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Application News

Monoclonal Antibody Characterization / LCMS-9030

Monoclonal Antibody (mAb) Intact Mass and Subunit Analysis Using Shimadzu Q-TOF LCMS-9030

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User Benefits

- Rapid and straightforward workflow for performing mAb characterization using Shimadzu Q-TOF LCMS-9030.

■ Introduction

Monoclonal Antibody (mAb) therapeutics are a growing group of biopharmaceuticals used to treat medical conditions such as cancer. The characterization of mAb is essential to assess the Critical Quality Attribute (CQA) for product development and regulatory approval.

mAbs are large, complex, and heterogeneous proteins which typically require analytical strategies to characterize them to ensure their safety and efficacy as drugs. The use of liquid chromatography coupled with mass spectrometry (LC/MS) is a powerful technique that can be used to characterize of mAb effectively.

Fig. 1 LCMS-9030

mAb have a molecular weight of approximately 150 kDa. They are made up of two identical heavy chains, which are each about 50 kDa, and two identical light chains, which are each about 25 kDa. These chains are linked together by disulfide bonds. Each mAb contains a fragment antigen-binding (Fab) region that binds to the antigen and fragment crystallizable (Fc) region at its tail, which contains the N-glycosylation site (Fig. 2).

Intact mass analysis is a simple and rapid method that can be used to determine the molecular weight of monoclonal antibodies (mAbs). To further characterize mAb, subunit analysis can be performed by cleaving the inter-chain disulfide bonds, which results in the formation of two identical light chains and two identical heavy chains (as shown in Fig. 2). These subunits can then be analyzed using LCMS to obtain valuable insights into the structural integrity of the mAb protein, which may not be evident from intact mass analysis alone.

Subunit analysis can also provide a quick overview of any post-translation modifications (PTMs) or changes in disulfide bonds on the individual light and heavy chains.

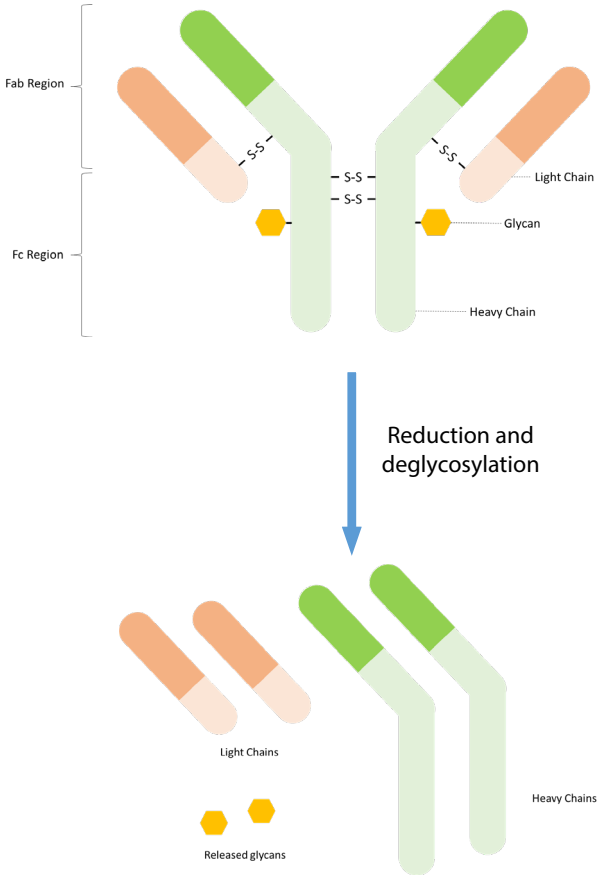

Fig. 2 Subunit Analysis of mAb to give light and heavy chains

■ Experimental

Reagents and Chemicals

NISTmAb Humanized IgG1k monoclonal antibody reference material 8671 was obtained from Merck Pte Ltd. Rapid™ PNGase F (non-reducing format) (P0711S) was purchased from New England BioLabs. Torast-H Bio Vial (P/N: 370-04350-00) was obtained from Shimadzu.

Sample preparation

Intact Mass Analysis

NISTmAb was diluted to a final concentration of 0.1 mg/ml using 0.1% formic acid in water and transferred to a Shimadzu Torast-H Bio Vial.

Samples were injected and analyzed by Shimadzu LCMS-9030 Q-TOF mass spectrometer. The analytical conditions are shown in Table 1.

