IMPROVEMENTS IN REVERSED-PHASE HPLC COLUMNS DESIGNED FOR POLAR COMPOUND RETENTION

Fang Xia, Paula Hong, Bonnie Alden, Cheryl Boissel, Diana Swanson, Erin Chambers, Diane Diehl, Pamela Iraneta, Jeffrey Mazzeo, Kevin Wyndham, Nicole Lawrence, Thomas Walter
Waters Corporation, Milford, MA 01757, USA

OVERVIEW

Reversed-phase LC columns designed for polar compound retention often have low ligand densities and/or novel ligands in order 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 above pH 5 and MS bleed. To solve these issues, we developed an improved C18 column for polar compound retention (Atlantis® T3) using a trifunctional ligand and a propriety endcapping processes. We have 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. MS response of column bleed was also measured and compared to other RPLC columns. In addition, these new columns exhibited similar selectivity to Atlantis® dC_{18} columns for easy method transfer. Lastly, we show additional applications that are now possible with this new column chemistry.

LOW pH STABILITY TEST

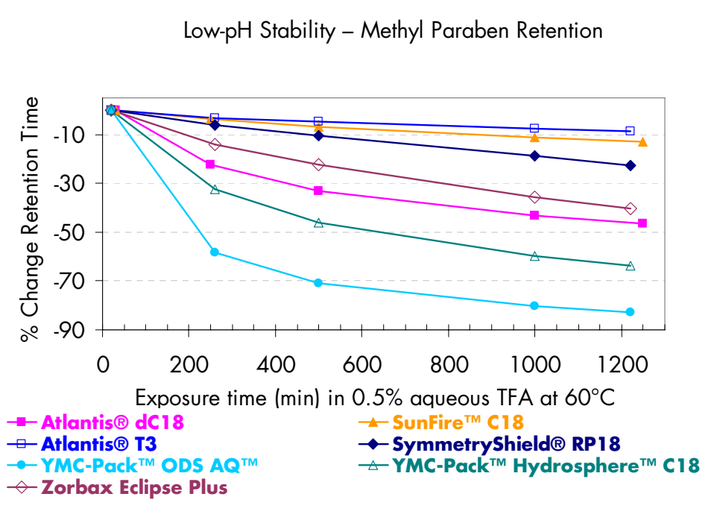

Figure 1: Comparison of efficiency loss for C18 stationary phases during exposure to **0.5% TFA** mobile phases at 60°C.

Trammell, B. C. et al J. Chromatogr. A, 1060 (2004) 153-163.

HIGH pH STABILITY TEST

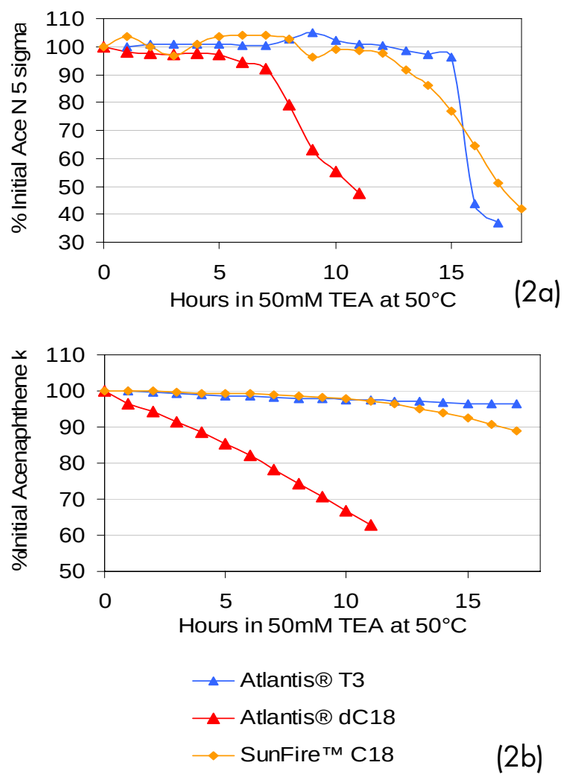

Figure 2: Comparison of stability of C18 stationary phases during exposure to **50 mM TEA pH 10** mobile phases at 50°C. (2a): plate count; (2b): capacity factor
Analyte: acenapthene
Test mobile phase: 65:35 (MeOH:20 mM K_2HPO_4, pH 7) at 50°C

Wyndham, K.D. et al Anal. Chem. 2003 (75) 6781-6788.

