

Dietary Biotin Interference in Hybrid LBA-LC-MS/MS Assays: Characterization, Impact, and Recommendations

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OVERVIEW

PURPOSE

The purpose is to mitigate the impact of free biotin on hybrid LBA-LC-MS/MS bioanalytical assays relying on the biotin-streptavidin interaction.

METHODS

Biotin interference was tested on two prototypical biotherapeutics quantified using hybrid LBA-LC-MS/MS approaches. Both assays used biotinylated antibodies and streptavidin-coated magnetic particles for analyte enrichment. The impact of free biotin on different assay parameters, including assay design, type, and volume of beads used, as well as choice of internal standard were evaluated.

RESULTS

The assay design was critical in mitigating biotin interference. Pre-coupling the beads with the biotinylated antibody was highly tolerant to biotin interference. The incorporation of a SILAC-labeled internal standard could, at least partially, compensate for biotin interference.

INTRODUCTION

Most hybrid LBA-LC-MS/MS assays rely on the interaction between a streptavidin-coated solid support and a biotinylated affinity reagent used for the capture of biotherapeutic agents (Figure 1). In 2017, the FDA warned the scientific community that samples from patients taking massive doses of biotin supplements can significantly interfere with laboratory tests based on the streptavidin-biotin interaction.

In this research, the recombinant hPTH analog Teriparatide (4.1kDa) and the monoclonal antibody Rituximab (145kDa) were used as model compounds to assess biotin interference in hybrid LBA-LC-MS/MS assays. The bioanalytical assay design, the type of streptavidin-coated particles and the internal

standard (IS) used, play a critical role in our evaluation of biotin interference.

Table 1. Examples of dietary sources of biotin. Recommended daily intake of biotin for adults is 30 µg.

| Source | Biotin (µg) |
|---|-------------|
| Beef liver, cooked, 3 ounces | 30.8 |
| Egg, whole, cooked | 10.0 |
| Salmon, pink, canned in water, 3 ounces | 5.0 |
| Centrum Multivitamins | 45.0 |
| Jamieson Multi Adults | 45.0 |
| Jamieson Clear Skin | 150.0 |
| Centrum MultiGummies Multi+Beauty | 2,500.0 |
| Jamieson Hair Skin Nail | 5,000.0 |
| Webber Naturals Extra Strength Biotin | 10,000.0 |

METHODS

BIOTIN INTERFERENCE

Rituximab and Teriparatide plasma samples were fortified with biotin from 0.1 to 50 µg/mL, a much higher level from the FDA-recommended concentration of 1.2 µg/mL for investigating interference from biotin. Fortified samples were analyzed using different hybrid assay coupling approaches to characterize the impact of biotin on biotherapeutics quantitation.

TERIPARATIDE (RECOMBINANT hPTH 1-34)

Sample Volume: 250 µL
 Internal Standard: ValD₆-Teriparatide
 Capture Antibody: Biotinylated anti-hPTH
 Detection: Teriparatide (intact) is monitored by LC-MRM on a SCIEX Triple Quad 5500 mass spectrometer.

RITUXIMAB

Sample Volume: 25 µL
 Internal Standard: Isotopically labeled peptide or SILAC Rituximab. The SILAC Rituximab was supplied by Promise Proteomics.
 Capture Antibody: Biotinylated anti-human IgG
 Detection: Following trypsin digestion, Rituximab signature peptide is monitored by LC-MRM on a SCIEX Triple Quad 5500 mass spectrometer.

MAGNETIC BEADS PROCESSING

Processing of the Streptavidin-coated magnetic beads was automated using the Thermo KingFisher Flex.

