Choosing the Right UHPLC Column for Intact Peptide Analysis

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Overview

For peptide analysis, including both intact peptides and peptide fragments arising from tryptic digests, reversed phase methods using C18 columns, followed by either UV or MS detection, are an integral component of any thorough characterization workflow. Under classical LC-UV method conditions using water/acetonitrile/TFA gradients, secondary silanol interactions are not problematic because TFA (trifluoroacetic acid) prevents strong secondary interactions between basic amino acids and residual silanols on the silica.

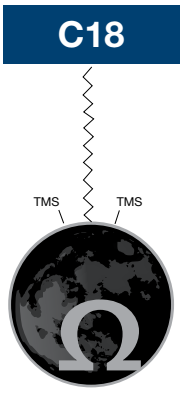
However, analysts are increasingly relying upon LC-MS to perform their peptide analyses, and TFA is rarely used in conjunction with LC-MS due to its strong signal-suppression properties. Thus, when performing peptide analysis by LC-MS using MS-compatible modifiers such as 0.1% formic acid, secondary interaction between basic amino acids in the peptide and the underlying silica can lead to broad peaks, peak tailing, and interfere with resolution between close-eluting peaks. To obtain optimal performance for peptide analysis using LC-MS, the ideal media should be highly efficient (narrow peaks) and highly inert (minimal peak tailing when using MS-compatible modifiers. In addition, a highly 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.

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 (using a proprietary technology) 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 show the analysis of a basic peptide (first large peak; MW = 1046 g/mol) as well as two isobaric interferences (the two smaller peaks). Under these conditions, you can see that both traditional C18 phases (**Figure 1** = Waters® ACQUITY® BEH C18 1.7 µm, **Figure 2** = Luna Omega 1.6 µm C18) provide essentially identical performance, with similar peak widths, peak shape, and resolution of the isobaric interferences from the main peptide. Clearly, either product is well-suited to this analysis, and other factors such as column lifetime, pricing, and customer service and support would need to be factored into a final column decision.

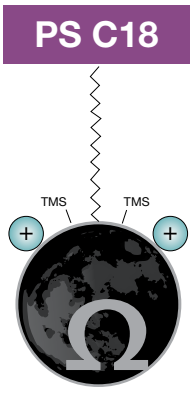
In contrast, when using the Luna Omega 1.6 µm PS C18 column (**Figure 3**), we get a significant improvement in the separation of the two isobaric interferences from the main peptide peak. As the underlying silica is the same as the Omega C18 phase, we must attribute this shift in retention and selectivity to the reduced bonding density of the PS C18 phase and the presence of the proprietary positive surface functional group. Clearly, under these conditions, the Luna Omega PS C18 provides the best performance of the three columns.

When developing methods for the analysis of peptides using either LC-UV or LC-MS conditions, Luna Omega C18 and Luna Omega PS C18 should be evaluated as part of any thorough method development process.



Luna Omega C18

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



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.

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 5 4 95
Flow Rate:	0.5 mL/min
Temperature:	50 °C
Detection:	MS/MS (SCIEX API 4000™)
Sample:	1. Angiotensin II 2. Isobaric Interference 1 3. Isobaric Interference 2

