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ALTERNATE SELECTIVITY FOR BASES IN ULTRAPERFORMANCE LIQUID CHROMATOGRAPHY USING A NON-ENDCAPPED HIGH STRENGTH SILICA STATIONARY PHASE

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A non-endcapped C_{18} stationary phase bonded to a 1.8 µm high strength silica (HSS) particle substrate was developed to provide different selectivities in UltraPerformance liquid chromatography (UPLC technology) separations. ACQUITY UPLC HSS C_{18} SB (Selectivity for Bases) columns provide alternate selectivity for bases under acidic conditions and provide an additional UPLC method development tool.

INTRODUCTION

Altering selectivity plays a major role in maximizing resolution in chromatographic separations. UPLC technology improves resolution through the use of sub-2 µm particle-packed columns in a chromatographic system designed specifically for operation at the optimal linear velocities (and resulting pressures) for these particles. Combining multiple UPLC particle substrate technologies with alternate chemistries for different selectivity is a powerful tool for methods development scientists.

Waters ACQUITY UPLC HSS columns contain the only 100% silica particles designed, tested and intended for use in applications up to 15000 psi (1000 bar). The most recent addition to this family of chemistries is the ACQUITY UPLC HSS C18 SB column, which is designed to provide different selectivity for basic compounds when compared to traditional high coverage, fully endcapped C_{18} chemistries. This is because of the increased silanol activity on the silica particle surface when bonded at intermediate ligand densities with no endcapping. Example separations were developed for a mixture of basic drugs and some tricyclic antidepressants. A comparison with other sub-2 µm chemistries is also shown.

EXPERIMENTAL CONDITIONS

ACQUITY UPLC Conditions

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System:	Waters ACQUITY UPLC System with PDA detector
Columns:	Indicated on figures; all columns tested in the 2.1 50 mm format
Mobile Phase A:	10 mM NH_4COOH, pH 3.0
Mobile Phase B:	MeOH (basic drug mixture) OR ACN (tricyclic antidepressants)
Gradient:	Indicated in figure captions
Flow-rate:	0.4 mL/min (basic drug mixture) OR 0.5 mL/min (tricyclic antidepressants)
Injection:	1 µL
Temperature:	30 °C
Detection:	Indicated in figure captions
Sampling rate:	40 Hz
Samples:	All compounds were prepared in water at a concentration of 10–60 µg/mL

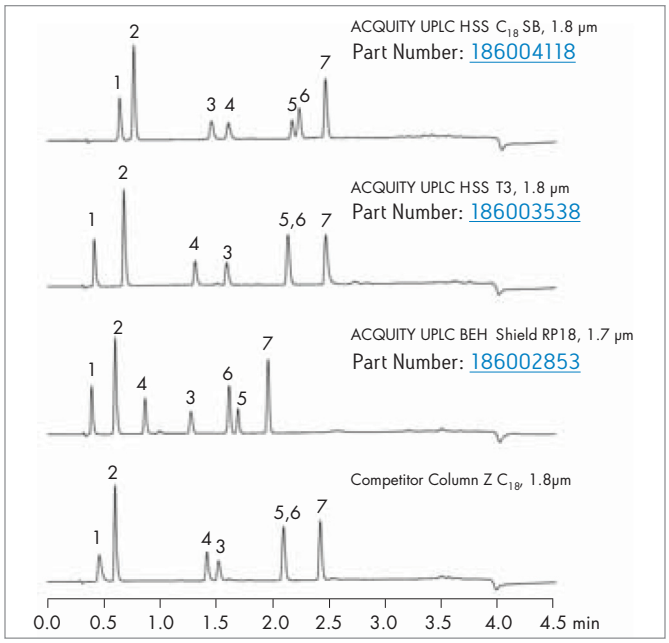

Figure 1: UPLC separation of seven basic drugs. All columns were in the 2.1 X 50 mm format. Gradient from 30–85% B in 3 min, hold at 85% B for 0.5 min, reset. UV 260 nm. Compounds: 1= aminopyrazine, 2= pindolol, 3 = labetalol, 4= quinine, 5= verapamil, 6= diltiazem, 7= amitriptyline.

RESULTS AND DISCUSSION

Figure 1 shows the separation of seven basic drug compounds on four different sub-2 µm chemistries. Note the differences in selectivity between the four columns. This is best seen with the labetalol and quinine peaks (switch in elution order on the HSS C_{18} SB column) and with verapamil and diltiazem, which are almost baseline resolved on the HSS C_{18} SB chemistry. Second, the HSS C_{18} SB column provides more retention for some extremely polar bases like aminopyrazine and pindolol. This is further illustrated in the isocratic separation of tricyclic antidepressants (Figure 2). The HSS C_{18} SB column retains these compounds much longer than the fully endcapped C_{18} stationary phase. In addition, it provides alternate selectivity and better resolves all compounds when compared to a competing non-endcapped C_{18} column.

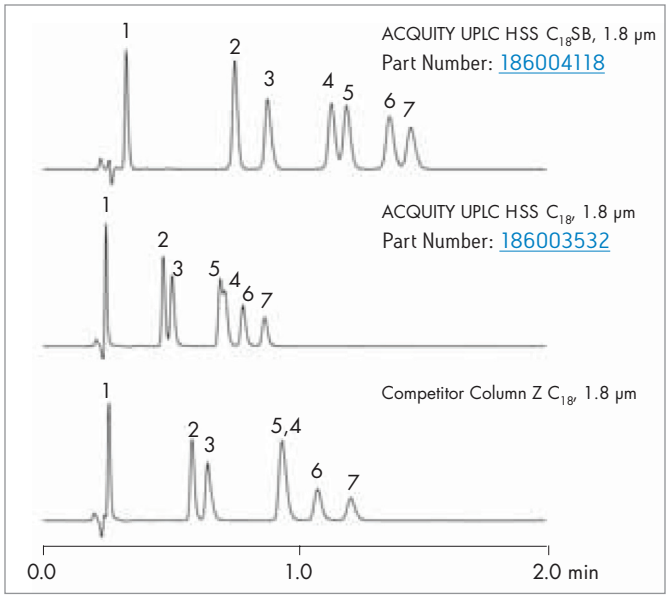

Figure 2: UPLC separation of tricyclic antidepressants. All columns were in the 2.1 X 50 mm format. Isocratic separation at 40% B. UV 254 nm. Compounds 1= trimethoprim, 2= nordoxepin, 3= doxepin, 4= nortriptyline, 5= imipramine, 6= amitriptyline, 7= trimipramine.

CONCLUSIONS

As a result of the increase in surface silanol interactions, the ACQUITY UPLC HSS C18 SB column provides different selectivity under acidic conditions, especially for bases. When combined with the increased speed, sensitivity, and resolution of UPLC technology, this additional bonded phase provides another powerful tool for rapid and robust method development.

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