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Utilizing HRAM Orbitrap MS to Quantify Therapeutic Monoclonal Antibodies (mAbs) in Human Serum for Clinical Research

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ABSTRACT

Purpose: To develop a middle-up approach for therapeutic monoclonal antibodies (mAbs) monitoring in human serum using protein L purification and LC-HRAM-MS.

Methods: Protein L purification was implemented to purify mAbs containing kappa light chains of a wide range of immunoglobulins. Through on-beads IdeS digestion and reduction steps, three subunits were generated. Thermo Scientific™ Orbitrap Exploris™ 240 MS was operated at a resolution of 120k to quantify the intact light chains.

Results: The method needed low volume sample of 10 mL per analysis within < 1.5 hours of sample preparation. The LOQs were determined to be between 1 to 5 µg/mL of the mAb concentration in human serum with great linearity of R^2 > 0.99. Different lots of MAbPac columns generated reproducible retention times and peak areas, providing coherent intact protein data for clinical research.

INTRODUCTION

Each year, more monoclonal antibodies (mAbs) are approved by regulatory agencies to treat a wide range of diseases with an expected market value of over $200 billion by 2026.[1,2] In clinical testing, the presence of endogenous immunoglobulins with almost identical structures from patients' samples adds another challenge to the accurate quantitation of therapeutic mAbs. Accordingly, mass spectrometry has gained substantial popularity for therapeutic mAb monitoring in clinical laboratories due to its great versatility to detect both tryptic peptides and intact light and heavy chains quantitatively.[3,4]

Here we present the intact light chain quantitation approach for measuring the concentrations of therapeutic mAbs in human serum using HRAM-MS for clinical research.

MATERIALS AND METHODS

Sample Preparation

The workflow is described in Figure 1. Different concentration points of target mAbs were prepared to range from 1 µg/mL to 100 µg/mL. For antibody purification, 50 µL of Thermo Scientific™ Pierce™ Protein L magnetic beads were used to purify 10 µL of different concentration points. The purified samples were directly subjected to IdeS digestion following the protocol provided by the vendor (FabRICATOR® Ides, Genovis) followed by the reduction step.

Test Method(s)

LC separation was performed on a Thermo Scientific™ Vanquish™ HPLC system using Thermo Scientific™ MAbPac™ RP HPLC column (2.1 x 50 mm, 4 µm, Part No. 088648). MS analysis was performed on a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer. LC and MS conditions are listed in Table 1. Two columns from two different lots were used for the column reproducibility evaluation (data shown in Figure 5 and Table 3).

Data Analysis

Thermo Scientific™ BioPharma Finder™ 4.1 software was used to confirm the intact masses of three subunits. Most of the default Xtract algorithm parameters remained unchanged.

Thermo Scientific™ TraceFinder™ 5.1 software was used from data acquisition to processing. Peak areas from 12 most abundant m/z values were summed for the intact light chain quantitation. Each data point of the calibration curve was analyzed in triplicate and then fitted with % accuracy between 80 and 120, % RSD < 15, % CV < 15, and R^2 > 0.95 to determine the LOQs for each mAb. The LODs and linear ranges were also determined.

