A Fast, Robust, and Generic IA-LC-MS/MS Method for Quantification of Monoclonal Antibody Therapeutics: Optimizing Immunoaffinity Capture, Proteolytic Digest, and LC Conditions

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Introduction

Monoclonal antibody (mAb) therapeutics have become one of the primary focuses for pharmaceutical industry. Hybrid methods, by combining traditional LBA immunoaffinity (IA) capture steps with LC-MS/MS, achieve efficient immunoaffinity capture and sample extraction of large molecules, followed by proteolytic digestion, and highly sensitive and selective data analysis. In this study, we report a fast, robust, and generic IA-LC-MS/MS method for quantification of human IgG antibody drugs in pre-clinical studies using SILuMab as internal standard. Universal signature peptides were selected from the IgG backbone and are suitable for both human IgG1 and IgG4 type mAbs. Selection of capture reagents, optimization of immunoaffinity capture procedure, optimization on proteolytic digestion, improvement on LC chromatography, as well as reducing the lower limit of quantification, will be discussed.

Method

Sample preparation

mAbs and SILuMab (IS) were spiked into monkey plasma in 96-well plates, followed by immunoaffinity capture using PureProteome Protein A Magnetic Beads. Next, cleanup to remove unbounded matrix proteins and other molecules were achieved utilizing the Tomtec automation system with a magnetic plate, while simultaneously unfolding and digesting the mAbs using immobilized Trypsin at 70°C. The resulting solution went through a multiscreen-HV 96 well filter plate before injection on LC-MS/MS (Sciex 6500+). A Halo Peptide ES-C18 2.7µm 2.1x50 mm column was used with 4 minutes run time. Two to three universal signature peptides were selected as "surrogate" peptides for quantification and qualification.

UHPLC: Nexera LC-30AD, Shimadzu

Mass Spectrometer: QTrap 6500+, AB Sciex

Analytical Column: Halo Peptide ES-C18 2.7µm 2.1x50 mm

Ionization Mode: Positive ion

Sample Volume: 10 µL

Assay Range: 100-25000 ng/mL in K_2EDTA Cynologous Monkey Plasma.

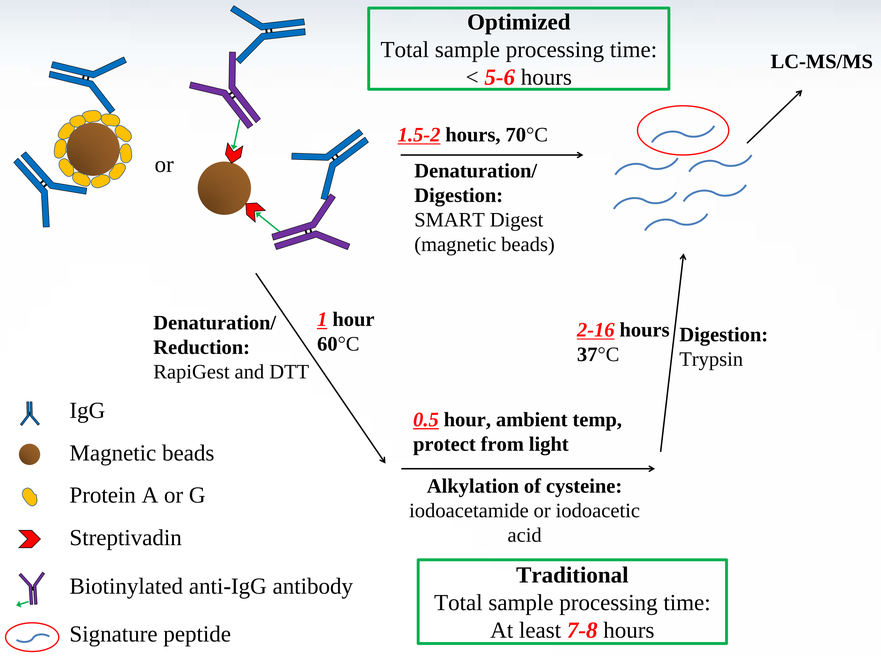
LC conditions

Mobile phase composition	A: Water:Formic Acid at 100:0.1 (v:v)
	B: Acetonitrile:Water:Formic Acid at 95:5:0.1 (v:v:v)
Flow rate	0.5 mL/min
Analysis time	~4.0 min

Gradient program

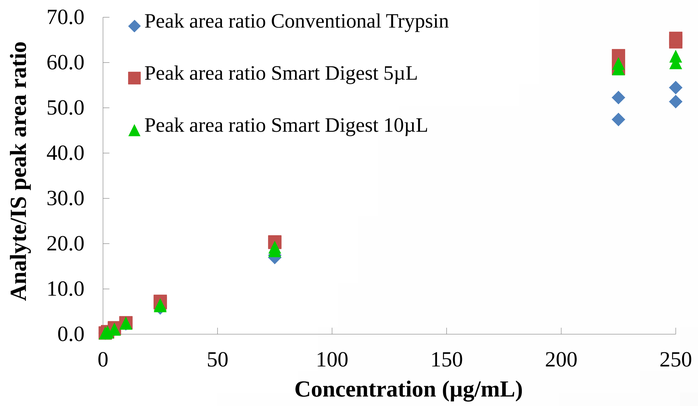
Time (min)	0	0.2	2.5	2.6	3.2	3.3	4.0
%B	20	20	40	90	90	20	20

