Simplified Strategy for Rapid Reproducible Quantitation of Monoclonal Antibodies in Plasma

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Overview

- Novel method for mAb quantitation in plasma matrices
- Sample to results in <2 hours
- Potential for method development in 1 week or less
- LLOQ of 125.2ng/mL achieved without sample cleanup

Introduction

Monoclonal antibodies (mAbs) such as Immunoglobulin Gs (IgGs) make up a large and rapidly growing portion of today's pharmaceutical market, including treatments for cancer and rheumatoid arthritis. As efficacy of these antibodies improves, smaller doses are needed to obtain desired outcomes. In order to accurately measure the kinetics of antibody degradation in biological systems it is necessary to have a sensitive, accurate and highly reproducible method of antibody quantitation in biological samples. However, the complexity of the sample matrix and increasing sensitivity requirements have created a significant analytical challenge. Here, by means of direct digestion of biological fluids using temperature stable immobilized enzyme technology followed by quantitative high resolution LC/MS analysis, we have demonstrated an accurate and reproducible method for obtaining a ten-fold improvement in sensitivity over traditional methods.

Methods

Human Immunoglobulin G (IgG) was obtained from Sigma Aldrich (St Louis, MO). Mass Check Antibody (mAb) from mouse wash obtained from Waters (Milford, MA). Plasmas were obtained from Bioreclamation (Hicksville, NY). Perfinity Flash Digest™ kits are made by Perfinity Biosciences, Inc (West Lafayette, IN).

Method development samples for the mouse mAb were prepared by adding 150 µL of Flash Digest buffer and 50 µL of mAb solution to each well of a strip of Flash Digest before incubation at 70 °C with shaking at 1400 rpm. These were sampled every 15 minutes, filtered to remove the resin, and analyzed to determine optimal peaks for quantitation.

Quantitation samples were prepared by adding 150 µL of Flash Digest Buffer and 50 µL of plasma spiked with varying concentrations of human IgG or mouse mAb to individual wells of Flash Digest. Samples were then incubated at 70 °C with shaking at 1400 rpm for 45 minutes or 2 hours, respectively, filtered to remove the resin, and analyzed by LC-high resolution (HR) MS/MS.

Mouse mAb detection:
LC system: Thermo Scientific Dionex XRS pump and OAS autosampler
MS System: Q Exactive™ MS interfaced with HESI source
Column: Halo C18 RP column (2.1x100mm, 2.7µm)
Column conditions: 40°C with a flow rate of 300 µL/min
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in acetonitrile
Gradient: 5%B kept for 1 minute then ramped to 40% B over 10 minutes
Injection volume: 5 µL digested plasma solution
MS Method: Target MS/MS (17,500 resolution) in positive mode; AGC=2e5; IT=400ms; NCE=20; Isolation window=m/z 2; Capillary temp: 320°C; Sheath gas: 30; Aux gas:10; Probe heater temp: 300°C; Spray volt: 3.8kv; S-lens:50

Human IgG detection:
LC system and column: As above
MS System: Q Exactive™ Plus MS interfaced with HESI source
Column conditions: 50°C with a flow rate of 500 µL/min
Solvent A: 98% water, 2% acetonitrile, 0.1% formic acid
Solvent B: 10% water, 90% acetonitrile, 0.1% formic acid
Gradient: 2% B kept for 1 minute then ramped to 70% B over 4 minutes
Injection volume: 1µL digested plasma solution
MS Method: Target MS/MS (35,000 resolution) in positive mode; AGC=2e5; IT=150ms; NCE=21; Isolation window=m/z 2; Sheath gas: 60; Aux gas:20; Probe heater temp: 500°C; other source parameters are same as above.

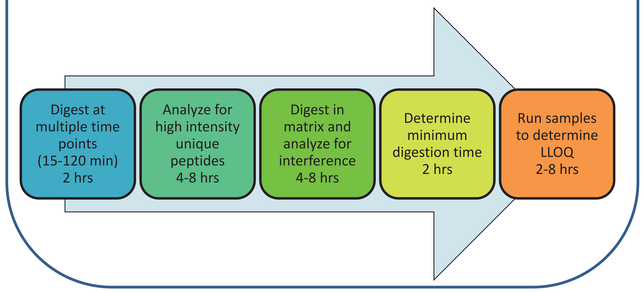
PepFinder 1.0 BioPharma software (Thermo Fisher Scientific) was employed to determine high signal, unique peptides from the mouse mAb. Unique peptides from the trypsin-digested mAb were verified by BLAST search. The quantitative analysis of monoclonal antibodies was performed by LCQuan 2.8.

