STATIONARY PHASE SELECTIVITY IN HILIC: THE IMPORTANCE OF IONIC INTERACTIONS

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INTRODUCTION

Hydrophilic interaction chromatography (HILIC) is a separation method that utilizes a polar stationary phase and a less polar mobile phase which typically contains a mixture of acetonitrile and an aqueous buffer [1, 2]. It provides greater retention of polar analytes than RP chromatography, and is often chosen when traditional reversed-phase chromatography does not display the ability to separate highly polar or ionic compounds from the column void volume.

The primary retention mechanism in HILIC is believed to be the partitioning of analytes into the water-rich layer that forms at the stationary phase surface [1, 2, 4–8]. Studies have shown that electrostatic and hydrogen bonding interactions [9–12] are contributing secondary interactions.

To expand on these prior studies, we evaluated 19 commercially available HILIC columns, including unbonded silica, cyano, diol, pentahydroxy, urea, amide, zwitterionic, and mixed-mode chemistries on either silica or BEH™ Hybrid Organic/Inorganic Particles. A number of these columns were not included in previous comparisons of column selectivity. Seventy-seven analytes were used, including acidic, basic, zwitterionic, and neutral species. The goal of this study was to investigate the contribution of electrostatic interactions to HILIC separation selectivity.

METHODS

The evaluation was performed using an ACQUITY™ UPLC™ H-Class Series System configured with a quaternary solvent manager, a sample manager with a flow-through needle, a column manager with column auxiliary compartment, both utilizing active pre-heaters. Detection was made using an ACQUITY UPLC Photodiode Array Detector, and either a Waters ACQUITY QDa™ Detector or a Waters Xevo™ TQD Mass Spectrometer. Column temperature was maintained at 30°C. Column configuration was 2.1 x 150 mm and particle size varied from 2 to 5 µm.

For each experiment, the following steps were used.

- Equilibrate column using starting conditions of 95% Mobile phase B and 5% mobile phase D for 20.6 min at a flow rate of 0.5 mL/min, ~ 30 column volumes (Cv)
- Make a zero volume injection using the gradient table below to condition the column and ensure adequate post-gradient equilibration. The mobile phases were A = 100% Milli-Q™ Water, B = 100% Acetonitrile LC/MS grade, D = 200 mM Ammonium Acetate pH 5.0. All compositional changes were made using a linear gradient at a flow rate of 0.5 mL/minute.
- The total run time of 18 minutes allowed for an equilibration of 10 minutes or 14.6 Cv, a sufficient equilibration according to a recent study [3].

Gradient Table				
Time	%A	%B	%C	%D
Initial	0.0	95.0	0.0	5.0
2.00	0.0	95.0	0.0	5.0
6.00	45.0	50.0	0.0	5.0
8.00	45.0	50.0	0.0	5.0
8.01	0.0	95.0	0.0	5.0

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RESULTS AND DISCUSSION

Separation selectivity was determined for each of the 19 HILIC columns evaluated in this study. Retention correlation plots were constructed and the Selectivity Factor (s) was determined. A panel of 77 polar analytes consisting of cations, anions, and 0 charge analytes were used for this determination. The analytes were separated using the described HILIC gradient conditions and the retention times were recorded. Selectivity factors (s) were calculated using equation (1), where r^2 represents the correlation coefficient for the retention times of a set of analytes on two different columns. In the comparison of a CORTECS™ HILIC Column to an Accucore™ HILIC Column shown in Figure 1, a high correlation of the retention times was observed and a low selectivity factor (s) of 12 was calculated indicating similar separation selectivity. As the r^2 values decrease, the s values increase to a maximum of 100, which would signify that the columns have highly different selectivity. S values were determined for column pairs and the data was tabulated in Figure 2.

