

APPLICATIONS

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

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Overview

Sequence confirmation is an important “conforms to standard” criteria for testing. Monoclonal antibodies are typically 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 eluting pieces and good separation of later eluting 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 show excellent sequence coverage at 100%, XB-C18 was found to be more retentive for early eluting peptide pieces, providing for improved quantitation. bioZen Peptide PS-C18 also gave overall comparatively better peak shape of later eluting 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 a Waters® CSH C18 column. Under the same conditions, on the CSH 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 eluting 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 Milli-Q® 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-MS Conditions

Column 1: bioZen 2.6 μm Peptide XB-C18
Dimensions: 150 x 2.1 mm
Part No.: 00F-4768-AN
Column 2: bioZen 1.6 μm Peptide PS-C18
Part No.: 00F-4770-AN
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 Digest, 1 μg

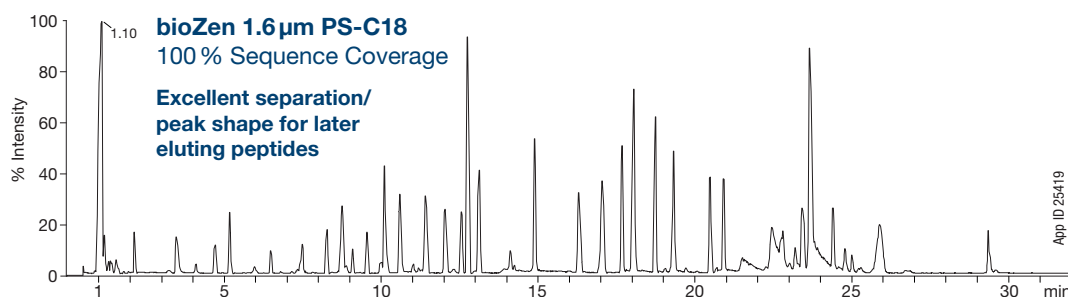
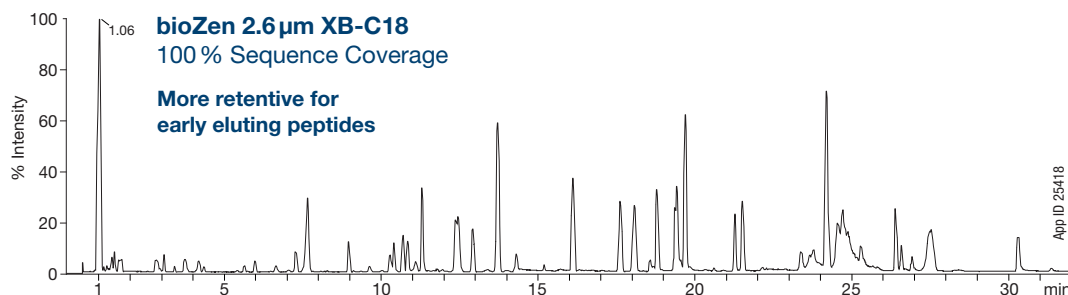
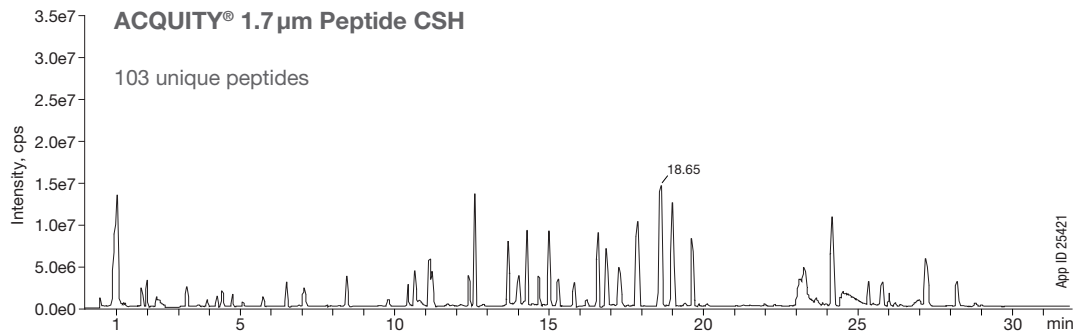
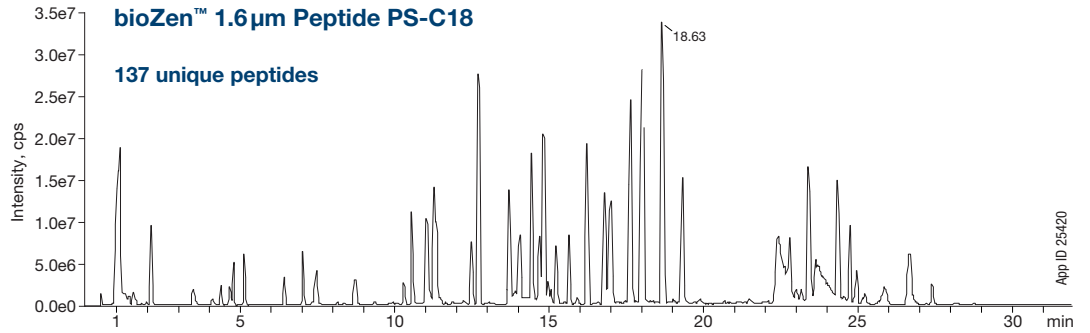


