



TN-1307

Scalability and Reproducibility of Luna™ Omega C18 for Acid, Base, and Neutral Mixture

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Introduction

The analysis of an acid, base, and neutral sample was used to assess the scalability and reproducibility for three different batches of Luna Omega C18 columns with either 1.6 μm , 3 μm or 5 μm matrix in three different dimensions. The sample contains acid (A), base (B), and neutral (N) compounds which are used to assess the chromatographic performance (retention and peak shape) for several different C18 bonded phases. The basic analyte, in particular, is quite sensitive to the presence of any active silanol groups in acidic mobile phase conditions, which is reflected as increased peak tailing and retention time reproducibility. The Luna Omega columns were compared to multiple batches of Waters® ACQUITY® BEH C18 and XBridge® C18, and Agilent® ZORBAX® SB-C18 RRHD and SB-C18 columns. 1.7 μm , 1.8 μm , 3.5 μm or 5 μm media were analyzed, in three different dimensions. In this technical note, we show the reproducibility and scalability of the Luna Omega C18 columns compared to other columns with similar dimensions and particle sizes.

Results and Discussion

Under the acidic mobile phase condition used, most of the silanol groups would be fully protonated; however, as the pH is ca. 2.7, there are still a percentage of silanols that will be deprotonated because we are not a full 2 pH units below the silanol pKa. As a result, the basic analyte, Nortriptyline, would be positively charged in these conditions and would be a good indicator as to the presence of any active silanol groups. In **Figure 1**, the Luna Omega 1.6 μm C18 columns provided complete separation of all analytes. One of the batches resulted in a small retention time shift for A and N, resulting in a higher retention time % Relative Standard Deviation (%RSD). This could result from a higher surface area or carbon load in that batch of Luna Omega 1.6 μm media. The Agilent ZORBAX SB-C18 columns showed consistent co-elution of peaks A and B, while N gave reproducible retention across all batches. The Waters ACQUITY BEH 1.7 μm C18 columns showed a change in the retention order for the N and B analytes relative to the Luna Omega C18 columns, with one batch exhibiting co-elution of the N and B peaks.

Figure 2 shows separation of all analytes on the three different sets of 150 x 4.6 mm columns. Overall, the Luna Omega 3 μm C18 columns had a retention time %RSD of 2.48 and 2.14 for peaks A and N, respectively. Peak B had a retention time %RSD of 5.51. The Agilent ZORBAX 3.5 μm SB-C18 columns showed a higher retention time %RSD for peak A, and a lower retention time %RSD for peak B and N, compared to the Luna Omega 3 μm C18 columns. Although the retention time %RSD was lower for peak B, there was more tailing observed for B resulting in a much higher symmetry factor. The Waters XBridge 3.5 μm C18 columns had similar reproducibility between batches and similar retention time %RSD for all peaks compared to the Luna Omega 3 μm C18 columns.

Finally, in **Figure 3**, the Luna Omega 5 μm C18 columns showed very good batch-to-batch reproducibility with retention time %RSDs lower than 3 for all peaks. The Waters XBridge 5 μm C18 columns showed similar retention time %RSDs compared to the Luna Omega 5 μm C18 columns. However, the Agilent ZORBAX 5 μm SB-C18 columns had higher retention time %RSDs for all peaks compared to the Luna Omega 5 μm C18 columns and the Waters XBridge 5 μm C18 columns. Peak B on the Agilent ZORBAX 5 μm SB-C18 columns showed considerable tailing, evidenced by higher symmetry factor values.