$$s = 100 \times \sqrt{1 - r^2} \qquad (1)$$

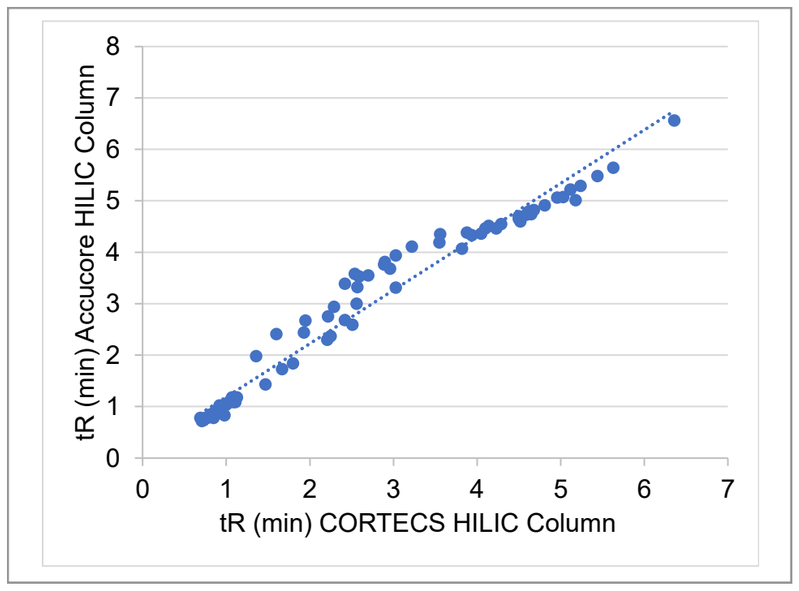

Figure 1. Retention Time Correlation Plot for Polar Analyte Panel using the Column Pair of CORTECS HILIC Column and Accucore HILIC Column.

In Figure 2, the columns were grouped according to the type of retention activity present in addition to HILIC retention. The cyano bonded, bare silica, or hybrid and mixed-mode HILIC-1 columns are listed first (A-G). In addition to HILIC retention, these stationary phases exhibit cation-exchange activity due to acidic silanols present on the surface of these materials. The second group are zwitterionic columns (H-K). The stationary phases in these columns have been bonded with a 1:1 molar ratio of positively and negatively charged functional groups. The expectation is that these stationary phases should be net neutral, however, they have been reported to exhibit weak ion-exchange retention behavior. The third grouping of HILIC columns (L-Q) were prepared with stationary phases that contained neutral functional groups. These groups include amide, diol, pentahydroxy, or urea. HILIC columns in this grouping exhibited lower ionic retention. The final two columns (R, S) contain stationary phases which have amine groups. These columns exhibit anion-exchange activity in addition to HILIC retention.

Columns packed with similar sorbent chemistries give similar separation selectivities. For the group of silica columns, Ascentis™ Si, Accucore HILIC, and CORTECS HILIC Columns, the s values are low, < 30, (shaded in red). While low s values are indicative of columns with similar selectivity, high s values tend to indicate dissimilar separation selectivities. The highest s values were observed for columns with opposite ion-exchange activities.

Selectivity factors were determined using the full complement of 77 polar analytes regardless of their charge state. One observation was that column pairs can present very similar s values and not have the same selectivity, as was observed in a comparison of CORTECS HILIC and Torus DEA Columns *vs* an Ascentis OH5 Column.

To further investigate the contribution of electrostatic interactions to retention, the analytes were classified as cationic (blue diamonds), zero charge (green triangles), and anionic (red squares). Clear patterns in the behaviors of these three groups of analytes were observed in many of the plots. For example, it can be seen in Figure 3 that the cations are strongly retained, and the anions are weakly retained on the CORTECS HILIC Column. The reverse is true for the Torus DEA Column, the anions are strongly retained, and the cations are weakly retained.

