

Nota applicativa

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

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Abstract

This application note details on new Atlantis T3 Columns which provide enhanced polar compound retention.

Benefits

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

Introduction

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.

Experimental

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

HPLC Conditions

Isocratic mobile phase:	10 mM NH ₄ COOH, pH 3
Flow rate:	1.0 mL/min
Column temperature:	30 °C
Detection:	UV @ 254 nm
Instrument:	Waters Alliance 2695 with 2996 PDA

Results and Discussion

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.

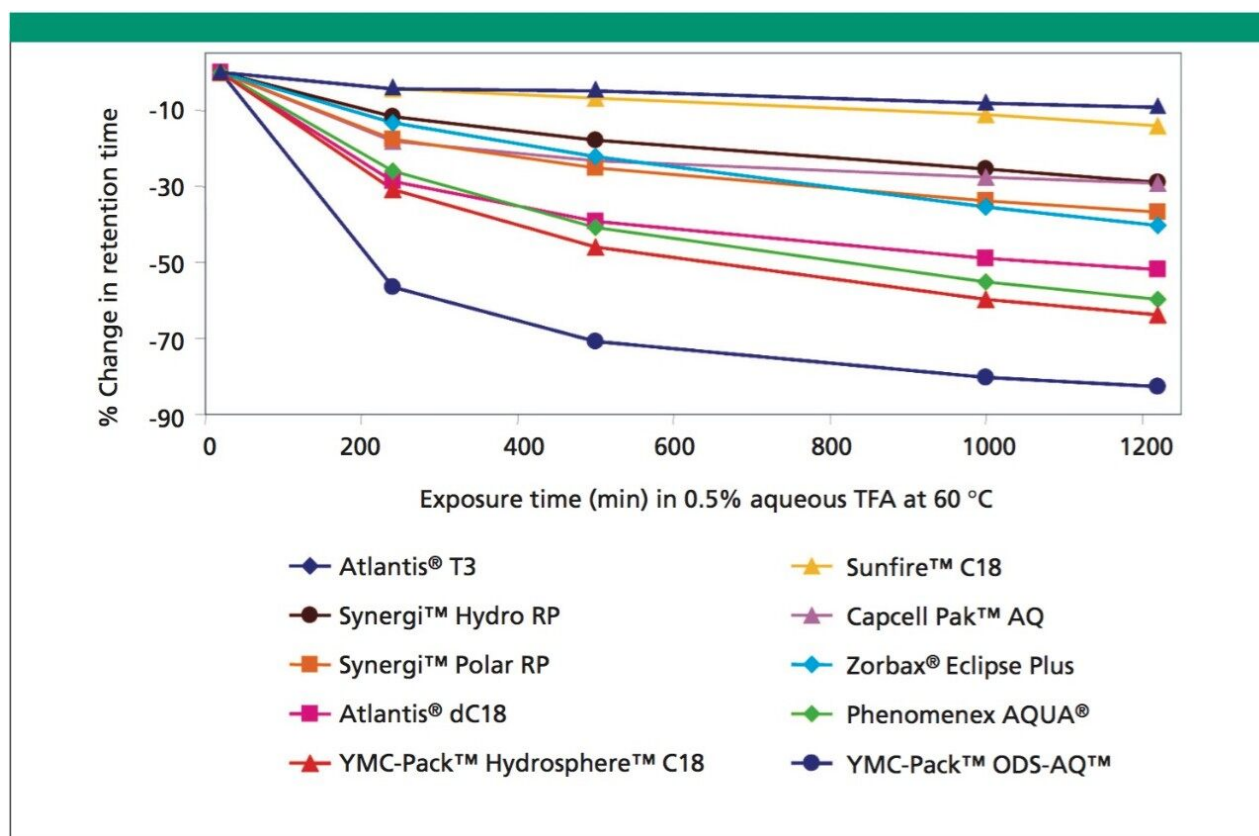


Figure 1. Low pH stability results. Comparison of retention loss for C_{18} stationary phases during exposure to 0.5% TFA mobile phases at 60 °C.

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.

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

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.

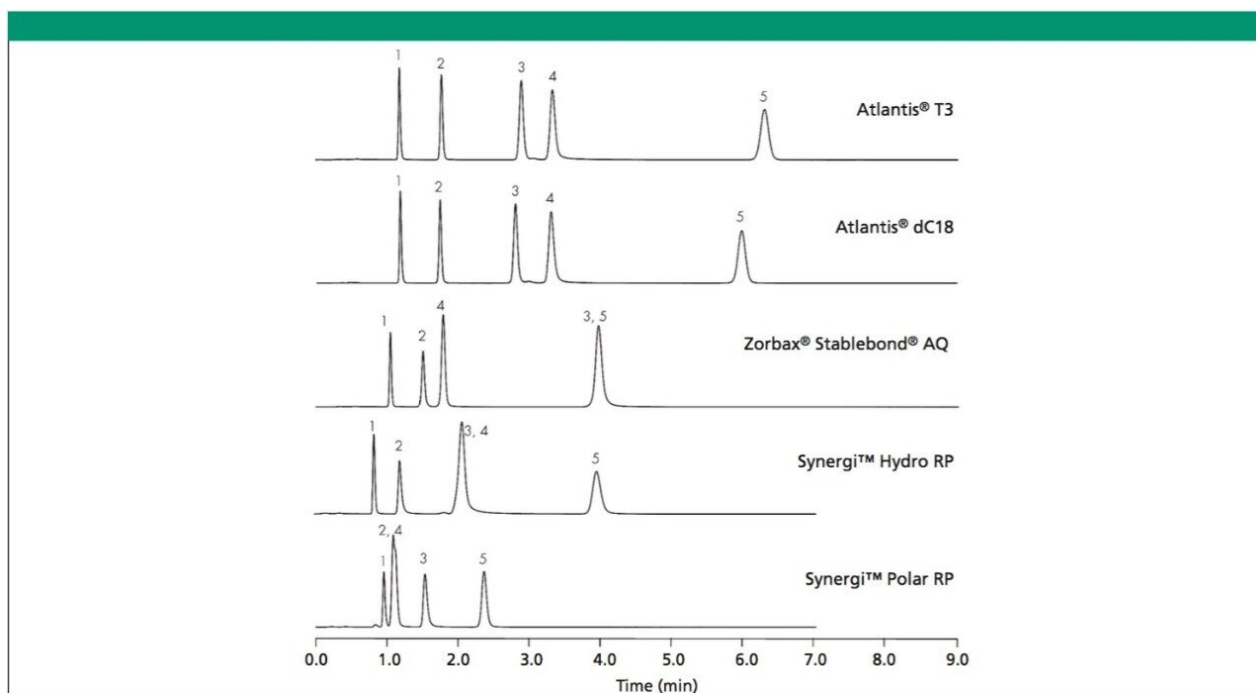


Figure 2. Comparison of the retention of polar analytes in 100% aqueous pH 3 mobile phase. Analytes: 1) thiourea, 2) 5-fluorocytosine, 3) adenine, 4) guanosine-5'-monophosphate, 5) thymine.

Conclusion

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.

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