APPLICATIONS

Complementary Selectivity of bioZen™ XB-C18 1.7 µm Peptide & bioZen™ PS-C18 1.6 µm Peptide Column for Improved Peptide Mapping

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Overview

Sequence confirmation is an important "conforms to standard" criteria for testing. Monoclonal antibodies are tryptically digested to its smaller, peptidic substituents to more easily see the sequence. A key component of sequence variant analysis is sequence coverage. Though enzymatic digestion such as with trypsin can allow us to see more predictable peptide pieces, there are still some challenging groups of peptide pieces for analysis. Smaller, peptide pieces, for example, can be difficult to retain. Larger peptide pieces on the other hand retains well but can be challenging when it comes to ionization when using MS detection. This tech note demonstrates the use of modified C18 phases as complementary selectivities for the better retention of typically early eluding pieces and good separation of later eluding peptide pieces under mass spectrometry conditions.

Though both bioZen Peptide PS C18 and XB-C18 are octadecylsilyl phases, surface chemistry modifications on each gives distinctly different selectivities. This difference is apparent in peptide mapping under LC-MS conditions with formic acid. **Figure 1** shows comparative chromatograms of bioZen peptide XB-C18 vs bioZen peptide PS-C18. While both shows excellent sequence coverage at 100 %, XB-C18 was found to be more retentive for early eluding peptide pieces, providing for improved quantitation. bioZen Peptide PS C18 also gave overall comparatively better peak shape of later eluding peaks under formic acid conditions.

The unique selectivity of the PS C18 column under formic acid conditions can also be seen when comparing with another modified C18 column. **Figure 2** compares a bioZen Peptide PS C18 column to another C18 column. Under the same conditions, on the other column, 103 unique peptides were identified and on the PS-C18 column, 137 unique peptides were identified. The greater sequence coverage is due in large part to improved peak shapes and better ionization of later eluding pieces with bioZen Peptide PS C18.

In summary, though both bioZen Peptide XB-C18 and bioZen Peptide PS C18 are both excellent options for sequence variant analysis, they can be used complementarily to gain improved sequence coverage for optimal quantitation.

Materials and Methods

The instrument that was used was an Agilent 1290 with a SCIEX® X500B Q-TOF. Trastuzumab and infliximab were purchased from Myoderm (Norristown, PA, USA). Acetonitrile was obtained from Honeywell. All reagents were obtained from Sigma Aldrich (St Louis, MO) and MilliQ Water was used.

Trastuzumab and infliximab digests were speedvac'ed to dryness and resuspended to 0.1 % formic acid in water.

Results

Figure 1.
TICs for infliximab, XB-C18 vs PS-C18

LC-UV conditions

Column 1:	bioZen 2.6 µm Peptide XB-C18
Part No.:	00F-4768-AN (XB-C18)
Column 2:	bioZen 1.6 µm Peptide PS-C18
Part No.:	00F-4770-AN (PS-C18)
Mobile Phase:	A. 0.1 % Formic Acid in Water B. 0.1 % Formic Acid in Acetonitrile
Gradient:	1-50 % B in 50 minutes
Flow-rate:	0.3 mL/min
Detection:	SCIEX® X500B Q-TOF
Sample:	Infliximab

