

# EVALUATION OF SUB-2 $\mu$ m SILICA AND HYBRID PARTICLES FOR SFC APPLICATIONS

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## INTRODUCTION

Supercritical Fluid Chromatography (SFC) has recently gained interest for achiral separations, in part due to improvements in instrumentation and software, providing more consistent and robust operating conditions, as well as for increased savings due to decreases in solvent consumption and analysis time. The recent release of the first commercially-available Ultra-Performance SFC (UPSFC) instrument provides the opportunity to make use of smaller particle sizes, smaller columns, and more robust chemistries for SFC applications. These opportunities take advantage of low system dispersion of the UPSFC system which has not been previously available. The greatest realization of these benefits can be observed with SFC particles that are less than 2 $\mu$ m in particle size.

In the development of new stationary phases, the goal of creating materials with orthogonal selectivities can be difficult to assess. Here we present our method of comparing column chemistries using Selectivity Values (S-Values)<sup>1</sup>.

In addition, we demonstrate the ability to scale to smaller column dimensions and smaller particle sizes, resulting in faster analyses.

## METHOD

### Chromatographic Method for Selectivity Evaluation

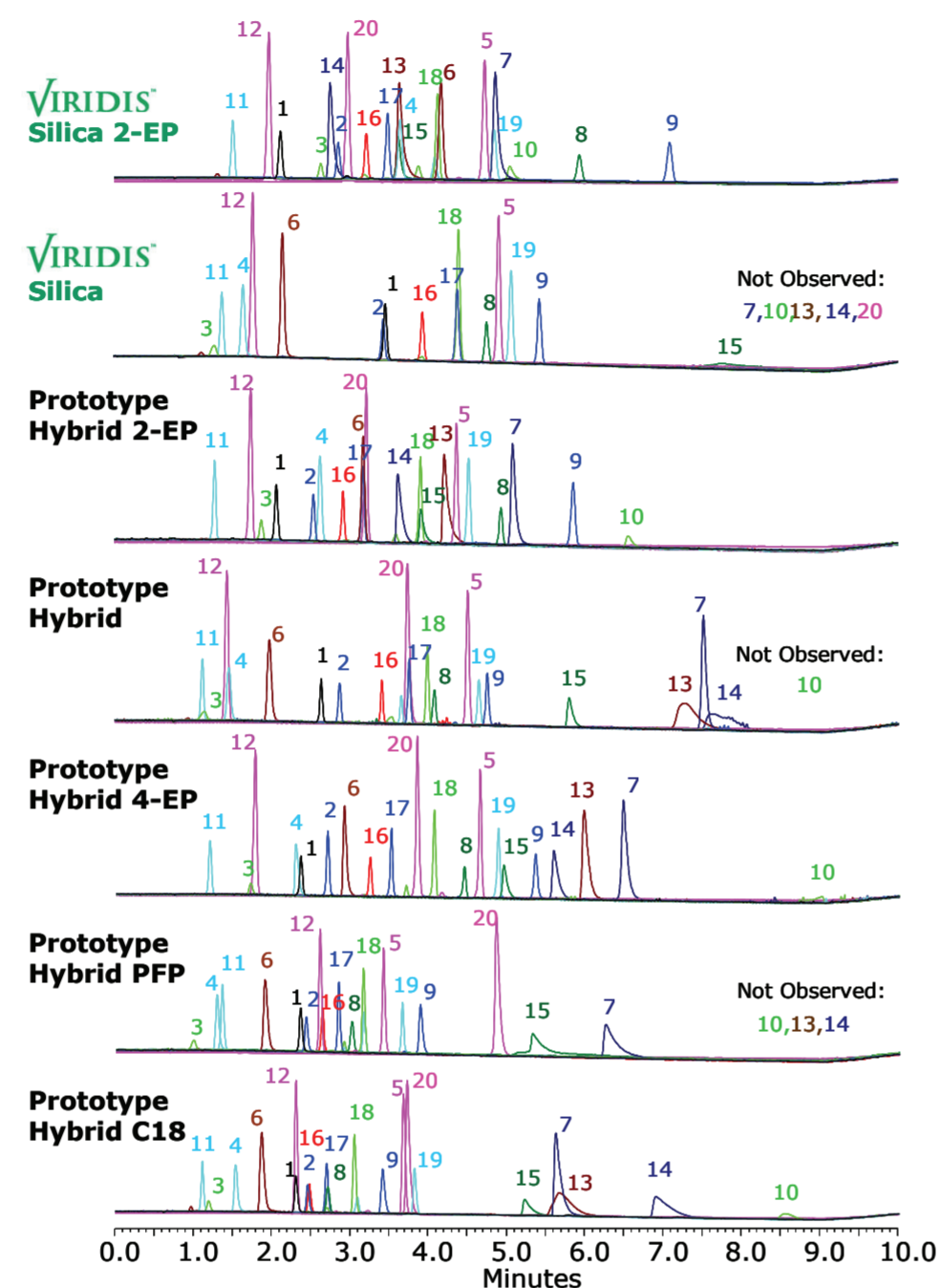
Instrument: Method Station Analytical SFC  
Gradient: 5-40% MeOH in 8 min, return to initial conditions in 1 min, re-equilibrate for 9 min.  
Column Configuration: 5  $\mu$ m, 4.6 x 150 mm  
Flow rate: 3 mL/min  
Pressure: 150 bar  
Temperature: 40 °C  
Injection volume: 3  $\mu$ L  
UV Detection: 235 nm

