Selectivity Characterization of Five Achiral Stationary Phases Using Supercritical Fluid Chromatography and Hydrophilic Interaction Chromatography

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Introduction

Supercritical fluid chromatography (SFC) is widely recognized as a preferred technique for preparative chiral applications. The use of compressed CO_2 as the primary mobile phase provides many benefits: it is readily available, relatively inexpensive, and safe. It can also be recycled, thus leading to SFC's designation as a "green" technology. Additionally, the viscosities of compressed CO_2 and mixtures with polar modifiers are much lower than those of aqueous mixtures. This allows for chromatographic run times, which are approximately one-third to one-fifth as long as typical HPLC runs. These advantages are clearly applicable to a broad range of separations, beyond just chiral separations. Five achiral SFC stationary phases (2-ethyl pyridine, 4-ethyl pyridine, propyl-amine, polyethylenimine, and arginine) are described with respect to the selectivity characteristics observed in SFC analyses of acidic, basic, and neutral compounds. These results are compared to the selectivities observed when using these same stationary phases in hydrophilic interaction (HILIC) mode.

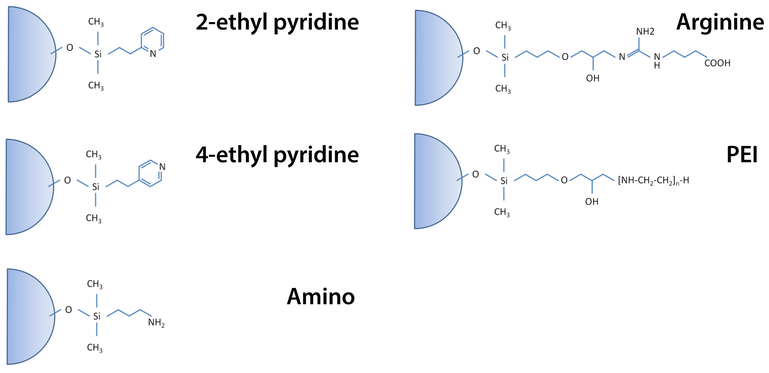

Figure 1: Achiral stationary phases tested in this study

Experimental

Each of the stationary phases shown in Figure 1 was tested in gradient SFC mode using the acidic, basic, and neutral probes shown in Figure 2. Column orthogonalities were characterized by the r^2 values of each phase pair for acidic/neutral and basic compound sets. Columns were also tested in HPLC mode, and retention profiles were characterized across a range of organic modifier content. All analyses were performed using a Shimadzu Nexera SFC/UHPLC system.

Column	250 mm x 4.6 mm; 5 µm		
Mobile phase A:	Carbon dioxide		
Mobile phase B:	Methanol		
Gradient:	Time (min.)	Flow (mL/min)	%B
	0.00	3.0	5
	10.00	3.0	40
	20.00	3.0	40
Oven Temp.:	30°C		
Pressure:	150 bar		
Detection:	212 nm		

