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Embedded Polar Group Columns: Do They Really Provide Increased Retention of Polar Analytes?

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Atlantis™ dC_{18} reversed-phase high performance liquid chromatography (HPLC) columns provide enhanced polar retention without an embedded-polar ligand.

Embedded-polar group (EPG) columns have been commercially available for many years. These columns were originally designed to provide better peak shapes for bases by shielding basic analytes from the silanol groups. An added benefit is that these columns can also be operated in fully aqueous mobile phases without the fear of pore dewetting. Pore dewetting occurs when a C_{18} stationary phase is used in a 100% aqueous mobile phase and flow is stopped. This forces the mobile phase out of the pores and retention is lost. Unfortunately, the use of the word "polar" in describing these phases has led chromatographers to believe these phases also provide increased retention for polar analytes. However, we observed less retention for polar compounds on EPG columns. The Atlantis™ dC_{18} is an alkyl-chain column created with an optimized pore size, ligand density, ligand type, and endcapping technique to achieve retention of polar analytes without overly retaining nonpolar compounds, as well as resisting pore dewetting, all without incorporating an EPG.

Experimental Conditions

Polar Retention Conditions

Columns: Waters Atlantis™ dC_{18} (no EPG), Agilent Zorbax® Bonus-RP (amide EPG), Supleco Supelcosil® ABZ+ (amide EPG), and Varian Inertsil® ODS-EP (proprietary EPG)
Column dimensions: 4.6 × 150 mm, 5 µm
Isocratic mobile phase: 10 mM ammonium formate, pH 3.0
Flow rate: 1.2 mL/min
Injection volume: 7.0 µL
Sample mixture: thiourea, 5-fluorocytosine, adenine, guanosine-5' monophosphate, and thymine dissolved in the mobile phase
Detection: UV at 254 nm

Pore Dewetting

Columns: Atlantis™ dC_{18} and traditional C_{18}, 4.6 × 150 mm, 5 µm
Isocratic mobile phase: 0.1% formic acid
Flow rate: 1.0 mL/min
Sample: amoxicillin dissolved in the mobile phase
Injection volume: 2.0 µL
Dewetting test: The columns were conditioned with organic and then equilibrated under 100% aqueous conditions. Several injections of the sample were made to characterize the retention. The flow was stopped on the column for 10 min and then restarted without rewetting the phase with organic. An injection of the sample was then made to observe the amount of retention loss.
Instrument: Waters Alliance® 2695 separations module with 2996 photo diode array detector.

Results

Enhanced polar retention on the Atlantis™ dC_{18} columns compared to several EPG columns can be seen in Figure 1. Zorbax® Bonus-RP and Supelcosil® ABZ+ contain amide groups. The Inertsil® ODS-EP contains a proprietary EPG. One possible reason for decreased retention of these EPG columns is the shorter ligand chain length of nearly all packings with incorporated polar groups. Clearly, EPG columns do not necessarily result in enhanced retention of polar analytes. Additionally, the peak shapes on the EPG columns are poor in comparison to the Atlantis™ dC_{18} column.

Figures 2a and 2b demonstrate the pore dewetting phenomenon on a traditional C_{18} packing. Figures 2c and 2d demonstrate that Atlantis™ dC_{18} columns are resistant to pore dewetting.

Conclusions

We have demonstrated that embedded polar group columns exhibit low polar retention compared to the Atlantis™ dC_{18} column. The Atlantis™ dC_{18} column was optimized to provide enhanced polar retention without overly retaining nonpolar analytes and to resist pore dewetting when operating under 100% aqueous mobile phase conditions, all without utilizing an embedded polar group ligand.