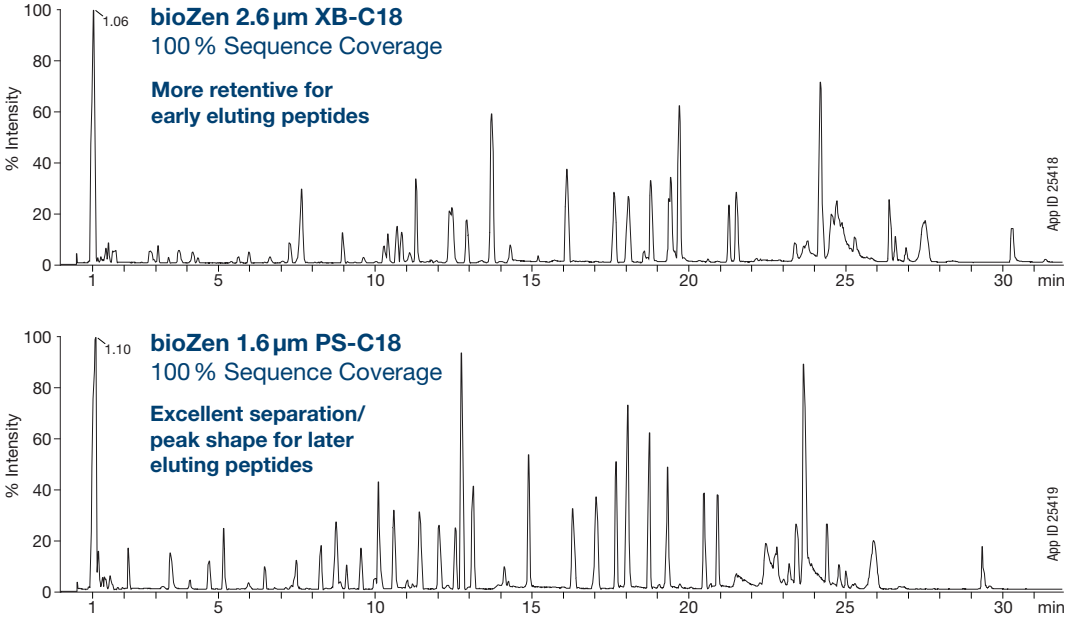
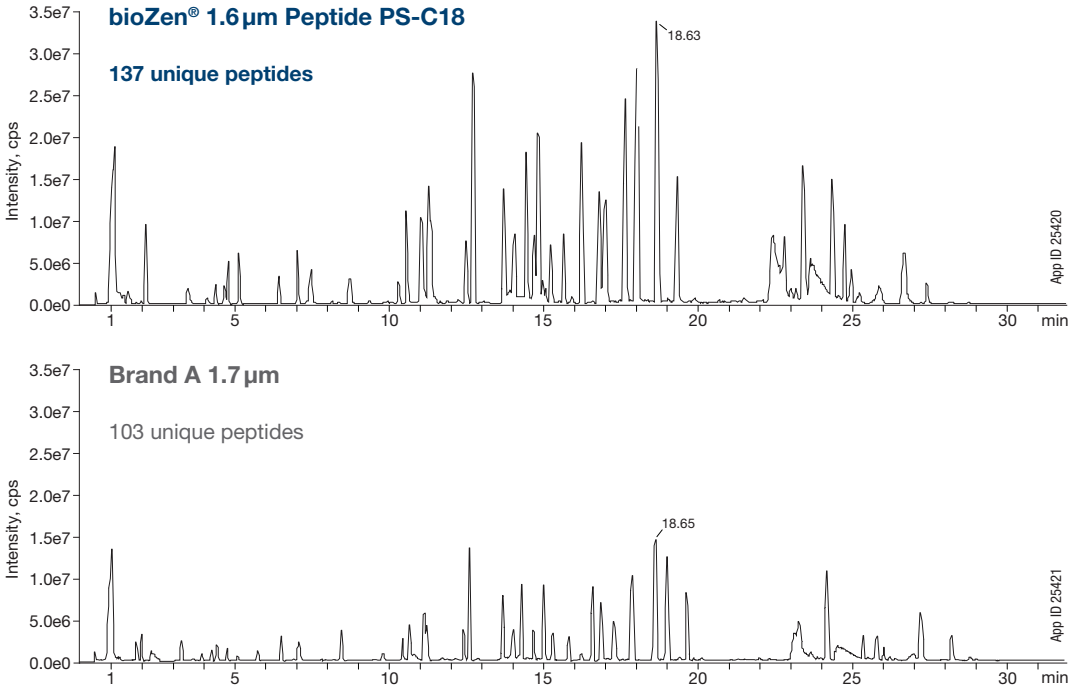


Figure 2.
TICs for Trastuzumab, PS-C18 vs sub 2 µm C18

LC-UV conditions

Column 1: bioZen 1.6 µm Peptide PS-C18
Part No.: 00F-4770-AN (PS-C18)
Column 2: Brand A 1.7 µm
Mobile Phase: A. 0.1 % Formic Acid in Water
B. 0.1 % Formic Acid in Acetonitrile
Gradient: 1-50 % B in 50 minutes
Flow-rate: 0.3 mL/min
Detection: SCIEX® X500B Q-TOF
Sample: Trastuzumab Digest, 1 µg



Comparative separations may not be representative of all applications.

bioZen Ordering Information

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
				—	—	/3pk	—	ea
bioZen 2.6 µm Glycan	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	AJ0-9800	—	AJ0-9000
				—	—	/3pk	—	ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJ0-9803	—	AJ0-9000
		—				/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJ0-7605	AJ0-7606	KJ0-4282
				—	—	/3pk	—	ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJ0-9806	—	AJ0-9000
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJ0-9806	AJ0-9808	AJ0-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJ0-9812	AJ0-9814	AJ0-9000
	50 x 4.6	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**300 x 4.6**		**for 4.6 mm**	**Holder**
	—			—		—	/3pk	ea
bioZen 1.8 µm SEC-2	—	—	00F-4769-E0	—	00H-4769-E0	—	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
					—	—	/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJ0-9400	KJ0-4282

Sample Preparation Ordering Information

Format	bioZen Solid Phase Extraction	Sorbent Mass	Part Number	Unit
Microelution 96-Well Plate				
	bioZen N-Glycan Clean-Up	5 mg/well	8M-S009-NGA	1/box

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