DEWETTING

Compounds:
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Guanosine-5'-monophosphate
5. Thymine

Isocratic Conditions
Mobile Phase: 10 mM NH_4COOH, pH 3.0
Flow Rate: 1.2 mL/min
Injection Volume: 7 µL
Temperature: Ambient
Detection: UV @ 254 nm
After proper equilibration, the first set of injections are made, the flow is stopped for 10-min, restarted and injections made again.

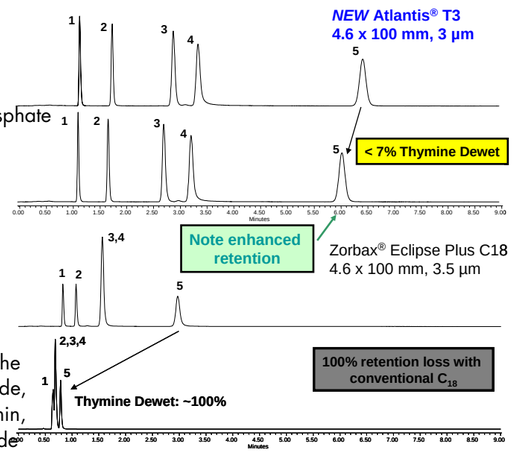

Figure 3: Results of the dewetting study on Atlantis® T3 and a conventional C18 column.

Walter, T. H. et al J. Chromatogr. A 1075 (2005) 177-183

MASS SPECTROMETRY

HPLC Conditions:
Mobile Phase A: 0.1% HCOOH in water
Mobile Phase B: 0.1% HCOOH in acetonitrile
Flow Rate: 1.5 mL/min with 250 µL/min flow split to mass spectrometer
Gradient: 10 min gradient from 5% to 100% B with 3 min initial hold
Column Temperature: 50°C
Column Dimension: 4.6 × 50 mm
Instrument: Waters Alliance® 2695

MS Conditions:
Ion Source: ES+
Capillary: 3.5 kV
Extractor: 3.0 V
Source Temp: 150 °C
Cone Gas: 60 L/hr
Ion Energy: 0.5
Cone: 33.0 V
RF Lens: 0.3 V
Desolvation Temp: 300 °C
Desolvation Gas: 509 L/hr
Instrument: Waters Micromass® ZQ™

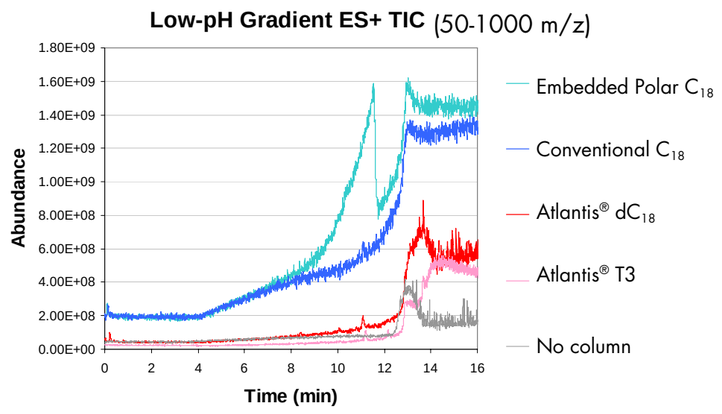

Figure 4: Comparison of column bleed in mass spectrometry.

SELECTIVITY COMPARISON

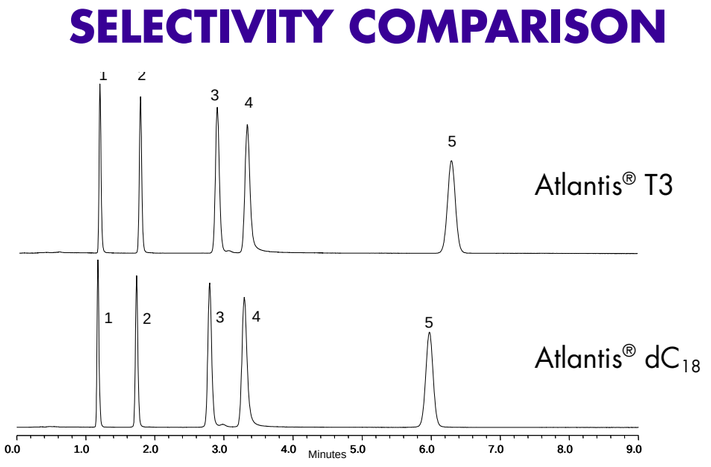

Figure 5: Comparison of column selectivity.
Column: 4.6 x 150 mm, 5 µm
Flow Rate: 1.0 mL/min
Analytes: (1) thiourea, (2) 5-fluorocytosine, (3)adenine, (4) guanosine-5'-monophosphate, (5) thymine
Test mobile phase: 10 mM NH_4COOH, pH 3 (v/v) at 30°C