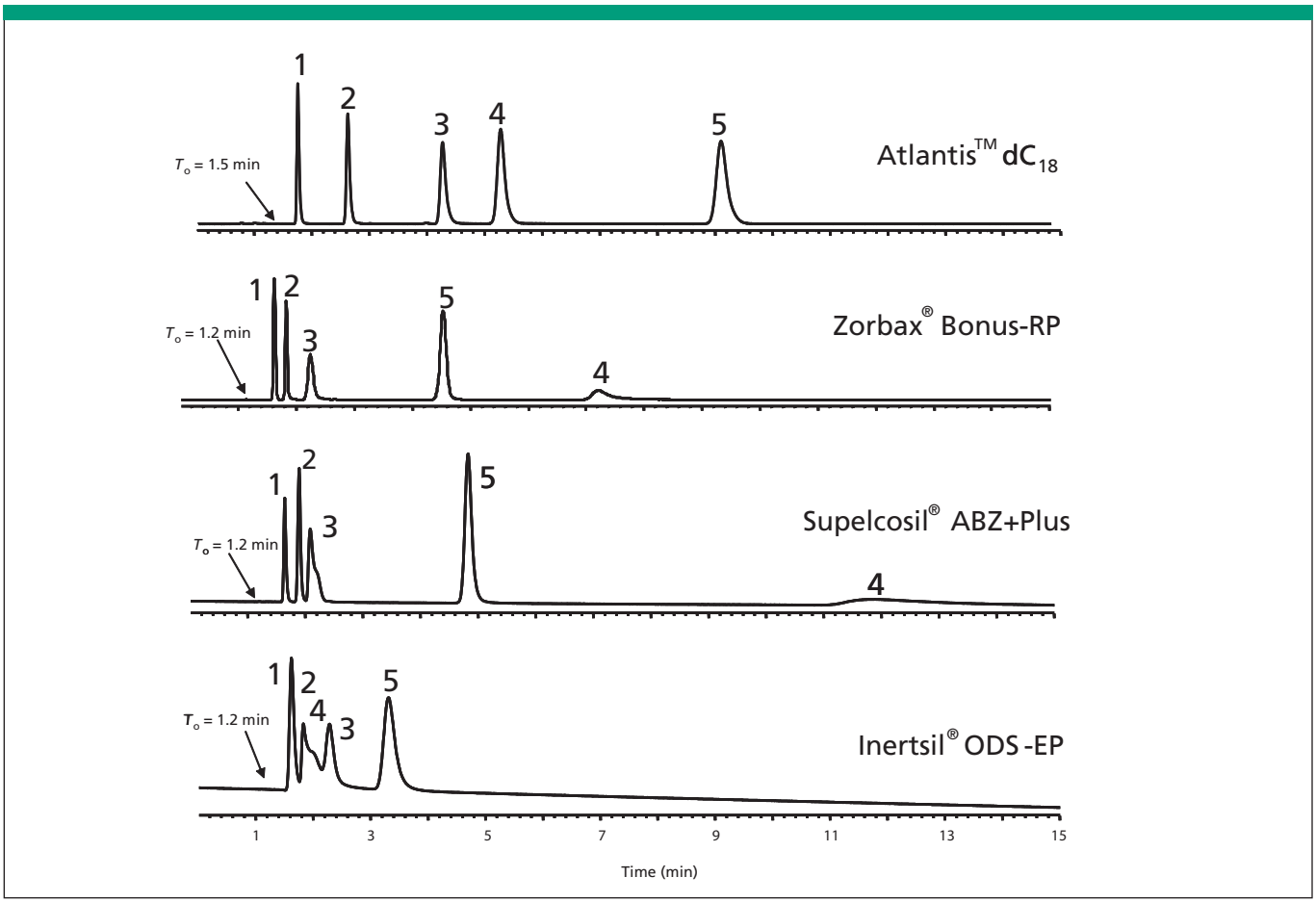

Figure 1: (Top) Comparison of the retention times of polar analytes on packing materials with embedded polar groups to the Atlantis™ dC_{18} packing. The Atlantis™ dC_{18} packing exhibits better retention and peak shapes. Peaks: 1 = thiourea, 2 = 5-fluorocytosine, 3 = adenine, 4 = guanosine-5′ monophosphate, 5 = thymine.

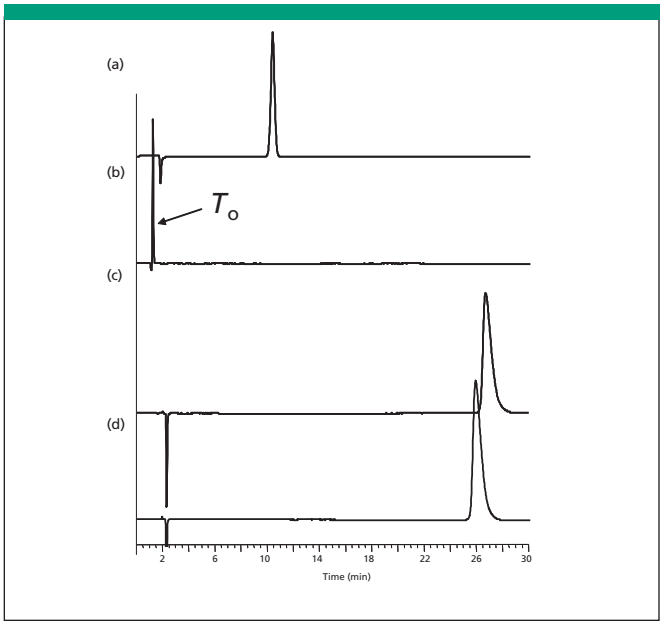

Figure 2: Pore dewetting in 100% aqueous mobile phase. (a) Classical C_{18} packing before flow stoppage, (b) classical C_{18} packing after flow stoppage exhibiting complete retention loss, indicative of pore dewetting, (c) Atlantis™ dC_{18} before flow stoppage, and (d) Atlantis™ dC_{18} after flow stoppage, exhibiting no retention loss.

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