Table 1: Gradient SFC method conditions

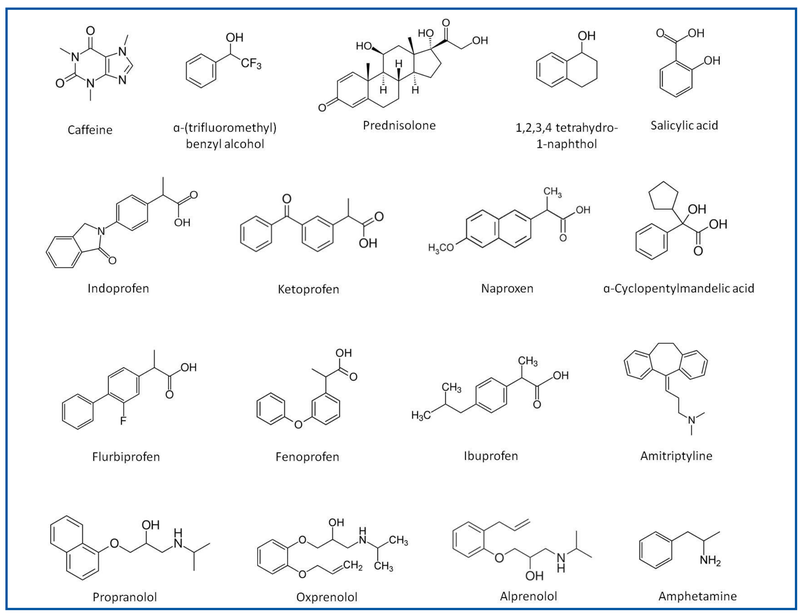

Figure 2: Structures of acidic, basic, and neutral probes

Results

Selectivity of Acidic and Neutral Compounds in Gradient SFC

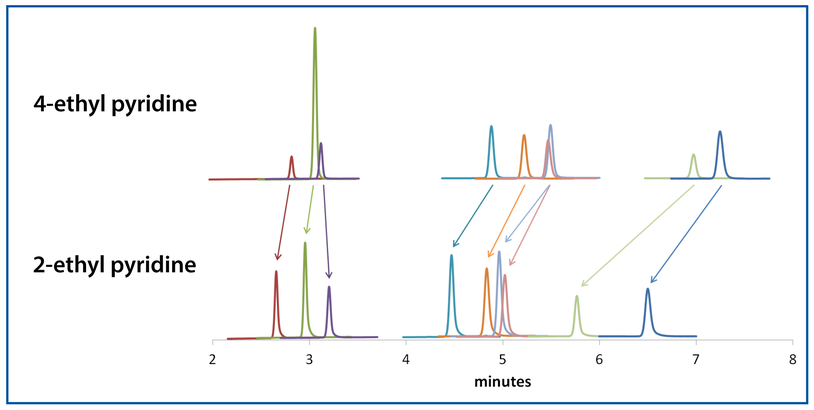

Figure 3: Acidic and neutral probes on 2EP and 4EP

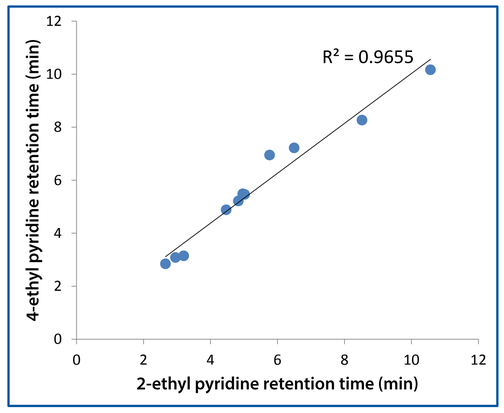

Figure 4: Retention times of acidic and neutral probes on 2EP and 4EP

Orthogonality between all combinations of phases was numerically characterized by obtaining the r^2 values of linear regression fits for the retention times on the two different phases. The lower the r^2 value, the greater the orthogonality. Acidic and neutral probes on 2EP and 4EP showed very similar elution orders with only minor shifts in retention time. r^2 = 0.966

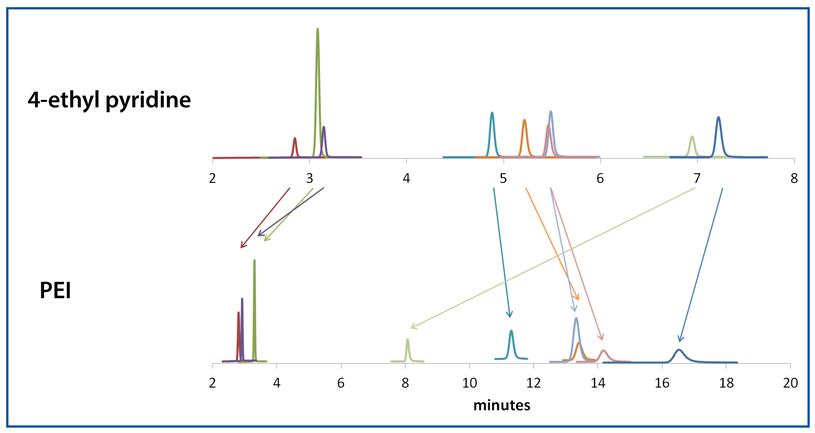

Figure 5: Acidic and neutral probes on 4EP and PEI

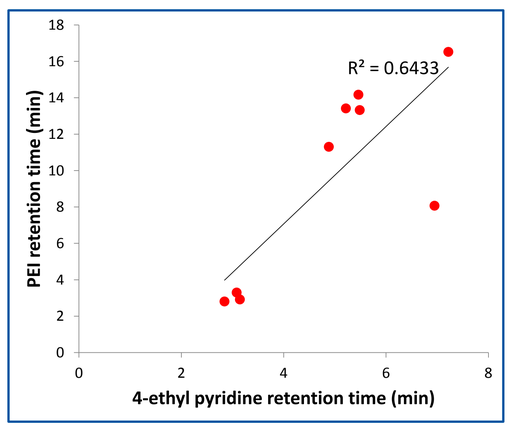

Figure 6: Retention times of acidic and neutral probes on 4EP and PEI

For acidic and neutral test probes, the r^2 value for the PEI/4EP combination is much lower than the 2EP/4EP combination, suggesting higher orthogonality. Figure 5 illustrates the differences in selectivity with a number of elution order changes as well as significant shifts in absolute retention times for acids. The selectivity of PEI is similar to a traditional aminopropyl phase, but more retentive of acids.

r^2 values for the linear regressions of the retention times for all combinations of the five phases using the conditions listed in Table 1 were calculated. Values, independently determined for acidic/neutral and basic probes, are shown in Tables 2 and 3.