Figure 1: Generic IA-LC-MS/MS Method for mAbs and Optimization



Results

Figure 2: Comparison of SMART Digest and Conventional Digest



- Conventional Digestion: including reduction (60°C for 1 hour), alkylation (0.5 hour), and digestion (2 hours)- **Cost: ~USD $300 / 96-well plate**
- SMART Digestion: 70°C for 1.5 hours- **Cost: ~USD $175 / 96-well plate**

Optimization of Liquid Chromatography for Surrogate Peptide

- Analytical columns tested:
  - BEH C18, 1.7 µm, 2.1 x 50 mm, Waters
  - Halo Peptide ES-C18, 2.7 µm, 2.1 x 50 mm, Advanced Materials Technology
  - Halo Peptide ES-CN, 2.7 µm, 2.1 x 50 mm, Advanced Materials Technology
- **Halo Peptide ES-C18 (2.7 µm, 2.1 x 50 mm)** was eventually selected due to better peak shape, better sensitivity, shorter run time, and reproducibility from different lots of columns for the selected surrogate peptide.

Figure 3: Representative Calibration Curves

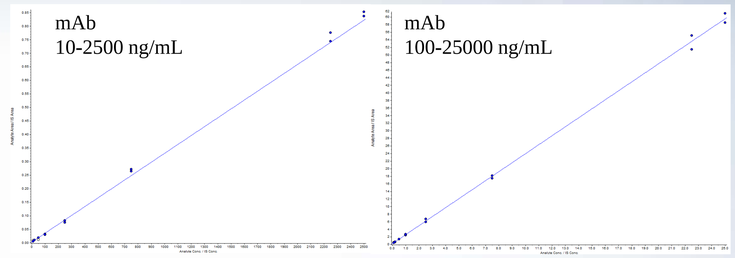
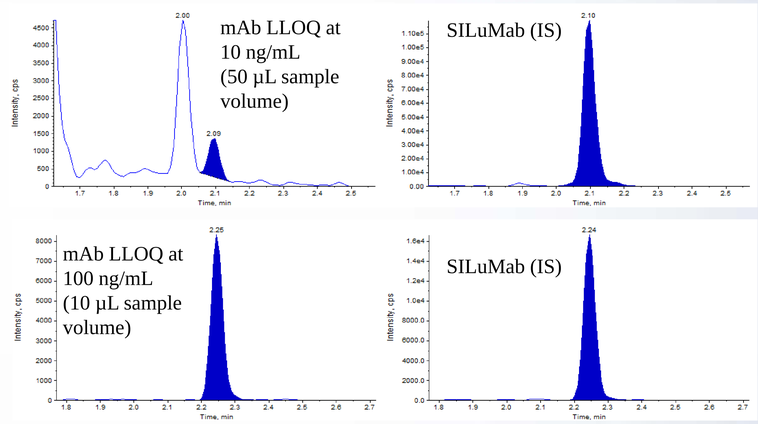


Figure 4: Representative Chromatograms



Validation Summary table

Analyte Name	mAb
Internal Standard (IS)	SILuMab
Mean Recovery of Analyte (%)	62.0
LLOQ QC Intra-run Precision Range (%CV)	4.5 to 20.6
LLOQ QC Intra-run Accuracy Range (%RE)	-15.6 to 9.0
Analytical QC Intra-run Precision Range (%CV)	1.2 to 7.7
Analytical QC Intra-run Accuracy Range (%RE)	-4.7 to 6.5
LLOQ QC Inter-run Precision (%CV)	19.5
LLOQ QC Inter-run Accuracy (%RE)	-1.8
Analytical QC Inter-run Precision Range (%CV)	3.4 to 5.2
Analytical QC Inter-run Accuracy Range (%RE)	3.3 to 4.0

Conclusion

- **Multiple-step optimization was performed to yield a fast, robust, and generic IA-LC-MS/MS method, which was then validated to quantify human IgG antibody drugs in pre-clinical studies.**
- **Immunoaffinity capture procedure and proteolytic digestion were optimized.**
- **Liquid chromatography was improved using Halo® Peptide ES-C18 analytical column.**
- **Lower limit of quantification (LLOQ) can be reduced to 10 ng/mL.**
- **This generic IA-LC-MS/MS method demonstrated adequate selectivity, sensitivity, accuracy, reproducibility, and is easily transferrable to human IgG1 and IgG4 type antibody therapeutics for nonclinical studies.**