Using Streptavidin Coated bioZen™ MagBeads for Improvements in an Insulin Aspart (Novolog) Immunocapture Workflow

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Overview

In this application, Streptavidin coated bioZen MagBeads are utilized for the fast and accurate immunocapture of insulin aspart (novolog). To properly analyze insulin from plasma, targeted extraction and proper matrix clean-up must occur for reproducible and reliable results. This has traditionally been achieved using an ELISA-based method which can provide narrow LDR and time-consuming method development. However, by combining immunocapture sample preparation with LC-MS/MS, this method demonstrates that it is capable of being multiplexed for higher throughput and lowering background complexity to provide a functionalized assay that yields greater selectivity and sensitivity. Because this method is highly reproducible with low variation between samples, it is ideal for implementation in laboratories.

Materials and Methods

Insulin Aspart (Novolog) was purchased from Myoderm (Part No.: 00169-7501-11) and plasma samples were spiked from 50 pg/mL – 10,000 pg/mL. Bovine Insulin was used as internal standard and was purchased from Sigma-Aldrich® (Part No.: I6634-50MG). Anti-insulin and proinsulin antibody (biotin) was purchased from Abcam® (Part No.: ab20756). Strata® Low-Bind 2 mL 96-Well Collection Plates **(Part No.: AH1-7036)** were used for all magnetic bead processing steps. All other reagents were purchased through VWR International LLC.

Sample Preparation

MagBead Activation

1. Aliquot 25 µL of bioZen MagBeads slurry for each sample, 20 mg/mL (**Part No.: KS0-9532**), and wash with 500 µL of PBS Buffer. Discard excess liquid using a magnetic stand (3x).
2. Reconstitute MagBeads to original volume from step 1 using PBS.
3. Add 5 µg of anti-insulin and proinsulin antibodies 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 PBS Buffer. Discard excess liquid using a magnetic stand (3x).
6. Reconstitute MagBeads to original volume from step 1 using PBS.

Immunocapture

1. Add 250 µL of plasma samples to the wells of a 96-well plate. 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 for 2 hours at 1200 RPM on a thermoshaker.
3. Discard excess liquid using a magnetic stand.

Sample Preparation *(cont'd)*

Washing and Elution

1. Add 200 µL of 0.5% CHAPS in PBS Buffer and mix. Centrifuge at 800 RPM for 3 seconds and discard using magnetic stand.
2. Wash with 200 µL of PBS, mix and shake for 10 minutes with a speed of 1200 RPM using a deep well plate thermoshaker.
3. Centrifuge at 800 RPM for 3 seconds and discard using a magnetic stand for 2 minutes.
4. Add 70 µL of Methanol/Water/Acetic acid (50:48:2), mix and shake for 10 minutes with a speed of 1200 RPM using a deep well plate thermoshaker.
5. Centrifuge at 800 RPM for 3 seconds and place on magnetic stand for 10 minutes. **DO NOT DISCARD THE LIQUID.**
6. Transfer the supernatant to another 96-well plate, add 45 µL of Water and mix.
7. Place on magnetic stand for 10 minutes and transfer supernatant to injection plate.

LC Conditions

Column: Kinetex® 2.6 µm C18
Dimension: 50 x 3.0 mm
Part No.: 00B-4462-Y0
SecurityGuard™ ULTRA: AJ0-8775
Mobile Phase: **A:** 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile

Gradient:

Time (min)	% B
0	10
0.2	10
3.7	45
4	95
4.4	95
4.5	10

Flow Rate: 0.7 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: SCIEX QTRAP® 6500+

MRM Transitions and MS Parameters
Curtain Gas: 40
Collision Gas: High
IonSpray Voltage: 5500
Temperature: 650
Ion Source Gas 1: 65
Ion Source Gas 2: 65

Compound and Internal Standard MRM Parameters

	Q1 (m/z)	Q3 (m/z)	DP (v)	EP (v)	CE (v)	CXP (v)	Dwell Time
Insulin Aspart 1	971.6	1133.2	80	10	32	22	70
Insulin Aspart 2	971.6	1110.5	80	10	35	24	70
Insulin Aspart 3	971.6	661.1	80	10	35	12	70
Bovine Insulin 1	956.6	1115.2	110	10	30	25	70
Bovine Insulin 2	956.6	637	80	10	32	17	70
CHAPs	615.7	412.3	55	10	15	20	70