### Selectivity Sample Mix

- |                     |                   |
|---------------------|-------------------|
| 1. Caffeine         | 11. Coumarin      |
| 2. Theophylline     | 12. Flavone       |
| 3. Ibuprofen        | 13. Propranolol   |
| 4. Fenopropfen      | 14. Amitriptyline |
| 5. Hydrocortisone   | 15. Adenine       |
| 6. Ketoprofen       | 16. Thymine       |
| 7. Cytosine         | 17. Uracil        |
| 8. Sulfamethoxazole | 18. Prednisone    |
| 9. Sulfamethizole   | 19. Prednisolone  |
| 10. Procainamide    | 20. Papaverine    |

Individual analytes each at 0.2 mg/mL in MeOH

## CHROMATOGRAPHY



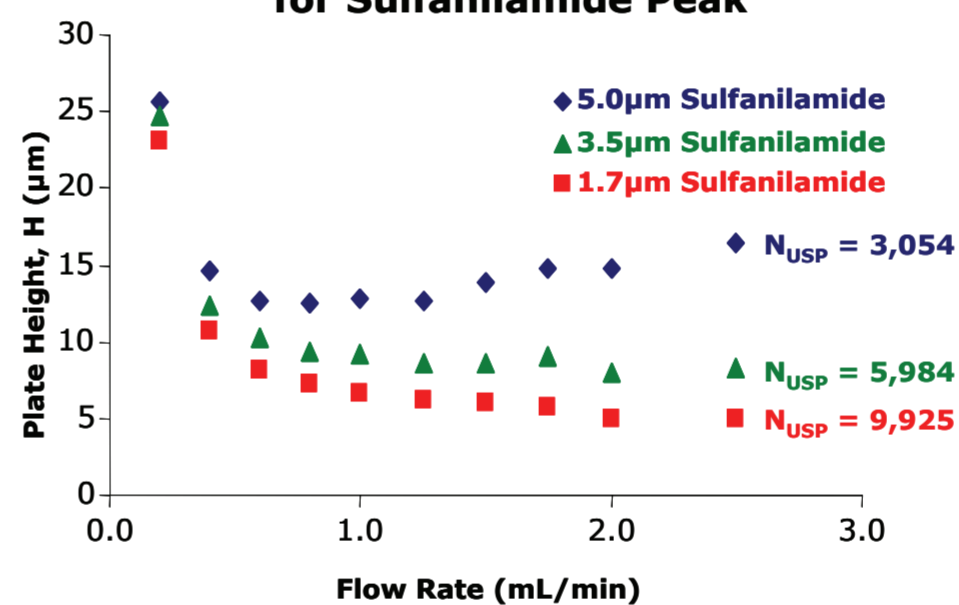
## EVALUATION OF SUB-2 $\mu$ m MATERIALS

### Chromatographic Method for Evaluation of Sub-2 $\mu$ m Materials

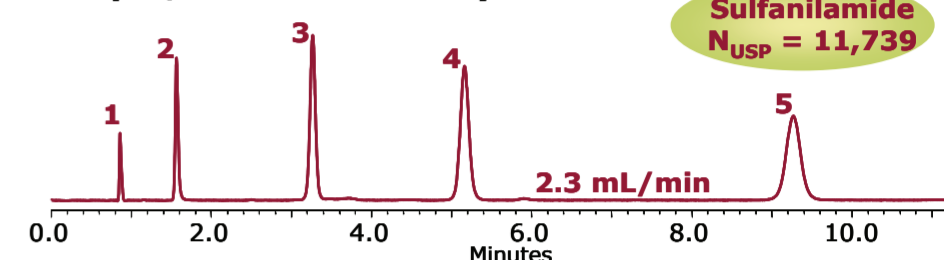
Instrument: Method Station Analytical SFC  
Prototype UPSFC Instrument  
Isocratic: 10% MeOH  
Column Chemistry: Viridis Hybrid  
Flow rate: Varied  
Pressure: 150 bar  
Temperature: 40 °C  
Injection volume: 2.0  $\mu$ L or 0.3  $\mu$ L  
UV Detection: 254 nm

