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Development of an Affinity Capture - LC-MS/MS Assay for the Quantitation of Adalimumab in Human Plasma using BioBA sample preparation kit

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OVERVIEW

PURPOSE

To evaluate a kit-based sample preparation solution for Adalimumab quantitation by affinity capture - LC-MS/MS in human plasma.

METHOD

Human plasma was spiked with Adalimumab over a range from 5.00 to 50,000 ng/mL. Infliximab was used as internal standard. Affinity purification using TNFa was processed using a SCIEX BioBA sample preparation kit, which includes magnetic beads, digestion enzymes along with different reagents and buffer solutions used for sample preparation. Magnetic sample processing was automated using the KingFisher Flex. LC-MS/MS analysis was performed on a SCIEX Triple Quad 6500+ system.

RESULTS

Surrogate peptides for Adalimumab and Infliximab were selected based on their specificity in human plasma, as well as their ionization and fragmentation characteristics. Affinity capture using biotinylated TNFa showed higher efficiency compared to immunocapture with anti-TNFa. BioBA kit reagents, mainly the magnetic beads and Tryp/LysC digestion mix, showed higher effectiveness in comparison to equivalent reagents used in the lab. For LC-MS/MS analysis, good sensitivity, linearity and high dynamic range were achieved using Sciex 6500+ system.

INTRODUCTION

Historically, immunoassays have been the golden standard for monoclonal antibody (mAb) quantitation in the pharmaceutical industry. However, in recent years, method development of LC-MS/MS based monoclonal antibody (mAb) quantitation assays have gained in popularity. Although hybrid immunocapture - LC-MS/MS workflows have proven to be highly sensitive and specific, validation of these complex methods in a regulated environment remains challenging due to the multiple assay steps, critical reagent management and the requirement for high levels of expertise to implement these intricate methodologies. In this research, we describe a robust and reliable affinity capture - LC-MS/MS workflow combining BioBA solution for integrated kit-based sample preparation and MS analysis to quantify Adalimumab (Humira), used for the treatment of rheumatoid arthritis.

METHODS

SAMPLE PREPARATION

- Add 200 µL human plasma spiked with Adalimumab to 96-well plate
- Add 25 µL of Infliximab internal standard in PBS
- Add 50 µL of BioBA Streptavidin magnetic beads preconjugated with biotinylated TNFa
- Incubate 1 hour with shaking.
- Process samples on KingFisher™ Flex
- Elute in 100 µL of BioBA Elution buffer
- Reduce with 5 mM TCEP (30 min at 60 °C)
- Alkylate with 10 mM IAM (30 min at RT)
- Digest with BioBA Trypsin/LysC mix (60 min at 37 °C)
- Acidify and analyze by LC-MS/MS

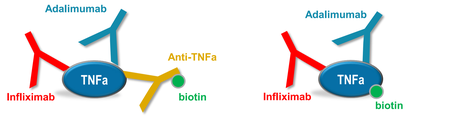

Figure 1. Adalimumab and Infliximab affinity capture approaches

LC-MS/MS DETECTION

- Shimadzu Nexera X2 UHPLC system
- Agilent Zorbax SB300 C_{18} column (50 x 2.1mm, 3.5 µm)
- Gradient elution with 0.1% CH_2O_2 in H_2O and ACN
- SCIEX Triple Quad API 6500+ operated in (+) ESI-MRM

Table 1. MRM transitions of Adalimumab and Infliximab.

Drug	Peptide	Q1 m/z	Q3 m/z	CE (eV)
Adalimumab	APYTFGQGTK	535.3 (2+)	738.3 (y7)	28
		535.3 (2+)	901.4 (y8)	30
Infliximab (IS)	YASESMSGIPSR	642.8 (2+)	359.1 (y3)	32

RESULTS

Affinity Capture Approach

Two different affinity capture approaches were evaluated. Enrichment using biotinylated TNFa showed higher capturing efficiency compared to biotinylated anti-TNFa used to immunocapture the TNFa, Adalimumab and Infliximab complex (Figure 2).

