## Waters™

Application Note

Alternate Selectivity for Bases in UltraPerformance Liquid Chromatography Using a Non-Endcapped High Strength Silica Stationary Phase

Erin E. Chambers, Diane M. Diehl

Waters Corporation



**Abstract** 

A non-endcapped  $C_{18}$  stationary phase bonded to a 1.8  $\mu m$  high strength silica (HSS) particle substrate was

developed to provide different selectivities in UltraPerformance liquid chromatography (UPLC technology)

separations. ACQUITY UPLC HSS C<sub>18</sub> 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 C<sub>18</sub> SB Column, which is designed to provide different selectivity for basic compounds

when compared to traditional high coverage, fully endcapped C<sub>18</sub> 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

**Experimental Conditions** 

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 <sub>4</sub> COOH, 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

## Results and Discussion

Sampling rate:

Samples:

Figure 1 shows the separation of seven basic drug compounds on four different sub-2  $\mu$ m 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

40 Hz

All compounds were prepared in water at a

concentration of 10-60 µg/mL

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.

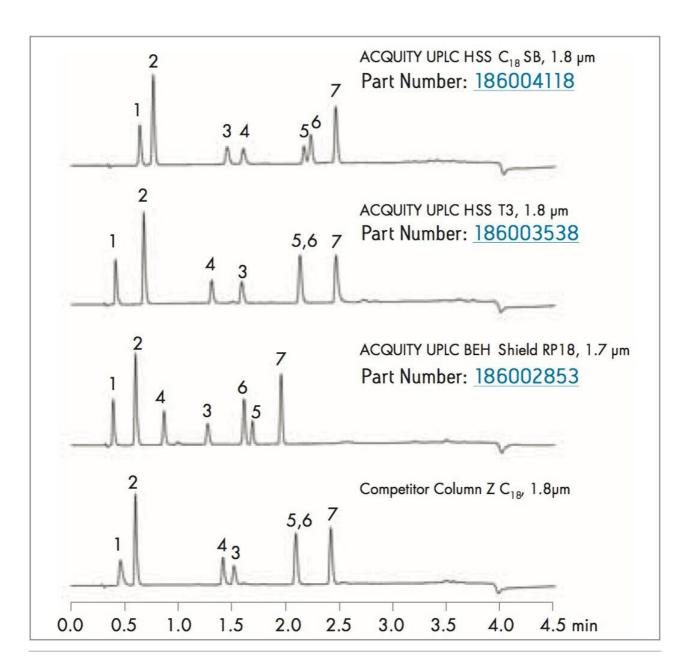


Figure 1. UPLC separation of seven basic drugs. All columns were in the 2.1 X 50 mm format. Gradient from 30–85% B in 3 min, hold at 85% B for 0.5 min, reset. UV 260 nm. Compounds: 1= aminopyrazine, 2= pindolol, 3 = labetalol, 4= quinine, 5= verapamil, 6= diltiazem, 7= amitriptyline.

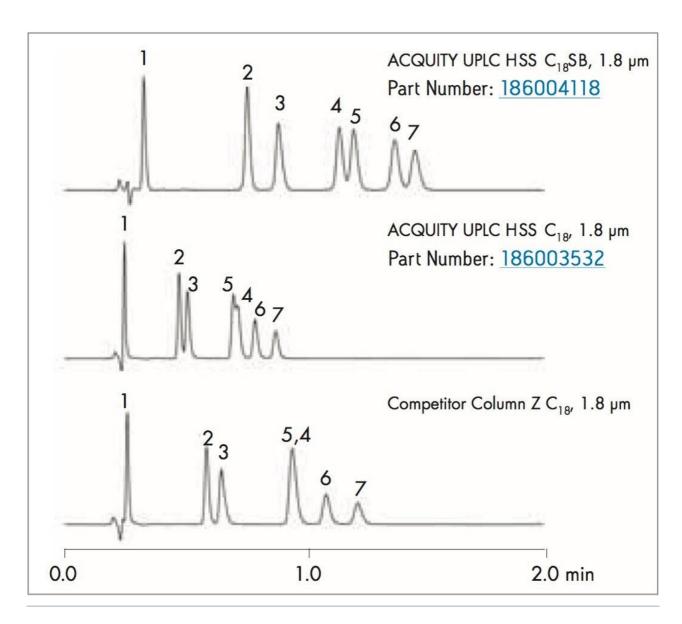


Figure 2. UPLC separation of tricyclic antidepressants. All columns were in the 2.1 X 50 mm format. Isocratic separation at 40% B. UV 254 nm. Compounds 1= trimethoprim, 2= nordoxepin, 3= doxepin, 4= nortriptyline, 5= imipramine, 6= amitriptyline, 7= trimipramine.

## Conclusion

As a result of the increase in surface silanol interactions, the ACQUITY UPLC HSS C<sub>18</sub> 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.

## **Featured Products**

ACQUITY UPLC System <a href="https://www.waters.com/514207">https://www.waters.com/514207</a>

ACQUITY UPLC PDA Detector <a href="https://www.waters.com/514225">https://www.waters.com/514225</a>

720002460, March 2009

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