

APPLICATION

Choosing the Right UHPLC Column for Analyzing Highly Polar Basic Molecules



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

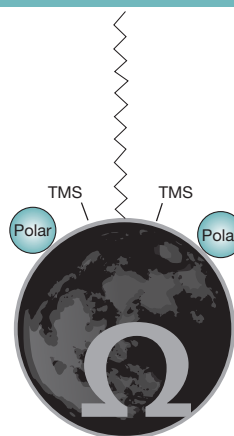
Overview

One of the most common challenges that chromatographers face is achieving acceptable retention for polar molecules under typical reversed phase mobile phase conditions. This problem is most often encountered when working with polar basic analytes, which are ionized under the low pH mobile phase conditions that are so common in reversed phase methods. When developing LC-UV methods, analysts can add various ion-pairing agents, such as hexane sulfonic acid or the like, to dramatically improve the retention of these types of analytes. However, ion-pairing agents are not compatible with mass spectroscopy due to the fact that they are non-volatile and also have strong ion-suppression effects. Thus, they are rarely, if ever, used in LC-MS methods. In addition, even for LC-UV methods, ion-pairing agents can be problematic in terms of method robustness and reproducibility, as the retention times of the analytes can be strongly affected by small variations in the concentration of ion-pairing agent in the mobile phase, and over time these ion-pairing agents can build up within the column itself and lead to gradual drifts in retention as the chemical nature of the sorbent becomes altered by the accumulated ion-pairing agent.

In response to this, many manufacturers have developed unique sorbents designed specifically to provide improved retention and selectivity for highly polar molecules. These types of phases typically contain a polar functional group embedded within the primary alkyl bonded phase ligand itself, or may utilize a polar functional group as an end-capping reagent. Either way, the presence of the polar modification generally provides two benefits relative to a standard alkyl (i.e. C18) bonded phase. First, the presence of the polar modification typically provides stability in 100% aqueous mobile phases. Typical C18 phases are not stable in mobile phases that contain less than 2-3% of organic solvent and will display a catastrophic loss of retention when exposed or stored to 100% buffer conditions. Secondly, the polar modifications often provide the phases with increased retention and/or unique selectivity for highly polar analytes as they can provide a secondary interaction beyond the standard hydrophobic mode of interaction provided by the primary alkyl ligand. The use of a highly aqueous mobile phase combined with the novel secondary interaction can often provide an enhancement in retention for hard-to-retain polar, basic species.

This property is illustrated in the figures inside, which show the separation of a group of highly polar, basic catecholamines (e.g. Log P for metanephrine = -0.68) analyzed using a “typical” sub 2µm C18 column (Waters[®] ACQUITY[®] BEH 1.7µm C18; **Figure 1**) and the same analytes chromatographed using a C18 column with a polar modified surface to increase its polar selectivity and provide stability in 100% aqueous conditions (**Figures 2 and 3**; Luna[®] Omega 1.6µm Polar C18). Mobile phase A was water with 0.1% formic acid, and mobile phase B was acetonitrile with 0.1% formic acid. In **Figures 1 and 2**, the gradient started 3% MP B, conditions suitable for a standard C18 phase. Under these conditions, you can see that the polar modified Luna Omega 1.6µm Polar C18 phase (**Figure 2**) provides a slight increase in retention compared to the standard C18 phase (**Figure 1**; Waters ACQUITY 1.7µm BEH C18). However, when we take advantage of the aqueous stability provided by the Luna Omega Polar surface modification (**Figure 3**), you can see the dramatic increase in retention that is achieved. That improvement in retention can be critical in moving target analytes away from early suppression zones to improve quantitation and sensitivity.