LC Conditions

Column: Luna Omega 1.6 μm C18, 50 x 2.1 mm ([00B-4742-AN](#))
Agilent ZORBAX 1.8 μm SB-C18 RRHD, 50 x 2.1 mm
Waters ACQUITY BEH 1.7 μm C18, 50 x 2.1 mm
Luna Omega 3 μm C18, 150 x 4.6 mm ([00F-4784-E0](#))
Agilent ZORBAX 3.5 μm SB-C18, 150 x 4.6 mm
Waters XBridge 3.5 μm C18, 150 x 4.6 mm
Luna Omega 5 μm C18, 250 x 4.6 mm ([00G-4785-E0](#))
Agilent ZORBAX 5 μm SB-C18, 250 x 4.6 mm
Waters XBridge 5 μm C18, 250 x 4.6 mm

Mobile Phase: A: 0.1 % Phosphoric acid in water
B: Water/Acetonitrile (70:30, v/v), premixed

Flow Rate: 400 $\mu\text{L}/\text{min}$ for 4 min (50 x 2.1 mm columns)
2 mL/min for 10 min (150 x 4.6 mm columns)
2 mL/min for 15 min (250 x 4.6 mm columns)

Injection Volume: 0.2 μL (50 x 2.1 mm columns)
1 μL (150 x 4.6 mm columns)
1 μL (250 x 4.6 mm columns)

Temperature: 30 °C

Detection: DAD @ 254 nm

Instrument: Agilent 1260 (Binary)

Analytes: Acid (A): 3-Methyl,4-Nitrobenzoic acid (1.0 mg/mL in Methanol)
Base (B): Nortriptyline (1.0 mg/mL in Methanol)
Neutral (N): 2-Hydroxy, 5-Methylbenzaldehyde (1.0 mg/mL in Methanol)



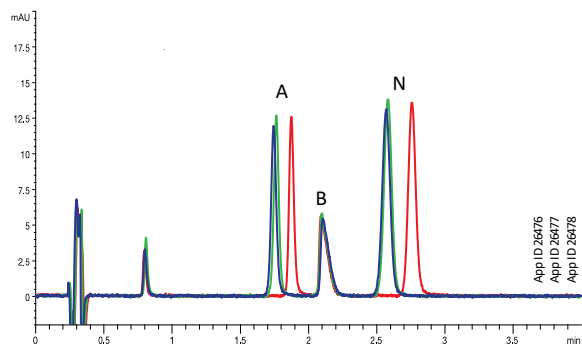
Zeshan Aqeel
Senior Application Scientist

Aside from the lab being his favorite place to be, Zeshan enjoys playing vintage videogames with his twin boys and loves every minute of reliving parenthood with his baby girl.



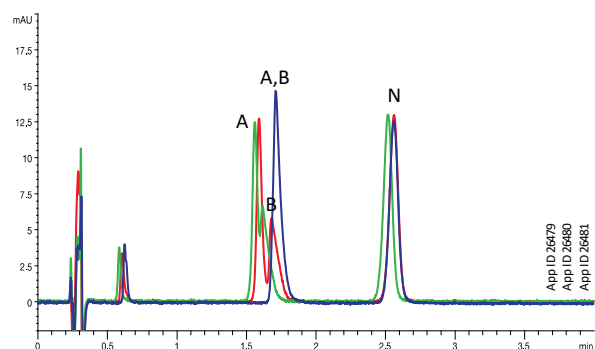
Figure 1.

Luna™ Omega 1.6 µm C18, 50 x 2.1 mm



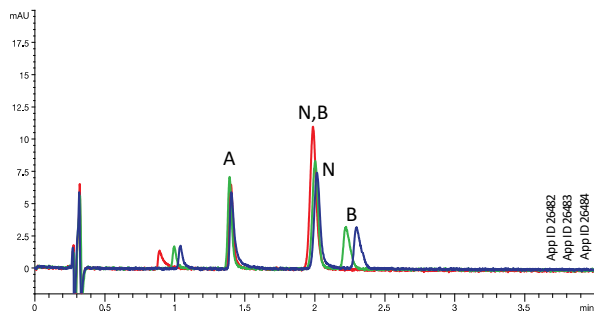
Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	3.94	0.0417	0.0357	0.0381	1.13	1.04	1.17
B	0.26	0.0576	0.0517	0.0464	2.02	1.82	2.52
N	4.01	0.0605	0.0517	0.0505	1.01	1.02	1.08