Sample Mix:  
(1) Toluene, (2) Naproxen, (3) Thymine, (4) Prednisone, and (5) Sulfanilamide

### HETP Curve for Sulfanilamide Peak

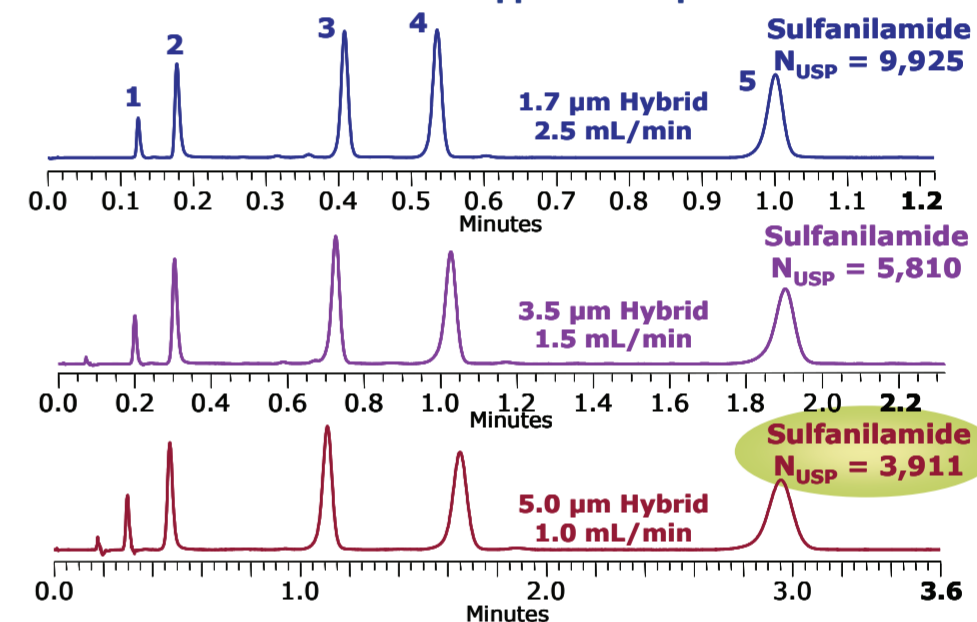


### Method Station Analytical SFC 5.0 $\mu$ m, 4.6 x 150 mm Hybrid

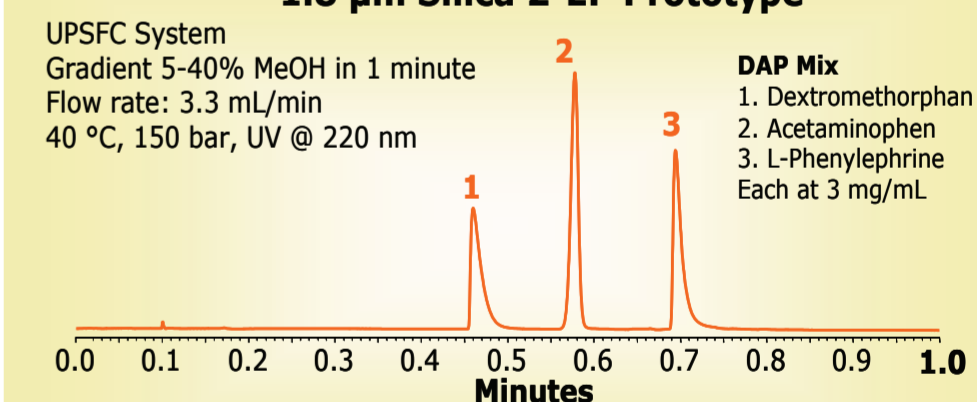


### Prototype UPSFC System 1.7 $\mu$ m, 3.5 $\mu$ m, and 5.0 $\mu$ m, 3.0 x 50 mm Hybrid

Separation scaled based on change in column dimension and approximate particle size



