APPLICATIONS

Peptide Quantitation with a bioZen™ Peptide PS-C18 Column Following Antibody Enrichment Through Ligand Binding with bioZen MagBeads

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In addition to chromatography, Brian also has a passion for ice cream-making, and enjoys experimenting with bold, new flavors.

Introduction

Biotherapeutics and specifically, monoclonal antibodies (mAbs), have increased in abundance in the drug development process. During development, sensitive, accurate, and high-throughput methods offering high-quality quantitative data for pharmacokinetic, pharmacodynamic, and toxicokinetic studies are important and essential to bring these therapeutic agents to market. Part of the analysis involves separating and quantitating peptides, often at low concentrations, which traditionally has used ligand-binding assays (LBA), such as enzyme-linked immunosorbent assay (ELISA). It is imperative when characterizing a biotherapeutic agent that it can be isolated from other interfering matrix proteins or components, however ELISA and other similar techniques are generally considered lengthy and provide low sensitivity. Due to this and to meet throughput demands in a discovery setting, more laboratories have begun to use magnetic beads in place of ELISA methods for the increased response, lower background, and more accurate results.

In this application, we present a LC-MS method for peptide quantitation which uses bioZen MagBeads Streptavidin Coated paramagnetic beads to capture and isolate biomolecules through a LBA. In this specific technical note, we look at the recovery of signature peptides from Rituximab comparing bioZen MagBeads and other streptavidin magnetic bead products for batch-to-batch reproducibility. Due to the properties of the LC-MS analysis, reproducibility of lot-to-lot recovery is significant when looking at robust peptide quantitation methods.

Materials and Method

Rituximab was purchased from Myoderm® (Norristown, PA). Trypsin was purchased from Promega® Corporation (Madison, WI). SiLuMab and Dulbecco's Phosphate Buffered Saline (DPBS) were purchased from Sigma-Aldrich® (St Louis, MO).

Sample Preparation

MagBead Activation

1. Aliquot 500 µg of bioZen MagBeads slurry for each sample, 20 mg/mL (Part No.: KS0-9533), and wash with 500 µL of DPBS Buffer. Discard excess liquid using a magnetic stand (3x).
2. Reconstitute MagBeads to original volume from step 1 using DPBS.
3. Add 20 µg of biotinylated goat anti-human IgG to the MagBeads for each sample and incubate at room temperature for 1 hour with a shaking speed of 1200 RPM using a deep well plate thermoshaker.
4. Discard excess liquid using a magnetic stand.
5. Wash with 500 µL of DPBS Buffer. Discard excess liquid using a magnetic stand (3x).
6. Reconstitute MagBeads to original volume from step 1 using DPBS.

Immunocapture

1. Add 250 µL of rat plasma samples spiked with 10 µL of internal standard (SiLuMab) to the wells of a low bind 96-well collection plate (Part No.: AH1-7036). Vortex the MagBeads to thoroughly mix and add 25 µL to each well containing sample.
2. Cover plate and spin down at 800 RPM for 3 seconds before incubating at least 2 hours (in this application, we incubated overnight). Discard excess liquid using a magnetic stand.

Washing and Elution

1. Add 300 µL DPBS buffer, cover with film, and vortex to mix. Centrifuge at 400 RPM for 2 minutes and discard using a magnetic stand.
2. Add 300 µL of 10 mM Ammonium bicarbonate and mix using a vortex or pipette. Centrifuge at 400 RPM for 2 minutes and discard using a magnetic stand.
3. Add 30 µL 0.1 % TFA in Water and vortex to mix. If applicable, ensure the pH is lower than 3, cover with polyester sealing tape, and shake the plate or tube at 1200 RPM for 10 minutes using a deep well thermoshaker.
4. Centrifuge at 400 RPM for 2 minutes. Place on a magnetic stand for 5 minutes and collect supernatant. **DO NOT DISCARD THE LIQUID.**