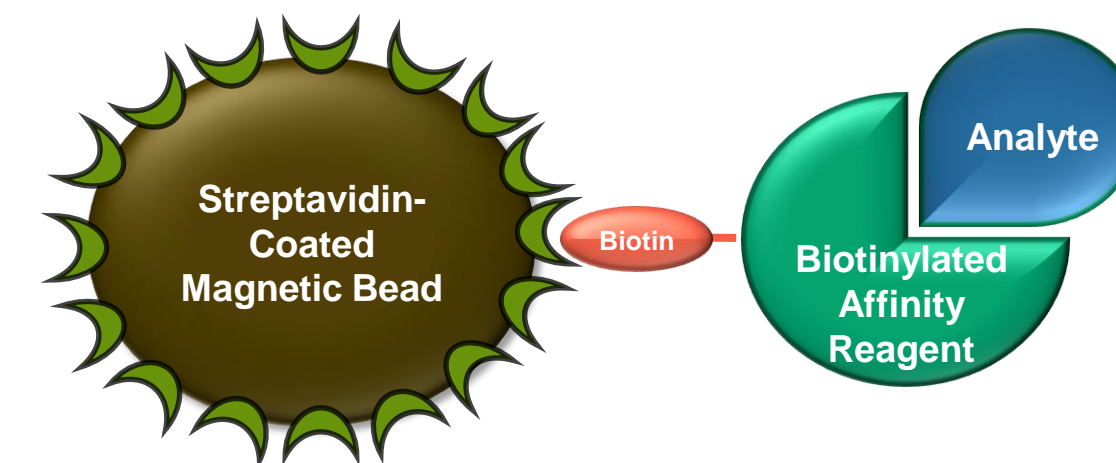


Figure 1. Biotin-streptavidin based hybrid bioanalytical assay.

Table 2. Streptavidin-coated magnetic particles used in this study.

| Brand | Vendor | Format | Capacity/mL |
|---------|---|---------------|-----------------------------|
| Brand A | Promega Streptavidin MagneSphere® | 1mg/ml slurry | 70 µg of biotinylated IgG |
| Brand B | GE Healthcare Streptavidin Mag Sepharose | 10% slurry | >300 µg of biotinylated BSA |
| Brand C | Promega High Capacity Magne® Streptavidin | 20% slurry | ≥1.8 mg of biotinylated IgG |

RESULTS

IMPACT OF ASSAY DESIGN

In-sample coupling and pre-coupling assay designs are summarized in Figure 2. Pre-coupling the beads was highly tolerant to biotin interference, suggesting that once the biotinylated probe is bound to streptavidin, it cannot be displaced by an excess of biotin. On the other hand, performing the coupling reaction in sample, where the free biotin competes with the biotinylated antibody for streptavidin binding, led to a significant loss in recovery (>95%) at elevated biotin concentrations (Figure 3).