Agilent® ZORBAX® 1.8 µm SB-C18 RRHD, 50 x 2.1 mm



Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	5.00	0.0551	0.0395	0.0416	1.66	0.88	1.21
B	2.96	0.0543	0.0583		3.76	2.24	
N	0.97	0.0625	0.0588	0.0655	1.00	0.97	0.97

Waters® ACQUITY® BEH 1.7 µm C18, 50 x 2.1 mm

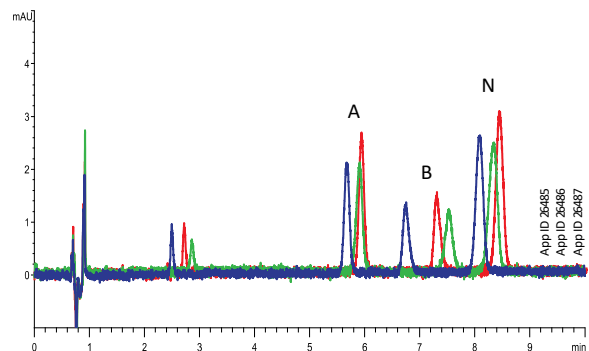


Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	0.62	0.0369	0.329	0.0324	3.07	1.45	1.57
N	0.55	0.0449	0.0412	0.048	1.24	1.06	1.21
B	7.38	0.0473	0.0474		1.52	1.33	



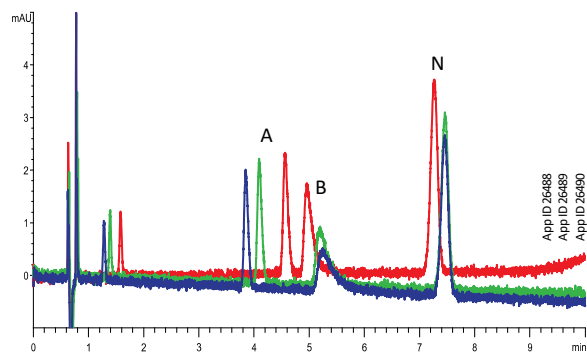
Figure 2.

Luna™ Omega 3 μm C18, 150 x 4.6 mm



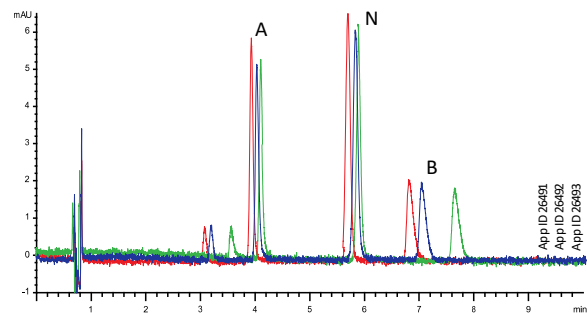
Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	2.48	0.0881	0.1007	0.0818	1.04	0.86	0.99
B	5.51	0.1004	0.1107	0.0949	1.07	0.99	1.16
N	2.14	0.1237	0.135	0.1111	0.92	0.89	1.03

Agilent® ZORBAX® 3.5 μm SB-C18, 150 x 4.6 mm



Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	8.80	0.0657	0.0718	0.078	1.25	1.23	1.24
B	2.85	0.3132	0.2737	0.1772	2.03	1.81	2.15
N	1.34	0.1102	0.1111	0.1083	0.94	0.97	1.14