Results and Discussion

Acids and Neutrals

	2EP	4EP	Amino	ARG	PEI
2EP	1	0.966	0.737	0.823	0.728
4EP	0.966	1	0.657	0.746	0.643
Amino	0.737	0.657	1	0.986	0.991
ARG	0.823	0.746	0.986	1	0.978
PEI	0.728	0.643	0.991	0.978	1

Table 2: r^2 values based on retention of acidic and neutral probes in SFC mode

Bases

	2EP	4EP	Amino	ARG	PEI
2EP	1	0.302	0.193	0.568	0.386
4EP	0.302	1	0.96	0.889	0.967
Amino	0.193	0.96	1	0.81	0.945
ARG	0.568	0.889	0.81	1	0.906
PEI	0.386	0.967	0.945	0.906	1

Table 3: r^2 values based on retention of basic probes in SFC mode

Peak Shape of Basic Probes in Isocratic SFC Mode with and without Additive

It is often necessary to add a co-solvent modifier (acid, base, or salt) to affect the selectivity of the separation or improve peak shape, especially for bases. Figure 7 shows the peak shape of alprenolol, oxprenolol, amphetamine, propranolol, and amitriptyline on 4EP and aminopropyl phases. Peak shape is greatly improved by including 0.2% diethylamine in the co-solvent, methanol, when using 4EP. Peak shape of bases is good on the aminopropyl phase, even without the addition of DEA.

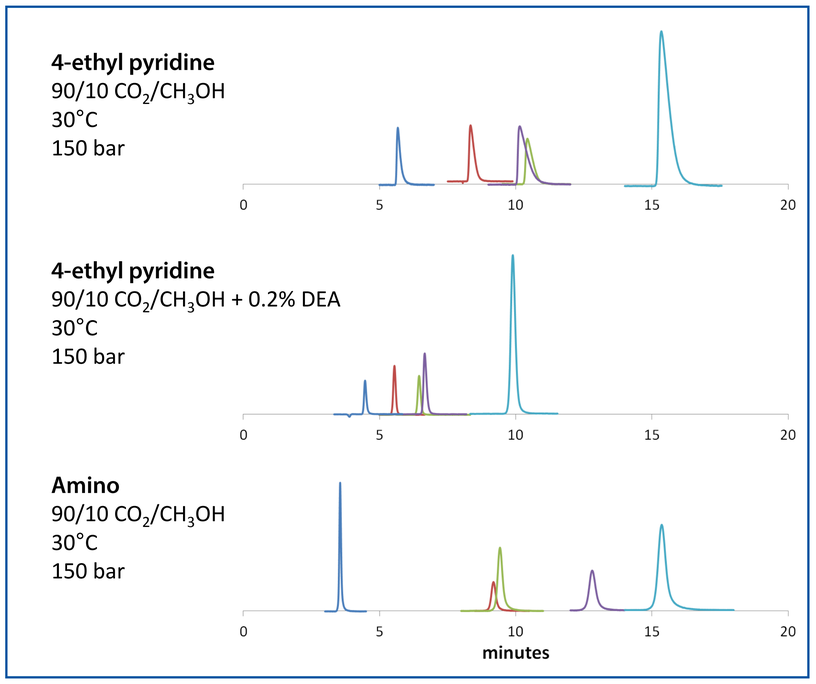

Figure 7: Peak shape of basic analytes in isocratic SFC mode with and without 0.2% DEA

Retention Profile of NSAIDs on PEI with H_2O/ACN + 10 mM Ammonium Acetate

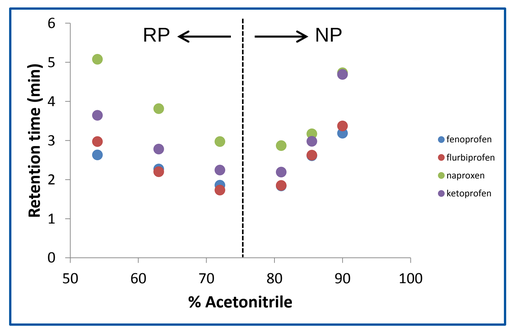

Figure 7: Retention of NSAIDs vs. % organic modifier

The U-shaped retention profiles of the NSAIDs with carboxylic acid moieties on the amine-based phases indicates a normal-phase, ion-exchange mechanism which can be affected by buffer concentration. As shown in Figure 7, below approximately 75% ACN, the reversed-phase mechanism dominates with the PEI phase.

Conclusions

A range of acidic, basic, and neutral probes were screened in gradient SFC mode. Retention changes amongst the classes reveals the dominant separation mechanisms and column orthogonalities which may aid in method development. Peak shape of primary, secondary, and tertiary amines can be affected through use of a co-solvent modifier. With the amine-based phases, aminopropyl and PEI, peak shape of basic analytes is good even without the addition of 0.2% DEA.