Subunit Analysis

10 µg of NISTmAb was denatured and reduced using 25 mM tris(2-carboxyethyl)phosphine (TCEP) by heating at 95ºC for 5 mins. Rapid™ PNGase F was added to the sample and incubated at 50 ˚C for 10 minutes to remove glycosylation on the heavy chain region. Samples were diluted to a final concentration of 0.1 mg/ml using 0.1% formic acid in water and transferred to a Shimadzu Torast-H Bio Vial. Samples were injected and analyzed by Shimadzu LCMS-9030 Q-TOF mass spectrometer. The analytical conditions are shown in Table 1.

Table 1 Analytical conditions on LCMS-9030 QTOF

LC Conditions	
Column	Shim-pack Scepter™ C4-300 2.1 × 150 mm, 1.9 µm (P/N: 227-31175-06)
Flow Rate	0.3 mL/min
Mobile Phase	A: 0.1% Formic acid in Water B: 0.1% Formic Acid in Acetonitrile
Elution mode	Gradient elution, 18 mins
Gradient Program	%B conc.: 0-2.0 min., 30%; 2.0-12.0 min.; 30% to 50%;12-14 min.,100%; 14 -18 min., 30%
Oven Temp.	80 ˚C
Injection Vol.	5 µL
Interface Conditions (LCMS-9030)	
Interface	Heated ESI at 4.50 kV
Interface Temp.	300 ˚C
DL Temp.	200 ˚C
Heat Block Temp.	400 ˚C
Nebulizing Gas	3 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Data acquisition (Q-TOF)	
MS Mode	MS positive
TOF m/z range	300-4500 m/z

Data files were uploaded onto Protein Metrics intact and reduced workflow with the following workflow parameters as per Table 2. Protein Metrics automatically deconvoluted the mass spectrum from the m/z acquired and calculated the molecular weight of the mAb with the added modifiers such as glycoforms.

Table 2 Protein Metrics workflow parameters

Sequence and masses	
Changed N-terminal Q to pyro Glu	☑
Clip off C-terminal k	☑
N-glycans removed by PNGase F	☑ only for subunit analysis
Mass Range	20,000 to 60,000 for subunit analysis, 100,000 to 160,000 for intact mass
m/z range	300 to 4,500

■ Results and Discussion

The total ion chromatogram (TIC) profile of the NISTmAb proves that the proteins can be separated on the LCMS (Fig. 3 and 4).

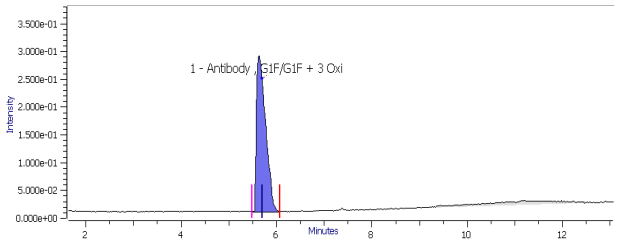

Fig.3 TIC profile of intact mass analysis

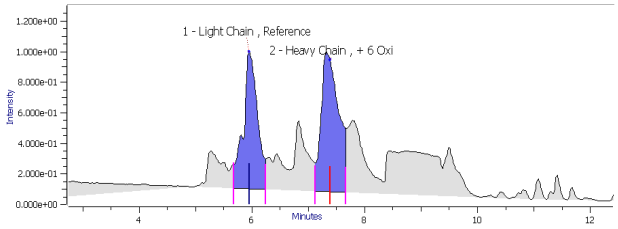

Fig.4 TIC profile of subunit analysis

Protein Metrics software automatically deconvolutes the m/z to give accurate molecular weight measurement of the intact mass and subunit analysis of the mAb, based on the default disulfide bond structure. By using high-resolution mass spectrometry, clear and distinguishable peak spectra can be obtained, which allows the protein metrics software to easily calculate the accurate molecular weight of the proteins with the glycoforms and modifiers (Fig, 5 to 7).

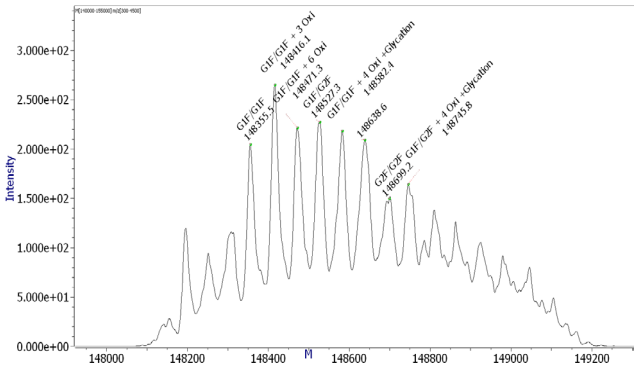

Fig. 5 Deconvoluted spectra of intact NISTmAb with modifiers.

