An Empirical Evaluation of HILIC and Monolithic Columns for Supercritical Fluid Chromatography (SFC) Applications

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Introduction

Separation of polar compounds by conventional reverse phase chromatography can be challenging due to their poor retention. Other HPLC approaches include normal phase chromatography (NPC) and its variation, hydrophilic interaction chromatography (HILIC). However, NPC typically suffers from solubility issues, poor reproducibility, and low MS detection sensitivity. As for HILIC, reasonable retention can only be realized when a high percentage of organic solvent (>70%), typically acetonitrile, is used.

More recently, supercritical fluid chromatography (SFC) has become an attractive alternative for the separation of polar compounds [1]. Similar to NPC and HILIC, SFC generally employs polar stationary phases and a less polar mobile phase, supercritical CO_2 in combination with methanol. Owing to the speed advantage as a result of the inherent low viscosity of supercritical CO_2, SFC has been used in high throughput analysis of drug-like compounds and bio-analysis [2-3]. To this end, monolithic columns are of interest in SFC applications for their potential to further improve throughput.

In this application note, we present our empirical evaluation on three different forms of silica columns for SFC applications: a particle packed silica column, an ethylene-bridged hybrid (BEH) silica column marketed as a HILIC column, and a monolithic silica column.

Experimental

All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA) and used as received. Stock solutions of 1 mg/mL were prepared for each compound as well as for the mixtures in 70:30 (v/v) methanol/water.

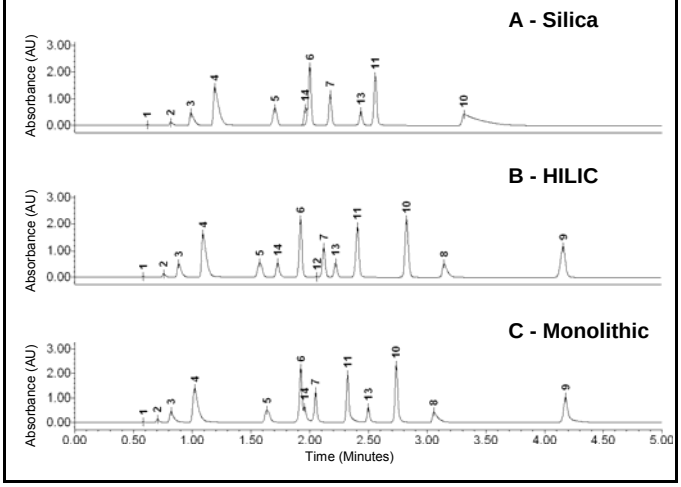
All experiments were conducted using a Waters TharSFC Method Station II controlled by Empower® software. A Spherisorb® particle packed silica column and an XBridge™ HILIC column (both 4.6 x 100 mm, 5 µm) were purchased from Waters (Milford, MA, USA). An Onyx™ monolithic silica column (4.6 x 100 mm) was purchased from Phenomenex (Torrance, CA, USA).

All experiments were run under the following conditions unless otherwise specified: flow rate: 4 mL/min; back pressure: 150 bar; temperature: 40ºC; injection volume: 5 µL (full loop); wavelength scan range: 220 to 300 nm; modifier: methanol; gradient: 5% to 40% in 5 min, 40% for 1 min, 40% to 5% in 2 min, and held at 5% for 2 min.

Results and Discussion

Figure 1 shows the SFC chromatograms obtained under the described gradient condition for each column. In summary, 12 of the 14 compounds eluted off the particle packed silica column and all 14 compounds eluted off the HILIC and monolithic columns. There appears to be more retention on the particle packed silica column as compared to the HILIC and monolithic columns. Two basic compounds, adenine and cytosine (peaks 8 and 9, respectively) did not elute off the particle packed silica column. In addition, compound 10, 6-amino-1,3-dimethyluracil, displayed significant tailing on the particle packed silica column, compared to the other two columns. This is possibly due to the presence of excessive acidic silanol groups on the surface of the particle packed silica column, and consequently, excessive retention for basic compounds. In SFC, it is a common practice to add small tertiary amines, such as dimethylethylamine and isopropyl amine, to shorten the retention time and improve the peak shape by blocking the active silanol sites; and a thorough column flushing is highly recommended afterwards. Both the HILIC and monolithic columns, on the other hand, demonstrated reasonable retention and more symmetrical peak shapes, especially for the BEH HILIC column, owing to the more controlled surface chemistry. It is speculated that the ethylene bridges within the silica matrix not only provide improved chemical and mechanical stability, but also effectively reduce the number of active silanol sites.

