faster and are cost-efficient for long term use. The most common mobile phase solvents for SFC are compressed CO_2 mixed with some organic modifier, typically an alcohol. The lower viscosity and higher diffusivity of this fluid offer less pressure drop across the column relative to aqueous-based solvents under similar flow and temperature conditions.[4]

Recent studies have found that the utilization of particles <3 µm in diameter for SFC provides an additional benefit to separation efficiency.[5-7] Compared to conventional fully porous particles (FPP), solid core particles (SCP) consist of solid core silica microspheres surrounded by a thin, porous outer shell. The advantages of using SCPs include faster solute mass transfer due to decreased diffusion distances, where molecules no longer have to traverse the longer distances previously found on the totally porous FPP.[8] Several groups suggest that SCPs are similar to sub-2 micron particles but without the pressure limitations of the micronized particles. For instance, Berger [9] concluded that SCP particles offered a 10-fold decrease in analysis time, with better resolution, at lower pressures than columns with fully porous, 3µm particles, under conventional pressure limitations (e.g. < 400 bar). To date, evaluation of SCP columns by SFC has been limited to those used primarily for reversed phase or bare silica. In this study, we created and evaluated a 2.6-µm core shell 2-ethylpyridine stationary phase made specifically for SFC applications. We compare its performance to that of two superficially porous bare silica phases, a fully porous sub-2µm silica phase, and a fully porous 2-ethylpyridine phase.

Experimental

Chemicals and Reagents

The compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared to a concentration of ~1mg/mL in methanol. The structure of the analytes are presented in Figure 1.

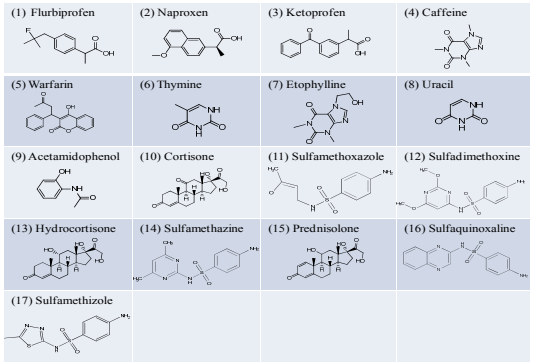

Figure 1: Seventeen drug-like compounds utilized to evaluate the columns in this study. They are numerically ordered based on elution from the Acquity UPC² Viridis BEH (silica) column using the conditions listed in the Analysis Conditions section.

Analysis Conditions

Analysis was performed using the columns listed below and under the following conditions unless specifically noted. The mobile phase consisted of carbon dioxide (A) and methanol (B) with a gradient profile from 5-20% B at a flow rate of 2.5 mL/min and outlet pressure at 150 bar. The columns were maintained at a temperature of 55˚C and sample injection volume was 1µL.

Fully Porous Particle (FPP) columns

The fully porous particle columns used were the Waters Acquity UPC² Viridis BEH (silica) and the Waters Acquity UPC² Viridis BEH 2-ethylpyridine (2-EP), with 1.7 µm diameter particles and column dimensions of 3.0 mm i.d. x 100 mm length.

Solid Core Particle (SCP) columns

The solid core particle columns used were the Ascentis Express HILIC (Supelco, Bellefonte, PA, USA) with 2.7 µm diameter particles; the SunShell 2-EP (Nacalai USA, San Diego, CA, USA) with 2.6 µm diameter particles; and the Phenomenex Kinetex HILIC (Torrance, CA, USA) with 2.6 µm diameter particles; The dimension of each column was 3.0 mm i.d. x 150 mm in length.

When using small particle columns, one must expect an increase in pressure relative to 3 or 5 µm particle columns. In order to avoid excessive column pressure for the 1.7 µm particles, the 100 mm length BEH columns were used.

Results and Discussion

Comparison of HILIC and 2-Ethylpyridine Columns

Three different silica columns and two 2-ethylpyridine columns were evaluated in the selectivity differences as shown in Figure 2. The 2-ethylpyridine columns demonstrated enhanced selectivity over the bare silica columns, as expected. The addition of the basic aryl functional group near the surface silanols enhance the selectivity of a wide variety of compounds and provide retention inversion relative to elution on HILIC or alkyl-bonded phases, which resulted in increased popularity of the 2-EP phase.