Trypsin Digest

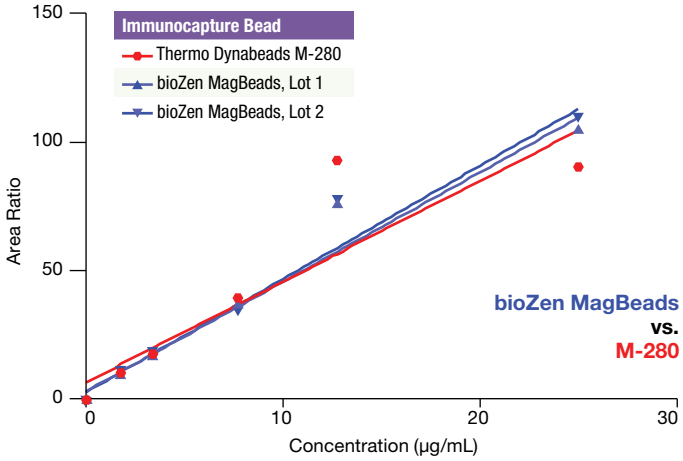
1. Transfer eluent (supernatant) into a new low bind 96-well collection plate (Part No.: AH1-7036) and discard MagBeads.
2. Add 10 µL of 1 M Ammonium bicarbonate and then heat to 95 °C for 5 minutes.
3. Vortex and check pH (should be higher than 7), proceed to cover plate and shake at 300 RPM for 5 minutes at 95°C using a deep well thermoshaker.
4. Set temperature of thermoshaker to 50 °C and allow plate to cool to < 50°C.
5. Add 4 µL of 0.25 µg/µL Trypsin in 0.1% Formic acid to each well.
6. Cover, incubate, and shake plate at 300 RPM for 1 hour at 50 °C using a deep well thermoshaker.
7. Centrifuge plate for 2 minutes at 400 RPM.
8. Place plate on 96-well magnetic stand for 10 minutes.
9. Transfer 140 µL of supernatant to an injection plate.
10. Cover and inject 20 µL of each sample for LC-MS/MS.

Results and Discussion

The correlation coefficient for 2 batches of bioZen™ MagBeads were compared with another magnetic bead product. For all beads, good correlation was seen and the results for the bioZen MagBeads displayed excellent reproducibility lot-to-lot with no evidence of any non-specific binding. Non-specific binding can be observed when the correlation coefficient line fails to pass through the origin of the chart. bioZen MagBeads also displayed excellent reproducibility between the different lots tested (**Figure 1**).

Magnetic Bead	Correlation Coefficient
Thermo® Dynabeads™ M-280	0.9176
bioZen MagBeads Lot 1	0.9914
bioZen MagBeads Lot 2	0.9941

Figure 1.
Correlation Coefficient Chart for Different Magnetic Beads

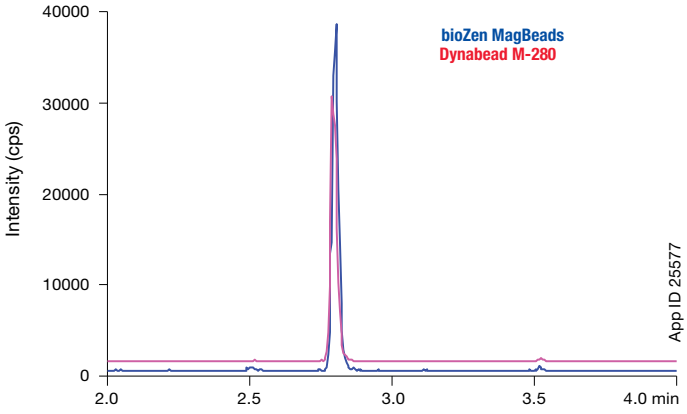


Comparing the recovery of a signature peptide for Rituxumab we found the bioZen Mag Beads offered superior recovery over the Thermo Dynabeads M-280 giving greater accuracy for the peptide quantitation (**Figure 2**).

