COMPARISON OF FUSED-CORE AND ETHYLENE BRIDGED HYBRID PARTICLES FOR THE SEPARATION OF BASIC COMPOUNDS

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INTRODUCTION

HPLC columns containing fused-core (superficially porous) particles have recently gained increasing notoriety, as manufacturers claim these columns are a replacement for UltraPerformance Liquid Chromatography (UPLC® technology). For neutral compounds, 2.7 µm superficially porous particles have 70% of the efficiency of 1.7 µm, fully porous particles, and approximately 50-60% lower backpressure. It is therefore implied that "UPLC-like" separations can be achieved without the need for instrumentation capable of operating at the pressures generated when using 1.7 µm particles (up to 15,000 psi).

A comparison of fused-core particles and ethylene bridged hybrid (BEH) particles was performed for the analysis of basic compounds. Unlike neutral compounds, bases are ionized at low pH, and secondary interactions can occur with the stationary phase, causing asymmetric peak shape and efficiency loss. Therefore, determining their chromatographic behavior on these stationary phases has practical implications.

METHODS

LC Systems: 1) ACQUITY UPLC® system with PDA detector (~ 80 µL system volume)
2) Agilent 1200SL system configured for lowest system volume (~ 183 µL)

Columns: ACQUITY UPLC® BEH C_{18}, 1.7 µm
XBridge™ C_{18}, 3.5 µm
Ascentis® Express C_{18}, 2.7 µm (Supelco)

Mobile phase A: 10 mM ammonium formate, pH 3.0
Mobile phase B: 100% acetonitrile

Injection volume: 2 µL (2.1 x 50 mm dimension)
Column temp.: 30-50 °C

UV detection @ 260 nm (40 Hz sampling rate, normal filter response)

Gradient conditions for 0.8 mL/min flow rate on ACQUITY UPLC® BEH C_{18} column, 2.1 x 50 mm, 1.7 µm: 15-65% B in 2.3 min, hold at 65% for 0.7 min, reset (4 min total cycle time)

Flow rate was scaled to particle size (d_p) at each gradient condition. The gradient was then recalculated to maintain constant column volumes during the separation. Injection volume was also scaled to column dimension.

LC SYSTEM COMPARISON

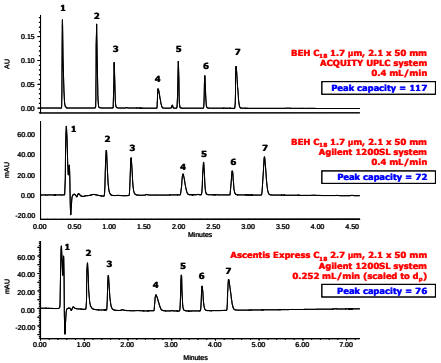

Figure 1. Comparison of fused-core and BEH particle column performance on the ACQUITY UPLC® system and Agilent 1200SL system. Peaks: (1) uracil, (2) pindolol, (3) quinine, (4) labetalol, (5) prednisone, (6) diltiazem, (7) amitriptyline. Separation temperature was 30 °C.

It is clear that system effects are responsible for the suboptimal performance of BEH 1.7 µm particle columns. When these columns are run on a system specifically designed for low system volume and high pressure, peak capacity is increased by over 60%.

COMPARISON BETWEEN 3.5 µm BEH AND 2.7 µm FUSED-CORE

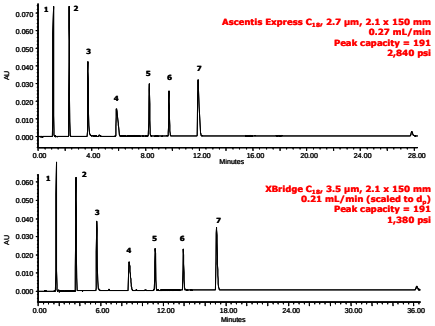

Figure 2. Comparison of 2.7 µm fused-core and 3.5 µm BEH particle column performance. Peaks: (1) uracil, (2) pindolol, (3) quinine, (4) labetalol, (5) prednisone, (6) diltiazem, (7) amitriptyline. Separation temperature was 50 °C.

MAXIMIZING PEAK CAPACITY

Method 1: Linking Columns Together

Because the backpressure of fused-core particle columns is ~ 50% of 1.7 µm BEH columns, it has been proposed that these columns can be linked together to achieve "UPLC-like" performance without the need for high pressure instrumentation.

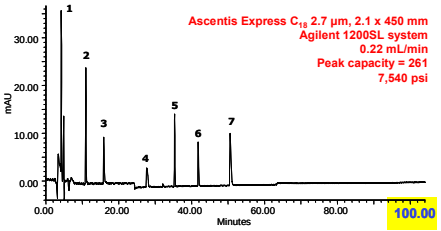

Figure 3. Separation of bases on three 150 mm linked fused-core columns. See Figure 2 for peak ID. Separation temperature was 50 °C.

Method 2: UPLC® Technology

The wide pH range (1-12) of BEH particles allows them to be operated under conditions that give excellent peak shape and retention for basic compounds. By combining high pH, elevated temperature, and longer 1.7 µm BEH columns, 40% higher peak capacities can be obtained in 5X less time than on linked fused-core particle columns.

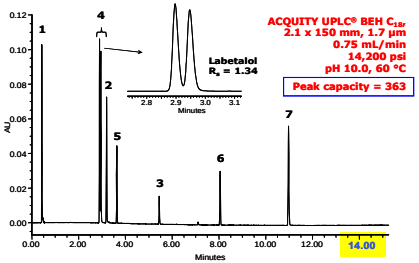

Figure 4. Separation of basic compounds at pH 10 on a 2.1 x 150 mm, 1.7 µm BEH C_{18} column. See Figure 2 for peak ID.

CONCLUSIONS

- **System effects are largely responsible for the suboptimal performance of BEH 1.7 µm columns on the 1200SL system.**
- **BEH 1.7 µm particle columns must be run on a system that is capable of operating at the pressures generated by running these columns at their optimal linear velocity.**
- **XBridge 3.5 µm columns gave the same performance as 2.7 µm fused-core columns, but had 50% lower backpressure.**
- **Using optimized conditions, 1.7 µm BEH particle columns have 40% higher peak capacity for bases in 5X less time than linked fused-core columns run on a lower pressure HPLC system.**

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