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ThP 407

Characterization of Monoclonal Antibody (mAb) using Shimadzu Q-TOF LCMS 9030

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1. Introduction

- Monoclonal antibody (mAb) (**Fig.1**) drugs are gaining traction in the biopharmaceutical industry. However, due to their complexity, they often require thorough characterization to assess Critical Quality Attributes (CQAs) during development stages and for regulatory approval. Typically, High Resolution Mass Spectrometry (HRMS) Quadrupole Time-of-Flight (Q-TOF) LCMS-9030 (**Fig.2**) is employed for mAbs characterization.
- During process development, mAbs undergo modifications like oxidation and deamination, impacting their safety and efficacy. Hence, post-translation modification (PTM) studies are crucial. Peptide mapping, a CQA method, confirms the amino acid sequence and identifies modification sites, aiding in assessing safety and efficacy..
- Intact and subunit analysis are conducted to ascertain the molecular weight and overall structure of the mAb. Additionally, peptide mapping is employed to achieve a thorough understanding of the sequence coverage of the mAb, while simultaneously identifying and locating post-translational modification sites.

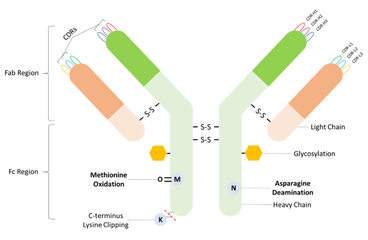

Fig. 1 Structure of mAb

Fig. 2 QTOF-9030

2. Methods

In this study, the designated reference mAb used was the NISTmAb reference material 8671, a humanized IgG1k monoclonal antibody. For subunit analysis, the Rapid^{TM} PNGase F enzyme was employed to effectively remove glycans from the heavy chain. For sequence confirmation and PTM analysis, the mAb underwent enzymatic digestion using trypsin to generate smaller peptide fragments. Analysis of the samples was performed using Shimadzu Q-TOF LCMS-9030 instrument, and Protein Metrics was employed for subsequent data processing.

3. Results

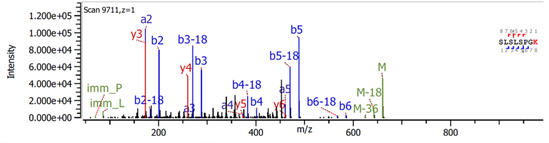

Fig. 3 MS Spectra of modified peptide with loss of amino acid Lysine (K) at heavy chain amino acid position 450

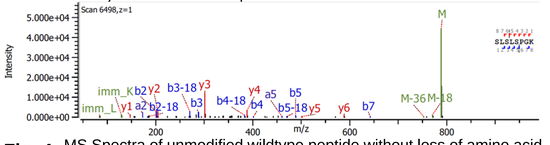

Fig. 4 MS Spectra of unmodified wildtype peptide without loss of amino acid Lysine (K) at heavy chain amino acid position 450

Protein name	Start AA ↕	Var. Pos. Protein ↕	Sequence (unformatted) ↕	Mod. Names	Mod. AAs ↕	MS Id ← MS Alias name	1 NIST (%)
Heavy Chain	1	1	QVTLR	Gln->pyro-Glu/-17.0265	Q		99
			QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIR	Gln->pyro-Glu/-17.0265	Q		1.04
	137	162	STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK	Deamidated/0.9840	N		2.81
		162,178	STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK	Deamidated/0.9840; Deamidated/0.9840	N,Q		12.4
	252	255	DTLMISR	Oxidation/15.9949	M		2.88
	278	289	FNWYVDGVEVHNAK	Deamidated/0.9840	N		2.5
	292	300	TKPREEQYNSTYR	NGlycan/1079.4017	N		5.57
				NGlycan/1241.4545	N		23.2
				NGlycan/1403.5073	N		9.23
				NGlycan/1444.5339	N		33.4
				NGlycan/1606.5867	N		17.2
				NGlycan/1768.6395	N		3.86
				NGlycan/876.3223	N		1.9
				NGlycan/1038.3751	N		5.61
	296	300	EEQYNSTYR	NGlycan/1241.4545	N		100
	305	314	VVSVLTVLHQDWLNGKEYKCK	Deamidated/0.9840	Q		0.659
			VVSVLTVLHQDWLNGKEYKCK	Deamidated/0.9840	Q		2.1
		318	VVSVLTVLHQDWLNGK	Deamidated/0.9840	N		0.942
			VVSVLTVLHQDWLNGKEYK	Deamidated/0.9840	N		26
			VVSVLTVLHQDWLNGKEYKCK	Deamidated/0.9840	N		2.2
	326	328	VSNKALPAPIEK	Deamidated/0.9840	N		0.767
	348	348	EPQVYTLPPSREEMTK	Glu->pyro-Glu/-18.0106	E		1.79
			EPQVYTLPPSREEMTKNQVSLTCLVK	Glu->pyro-Glu/-18.0106	E		1.59
	364		EPQVYTLPPSREEMTKNQVSLTCLVK	Deamidated/0.9840	N		2.1
	364	364	NQVSLTCLVK	Deamidated/0.9840	N		3.14
		365	NQVSLTCLVK	Deamidated/0.9840	Q		3.14
	374	387	GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840	N		13.8
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	Deamidated/0.9840	N		4.95
		387,389	GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840; Deamidated/0.9840	N,Q		6.51
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	Deamidated/0.9840; Deamidated/0.9840	N,Q		5.57
		389	GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840	Q		11.7
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	Deamidated/0.9840	Q		3.8
		389,392	GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840; Deamidated/0.9840	Q,N		6.51
		392	GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840	N		11.7
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	Deamidated/0.9840	N		3.8
	413	437	LTVDKSRWQQGNVFSCSVMHEALHNHYTQK	Deamidated/0.9840	N		3.1
	420	437	WQQGNVFSCSVMHEALHNHYTQK	Deamidated/0.9840	N		5.4
	443	450	SLSLSPGK	Lys-loss/-128.0950	K		76.4
Light Chain	108	136	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR	Deamidated/0.9840	N		4.26
	126	136	SGTASVVCLLNNFYPR	Deamidated/0.9840	N		5.16
	145	151,154	VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK	Deamidated/0.9840; Deamidated/0.9840	N,Q		21.3
		157	VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK	Deamidated/0.9840	N		4.03
	207	209	SFNRGEC	Deamidated/0.9840	N		2.33