Figure 1: SFC chromatograms at 254 nm obtained under gradient conditions. Test compounds are listed below. Acids: 1. Ibuprofen; 2. Fenoprofen; 3. Naproxen; 4. Ketoprofen. Bases: 5. Theophylline; 6. Thymine; 7. Uracil; 8. Adenine; 9. Cytosine; 10. 6-amino-1,3-dimethyluracil. Neutral polar compound: 11. Sulfamethoxazole. Neutral non-polar compounds: 12. Estradiol; 13. Cortisone; 14. Amcinonide.



A mixture consisting of compounds 5 through 8 and 10 through 14 was then injected under isocratic conditions (10% methanol) on all three columns. The resulting chromatograms are shown in Figure 2. Similar to the gradient experiments, 8 of the 9 compounds eluted off the particle packed silica column, whereas all 9 compounds eluted off the HILIC and monolithic columns. Compared to the particle packed silica and HILIC columns, the monolithic column displayed a somewhat different selectivity, especially for relatively bulky compounds, such as cortisone (compound 13) and amcinonide (compound 14). The increased retention is likely due to steric hindrance that impedes their passage through the deep mesopores of the monolithic column. Capacity factor (k'), plate number (at $w_{1/2}$), and USP tailing factor for baseline resolved peaks were calculated and are listed in Table 1. In summary, under SFC conditions, both HILIC and monolithic columns offered sufficient retention for all tested compounds, including polar basic compounds. The HILIC column offered comparable plate numbers with the monolithic column and more symmetrical peak shapes.

Figure 2: SFC chromatograms at 254 nm obtained under isocratic conditions (10% methanol) for 9 selected compounds. Compounds are listed below. 5. Theophylline; 6. Thymine; 7. Uracil; 8. Adenine; 10. 6-amino-1,3-dimethyluracil; 11. Sulfamethoxazole; 12. Estradiol; 13. Cortisone; and 14. Amcinonide.

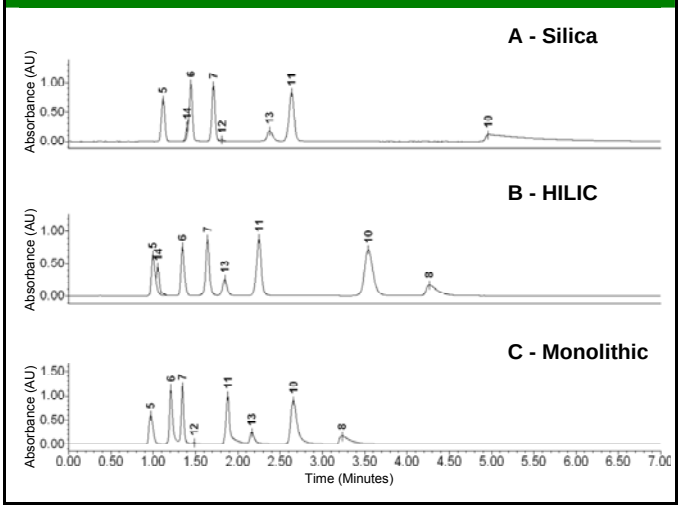


Table I: Selected system suitability parameters. S: Spherisorb® silica; H: XBridge™ HILIC; and M: Onyx™ monolithic.

Peak	Capacity factor (k')			Plate number			USP tailing		
	S	H	M	S	H	M	S	H	M
5	2.22	N/A	1.21	3483	N/A	1876	1.03	N/A	1.35
6	3.17	2.52	N/A	6461	5493	N/A	N/A	1.22	N/A
7	3.93	N/A	N/A	9297	N/A	N/A	N/A	N/A	N/A
8	N/A	10.13	6.35	N/A	5738	3374	N/A	2.28	3.21
9	13.3	8.25	5.04	1015	6262	6749	9.06	1.18	1.56
10	6.60	4.88	3.28	8857	7766	9863	0.98	0.98	1.64
11	5.85	3.82	3.92	6446	6156	9270	1.23	1.10	1.24
13	2.22	N/A	1.21	3483	N/A	1876	1.03	N/A	1.35

Conclusions

A mixture of 14 compounds, including 6 polar basic compounds, were baseline resolved on the HILIC column and partially resolved on the monolithic column, under generic SFC gradient conditions without the addition of additives. The monolithic column displayed a somewhat different selectivity compared to the particle packed and HILIC columns, which can be ascribed to steric hindrance from the analytes. Both XBridge™ HILIC and Onyx™ monolithic silica based columns have great potential for routine use in SFC applications.

References

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