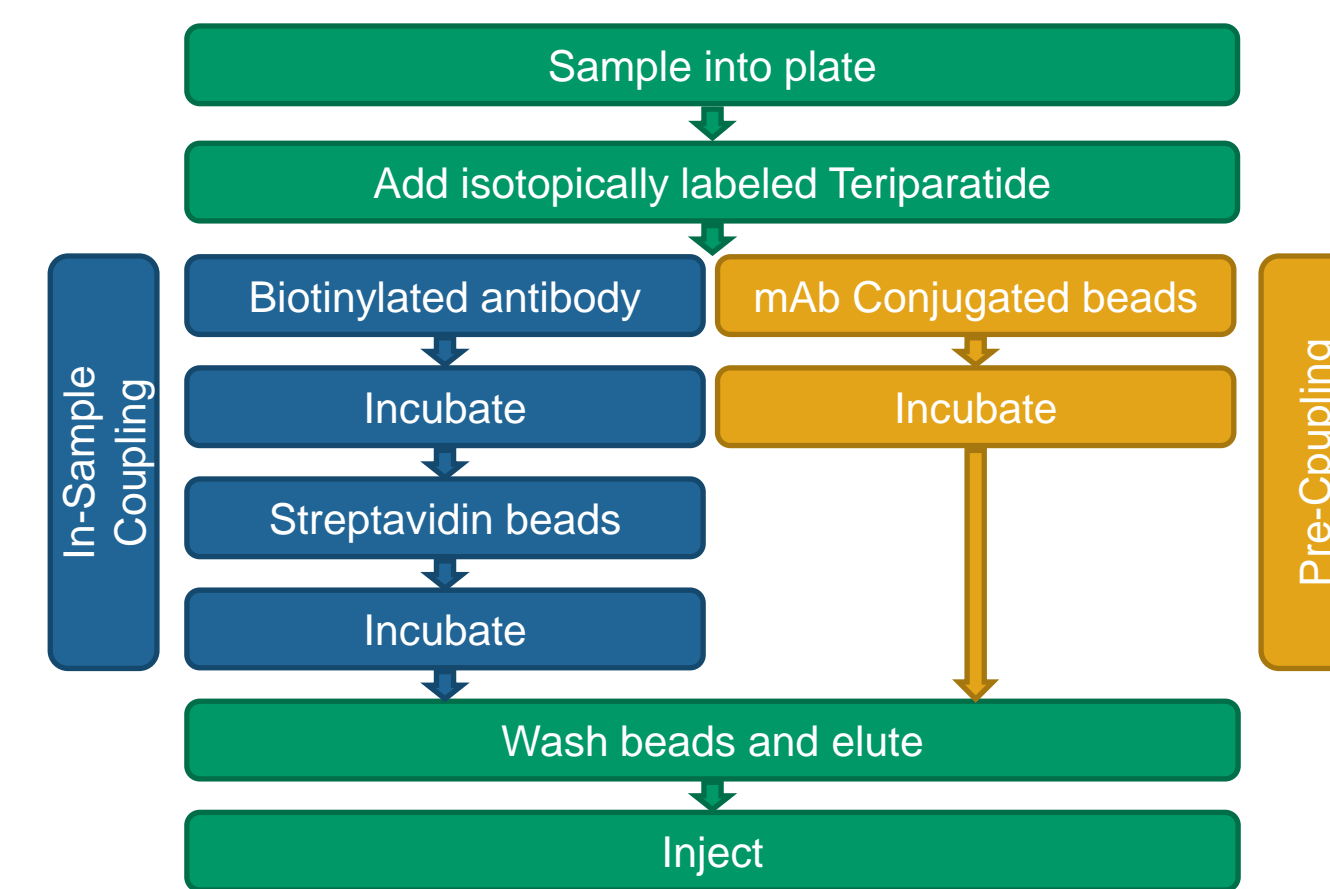


Figure 2. In-sample coupling and pre-coupling bioanalytical assay designs for Teriparatide.