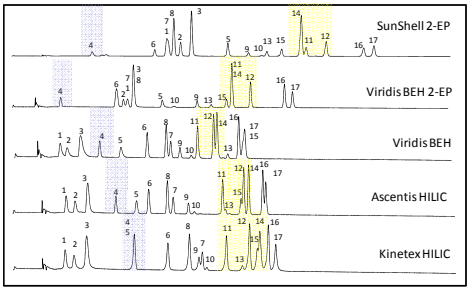

Figure 2: Chromatogram of the 17-component mix run. Gradient elution of 5-20% methanol over 4 minutes at 2.5mL/min flow rate; outlet pressure 150 bar and column temperature 55ºC.

The retention order of sulfamethazine (14) on the 2-EP columns is inverted compared to that of sulfamethoxazole (11) and sulfadimethoxine (12) on the silica columns, in addition to the significant increase in selectivity. On the 2-EP columns, caffeine (4) and thymine (6) elute before the acidic compounds flurbiprofen (1), naproxen (2) and ketoprofen (3)

The SunShell 2-EP column demonstrated the best overall baseline separation with respect to the columns tested.

An optimized gradient separations of the 17-component mix for the SunShell 2-EP is presented in Figure 3, where all 17 components are separated in less than 2 minutes. A similar separation was achieved using the Acquity Viridis BEH 2-EP (Figure 4) with the exception of co-eluting sulfamethazine (13) and sulfamethoxazole (14) peaks.

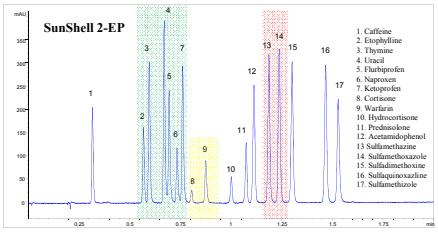

Figure 3: Chromatogram of the separation for the 17-component mix using the **Sun Shell 2-EP** column. A methanol gradient of < 2 minutes was used on the Agilent 1260 Infinity SFC system. SFC conditions: flow rate: 4.0mL/min; outlet pressure 160 bar; column temperature 55^{0}C. Gradient program: 5.0-7.5% in 0.20 min, then 7.5-20% in 1.3 min and held at 20% for 0.2 min.

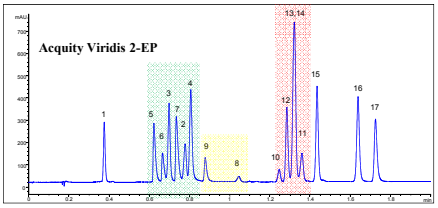

Figure 4: Chromatogram of the separation for the 17-component mix using the **Acquity UPC² Viridis 2-EP** column. 16 of the 17 components were resolved. A methanol gradient of < 2 minutes was used on the Agilent 1260 Infinity SFC system. SFC conditions: flow rate 3.5 mL/min; outlet pressure 160 bar; and column temperature 70^{0}C. Gradient program: 5.0-12.5% in 1.0 min, 12.5% for 0.25 min, then 12.5-20% in 0.75 min.

Conclusions

In this study, we have demonstrated the advantages of using the superficially porous particle-type 2-ethylpyridine column on retention, selectivity, and peak shape in comparison to comparable sub-2µ columns. Further, the superficially porous particles exhibit lower column pressure drops and have the potential to produce highly efficient separations without specialized high pressure pumping systems (> 400 bar). The superficially porous particle-type 2-ethylpyridine phase has demonstrated significant advantages over bare silica phases for fast, efficient SFC analysis.

References

1. C. Brunelli, Y. Zhao, M.H. Brown, P. Sandra, J. Chromatogr. A, 1185 (2008) 263.
2. C.M. Harris, Anal. Chem., 74, (2002) 89-90.
3. T. Berger, Supercritical Fluid Technology for Drug Product Development, Marcel Dekker Inc., New York, 2004, pp. 460.
4. Y. Zhao, W. Pritts, S. Zhang, J. Chromatogr. A, 1189 (2008) 245.
5. E. Lesellier, J. Chromatogr. A, 1228 (2012) 89.
6. T. Berger, J. Chromatogr. A, 1218 (2011) 4559.
7. F. Lestremau, A. Villiers, F. Lynen, A. Cooper, R. Szucs, P. Sandra, J. Chromatogr. A, 1138 (2007) 120.
8. J. Kirkland, F. Truszkowski, C. Dilks Jr., G. Engel, J. Chromatogr. A, 890 (2000) 4.
9. T. Berger, J. Chromatogr. A, 1218 (2011) 4559.