INVESTIGATION OF SELECTIVITY FOR A NEW FAMILY OF REVERSED-PHASE HPLC COLUMNS

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INTRODUCTION

Traditionally in method development the analyst needs to select the pH, organic solvent, temperature, and column that provide the best separation. The bonded phase chosen most often is a C_{18}. There are times when an adequate separation is not achieved using a C_{18} column. A number of reversed-phase columns offering different selectivity to C_{18} columns are available, but most suffer from problems with poor batch-to-batch reproducibility, poor peak shape, poor hydrolytic stability, and/or severe column bleed. We have developed a new family of reversed-phase columns that does not suffer from these problems. The family includes three bonded phase that were developed to offer selectivity differences needed to solve tough separation challenges. We will show the selectivity differences within this new family of bonded phases using sample mixes of acidic, basic, and neutral analytes. The results will be compared to other existing families of columns.

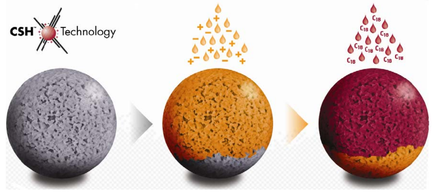

Figure 1: Schematic depiction of the CSH™ Technology process. Starting with an unbonded BEH particle [left], a small controlled charge is applied to the BEH particle surface [middle]. The CSH particle is then bonded and sometimes end capped [right]. CSH Technology is incorporated into the ACQUITY CSH™ and XSelect™ column families.

INSTRUMENT / COLUMNS

LC System: ACQUITY UPLC® system with PDA Detector and a Column Manager

Columns: All columns were 3.5µm materials, and evaluated in 2.1 x 50 mm configurations.

METHODOLOGY

Snyder-Dolan Classification Scheme

One method in measuring selectivity is the Snyder-Dolan (S-D) classification scheme.[1] which is based on the Hydrophobic-Subtraction Model. The S-D classification scheme entails a quantitative description of column selectivity which allows the chromatographer to select columns with either equivalent or a different selectivity, and is based on the following equation.

$$\text{Log}\,\alpha = k'/k'_{EB} = \eta' H - \sigma' S^* + \beta' A + \alpha' B + \kappa' C$$

k′ is the retention factor of a given solute, and k'_{EB} is the retention factor of the non-polar solute ethyl benzene evaluated on the same column under the same conditions. The relative retention factor, α, is related to five terms which represent the five predomominant solute-column interactions. The column properties are denoted as H, S*, A, B, and C where H refers to the hydrophobicity, S* refers to steric resistance, A refers to hydrogen-bond acidity, B refers to hydrogen-bond basicity, and C refers to cation-exhange activity. The corresponding complementary solute properties are denoted as η′, σ′, β′, α′, κ′.

The advantage of the S-D classification scheme is that a chromatographer can calculate a single parameter called the column selectivity function (F_s) which can be used to quantitatively compare the overall selectivity of any two columns. The column selectivity function is defined below

$$F_s = \{[12.5(H_2 - H_1)]^2 + [100(S^*_2 - S^*_1)]^2 + [30(A_2 - A_1)]^2 + [143(B_2 - B_1)]^2 + [83(C_2 - C_1)]^2\}^{0.5}$$

Each term of the equation is weighed differently due to the column parameters that vary in their relative contribution to the overall selectivity. The smaller the F_s value the more similar the two columns are. Conversely, columns that have large F_s values are more different in terms of selectivity.

Snyder-Dolan Evaluations:
Injection volume: 1.0 µL
Flow Rate: 210 µL/minute
Mobile Phase: Acetonitrile/60 mM Potassium Phosphate Buffer pH 2.8 (50/50)
Week Needle Wash: 100% Water
Strong Needle Wash: Acetonitrile/Water (20/80)
Seal Wash: Acetonitrile/Water (20/80)
Column Temperature: 35 ºC
Detection: 254 nm

The Snyder-Dolan evaluations use seventeen solutes[2] that were prepared in the mobile phase at various concentrations to match peak heights.

Selectivity Value Method

A second way to quantitatively determine the selectivity differences is by using the selectivity value method.[4] The selectivity value method is performed by plotting the retention factors for analytes on column pairs. Both columns have to be evaluated under identical test conditions.

A linear regression gives a correlation coefficient close to one for columns that have essentially the same selectivity; correlation coefficients that are close to zero shows columns that have orthogonal selectivity.

A measure of the selectivity differences uses the square of the correlation coefficient (R^2) to calculate the selectivity distance (S) between column pairs. Like the F_s Values, the smaller the S-value the more similar the two columns are; the larger the S-value the more different the two columns are.

$$S - Value = 100\sqrt{1 - R^2}$$

Selectivity Value Gradient Evaluations:
Injection volume: 5.0 µL
Flow Rate: 500 µL/minute
Mobile Phase A: 15.4 mM Ammonium Formate Buffer pH 3.0
Mobile Phase B: Acetonitrile
Gradient Conditions: 5% B to 90% B in 5 minutes using a linear gradient; 0.5 minute hold at 90% B; re-equilibrate in 5%B for 2 minutes.
Week Needle Wash: Acetonitrile/Water (10/90)
Strong Needle Wash: Acetonitrile/Water (50/50)
Seal Wash: Acetonitrile/Water (20/80)
Column Temperature: 30 ºC
Detection: 254 nm