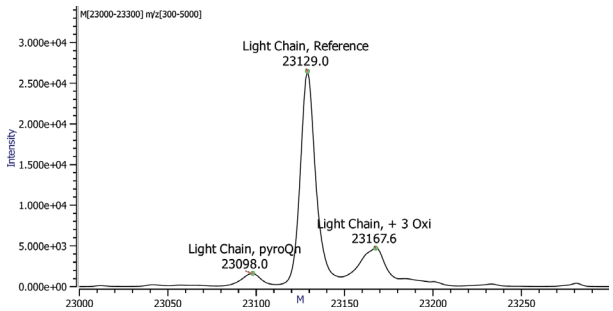

Fig. 6 Deconvoluted spectra of NISTmAb light chain with the modifiers.

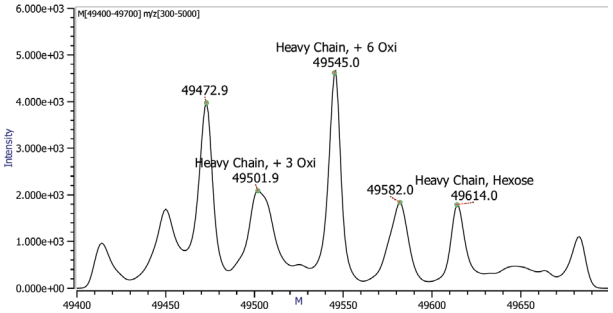

Fig.7 Deconvoluted spectra of NISTmAb heavy chain with the modifiers.

Tables 3 and 4 give the overall summary of the NISTmAb measured mass compared to the expected delta mass differences, which is automatically generated by protein metrics software. The data shows the overall calculated delta mass difference is less than 20 delta.

Table 3 Summary of NISTmAb intact mass analysis of theorical vs. observed mass

		Sample name	NIST Intact Mass Analysis
		Peak #	1
Name	Mass	Expected mass	Delta mass from calculated
Antibody G2F/G2F	148699.234	148685.875	13.36
Antibody G1F/G2F + 4 Oxi +Glycation	148745.795	148749.775	-3.98
Antibody G1F/G2F	148527.284	148523.775	3.51
Antibody G1F/G1F + 6 Oxi	148471.338	148457.575	13.76
Antibody G1F/G1F + 4 Oxi +Glycation	148582.439	148587.575	-5.14
Antibody G1F/G1F + 3 Oxi	148416.051	148409.575	6.48
Antibody G1F/G1F	148355.508	148361.575	-6.07

Table 4 Summary of NISTmAb subunit analysis of theorical vs. observed mass

		Sample name	NIST Subunit Analysis	
		Peak #	1	2
Name	Mass	Expected mass	Delta mass from calculated	
Light Chain Reference	23128.974	23123.593	5.38	
Light Chain pyroQn	23097.992	23105.593	-7.60	
Light Chain + 3 Oxi	23167.580	23171.593	-4.01	
Heavy Chain Hexose	49613.982	49614.645		-0.66
Heavy Chain + 6 Oxi	49544.999	49548.645		-3.65
Heavy Chain + 3 Oxi	49501.900	49500.645		1.25

■ Conclusion

Intact mass and subunit analysis are methods used to determine the average mass of monoclonal antibodies (mAbs). This information is crucial during the development of drug products as it provides insight into the structural integrity of the mAbs. Particularly, subunit analysis measures the molecular weight of the light and heavy chains of the mAb resulting from the breakdown of inter-chain disulfide bonds. Any alterations in the structural integrity of the mAb, such as a mismatch in disulfide bonds, will show a significant change in the mass measurement of the light and heavy chains. High-resolution mass spectrometry is well-suited to analyze multiple charged ions generated by mAb. Our report demonstrates that the Shimadzu Q-TOF LCMS-9030, together with protein metrics software, is capable of accurately measuring the mass of mAb, making it an effective tool for their characterization.

■ References

1. Interlaboratory Study for Characterizing Monoclonal Antibodies by Top-Down and Middle-Down Mass Spectrometry , Srzentić *et. al.* (2020). Journal of the American Society for Mass Spectrometry, 31(9), 1783–1802. https://doi.org/10.1021/jasms.0c00036

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