Figure 2.
TICs for Trastuzumab, PS-C18 vs. sub 2µm C18

LC-MS Conditions

Column 1: bioZen 1.6µm Peptide PS-C18
Dimensions: 150 x 2.1 mm
Part No.: 00F-4770-AN
Column 2: ACQUITY 1.7µm Peptide CSH
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 |
| bioZen 2.6µm Glycan | 00B-4773-AN | 00D-4773-AN | 00F-4773-AN | — | — | AJO-9800 /3pk | — | AJO-9000 ea |
| bioZen 1.6µm Peptide PS-C18 | 00B-4770-AN | 00D-4770-AN | 00F-4770-AN | — | — | AJO-9803 /10pk | — | AJO-9000 ea |
| bioZen 3µm Peptide PS-C18 | 00B-4771-AN | — | 00F-4771-AN | 00B-4771-E0 | 00F-4771-E0 | AJO-7605 /3pk | AJO-7606 /10pk | KJO-4282 ea |
| bioZen 1.7µm Peptide XB-C18 | 00B-4774-AN | 00D-4774-AN | 00F-4774-AN | — | — | AJO-9806 /3pk | — | AJO-9000 ea |
| bioZen 2.6µm Peptide XB-C18 | 00B-4768-AN | 00D-4768-AN | 00F-4768-AN | 00B-4768-E0 | 00F-4768-E0 | AJO-9806 /3pk | AJO-9808 /3pk | AJO-9000 ea |
| bioZen 3.6µm Intact C4 | 00B-4767-AN | 00D-4767-AN | 00F-4767-AN | 00B-4767-E0 | 00F-4767-E0 | AJO-9809 /3pk | AJO-9811 /3pk | AJO-9000 ea |
| bioZen 3.6µm Intact XB-C8 | 00B-4766-AN | 00D-4766-AN | 00F-4766-AN | 00B-4766-E0 | 00F-4766-E0 | AJO-9812 /3pk | AJO-9814 /3pk | AJO-9000 ea |
| | 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 |
| bioZen 1.8µm SEC-2 | — | — | 00F-4769-E0 | — | 00H-4769-E0 | — | AJO-9850 /10pk | AJO-9000 ea |
| bioZen 1.8µm SEC-3 | — | 00D-4772-E0 | 00F-4772-E0 | — | 00H-4772-E0 | — | AJO-9851 /10pk | AJO-9000 ea |
| bioZen 6µm WCX | 00B-4777-E0 | 00D-4777-E0 | 00F-4777-E0 | 00G-4777-E0 | — | — | AJO-9400 /10pk | KJO-4282 ea |

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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