Solutes [µg/mL]: prepared in 10% Acetonitrile/90% Buffer: 2-nitrobenzoic acid [5], 2-chlorobenzoic acid [125], pyrenesulfonic acid [18.75], fenoprofen [100] metoprolol [100], papaverine [2], propranolol [20], amitriptyline [10], resorcinol [50], 2-nitrobenzyl alcohol [10], 2-Chlorophenol [100], fluoxetine [500], thiourea [5] caffeine [10], diethylphthalate [25] dipropylphthalate [25] 2-nitrophenol [25] 3-nitrophenol [25] 4-nitrophenol [25]

DISCUSSION

Four column families: XSelect, XBridge™, XTerra® and High Strength Silica (HSS) columns were evaluated under the S-D classification scheme and the selectivity value method to characterize and compare their selectivity. All columns were compared to an XBridge C_{18} 3.5 µm column. Table 1 has the columns evaluated.

Table 1	End Capped	Hydrophobicity H	Steric resistance S*	Hydrogen-bond acidity A	Hydrogen-bond basicity B	Cation exchange capacity C	F_s value to XBr. C_{18}	S-Value to XBr. C_{18}
XBridge (XBr.)								
C_{18}	Yes	0.990	0.033	0.023	0.003	0.173	0.0	0.0
Phenyl	Yes	0.716	-0.073	-0.235	0.034	0.102	15.4	10.3
Shield RP18	Yes	0.828	-0.024	-0.209	0.092	-0.111	28.3	18.1
XTerra								
MS C_{18}	Yes	0.962	0.006	-0.002	-0.002	0.129	4.6	4.9
Phenyl	Yes	0.675	-0.065	-0.228	0.007	0.102	14.2	10.0
RP18	Yes	0.788	-0.038	-0.256	0.099	-0.249	39.2	19.0
HSS								
C_{18}	Yes	1.032	0.038	0.072	-0.014	0.112	5.8	8.7
T3	Yes	0.963	-0.024	-0.023	0.002	0.108	8.0	10.2
C_{18} SB	No	0.741	-0.119	0.416	0.027	0.357	25.0	19.6
XSelect CSH								
C_{18}	**Yes**	**0.950**	**-0.006**	**0.004**	**0.137**	**0.140**	**19.8**	**10.6**
Phenyl-Hexyl	**Yes**	**0.532**	**-0.014**	**-0.646**	**0.108**	**-0.331**	**49.3**	**19.7**
Fluoro-Phenyl	**No**	**0.363**	**-0.102**	**-0.318**	**0.037**	**-0.555**	**63.4**	**36.4**

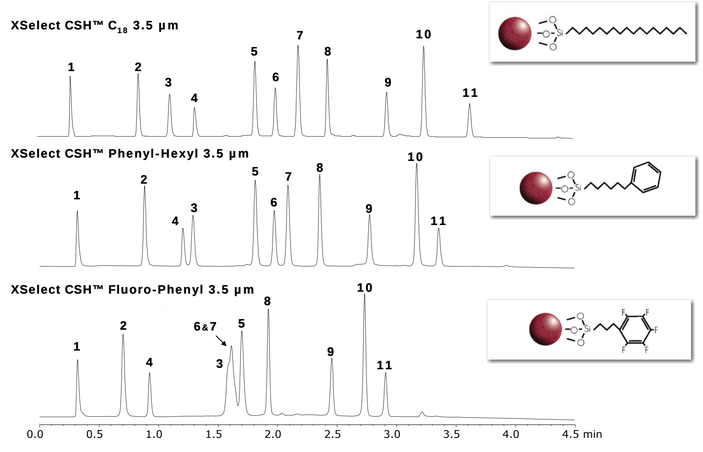

Figure 2: Selectivity value gradient separations on 2.1 x 50 mm columns, Analytes: (1) thiourea; (2) resorcinol; (3) 2-nitrobenzoic acid; (4) metoprolol; (5) 2-chlorobenzoic acid; (6) 3-nitrophenol; (7) 2-nitrophenol; (8) amitriptyline; (9) diethylphthalate; (10) fenoprofen; (11) dipropylphthalate.

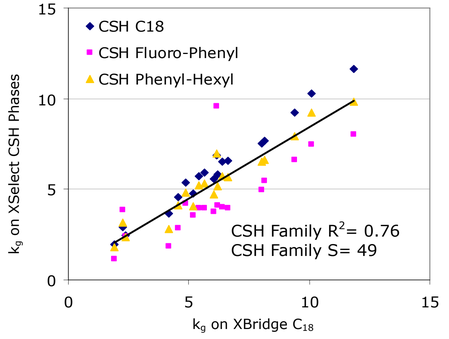

Figure 3: Selectivity Value gradient retention factors (k_g) for the XSelect CSH Family of columns versus an XBridge C_{18} column. The greater the scatter around the regression line, the greater the selectivity difference.

CONCLUSIONS

- With a need for different separation materials, the XSelect family of HPLC columns offers the chromatographer a wide range of selectivity over these currently available families of columns.
- Both the Snyder-Dolan classification scheme and the selectivity value method produce similar results. There is a strong correlation, R^2= 0.866, between the F_s values and the S values.
- All ACQUITY CSH and XSelect CSH materials provide excellent peak shape (even in low ionic strength acidic mobile phases), good hydrolytic stability, and excellent batch to batch reproducibility.[3]

References

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