APPLICATIONS

Recommendations for Transferring Peptide Mapping Methods to LC/MS Using Core-Shell Columns

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With the emergence of new protein therapeutics, including biosimilars, biobetters and ADCs, peptide mapping by LC/MS is becoming more commonly used for the identification of post-translational modifications (PTMs), glycosylation, and conjugation sites, as well as primary sequence confirmation.

Introduction

Because of the complexity of the analysis, the LC column used for this application must provide narrow peak widths, permeability for the largest peptide fragments, moderate hydrophobicity, and high peak capacity that will determine sequence coverage[1].

Traditionally, Trifluoroacetic Acid (TFA) is used as an ion-pairing reagent in peptide maps and is the preferred choice for UV detection. However, because TFA is ion suppressing, it is not preferred for LC/MS applications. Instead, many LC/MS methods use a volatile acid such as 0.1% formic acid. There is also a concern of loss in retention and poor peak shape with peptide mapping methods using low pH in the mobile phase.

The purpose of this study is to provide guidelines for developing optimal LC running conditions with Aeris™ PEPTIDE core-shell HPLC/UHPLC columns and determine the feasibility of transferring a method developed on UV to MS simply by adjusting the acidic modifier in the mobile phase.

Materials and Methods

Phosphorylase B Tryptic Digest Standard was obtained from Waters® (Waltham, MA, USA).

LC/UV Chromatographic Conditions

An Agilent® 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler, and UV-Vis detector was used for initial method development and gradient optimization.

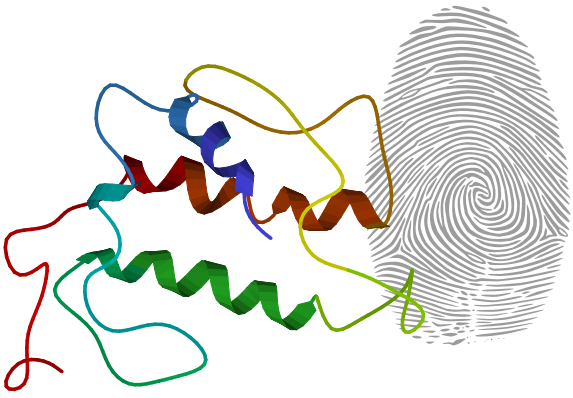
Column:	Aeris 2.6 µm PEPTIDE XB-C18
Dimensions:	150 x 2.1 mm
Part No.:	00F-4505-AN
Mobile Phase:	A: 0.1 % TFA/Acetonitrile (98:2) B: Acetonitrile/Water/TFA (90:10:0.1)
Gradient:	2-70 % B, 68 minutes
Flow Rate:	0.4 mL/min
Temperature:	60 °C
Detection:	UV @ 214 nm
Injection:	50 µL

The gradient initially started at 2% Mobile Phase B to focus the digested peptide fragments to the front of the column. A slope of 1% B/min was used for optimal peak capacity. Although not utilized for this method, multi-step gradients could be used to achieve further resolution, depending on the demands of the method.

LC/MS Chromatographic Conditions

As a proof-of-concept for MS detection, an Advion expression® CMS (Advion, Ithaca, NY) was used, with a Q1 scan range of 175-1200 amu. The ionization source was electrospray ionization (ESI) analyzed in positive ion mode. Mobile phase was modified by replacing the 0.1% TFA with 0.1% formic acid. All other parameters (e.g. flow rate, gradient program) remained the same.

Column:	Aeris 2.6 µm PEPTIDE XB-C18
Dimensions:	150 x 2.1 mm
Part No.:	00F-4505-AN
Mobile Phase:	A: 0.1 % Formic acid/Acetonitrile (98:2) B: Acetonitrile/Water/Formic acid (90:10:0.1)
Gradient:	2-70 % B, 68 minutes
Flow Rate:	0.4 mL/min
Temperature:	60 °C
Detection:	Advion single-quad MS
Injection:	50 µL



Results and Discussion

Because peak capacity correlates to protein sequence coverage, this was the primary parameter for method optimization. **Figure 1** shows good peak capacity using UV-Vis as the detection method, with peak capacity of 524.

It is a common practice to transfer a method from UV to MS detection by simply replacing the ion pair (i.e. TFA) with a volatile acid (i.e. formic acid, FA). Typically, this gives acceptable ionization efficiency for peptide fragments and is most convenient for the analyst. However, running the same method and gradient program with MS-friendly buffers may also lead to a decrease in retention as well as slight broadening of peaks, and this is observed in **Figure 2**. This result is expected since TFA acts as an ion-pair to both improve tailing and peak shape with peptide fragments. However, the method still gave reasonably good peak capacity at 305. Additionally, further gradient optimization could be performed to improve resolution if needed.

Finally, it is worth noting that some studies suggest running ammonium formate at moderate pH to improve both ionization efficiency and peak shapes for peptide maps[2], though further experiments would need to be adjusted to optimize MS parameters such as ion-source voltage, which would be effected by the change in mobile phase.

