Choosing the Right UHPLC Column for Peptide Mapping

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

Overview

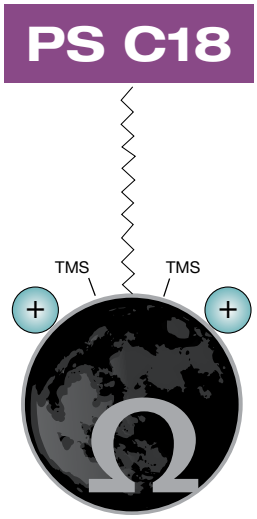
Analysts are increasingly relying upon LC-MS to perform their peptide and protein digest analyses, and TFA is not often used in conjunction with LC-MS due to its strong signal-suppression properties. Thus, when performing peptide analysis by LC-MS using typical mobile phases such as water/acetonitrile/formic acid, secondary interactions between basic amino acids on the peptide and the underlying silica can lead to broad peaks and peak tailing, and can interfere with optimal resolution between close-eluting peaks. To obtain optimal performance for peptide analysis using either LC-UV or LC-MS, the ideal media should be highly efficient (narrow peaks) and highly inert (minimal peak tailing when using MS-compatible modifiers like formic acid). In addition, a more retentive media is often favorable, particularly for peptide mapping, to maximize retention of small, polar peptide fragments that may not have a high affinity for a typical C18 phase, particularly in the absence of TFA (trifluoroacetic acid).

Luna® Omega HPLC and UHPLC products, ranging in size from 1.6 µm for UHPLC work up to 5 µm for conventional HPLC as well as preparative HPLC, are the ideal choice for the analysis of small peptide and tryptic mapping. The high surface area and dense C18 bonding provide Luna Omega with excellent retention for small, more polar peptides and fragments. The thermally modified silica gives Luna Omega an efficiency advantage over similarly-sized fully porous silica products, which can translate into narrower peaks, improved sensitivity, and improved resolution in many cases. This thermal treatment process also renders the silica highly inert for minimal secondary interactions, even when using LC-MS mobile phase modifiers such as 0.1% formic acid. Lastly, the unique Luna Omega PS C18 chemistry contains a proprietary, positively-charged functional group on the surface that makes the surface even less likely to exhibit peak tailing when analyzing basic peptides, and can also provide a selectivity quite distinct from a standard C18 phase, which may be useful for separating a target peptide from a matrix interference that co-elutes on a traditional C18.

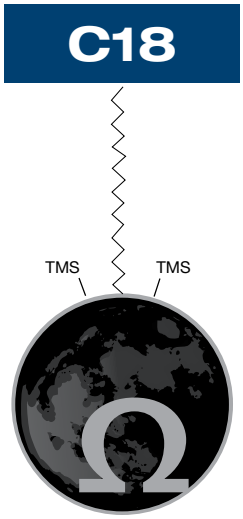
The three figures inside contain representative chromatograms of a tryptic digest of BSA obtained using three different UHPLC columns (Waters® ACQUITY® BEH C18 1.7 µm, **Figure 1**; Luna Omega 1.6 µm C18, **Figure 2**; and Luna Omega 1.6 µm PS C18, **Figure 3**). We do recognize that the use of a 50 x 2.1 mm format for analyzing peptide mapping is not ideal, and one would expect a more conventional format to be 100 x 2.1 mm or 150 x 2.1 mm to obtain maximal information. However, our goal in this comparison is to focus on the comparative differences in chromatographic performance between the three columns, as this information can be expected to scale directly to other column dimensions as well.

As you can see, the "conventional" C18 phases (**Figures 1** and **2**) provide very similar performance for this map under these running conditions. Overall retention times are similar, and the profile of the eluting peaks is very similar as well. One could argue that certain fragments are better resolved on one column versus the other, but overall one could conclude that an analyst should be relatively confident that both columns would be equally successful for this given separation. In contrast, with the unique Luna Omega PS C18 phase (**Figure 3**), we obtain a completely distinct elution profile for the peptide fragments, particularly in the region highlighted in the yellow box. You can see for that region in particular, the Luna Omega PS C18 appears to provide significantly better separation between the peaks, a shift in selectivity that could prove useful in separating a target peptide fragment from isobaric interferences.