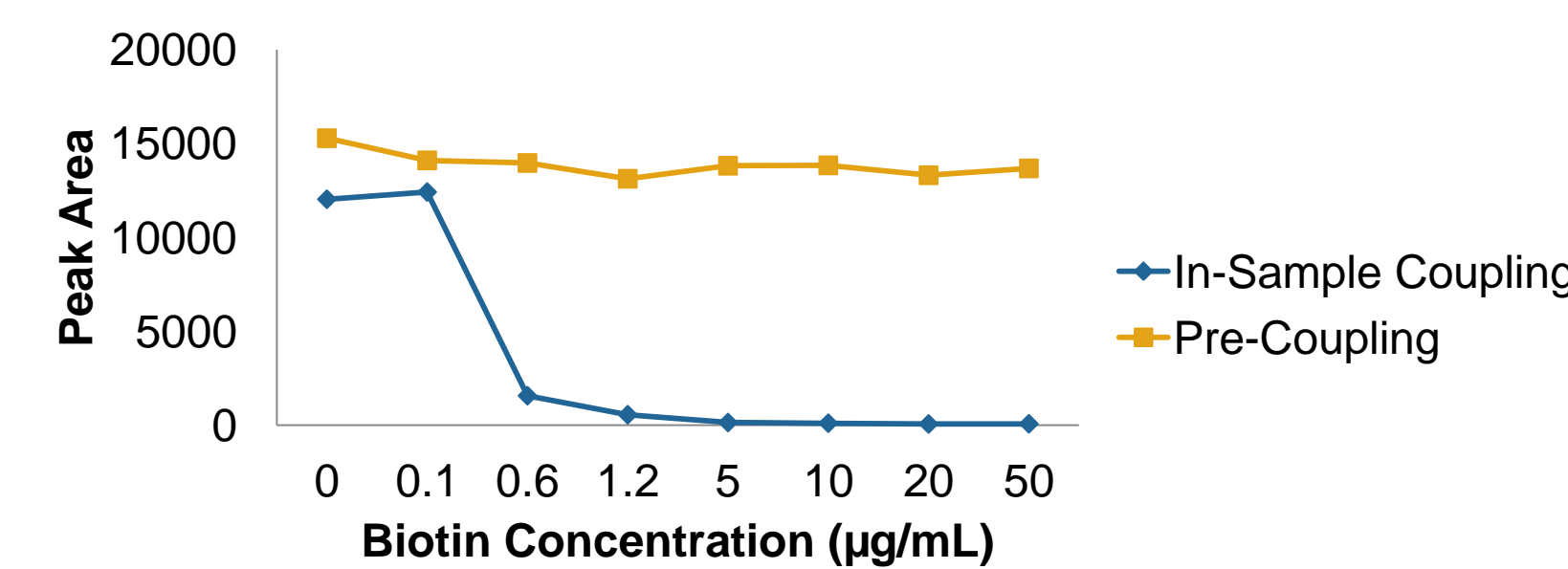


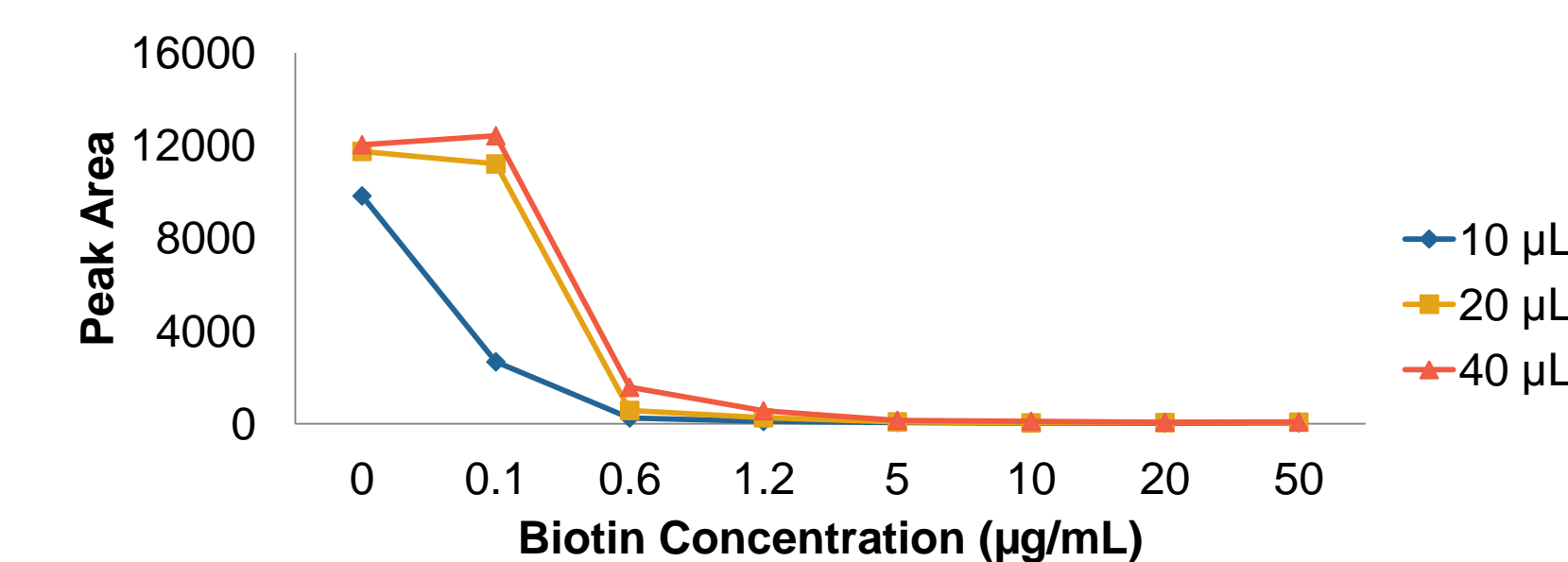
Figure 3. Teriparatide recovery from plasma fortified with biotin from 0.1 to 50 µg/mL. In-sample coupling and pre-coupling assay designs were tested using 40 µL of beads slurry (Brand A) and 1 µg of biotinylated anti-hPTH antibody per sample.