### 1.8 $\mu$ m Silica 2-EP Prototype

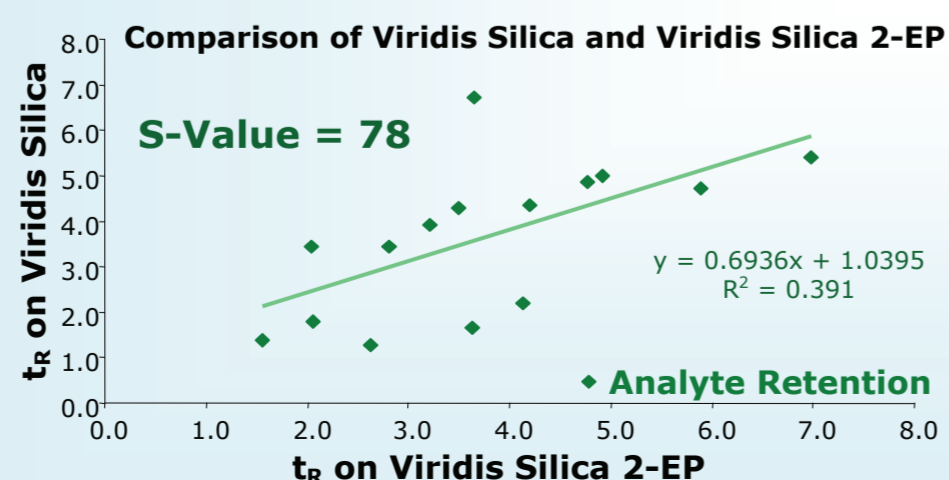


Separation of ingredients from common cold medication on a Prototype 1.8  $\mu$ m Silica 2-EP Column (3.0 x 50 mm)

## ASSESSING SELECTIVITY

### Calculation of Selectivity Values<sup>1</sup>

- The retention times for each compound of the *Selectivity Sample Mix* on one column are used as y-axis data points. The retention times for the same compounds on the comparison column are used as the x-axis data points.
- A linear regression through the x-y data gives the coefficient of determination,  $R^2$  value, that is used to calculate the S-value for a given method between two columns.
- $S = 100 \times \sqrt{1 - R^2}$ , where S is a measure of the selectivity difference between two columns.

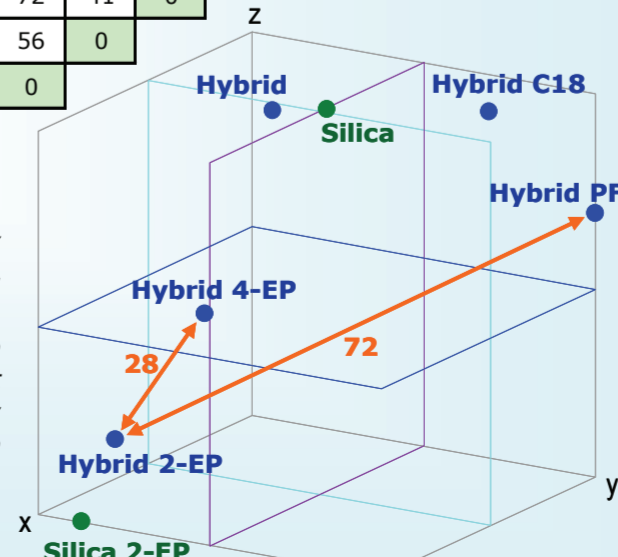


- Because the strong bases were not eluted on all columns under this method, the retention of bases was not used in these calculations of selectivity values
- Using this method, the selectivity of each column may be compared to each of the other columns, generating a matrix of selectivity values:

	Hybrid C18	Hybrid 4-EP	Hybrid 2-EP	Hybrid	Hybrid PFP	Silica	Silica 2-EP
Silica 2-EP	86	51	25	75	86	78	0
Silica	36	39	60	11	38	0	
Hybrid PFP	27	55	72	41	0		
Hybrid	36	33	56	0			
Hybrid 2-EP	72	28	0				
Hybrid 4-EP	52	0					
Hybrid C18	0						

S Value = 0 Indicates Identical Selectivity  
Greater S Value = Greater Selectivity Difference

"Selectivity Space"  
Column chemistries organized spatially, with the distance between each point equal to the selectivity value between those two columns



## CONCLUSION

- The evaluation of S-Values provides a simple method for assessing relative column selectivities, simplifying selection of orthogonal column chemistries
- Transfer of methods to smaller dimension, smaller particle size columns enables faster separations, resulting in savings in time and resources
- Low system dispersion of the UPSFC system permits the use of smaller diameter, shorter length columns resulting in expected efficiency when going from the 5  $\mu$ m, 4.6 x 150 mm to the 5  $\mu$ m, 3.0 x 50 mm column dimension

### Reference

- Uwe Neue, et al., Journal of Chromatography A, 1127 (2006), "Selectivity in reversed-phase separations—Influence of the stationary phase"