Thus, when developing methods for the tryptic mapping using either LC-UV or LC-MS, Luna Omega C18 and Luna Omega PS C18 should be evaluated as part of any thorough method development process, with the Luna Omega C18 column providing a more conventional elution profile and the Luna Omega PS C18 providing a unique and distinct selectivity solution.



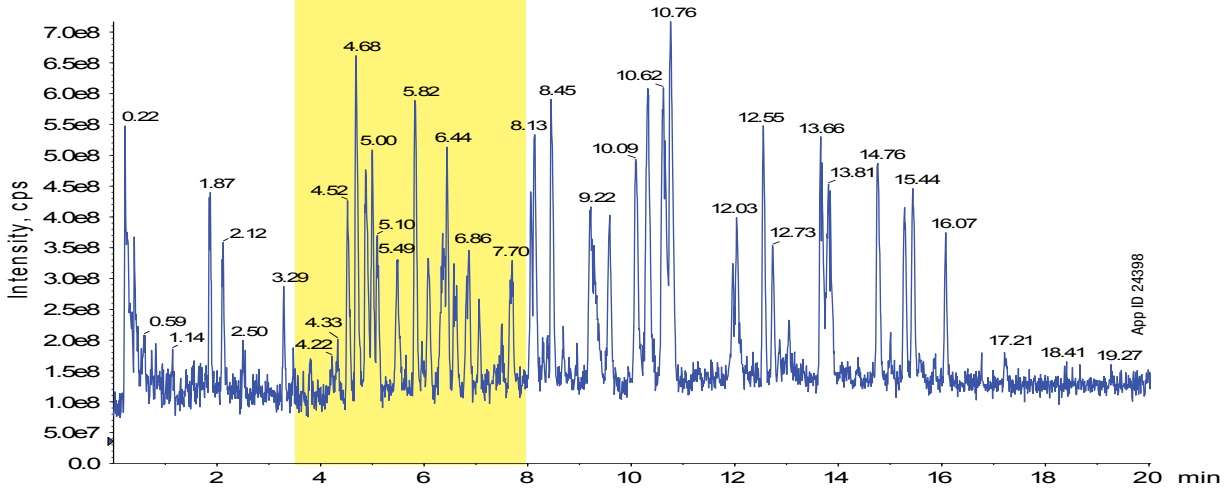
Luna Omega PS C18
Unique, 100% aqueous stable mixed-mode phase that provides both polar and non-polar retention. The surface contains a positive charged ligand which aids in the retention of acidic compounds through ionic interactions, while the C18 ligand promotes general reversed phase retention. The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



Luna Omega C18
Rugged and highly efficient C18 with strong focus on hydrophobic retention of non-polar and polar compounds.

APPLICATIONS

Figure 1
Waters® ACQUITY® BEH 1.7 µm C18; 420 Bar



UHPLC Conditions

Conditions same for all columns

Column:	As specified
Dimensions:	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
	20 / 35
Flow Rate:	0.5 mL/min
Temperature:	40 °C
Injection:	0.5 mL
Detection:	MS/MS (SCIEX API 4000™)
Sample:	BSA Tryptic Digest