Figure 1. Experimental workflow and LC-MS system

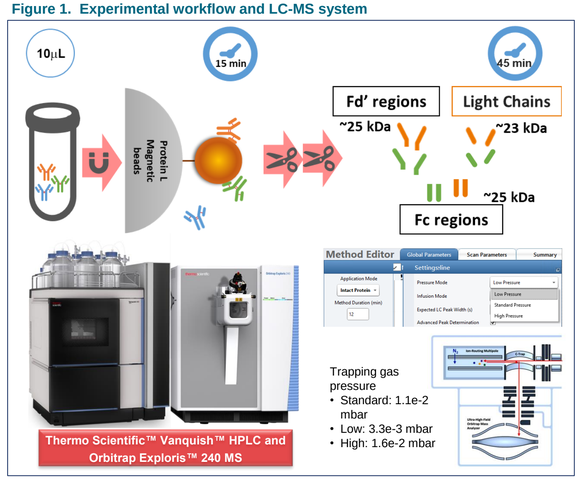

Table 1. LC and MS conditions

LC gradient			
Time (min)	% A	% B	Curve
0.0	80	20	5
0.5	80	20	5
0.7	68	32	5
8.5	60	40	5
9.2	20	80	5
9.7	20	80	5
9.8	80	20	5
12	80	20	5
Separation conditions			
Mobile phase A	0.1 % formic acid and 0.02 % trifluoroacetic acid in water		
Mobile phase B	0.1 % formic acid and 0.02 % trifluoroacetic acid in 10: 20: 70 water: isopropanol: acetonitrile (v/v/v)		
Flow rate	0.4 mL/min		
Column temperature	80 °C		
Injection volume	10 µL		
MS global parameters			
Application mode	Intact protein		
Pressure mode	Low pressure		
Source type	Heated electrospray ionization (H-ESI)		
Polarity	Positive		
Spray voltage (V)	3800		
Sheath gas (Arb)	25		
Aux gas (Arb)	5		
Sweep gas (Arb)	1		
Ion Transfer tube temp (°C)	320		
Vaporizer temp (°C)	150		
Divert Valve A	0.0 min: position 1-6 (waste) 1.0 min: position 1-2 (MS) 9.5 min: position 1-6 (waste)		
MS scan parameters			
Orbitrap resolution	120,000		
Scan range (m/z)	700-2000 for intact mass confirmation 1000-1400 for targeted quantitation		
RF lens (%)	60		
AGC target	Standard		
Maximum injection time mode	Custom		
Maximum injection time (ms)	200		

RESULTS

Figure 2. Retention times of bevacizumab subunits, Fc/2, LC (light chain), and Fd' (A), full MS spectrum for each subunit (B), assigned subunits from BioPharma Finder (C), and isotopic clusters of bevacizumab light chain at a charge state of 21 using different resolutions on Orbitrap (D)

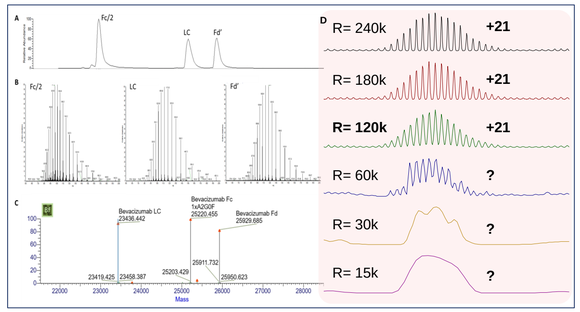

Figure 3. Observed retention times of two IS mAbs over the entire calibration curve analysis (A) and overlaid XICs of pembrolizumab at m/z 1033.3051 from rituximab analysis (B)

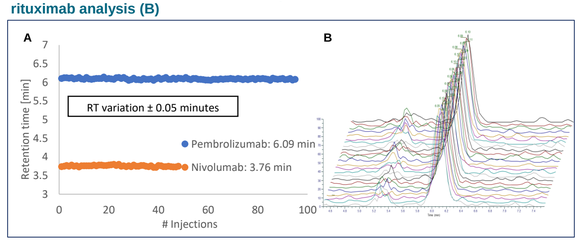

Figure 4. Calibration curves of six target mAbs (A) and peak areas of two IS mAbs, nivolumab and pembrolizumab, from golimumab and bevacizumab calibration curve analyses (B)

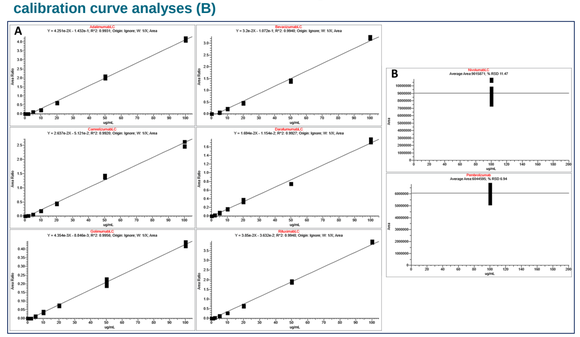

Table 2. Determined analytical properties of the method

MAb	LOD (mg/mL)	LOQ (mg/mL)	Linear Range (mg/mL)	R^2
Adalimumab	2	2	2 - 100	0.9931
Bevacizumab	5	5	5 - 100	0.9940
Camrelizumab	2	2	2 - 100	0.9920
Daratumumab	2	2	2 - 100	0.9927
Golimumab	2	5	5 - 100	0.9956
Rituximab	1	1	1 - 100	0.9948

Figure 5. Overlaid XICs of adalimumab, bevacizumab, and camrelizumab from column reproducibility evaluation

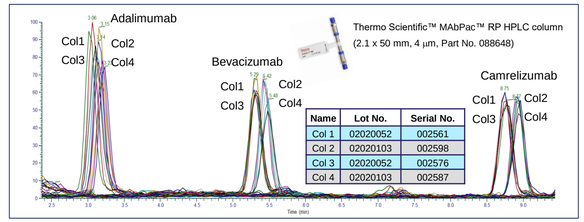

Table 3. Results of column reproducibility evaluation

	Adalimumab	Bevacizumab	Camrelizumab	Daratumumab	Golimumab	Rituximab
Ave. RT (minutes)	3.12	5.38	8.85	3.98	5.20	3.67
RT |Max - Min| (minutes)	0.15	0.18	0.18	0.14	0.16	0.15
Ave. peak area	3.5E+06	3.1E+06	3.1E+06	1.9E+06	1.3E+06	3.0E+06
% Peak area |Max - Min|	19%	20%	16%	14%	9%	13%

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