Figure 1.
Waters® ACQUITY® BEH 1.7 µm C18

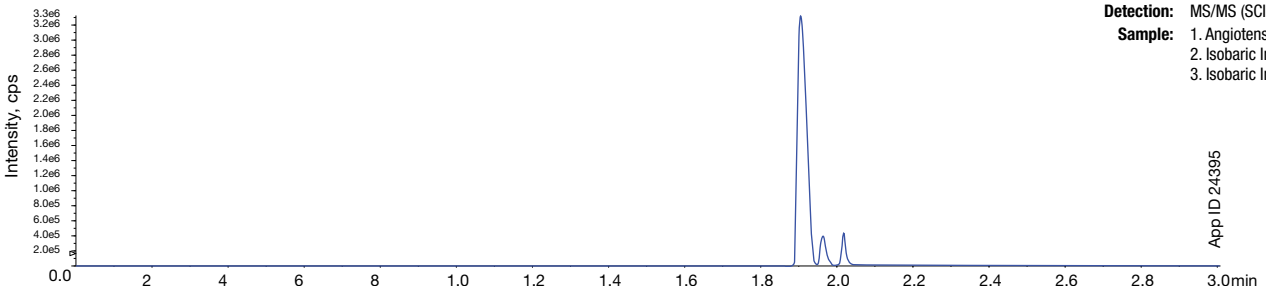


Figure 2.
Luna® Omega 1.6 µm C18

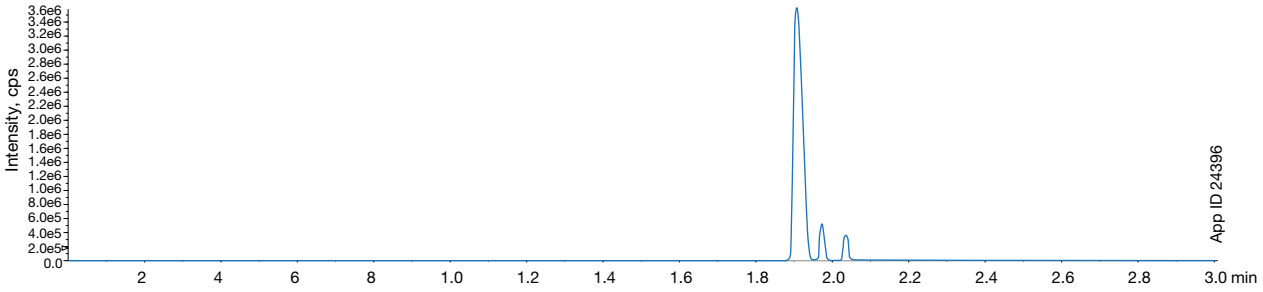
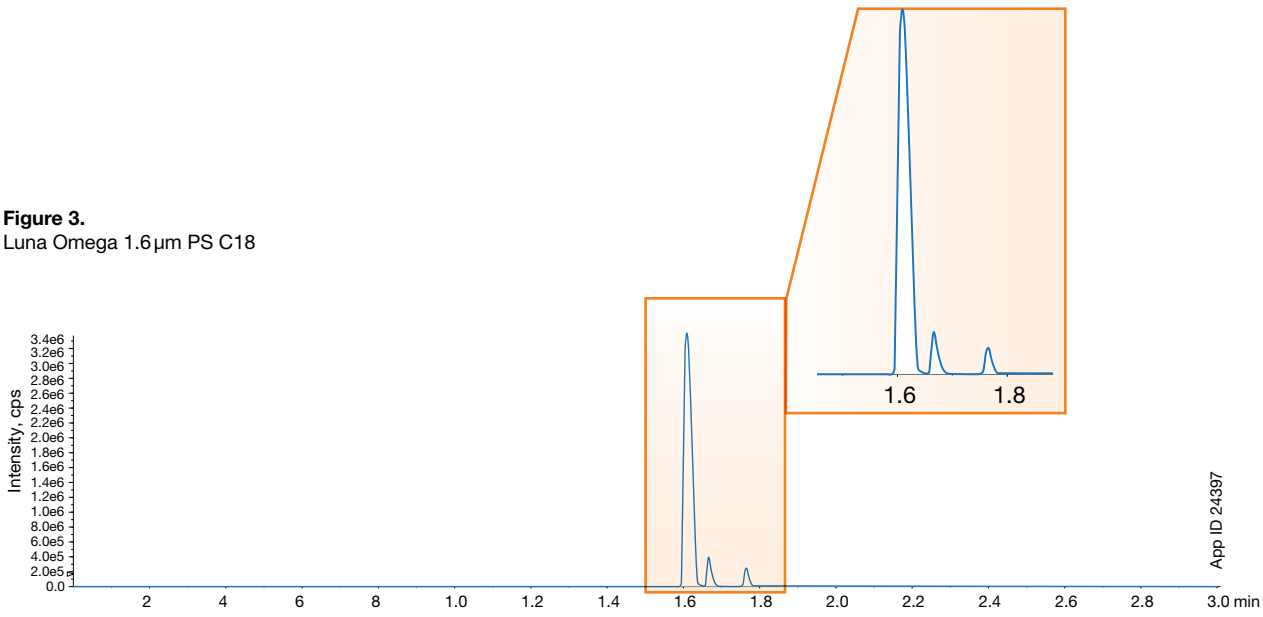


Figure 3.
Luna Omega 1.6 µm PS C18



Phenomenex is not affiliated with Waters Technologies Corporation. Comparative separations may not be representative of all 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

guarantee

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.

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