LC Conditions

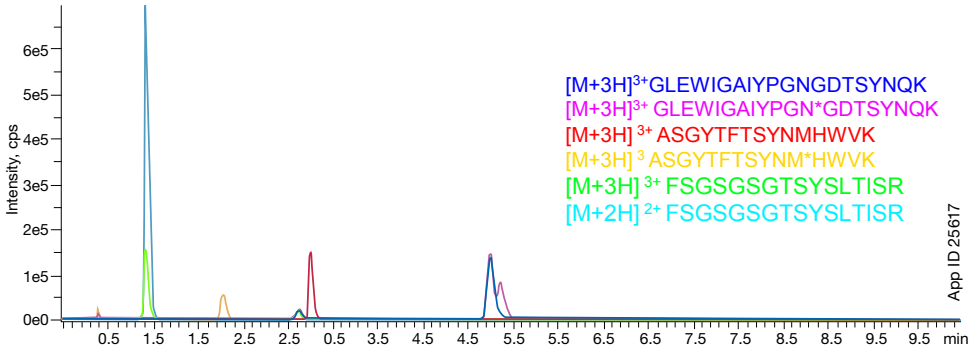
Column: bioZen 3 µm Peptide PS-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4771-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient: 30-50 % B over 4.5 minutes
Flow Rate: 0.3 mL/min
Temperature: 40 °C
Injection Volume: 20 µL
Detection: SCIEX® X500B QTOF

Figure 2.
Comparison of a Signature Peptide of Rituximab (ASGYTFTSYN-MHWVK) using bioZen 3 µm Peptide PS-C18



By switching to the bioZen 2.6 µm Peptide XB-C18 we were able to quantify 6 signature peptides from the digestion of Rituximab with excellent recovery and sensitivity for all peptides using only a 50 mm bioZen Peptide column. The chromatogram below displays the recoveries seen for a 1 µg injection of digested sample (**Figure 3**).

Figure 3.
XIC of the Signature Peptides of Rituximab on a bioZen Peptide XB-C18 column



LC Conditions

Column: bioZen 2.6 µm Peptide XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4768-AN
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient: 15-25% B over 4.5 minutes
Flow Rate: 0.3 mL/min
Temperature: 40 °C
Injection: 1 µg
Detection: SCIEX® X500B QTOF

Conclusion

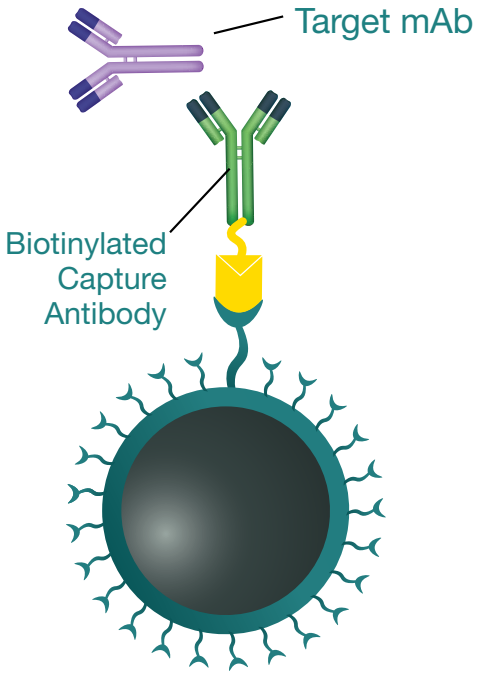
The ability to accurately quantify signature peptides from a mAb selectively isolated from other matrix proteins in biological fluids is essential for analyzing biotherapeutic drugs. Streptavidin coated bioZen™ MagBeads are a flexible sample preparation platform to be used for a fast and effective immunocapture and LBA LC-MS/MS workflows for peptide mapping and biotransformation. In this technical note, the bioZen MagBeads showed good reproducibility and correlation coefficients for different lots. When coupled with bioZen Peptide LC Columns, excellent peak shapes and efficiencies were observed when used for LC-MS of signature peptides, which improves method sensitivity and robustness.

Ordering Information

bioZen Columns (mm)				Biocompatible Guard Cartridges	
	50 x 2.1	100 x 2.1	150 x 2.1	for 2.1 mm	Holder
				3pk	ea
bioZen 1.6µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	AJ0-9803	AJ0-9000
				10pk	ea
bioZen 3µm Peptide PS-C18	00B-4771-AN		00F-4771-AN	AJ0-7605	KJ0-4282
				3pk	ea
bioZen 1.7µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	AJ0-9806	AJ0-9000
				3pk	ea
bioZen 2.6µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	AJ0-9806	AJ0-9000

bioZen MagBeads Streptavidin Coated

Formats	Part No.	Concentration	Bead Size
25 mg (1.25 mL)	KS0-9531	20 mg/mL	1.0 µm
50 mg (2.5 mL)	KS0-9532		
500 mg (25 mL)	KS0-9533		



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