Guest Author: Dr. Jeff Layne

It seems like more and more companies are shifting their focus towards the development of **biopharmaceuticals** these days. Peptide analysis, whether it be intact therapeutic peptides or peptide fragments arising from enzymatic digestions of larger proteins, is a crucial component in most any biological analysis workflow. Analysts are increasingly relying upon LC-MS to perform their peptide analyses due to its sensitivity and specificity. However, one of the most widely-used mobile phase modifiers, TFA (trifluoroacetic acid), is incompatible (for the most part) with LC-MS due to its strong signal-suppression properties. Although it is fantastic from a chromatographic point of view, due to its ion-pairing capacity and ability to mask unwanted secondary silanol interactions. 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 underlying silica can lead to broad peaks and peak tailing. It can also 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.

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 of **Luna Omega** phases provide 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 column even less likely to exhibit peak tailing when analyzing basic peptides, and can also provide a selectivity quite distinct from a standard C18 phase. This also may be useful for separating a target peptide from a matrix interference that co-elutes on a traditional C18.

The three figures below contain representative chromatograms of a tryptic digest of BSA obtained using three different UHPLC columns (**Figure 1.** Waters® ACQUITY® BEH C18 1.7 µm, **Figure 2.** Luna Omega 1.6 µm C18, **Figure 3**. Luna Omega 1.6 µm PS C18). 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, and this information can be expected to scale directly to other column dimensions as well.