Table 1 Amino acid site relative abundance %

- **Table 1** gives the summary of the overall calculated amino acid site relative abundance percentage.
- Protein Metrics software automatically calculates the relative percentage of PTM of each individual modified amino acid on the peptides based on the ratio of extracted ion chromatograms (XICs) corresponding to the modified and unmodified wildtype peptides.

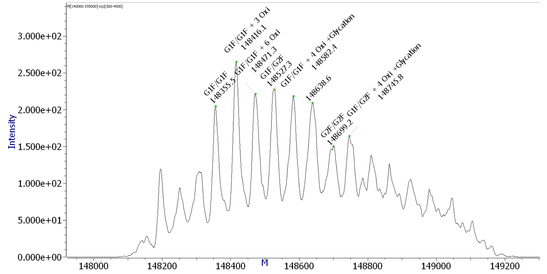

Fig.5 Deconvoluted spectra of intact NISTmAb with modifiers

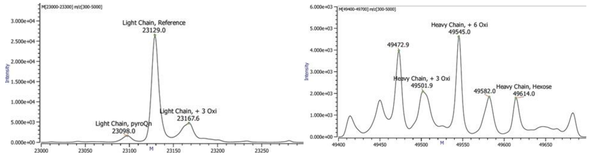

Fig.6 & 7 Deconvoluted spectra of NISTmAb light and heavy chain with modifiers

Name	Mass	Sample name / Peak #	NIST Intact / 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 2 Summary of NISTmAb intact mass analysis of theorical vs. observed mass

Name	Mass	Sample name / Peak #	NIST Subunit Analysis / Expected mass	NIST Subunit Analysis / Delta mass from calculated
Light Chain , Reference	23128.974	1	23123.593	5.38
Light Chain , pyroQn	23097.992		23105.593	-7.6
Light Chain , + 3 Oxi	23167.58		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	49501.9		49500.645	1.25

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

- 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**).
- **Tables 2** and **3** provide a comprehensive summary of the NISTmAb measured mass compared to the theorical delta mass differences, which is generated by protein metrics software. The data demonstrates the overall calculated delta mass difference is less than 20 delta ensuring accurate characterization of the mAb.

4. Conclusion

- During drug development, it's vital to monitor alterations in PTM amino acid site relative abundance percentages, like methionine (M) oxidation or asparagine (N) deamidation, as they can affect mAbs' efficacy, stability, and immunogenicity.
- Intact mass and subunit analysis determine the average mass of mAb, offering insights into their structural integrity crucial for drug product development. Subunit analysis assesses the light and heavy chain molecular weight following intra-chain disulfide bond reduction.
- The Shimadzu Q-TOF LCMS-9030 instrument is highly effective in generating precise data, which is crucial for the thorough characterization of mAb.

Reference
1) Monoclonal Antibody (mAb) Intact Mass and Subunit Analysis Using Shimadzu Q-TOF LCMS-9030, 04-AD-0296-EN
2) A Fast and Simple Workflow for Monoclonal Antibody (mAb) Post-Translation Modifications (PTM) Study Using Shimadzu LCMS-9030 Q-TOF, AD-0295-EN

********** All authors declare that they have no conflicts of interest. **********