Figure 1. Calibration Curve from 50 pg/mL-10000 pg/mL

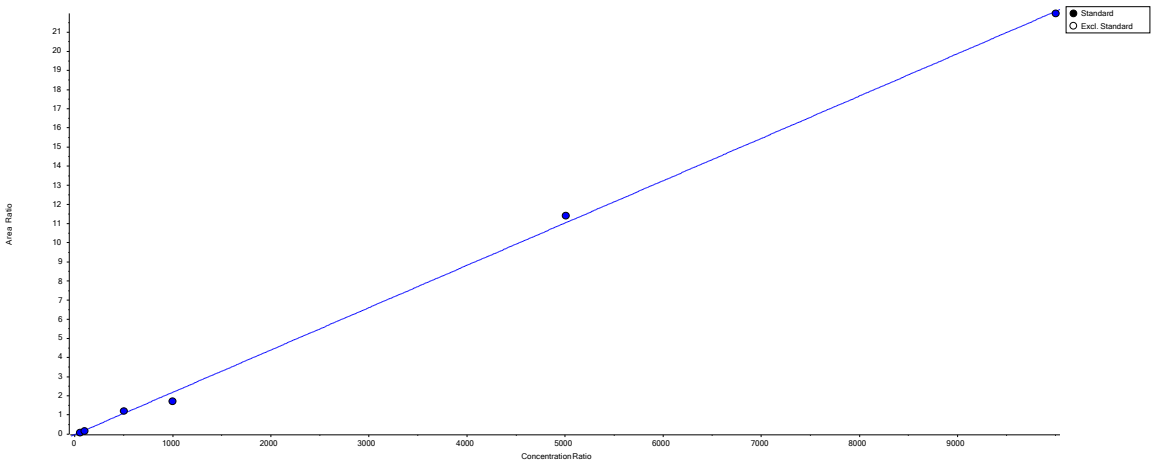


Figure 2. 50 pg/mL Extracted Insulin Aspart Standard Using bioZen™ MagBeads

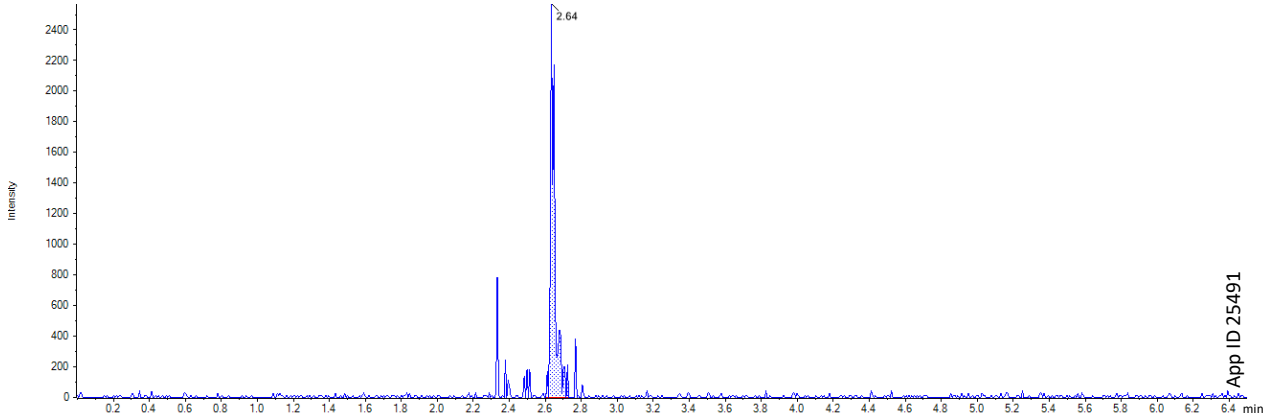
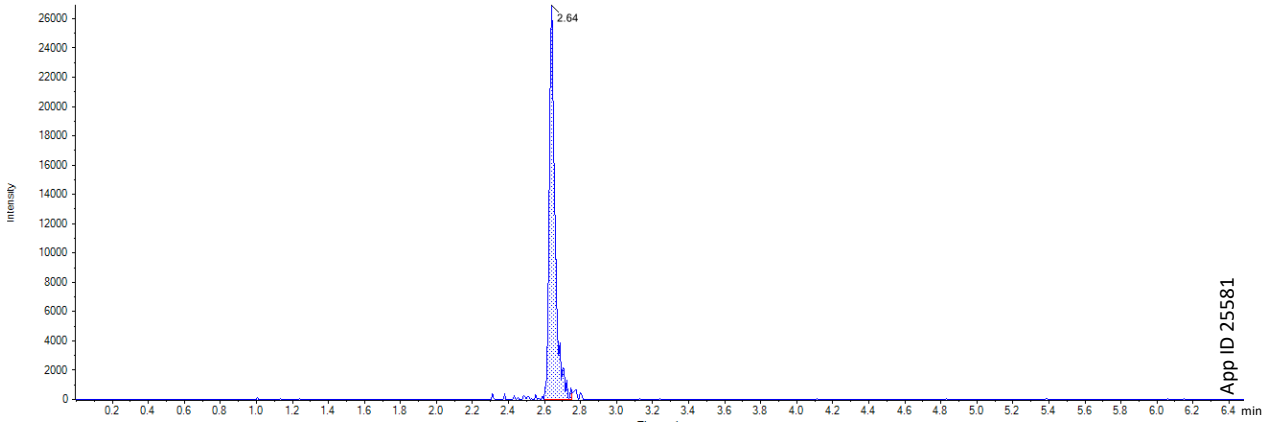


Figure 3: 500 pg/mL Extracted Insulin Aspart Standard Using bioZen MagBeads



Results and Discussion

In this application note, a hybrid ligand binding LC-MS/MS assay was demonstrated that yielded a correlation coefficient of 0.99814 for a LDR from 50-10000 pg/mL **(Figure 1).** Included are representative chromatograms at 50 pg/mL **(Figure 2)** and 500 pg/mL **(Figure 3)**. bioZen MagBeads Streptavidin coated are a beneficial immunocapture addition that uses high binding capacity magnetic beads to isolate and clean-up insulin fast and effectively. While a Kinetex® 2.6 µm C18 LC Column was used in the application, a bioZen Peptide PS-C18 or XB-C18 would provide better chromatography due to the titanium hardware that minimizes the need for priming, reducing unwanted secondary interactions, problematic carryover, and recovery issues between injection to detection.

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