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

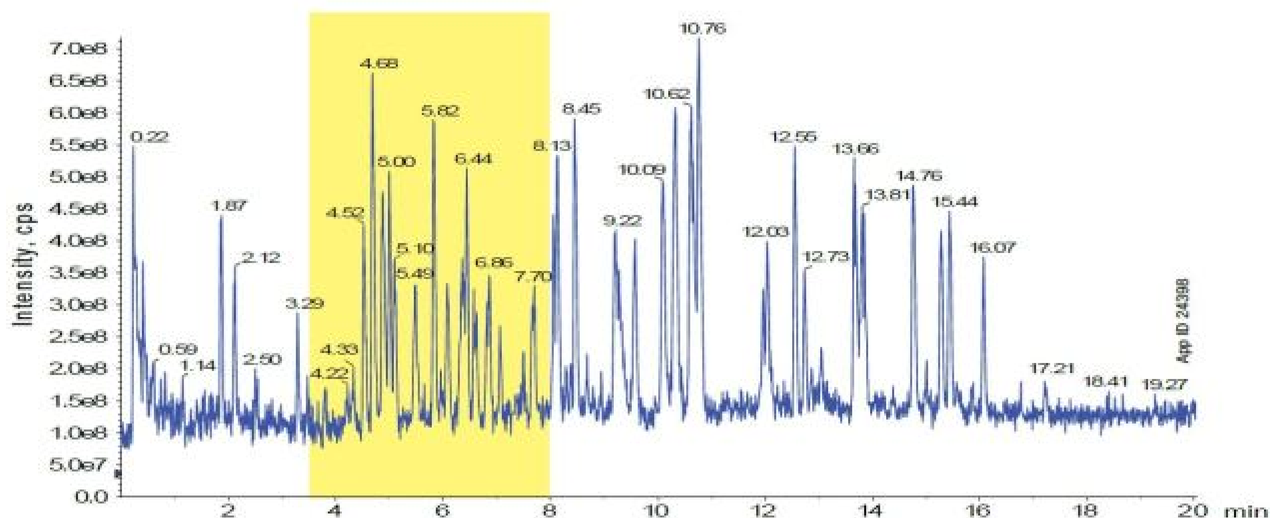


Figure 2
Luna® Omega 1.6 µm C18; 413 Bar

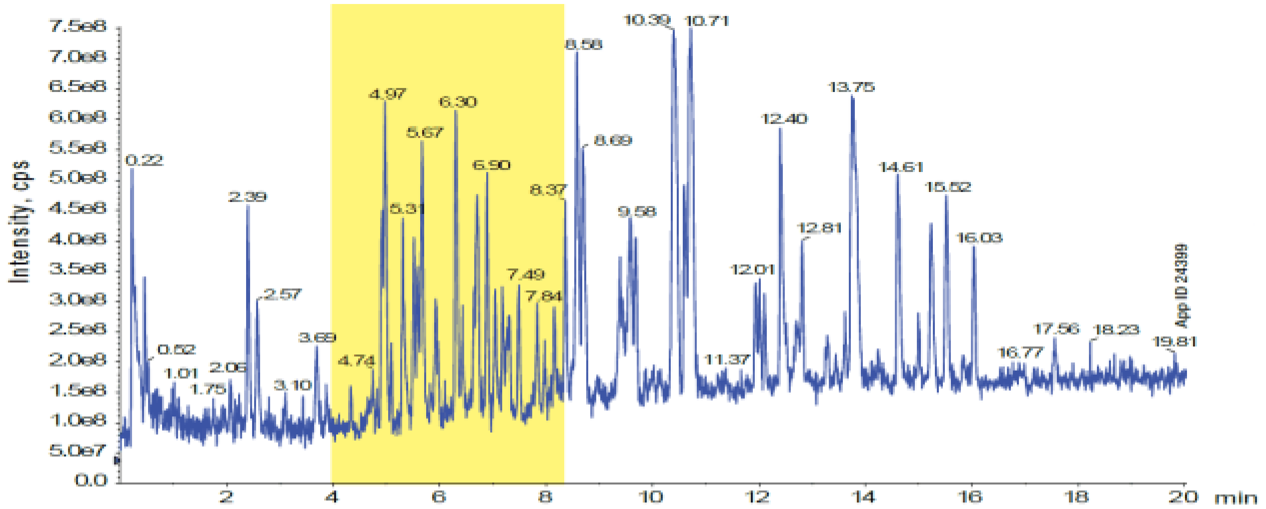
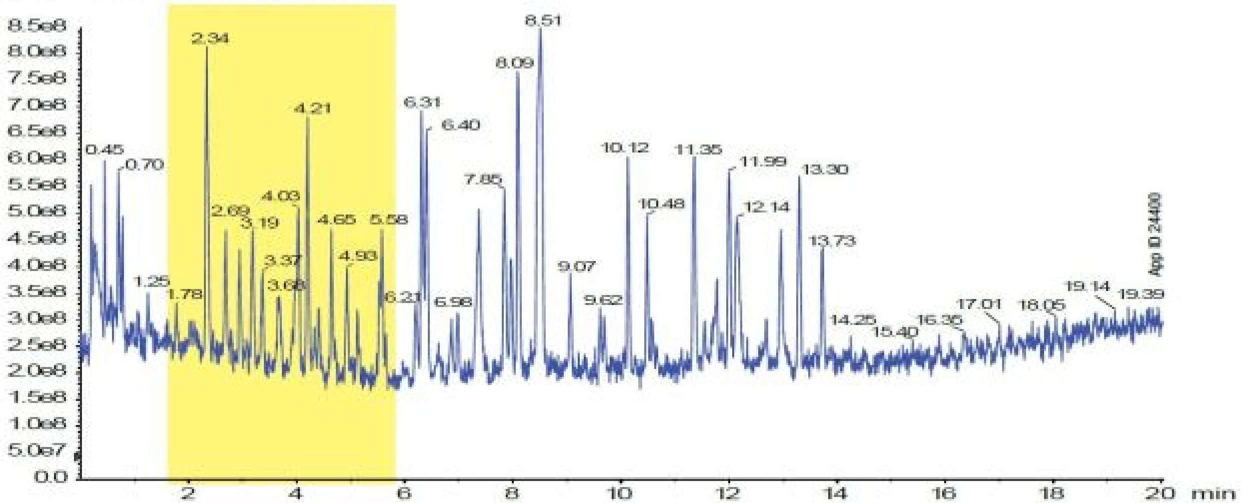


Figure 3
Luna Omega 1.6 µm PS C18; 301 Bar



Unique and distinct elution profile of peptides in this region

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.

Did you find this helpful? Then you might as like, “**Why 100% Aqueous Stability Is Important and Fun**“, “**Selectivity For Polar Acids**“, and “**Can an UHPLC Column Have Too Much Efficiency?**“

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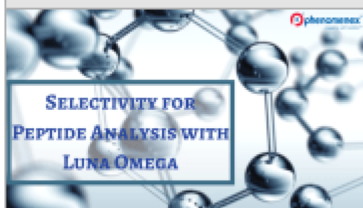
Summary



Article Name

Complementary Selectivity for Peptide Analysis

Description

Peptide analysis (intact therapeutic peptides or peptide fragments from enzymatic digestions) is a crucial in biological analysis workflow.