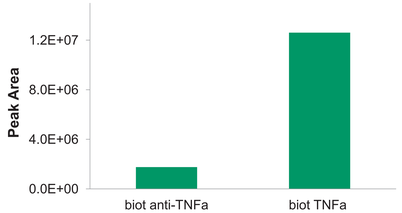

Figure 2. Enrichment of Adalimumab in human plasma using either biotinylated anti-TNFa or biotinylated TNFa for affinity capture.

BioBA High-Capacity Streptavidin Beads

BioBA High-Capacity Streptavidin beads were compared to Streptavidin Mag Sepharose beads (GE Healthcare) used routinely in the lab. Increasing volumes of 10% slurry of magnetic beads were used to extract Adalimumab ULOQ (50 µg/mL) samples. Higher recovery was obtained with10 and 25 µL of BioBA beads compared to the GE beads.

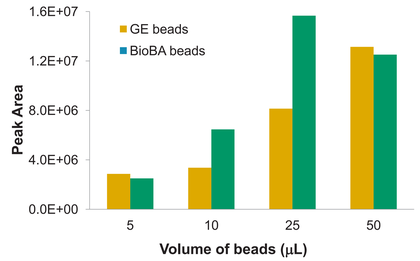

Figure 3. Affinity capture of Adalimumab using increasing volumes of GE beads versus BioBA high capacity beads.

BioBA Tryp/LysC digestion mix

Digestion yield was evaluated using BioBA Tryp/LysC mix and Trypsin Gold (Promega) at three different concentrations and three incubation times at 37 °C. Maximum digestion yield was reached after 1 hour of digestion with BioBA Tryp/LysC (1 µg) compared to 3 hours with Trypsin Gold (1 µg) (Figure 4).

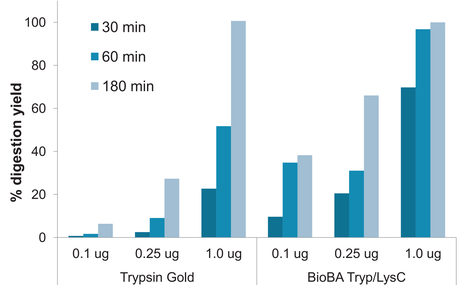

Figure 4. Digestion of extracted Adalimumab ULOQ samples using Trypsin Gold (Promega) and BioBA kit Trypsin/LysC mix.

Method Sensitivity

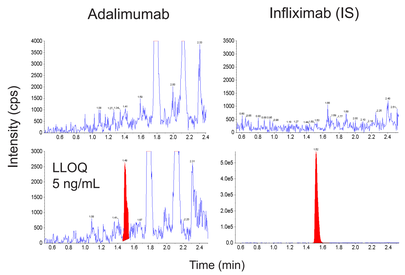

Figure 5. Representative chromatograms of Double Blank and LLOQ (5 ng/mL) extracted Adalimumab (left) and Infliximab (right).

Linearity and Dynamic Range

Extracted Adalimumab calibration curve showed excellent linearity in the analytical range (5 - 50,000 ng/mL) (Figure 6).

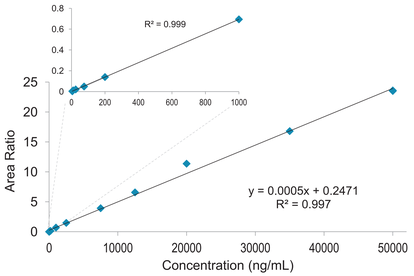

Figure 6. Calibration curve of Adalimumab from 5 ng/mL to 50,000 ng/mL in human plasma.

Automation using KingFisher™ Flex

Extraction procedure was automated using the KingFisher™ Flex, resulting in excellent precision for the IS peak area (Figure 7).

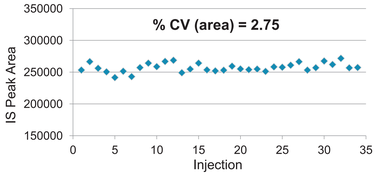

Figure 7. Internal standard precision with automated BioBA beads processing using KingFisher™ Flex.

CONCLUSION

The BioBA sample preparation kit simplifies the hybrid assay workflow for the quantitation of Adalimumab by providing ready-to-use reagents and consumables for every step of the sample preparation process, thereby minimizing the occurrence of inconsistent results inherent with manual assay processing.

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