

APPLICATION

Choosing the Right UHPLC Column for Analyzing Highly Polar Acidic Molecules



Zeshan Aqeel and Jeff Layne
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

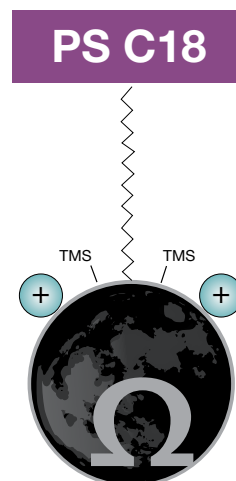
Overview

Developing robust methods for the analysis of small, polar, acidic compounds can be very challenging due to the fact that many simply do not retain well using typical reversed phase columns. When analyzing highly polar basic molecules, analysts have access to a range of ion-pairing agents (such as TFA, or hexane sulfonic acid) that can increase the retention of polar, basic species. In addition, many column manufacturers offer unique reversed phase stationary phases that are specifically designed to improve the retention of these types of molecules. But ion-pairing agents for polar acids are rarely used, and few LC media manufacturers offer reversed phase products specifically made for analyzing polar acids.

To address the gap, we have recently created a novel C18 phase which contains a proprietary positively charged functional group (weakly basic in character) – our Luna[®] Omega PS C18 phase (available in 1.6 μm , 3 μm , and 5 μm particle sizes). The presence of the positively charged functional group, combined with the standard C18 bonding, results in a true mixed-mode interaction that can provide a unique selectivity and enhanced retention for many polar acidic analytes. This surface modification also allows the Luna Omega PS C18 column to be used in 100% aqueous conditions so that analysts can maximize polar acid retention by using purely aqueous mobile phases.

These properties are illustrated in the separation of a group of polar organic acids in the included figures. The challenge in this separation is the resolution of methylsuccinic acid from an isobaric related analyte, ethylmalonic acid (Peaks 1 and 2). The unique selectivity afforded by the positively charged functional group of the Luna Omega PS C18 dramatically increases the separation between methylsuccinic acid and ethyl malonic acid (**Figure 2**).

The improved resolution gives analysts the confidence that slight variations in performance over time will not negatively impact accurate quantitation of these isobaric species. We can further improve this method by utilizing the aqueous stability of the Luna Omega PS C18 phase beginning our gradient in 100% buffer (**Figure 3**). You can now see that we now have the option to sacrifice a small amount of separation between the isobars to gain a dramatic improvement in retention (**Figure 3**). Together, by utilizing the unique properties of the Luna Omega PS C18 chemistry, we increase the overall retention for these polar acids and greatly improve the resolution between isobaric molecules.

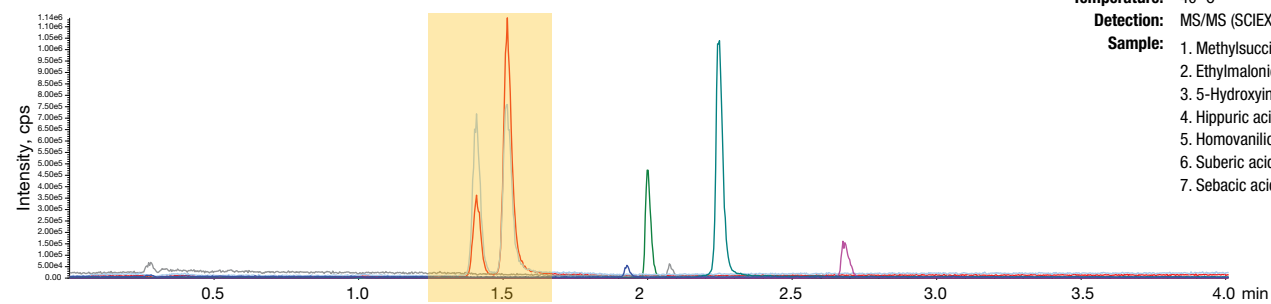


Luna Omega PS C18

Unique, 100% aqueous stable mixed-mode phase that provides both polar and non-polar retention. The surface contains a positive charged ligand which aids in the retention of acidic compounds through ionic interactions, while the C18 ligand promotes general reversed phase retention. The positively charged surface also improves basic compound peaks shape through ionic repulsion.