Waters® XBridge® 3.5 μm C18, 150 x 4.6 mm

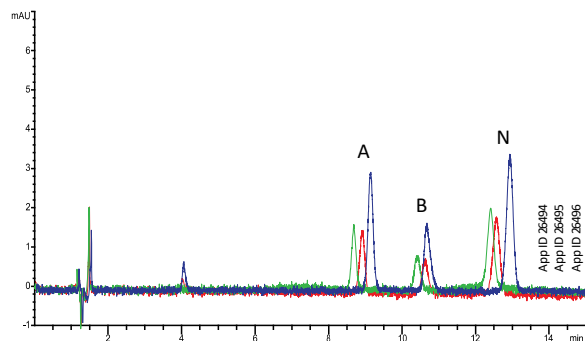


Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	2.15	0.0609	0.0599	0.0551	1.43	1.47	1.24
N	1.54	0.0747	0.0759	0.0752	1.16	1.16	1.05
B	6.18	0.0966	0.1542	0.0997	1.56	1.52	1.90



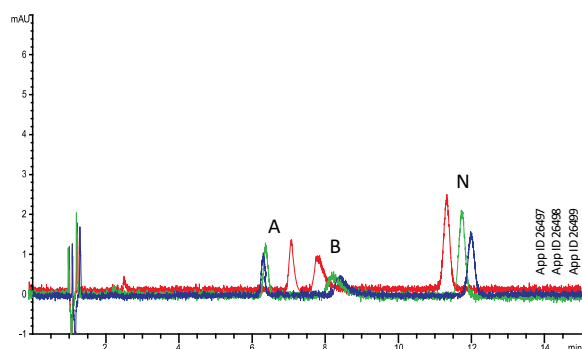
Figure 3.

Luna™ Omega 5 μm C18, 250 x 4.6 mm



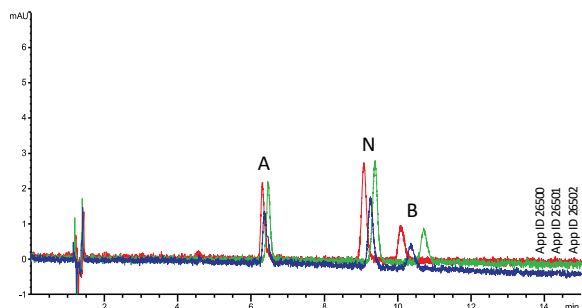
Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	2.51	0.1742	0.1472	0.1662	0.99	1.01	0.86
B	1.40	0.2052	0.198	0.2037	1.27	1.26	0.96
N	2.10	0.2126	0.208	0.2177	0.95	0.90	0.96

Agilent® ZORBAX® 5 μm SB-C18, 250 x 4.6 mm



Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	6.64	0.1012	0.1042	0.1494	1.15	1.37	1.17
B	3.37	0.361	0.4517	0.2885	1.63	1.67	1.48
N	2.77	0.2351	0.1398	0.1937	1.14	1.18	0.96

Waters® XBridge® 5 μm C18, 250 x 4.6 mm



Peak	Retention Time %RSD (N=3)	Peak Width			Symmetry Factor		
		Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
A	1.41	0.1413	0.0892	0.1262	1.37	1.24	1.66
N	1.73	0.1699	0.1639	0.1182	1.24	1.00	1.15
B	3.04	0.2167	0.1895	0.206	1.13	1.07	1.35

Conclusion

The data presented in this technical note shows that, while there is a small amount of batch-to-batch reproducibility for each set of columns from the three different manufacturers, the overall batch-to-batch reproducibility across the three batches tested is comparable. Likewise, the overall scalability of Luna Omega from 1.6 μm to 3 μm to 5 μm particle size is comparable to that observed for the Waters and Agilent columns.



Luna™ Omega Ordering Information

1.6 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges (mm) ‡		
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502

for 2.1 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#)

3 µm Analytical Columns (mm)			SecurityGuard Cartridges (mm) *		
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* /10 pk
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
C18	00B-4784-E0	00D-4784-E0	00F-4784-E0	00G-4784-E0	AJ0-7612
SUGAR	—	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495

for ID: 3.2-8.0 mm

5 µm Analytical Columns (mm)			SecurityGuard Cartridges (mm) *		
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* /10 pk
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJ0-7612

for ID: 3.2-8.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

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