Figure 1.
Peptide Map of Phosphorylate B Standard, UV Detection (214 nm)

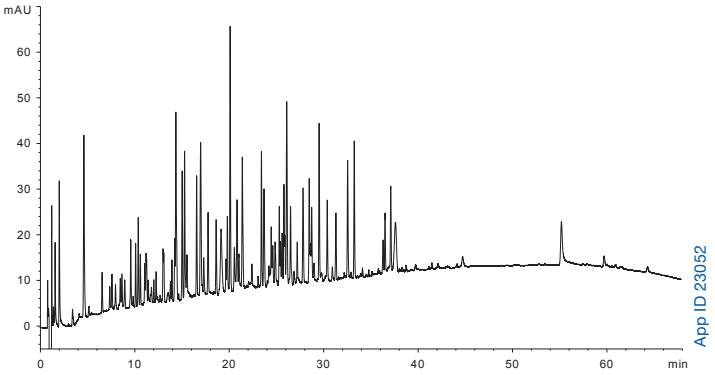
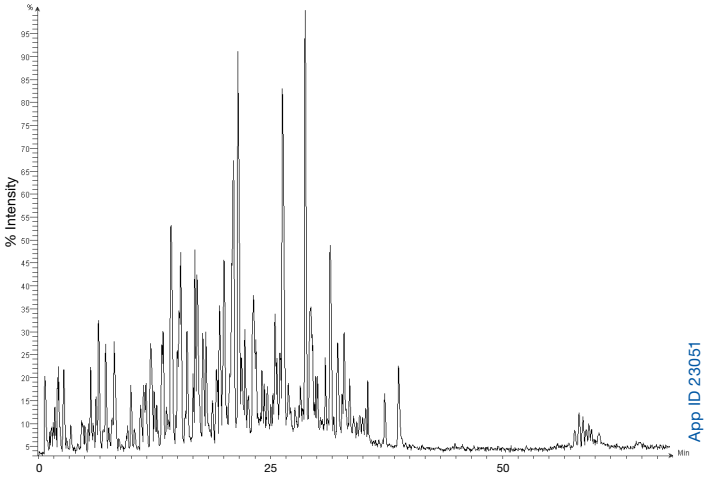


Figure 2.
Peptide Map of Phosphorylate B Standard, MS Detection



Conclusion

Aeris™ PEPTIDE is a core-shell HPLC/UHPLC column with high selectivity for peptide fragments and narrow peak widths. Because of its core-shell particle morphology, which provides a different selectivity than traditional fully porous C18 columns, special considerations need to be taken into account for LC/MS applications.

In this study, we demonstrated that Aeris PEPTIDE can be used to develop peptide mapping methods with high peak capacity, for both UV and MS applications.

References

1. Fairchild JN, Walworth MJ, Horváth K, Guiochon G. Correlation between peak capacity and protein sequence coverage in proteomics analysis by liquid chromatography-mass spectrometry/mass spectrometry. Journal of Chromatography A (2010);1217:4779–4783

2. Yang, Yuanzhong, Reinhard I. Boysen, Simon J. Harris, and Milton T.W. Hearn. "Peptide Mapping with Mobile Phases of Intermediate PH Value Using Capillary Reversed-phase High-performance Liquid Chromatography/electrospray Ionisation Tandem Mass Spectrometry." Journal of Chromatography A (2009): 3767-773.

Ordering Information
Aeris™ Core-Shell HPLC/UHPLC Columns

Aeris PEPTIDE 1.7 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges*
Phase	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
XB-C18	00B-4506-AN	00D-4506-AN	00F-4506-AN	AJ0-8948
				for 2.1 mm ID

Aeris PEPTIDE 2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges*
Phase	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	3/pk
XB-C18	00B-4505-AN	00D-4505-AN	00F-4505-AN	00G-4505-AN	AJ0-8948
					for 2.1 mm ID

Aeris PEPTIDE 2.6 µm MidBore™ and Analytical Columns (mm)				SecurityGuard ULTRA Cartridges*	
Phases	150 x 3.0	150 x 4.6	250 x 4.6	3/pk	3/pk
XB-C18	00F-4505-Y0	00F-4505-E0	00G-4505-E0	AJ0-8947	AJ0-8946
				for 3.0 mm ID	for 4.6 mm ID

Aeris PEPTIDE 3.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges*
Phases	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	3/pk
XB-C18	00B-4507-AN	00D-4507-AN	00F-4507-AN	00G-4507-AN	AJ0-8948
					for 2.1 mm ID

Aeris PEPTIDE 3.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges*
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00B-4507-E0	00D-4507-E0	00F-4507-E0	00G-4507-E0	AJ0-8946
					for 4.6 mm ID

Aeris PEPTIDE 5 µm Analytical Columns (mm)			SecurityGuard ULTRA Cartridges*
Phase	150 x 4.6	250 x 4.6	3/pk
XB-C18	00F-4632-E0	00G-4632-E0	AJ0-8946
			for 4.6 mm ID

Aeris WIDEPORE 3.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges*
Phase	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	3/pk
XB-C18	00B-4482-AN	00D-4482-AN	00F-4482-AN	00G-4482-AN	AJ0-8783
XB-C8	00B-4481-AN	00D-4481-AN	00F-4481-AN	00G-4481-AN	AJ0-8785
C4	00B-4486-AN	00D-4486-AN	00F-4486-AN	00G-4486-AN	AJ0-8899
					for 2.1 mm ID

Aeris WIDEPORE 3.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges*
Phases	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00D-4482-E0	00F-4482-E0	00G-4482-E0	AJ0-8769
XB-C8	00D-4481-E0	00F-4481-E0	00G-4481-E0	AJ0-8771
C4	00D-4486-E0	00F-4486-E0	00G-4486-E0	AJ0-8901
				for 4.6 mm ID

* SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

guarantee

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

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