Polar C18

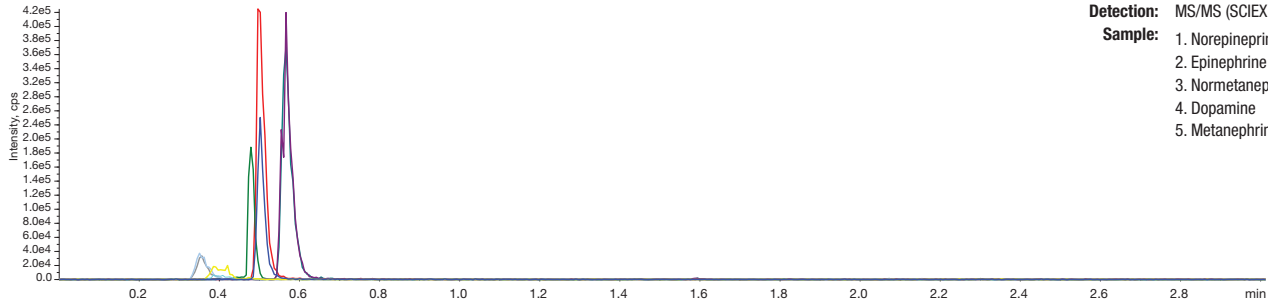


Luna Omega Polar C18

100% aqueous stability and enhanced selectivity/retention for polar analytes without diminishing useful non-polar retention. The C18 ligand provides general hydrophobic interactions while a polar modified particle surface provides enhanced polar compound retention.

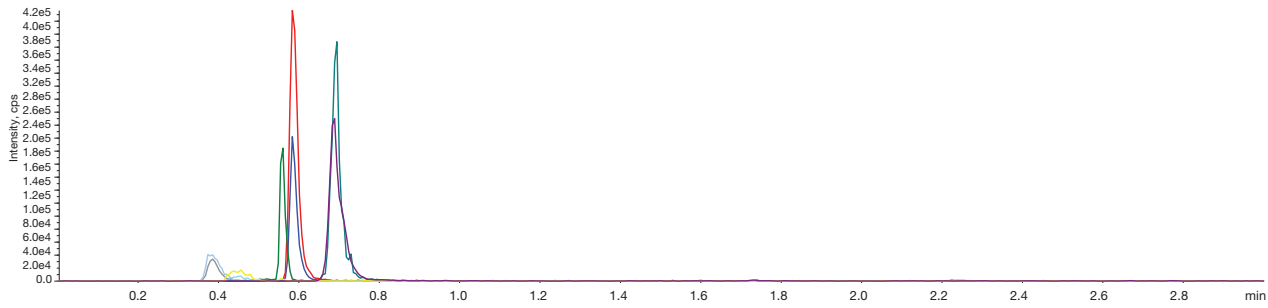


Figure 1.
Waters® ACQUITY® BEH 1.7µm C18



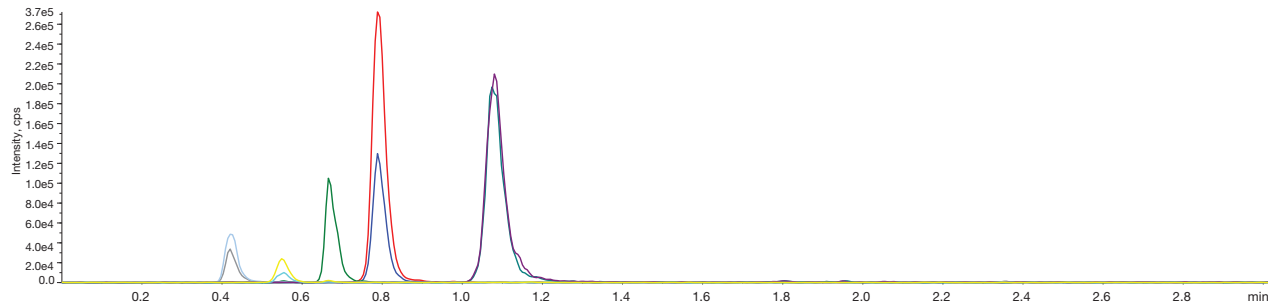
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Figure 2.
Luna® Omega 1.6µm Polar C18



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



App ID24408

Luna® Omega Ordering Information

1.6 µm 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

1.6 µm 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

3 µm 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

3 µm 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

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*
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

5 µm 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

5 µm 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

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*
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

5 µm 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

5 µm 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



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

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@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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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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