RESULTS (CONT.)

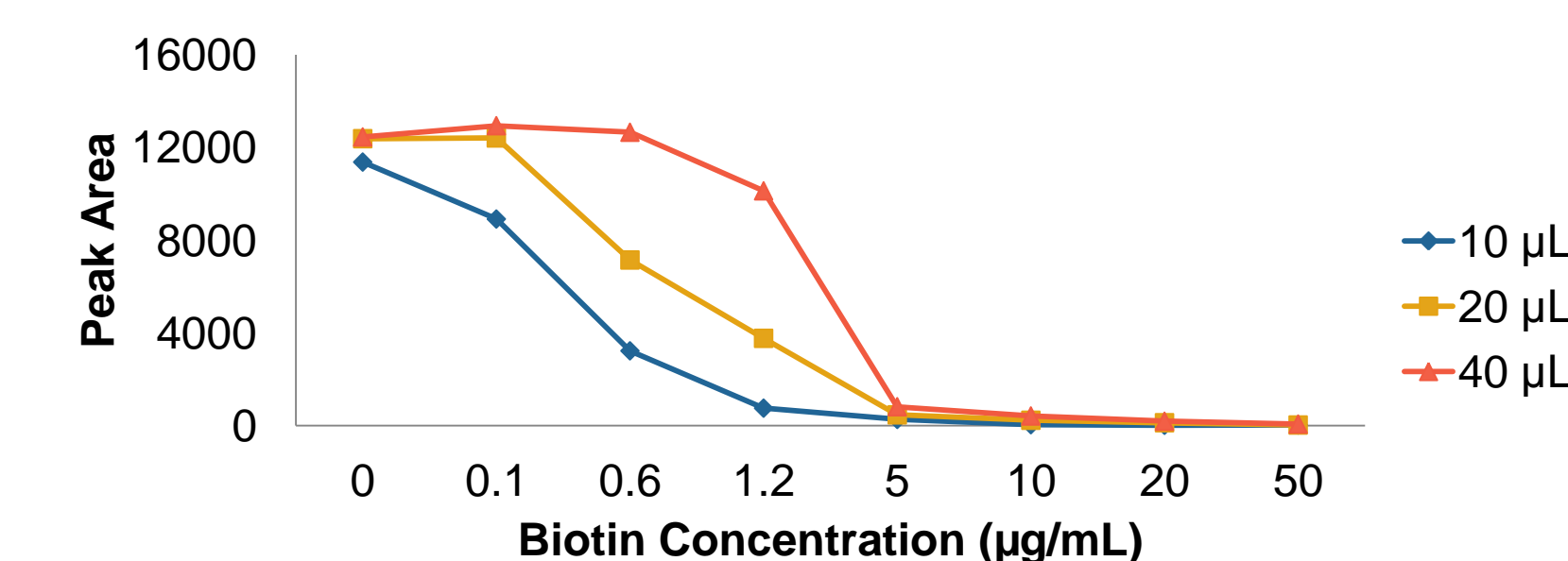
AMOUNT AND BINDING CAPACITY OF BEADS

The binding capacity and number of beads used to improve the biotin tolerance of the in-sample coupling assay was evaluated next. Increasing the number and/or binding capacity of the beads greatly influenced the assay tolerance to biotin, up to 50-fold (Figure 4). Regardless of the type and number of beads, the pre-coupling assay was highly tolerant to biotin competition, confirming that once the biotinylated probe is bound to streptavidin, it cannot be displaced (data not presented).

Brand A (1 mg/mL slurry, capacity 70 µg of biotinylated IgG)



Brand B (10% slurry, capacity 300 µg of biotinylated BSA)



Brand C (20% slurry, capacity ≥1.8 mg of biotinylated IgG)

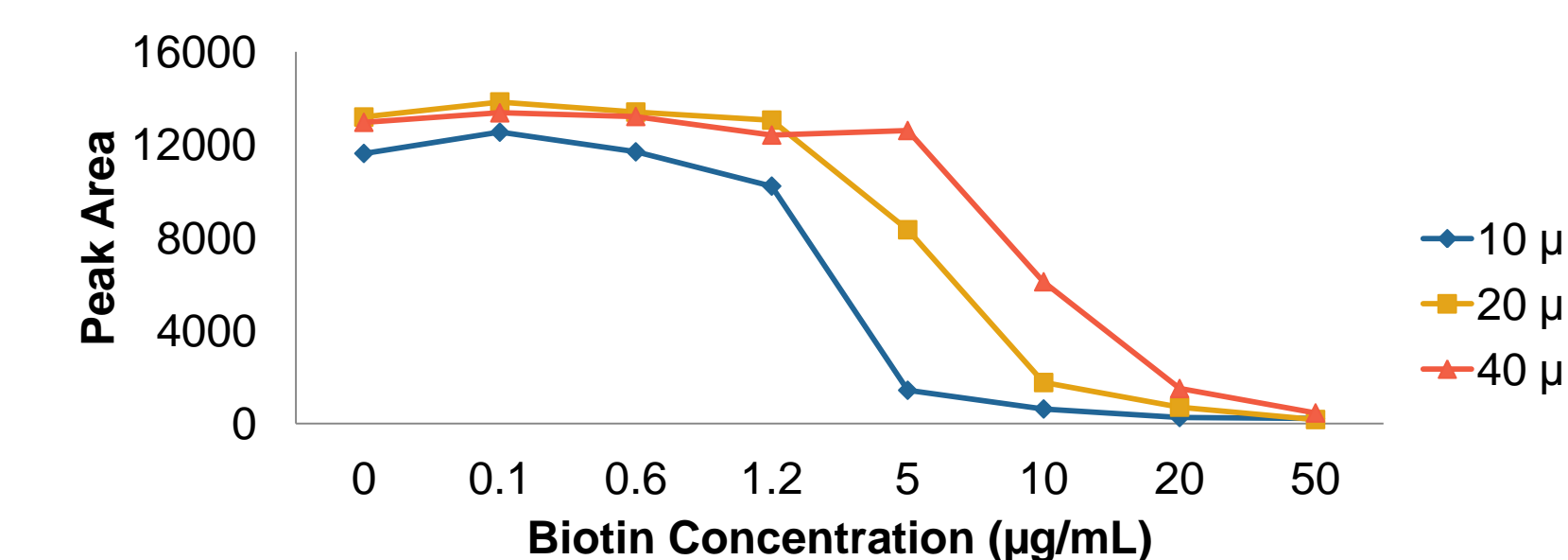


Figure 4. Teriparatide recovery from plasma fortified with biotin from 0.1 to 50 µg/mL. In-sample coupling assay was tested using 10, 20 and 40µL of beads slurry from 3 brands and 1µg of biotinylated anti-hPTH antibody per sample.

RESULTS (CONT.)

INTERNAL STANDARD

Monoclonal antibodies are typically analyzed using a surrogate peptide approach. It is common practice to use isotopically labeled peptide internal standards added post-extraction. SILAC proteins offer a valuable alternative since they can be added early in the sample processing and will therefore compensate for any variability in the extraction and digestion processes. As expected, the incorporation of a SILAC antibody helped to compensate for biotin competition in a hybrid assay for Rituximab (Figure 5).

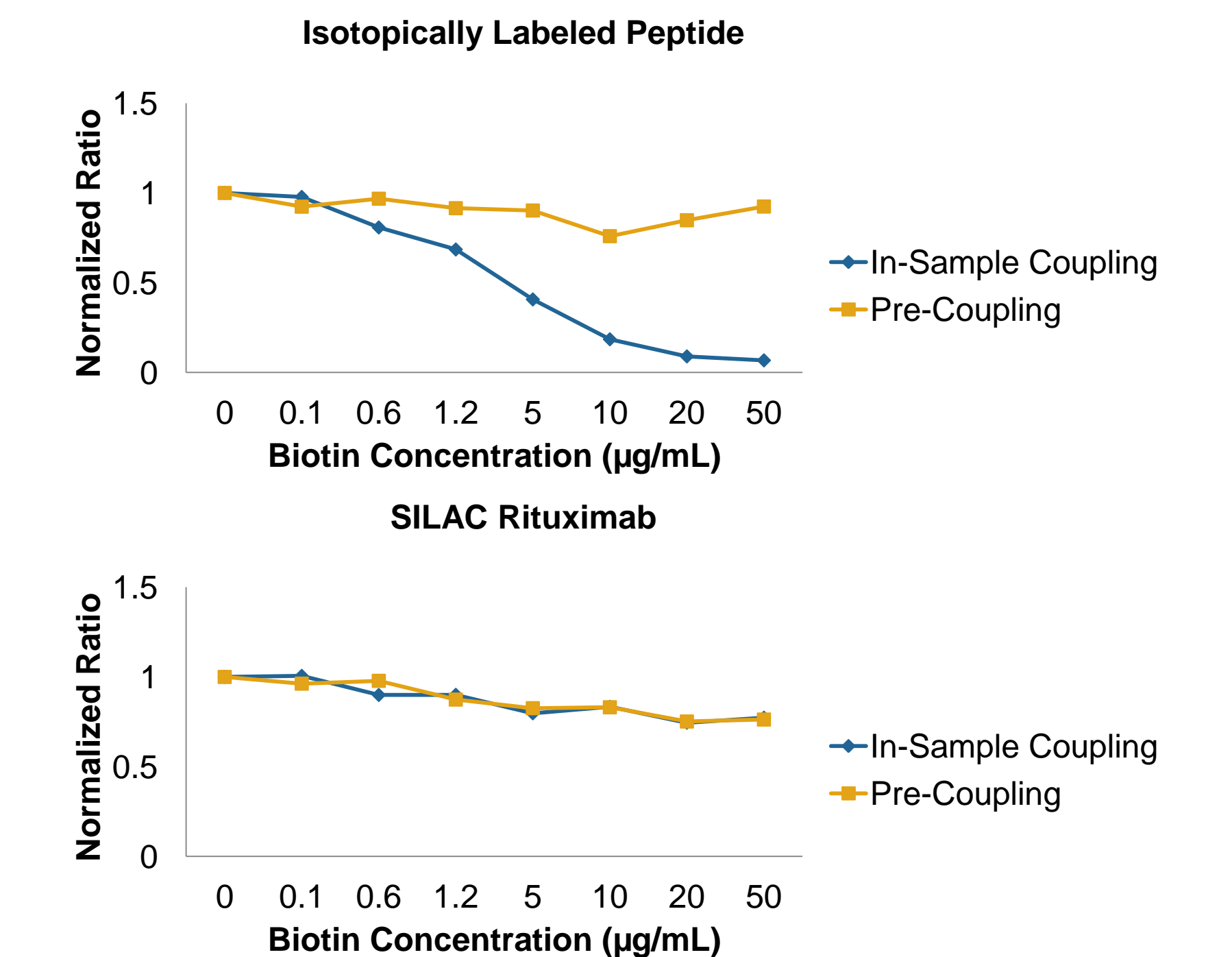


Figure 5. Rituximab/internal standard peak area ratio from plasma fortified with biotin from 0.1 to 50 µg/mL. Isotopically labeled peptide internal standard (top) and SILAC Rituximab internal standard (bottom).

CONCLUSION

Based on these findings, a set of recommendations can be defined for the development of bioanalytical assays using the streptavidin-biotin workflow. When applicable, the pre-coupling assay design is recommended. If not, using an excess of high binding capacity beads and incorporating a suitable internal standard, such as SILAC, should be investigated. In all cases, interference of biotin should be considered early in method development for a better understanding of assay limitations.

ACKNOWLEDGEMENTS

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