APPLICATIONS

Water Soluble Vitamins

	Compound	USP Tailing	Sample Concentrations
1.	L-ascorbic acid	1.05	25 (µg/mL)
2.	Nicotinic Acid	1.23	12.5 (µg/mL)
3.	Thiamine (vitamin B1)	1.22	25 (µg/mL)
4.	Pyridoxal	1.19	50 (µg/mL)
5.	Pyridoxine	1.28	50 (µg/mL)
6.	Folic Acid (vitamin B11)	1.17	40 (µg/mL)
7.	Caffeine	1.11	12.5 (µg/mL)
8.	Riboflavin (vitamin B2)	1.11	6 (µg/mL)

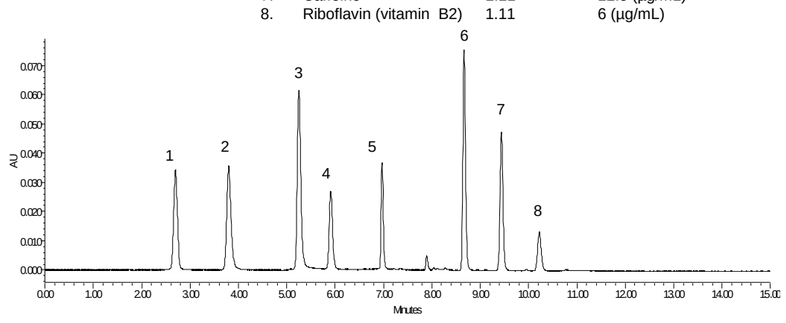

Figure 6: Separation of water soluble vitamins
Column: Atlantis® T3 4.6 x 150 mm, 5 µm
Mobile Phase A: 0.1% CF_3COOH in water
Mobile Phase B: Acetonitrile
Flow Rate: 1.4 mL/min

Gradient: Time	%A	%B
0	100	0
4	97	3
6	85	15
15	80	20
24	80	20

Injection Volume: 10.0 µL
Sample: As indicated in the chromatogram
Detection: UV @ 260 nm

Catecholamines and Metabolites

	Compound	UPS Tailing	Sample Concentration
1.	Norepinephrine	1.37	25 (µg/mL)
2.	Epinephrine	1.34	25 (µg/mL)
3.	Dopamine	1.29	10 (µg/mL)
4.	3,4- Dihydroxyphenylacetic acid	1.08	25 (µg/mL)
5.	Serotonin (5-HT)	1.49	30 (µg/mL)
6.	5-Hydroxy-3-indoleacetic acid	1.18	25 (µg/mL)
7.	4-Hydroxy-3-methoxyphenyl acetic acid (HVA)	0.9	25 (µg/mL)

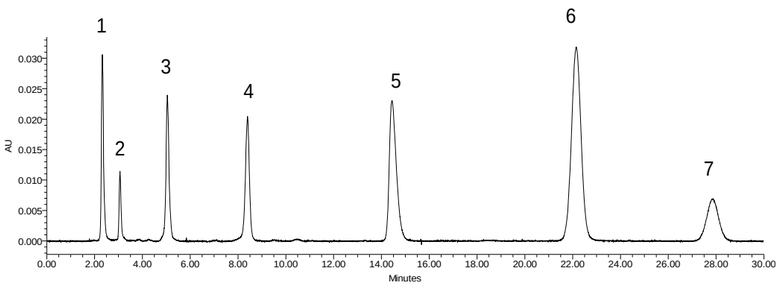

Figure 7: Separation of catecholamines and metabolites
Column: Atlantis® T3 4.6 x 150 mm, 5 µm
Mobile Phase A: 10 mM NH_4COOCH_3, pH 5.0
Mobile Phase B: Acetonitrile
Flow Rate: 1.0 mL/min
Isocratic: 98/2 (A/B)
Injection Volume: 10.0 µL
Sample: As indicated in the chromatogram above
Column Temp: 30 °C
Detection: UV @ 280 nm
Instrument: Waters Alliance® 2695 with 2996 PDA

CONCLUSIONS

Atlantis® T3 columns provide:
- Enhanced retention for polar analytes
- Superior stability under low and high pH conditions
- Minimal dewetting when the flow is stopped, then restarted, when using 100% aqueous mobile phases
- Low MS bleed
- Comparable selectivity to Atlantis® dC_{18} columns
- Good peak shapes
- Available in a variety of dimensions and particle sizes

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS