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Improvements in Reversed-Phase HPLC Columns Designed for Polar Compound Retention: Introducing Atlantis® T3 Columns

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New Atlantis® T3 columns provide enhanced polar compound retention with improved low pH lifetimes and better peak shapes at pH 7.

Reversed-phase LC columns designed for polar compound retention often have low ligand densities and/or novel ligands to maintain pore wetting and enhance retention. Potential weaknesses of these types of columns include shortened lifetimes at low pH (e.g., TFA-containing mobile phases), poor peak shapes and lifetime above pH 5 and mass spectrometric bleed. To solve these issues, Waters® developed an improved C18 column for polar compound retention using a trifunctional ligand and a propriety endcapping process: Atlantis® T3. Waters has seen dramatic improvements in low pH lifetimes (up to a 5-fold improvement) and high pH lifetimes (up to 2.5-fold improvement). Minimal dewetting is observed when the flow is stopped, then restarted, when using 100% aqueous mobile phases. In addition, these new columns exhibit similar selectivity to Atlantis® dC18 columns for straightforward method transfer.

Low pH Lifetime Conditions

Mobile Phase: 0.5% TFA in water
Flow Rate: 1.4 mL/min
Column Temperature: 60 °C
Detection: UV @ 230 nm
Instrument: Waters Alliance® 2695 Separations Module with 2487 UV

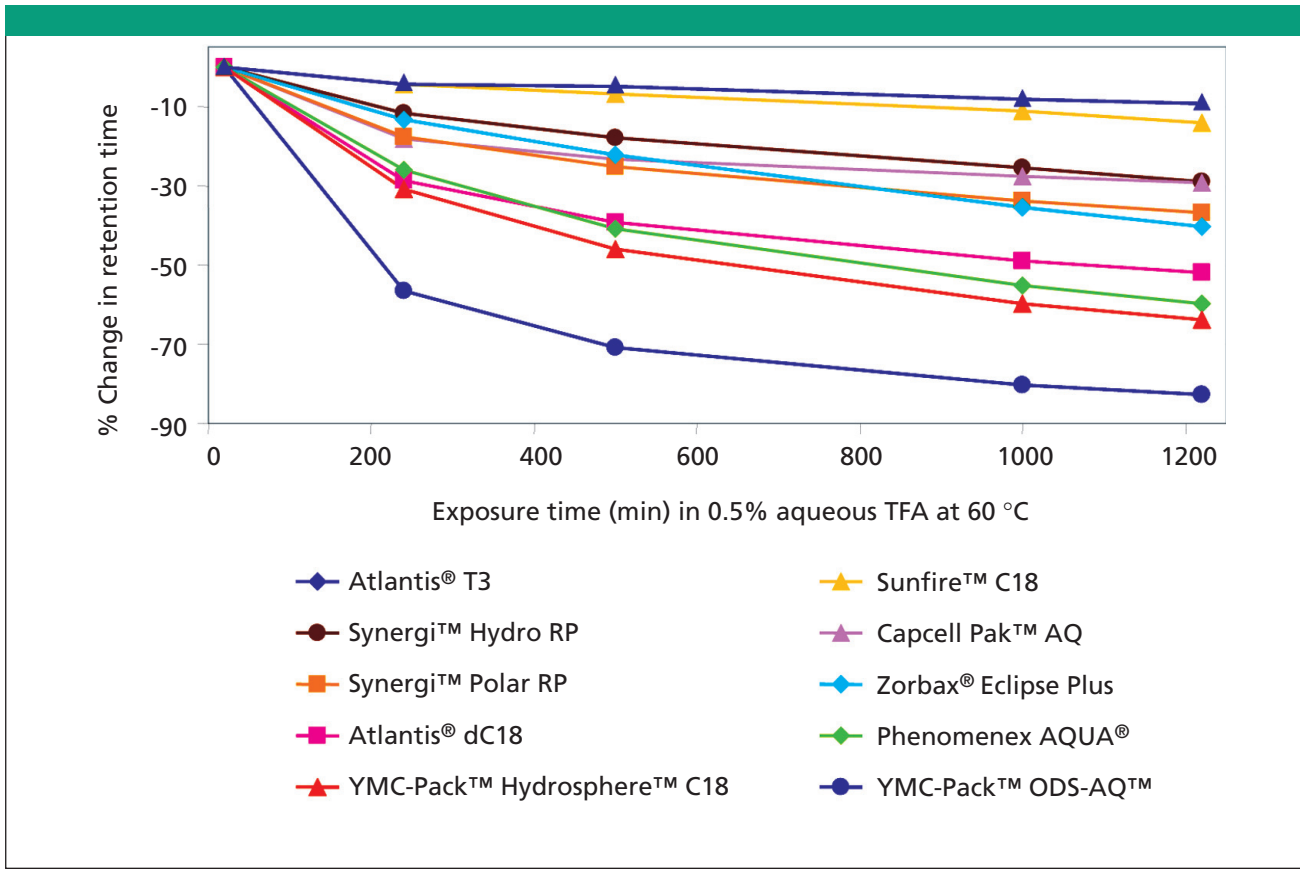

Figure 1: Low pH stability results. Comparison of retention loss for C18 stationary phases during exposure to 0.5% TFA mobile phases at 60 °C.

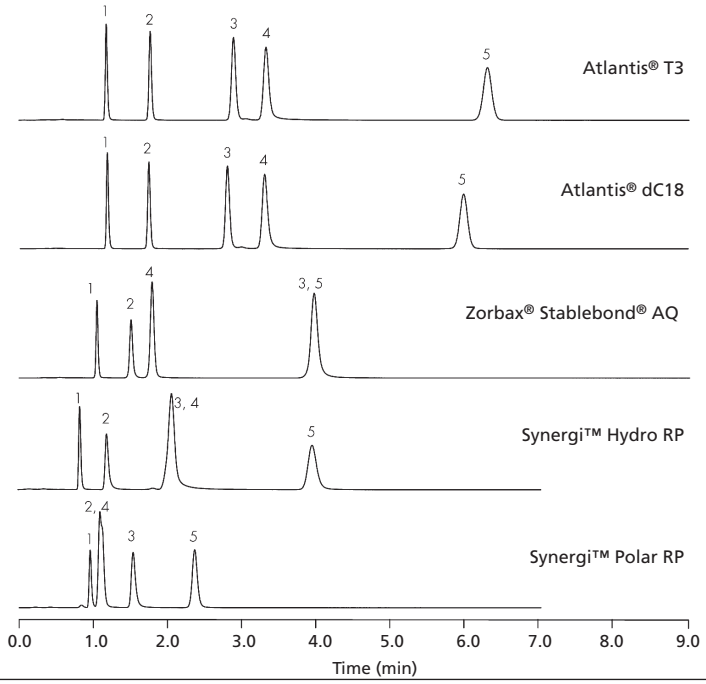

Figure 2: Comparison of the retention of polar analytes in 100% aqueous pH 3 mobile phase. Analtyes: 1) thiourea, 2) 5-fluorocytosine, 3) adenine, 4) guanosine-5′-monophosphate, 5) thymine.

HPLC Conditions

Isocratic Mobile Phase: 10 mM NH_4COOH, pH 3
Flow Rate: 1.0 mL/min
Column Temperature: 30 °C
Detection: UV @ 254 nm
Instrument: Waters Alliance® 2695 with 2996 PDA

Results

The combination of the trifunctional ligand and the proprietary endcapping process has improved the low pH stability of the Atlantis® T3 columns. In Figure 1, the results from our accelerated lifetime testing experiments are plotted. The percent change in initial retention time of methyl paraben (a neutral probe analyte) is plotted versus the exposure time to 0.5% TFA at 60 °C. The Atlantis® T3 material has a significantly improved lifetime over not only the original Atlantis® dC18 material, but also over several other commercially available reversed-phase columns. Therefore, for applications using the typical 0.1% TFA modifier, the new Atlantis® T3 columns will have longer column lifetimes than the other materials tested.

The dewetting values for both Atlantis® columns under the 100% aqueous pH 3 mobile phase conditions are both under 10% as listed in Table 1. The USP tailing factors for amitriptyline at pH 7 are also listed in Table 1. Clearly, the new bonding and endcapping procedures for the Atlantis® T3 material resulted in improvement in peak shapes for basic analytes above pH 5.

In Figure 2, the results from the 100% aqueous mobile phase Atlantis® batch test are shown. These results indicate that both Atlantis® columns provide excellent retention and peak shapes for a range of polar analytes over a variety of commercially available polar retention columns. Additionally, under these conditions, the selectivity between the Atlantis® T3 and Atlantis® dC18 materials are similar.

Conclusions

Improvements in the ligand-type and endcapping process for the Atlantis® T3 columns resulted in a column for polar compound retention with improved low pH lifetimes and improved peak shapes at neutral pH.

Table I: Dewetting values (pH 3) and USP tailing factors (pH 7)

Atlantis® T3	Atlantis® dC18
Dewetting: < 7%	Dewetting: < 5%
Tailing factor for amitriptyline: 1.77	Tailing factor for amitriptyline: 3.76

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