Figure 1.
Waters[®] ACQUITY[®] 1.7µm BEH C18

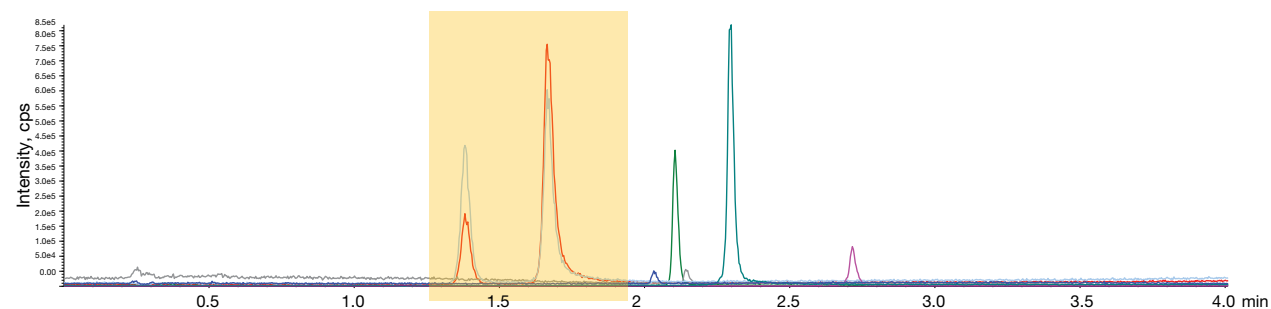


Conditions for all columns

Column: As specified
 Dimension: 50 x 2.1 mm
 Mobile Phase: A: 0.1 % Formic acid in water
 B: 0.1 % Formic acid in Acetonitrile
 Gradient: Time (min) % B
 0 3 (except where noted)
 3 90
 Flow Rate: 0.4 mL/min
 Temperature: 40 °C
 Detection: MS/MS (SCIEX API 4000™)
 Sample: 1. Methylsuccinate
 2. Ethylmalonic acid
 3. 5-Hydroxyindoleacetic acid
 4. Hippuric acid
 5. Homovanillic acid
 6. Suberic acid
 7. Sebaccic acid

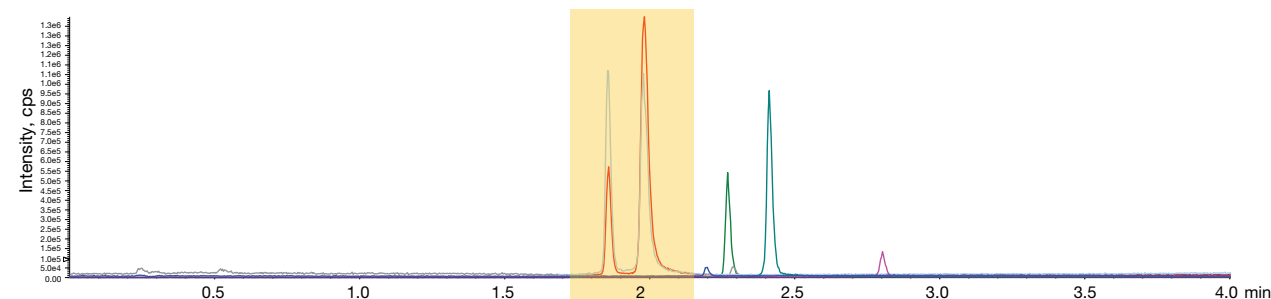
App ID 24411

Figure 2.
Luna[®] Omega 1.6µm PS C18



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Figure 3.
Luna Omega 1.6µm PS C18 (starting mobile phase = 100 % 0.1 % Formic acid in Water)



App ID24413

Phenomenex is not affiliated with Waters Corp. Comparative separations may not be representative of all applications.

Luna[®] Omega Ordering Information

1.6µm Microbore Columns (mm)			
Phases	50 x 1.0	100 x 1.0	150 x 1.0
Polar C18	00B-4748-A0	00D-4748-A0	00F-4748-A0
C18	00B-4742-A0	00D-4742-A0	00F-4742-A0

1.6µm Minibore Columns (mm)					SecurityGuard [™] ULTRA Cartridges [†]
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	AJO-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJO-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJO-9502
					for 2.1 mm ID

3µm Minibore Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	AJO-7600
PS C18	00A-4758-AN	00B-4758-AN	00D-4758-AN	00F-4758-AN	AJO-7605
					for ID: 2.0 - 3.0 mm

3µm MidBore [™] Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*	
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJO-7600	
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJO-7605	
					for ID: 2.0 - 3.0 mm

3µm 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*
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJO-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJO-7606
					for ID: 3.2-8.0 mm

5µm Minibore Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	AJO-7600
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJO-7605
					for ID: 2.0 - 3.0 mm

5µm MidBore [™] Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*	
Polar C18	00B-4754-Y0	00D-4754-Y0	00F-4754-Y0	AJO-7600	
PS C18	00B-4753-Y0	00D-4753-Y0	00F-4753-Y0	AJO-7605	
					for ID: 2.0 - 3.0 mm

5µm 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*
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJO-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJO-7606
					for ID: 3.2-8.0 mm

5µm Axia [™] Packed Preparative Columns (mm)				SecurityGuard [™] Cartridges (mm)
Phases	150 x 21.2	250 x 21.2	15 x 21.2**	
Polar C18	00F-4754-P0-AX	00G-4754-P0-AX	AJO-7603	
PS C18	00F-4753-P0-AX	00G-4753-P0-AX	AJO-7608	
				for ID: 18-29 mm

5µm Axia [™] Packed Preparative Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	150 x 30	250 x 30	250 x 50	15 x 30.0*	
Polar C18	00F-4754-U0-AX	00G-4754-U0-AX	00G-4754-V0-AX	AJO-7604	
PS C18	00F-4753-U0-AX	00G-4753-U0-AX	00G-4753-V0-AX	AJO-7609	
					for ID: 30-49 mm

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000
^{*} SecurityGuard Analytical Cartridges require holder, Part No.: KJO-4282
^{**} PREP SecurityGuard Cartridges require holder, Part No.: AJO-8223
[◆] PREP SecurityGuard Cartridges require holder, Part No.: AJO-8277

For additional technical notes, visit www.phenomenex.com



If Luna analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

APPLICATION

Australia

t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China

t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark

t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico

t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain

t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

**All other countries
Corporate Office USA** 

t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

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Comparative separations may not be representative of all applications. Phenomenex is not affiliated with Waters Technologies Corporation. Axia column and packing technology is patented by Phenomenex. U.S. Patent No. 7, 674, 383

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6, 162, 362

CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

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