Results

Method development and detection of mouse mAb in human plasma

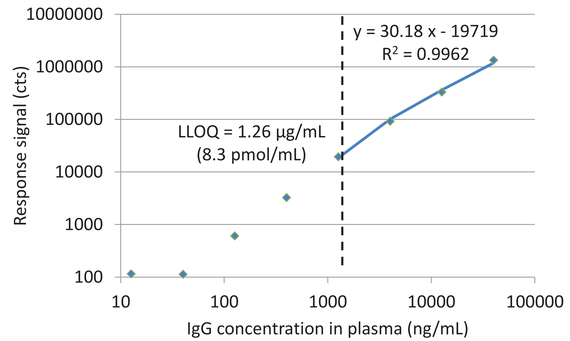
Rapid trypsin digestion without reduction and alkylation dramatically increases the speed of method development. Without the need to optimize denaturation, reduction, or alkylation and with minimal pressure on optimizing digestion time due to lack of peak decay, method development is composed almost exclusively of peptide target selection for LC-HR MS/MS analysis.

Total Method Development Time (est): 14-28 hours



With the relative quantitation feature in PepFinder 1.0, we determined peptide sequences that demonstrated better ionization. BLAST search was used to verify unique peptides from the trypsin-digested mAb and MS/MS spectra were acquired to confirm peptide identity and determine those with the best signal/noise for target MS/MS analysis. Following this approach we were able to take a protein through the entire method development process, achieving an LLOQ of 1.26 µg/mL in plasma within 1 week.

Calibration Curve of Mouse mAb in Human Plasma

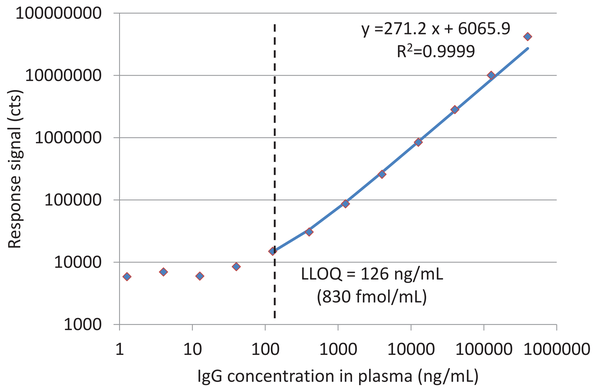


Regression: Linear, weighted to 1/x where x = analyte concentration

Detection of human IgG in plasma samples

Using a previously investigated tryptic peptide from the Fc region of human IgG [1] we were able to detect sub-µg/mL levels in mouse plasma consistently and without the use of an internal standard, still maintaining CVs below 7% for all points, including our LLOQ (3x noise).

Calibration Curve of Human IgG in Mouse Plasma



Regression: Linear, weighted to 1/x where x = analyte concentration

Conclusions

Method Development

A critical part of protein analysis in pharmaceuticals, food, agriculture, and many other industries is developing detection methods with good sensitivities. Our ability to take a protein with minimal prior knowledge of digestion or detection and develop a method capable of achieving 1.26 µg/mL sensitivities in less than 1 week demonstrates the power of an immobilized trypsin system coupled with a high resolution accurate mass MS system to dramatically shorten method development times, creating effective assays with a very rapid turnaround time.

IgG Detection Capabilities

Previously reported sensitivities for IgG in plasma without the application of affinity or other sample cleanup steps is limited to LLOQs at the µg/mL level [2,3]. Here we have shown that by achieving a complete digestion without the peak decay caused by autolysis we are able to quantitatively detect human IgG in mouse plasma down to levels of 126ng/mL, more than an order of magnitude lower than previously reported.

As pharmaceuticals grow more efficacious, reporting regulations on food and agriculture grow more rigorous, and more low abundance proteins are shown to be key biomarkers, effective digestion and detection strategies are becoming increasingly critical to effective work flows in proteomics analysis.

The work here shows that a well designed immobilized trypsin such as the Flash Digest kits coupled with a high resolution mass spectrometer such as the Q Exactive or Q Exactive Plus is capable of significantly increasing assay sensitivity. This should also hold true with the addition of an affinity step or depletion step to further purify samples and achieve even lower limits of detection.

References

1. Kaur S, Saad O, Xu K (2012) US Patent No. 2012155019. Washington, DC: U.S. Patent and Trademark Office.
2. Ouyang Z, Furlong MT, We S, Sleczka B, Tamura J, Wang H, Suchard S, Sure A, Olah T, Tymiak A, Jemal M (2012) Pellet digestion: a simple and efficient sample preparation technique for LC-MS/MS quantification of large therapeutic proteins in plasma. Bioanalysis 4:17-28.
3. Jiang H, Zeng J, Titsch C, Voronin K, Akinsanya B, Luo L, Shen H, Desai DD, Allentoff A, Aubry AF, DeSilva BS, Arnold ME (2013) Fully validate LC-MS/MS assay for the simultaneous quantitation of coadministered therapeutic antibodies in cynomolgus monkey serum. Analytical Chemistry 85:9859-9867.