Figure 2
Luna® Omega 1.6 µm C18; 413 Bar

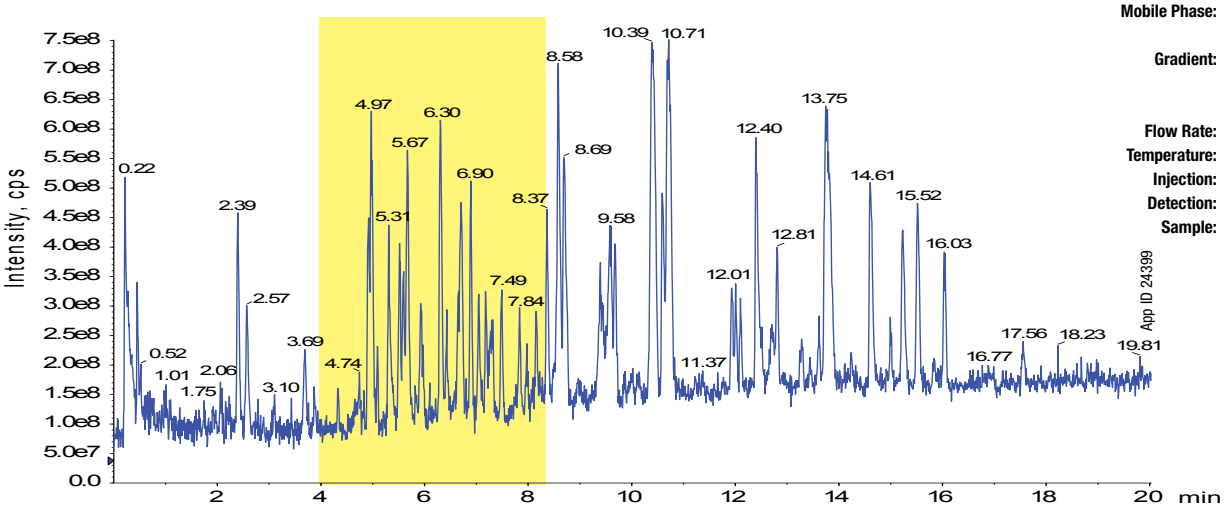
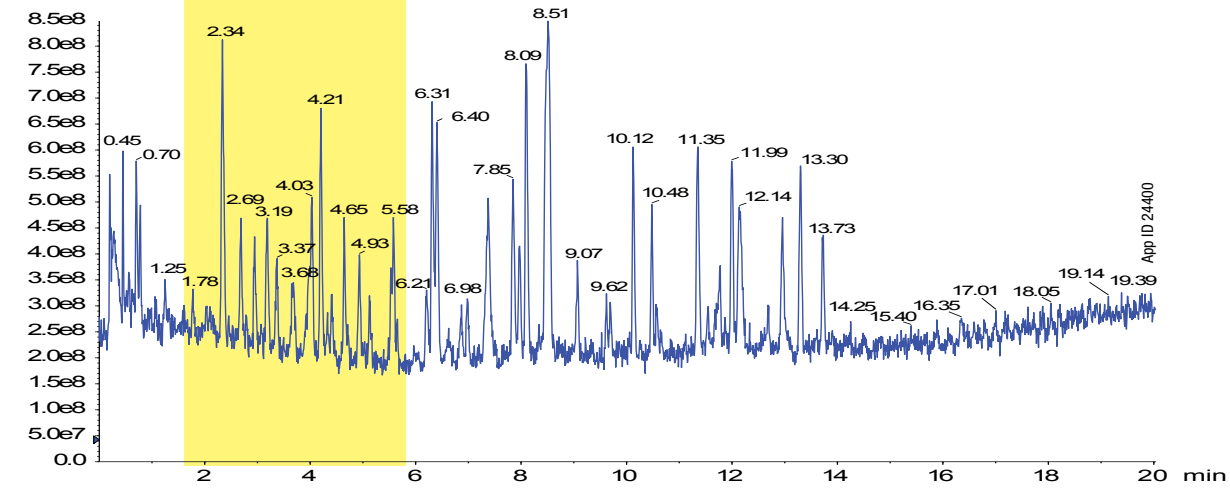


Figure 3
Luna Omega 1.6 µm PS C18; 301 Bar



Unique and distinct elution profile of peptides in this region

APPLICATIONS

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	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

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	AJ0-7600
PS C18	00A-4758-AN	00B-4758-AN	00D-4758-AN	00F-4758-AN	AJ0-7605
				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	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
			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	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
				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	AJ0-7600
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJ0-7605
				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	AJ0-7600
PS C18	00B-4753-Y0	00D-4753-Y0	00F-4753-Y0	AJ0-7605
			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	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
				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	AJ0-7603
PS C18	00F-4753-P0-AX	00G-4753-P0-AX	AJ0-7608
			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	AJ0-7604
PS C18	00F-4753-U0-AX	00G-4753-U0-AX	00G-4753-V0-AX	AJ0-7609
				for ID: 30-49 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
♦ PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277

APPLICATIONS

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

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

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

TN52590617_W