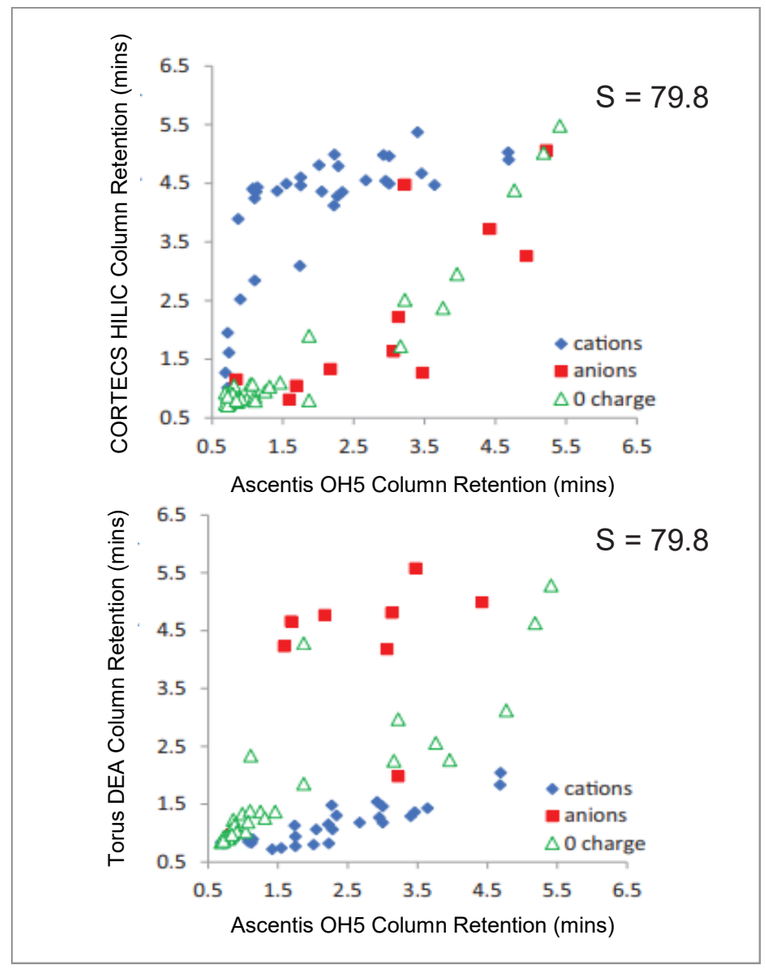

Figure 3. Selectivity Differences for Ionizable Analytes using CORTECS HILIC and TORUS DEA Columns.

CONCLUSIONS

- Electrostatic interactions were most prominently observed for unbonded silica, hybrid materials, cyano bonded silicas and amine containing stationary phases. The greatest selectivity differences were observed when comparing columns with cation exchange activity to those with anion exchange activity (s ranging from 93 to 100).
- Comparisons of columns with neutral chemistries (amide, diol, pentahydroxy, and urea) to those with ion-exchange activity gave reduced selectivity differences (s between 56 and 99).
- Comparisons among zwitterionic columns showed moderate selectivity differences, with s ranging from 35 to 79.
- For more information, see J. Sep. Sci. 2022, 45: 3264.

			A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
A	Kromasil™ Cyano	Column																			
B	HSS Cyano	Column	16.4																		
C	Acclaim™ MM	Column	41.5	37.5																	
D	Ascentis™ Si	Column	61.6	60.8	55.5																
E	Accucore HILIC	Column	58.1	57.1	50.6	17.5															
F	CORTECS HILIC	Column	59.7	58.4	48.9	26.5	12.0														
G	BEH HILIC	Column	61.0	58.3	38.9	48.0	41.1	35.1													
H	Obelisc N	Column	71.8	71.1	62.1	38.6	33.7	31.7	43.9												
I	ZIC™-HILIC	Column	76.1	75.7	73.0	57.2	52.3	51.8	60.4	44.5											
J	ZIC-cHILIC	Column	97.9	97.8	93.7	87.5	85.5	83.6	81.1	71.9	62.3										
K	BEH Z-HILIC	Column	99.3	99.1	96.2	92.2	90.6	88.4	84.1	79.0	71.7	35.9									
L	AdvanceBio™ MS	Column	98.0	97.0	92.0	88.5	86.1	82.5	75.5	73.4	74.6	50.8	40.8								
M	Acclaim HILIC	Column	87.8	85.2	72.6	71.3	67.7	62.3	47.2	55.3	66.8	62.9	64.3	45.5							
N	Accucore Urea	Column	91.8	89.4	79.0	77.1	73.3	67.9	56.6	61.6	71.5	64.3	62.2	39.6	24.4						
O	BEH Amide	Column	95.3	94.0	86.5	81.9	79.6	75.5	65.6	67.7	69.6	55.8	48.6	31.3	38.3	37.7					
P	Ascentis OH5	Column	97.6	96.3	89.0	86.2	83.9	79.8	71.1	72.2	76.4	58.7	54.0	25.1	36.9	32.9	29.3				
Q	Torus™ Diol	Column	98.7	97.3	92.0	89.5	87.7	84.4	77.5	77.9	81.7	67.0	61.3	30.4	48.8	39.1	41.3	27.9			
R	Torus 2-PIC	Column	99.5	100	98.9	98.4	97.9	96.4	93.2	93.0	92.6	76.3	71.9	53.1	72.7	67.4	67.8	53.9	41.2		
S	Torus DEA	Column	96.4	97.3	99.5	99.5	99.7	100	99.8	99.8	99.2	89.2	87.2	79.2	91.7	89.3	90.3	79.8	76.5	44.2	

Cation-exchange activity

Zwitterionic

Neutral

Anion-exchange activity

Figure 2. Selectivity values (s) for pairwise comparisons of the 19 columns evaluated. The cells are shaded to indicate the magnitude of the s values, ranging from red for the smallest values to green for the largest.

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