Poster Note 64397

Characterization of Monoclonal Antibodies and ADCs using a Benchtop Orbitrap Mass Spectrometer

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Overview

Purpose: To characterize mAbs and ADCs with high resolution orbitrap mass spectrometer.

Methods: Coupling High Performance Liquid Chromatography with high resolution Orbitrap mass spectrometer (HPLC-MS) enables accurate mass measurement of mAb, ADCs, and peptide mapping experiments.

Results: For Herceptin, 7 major glycoforms were detected; 100% sequence coverage of the ADC sample was achieved, and the modifications, including the drug-conjugated site information were determined; K42 and K103 were 100% modified by DM1 with good reproducibility were observed.

Introduction

The biopharmaceutical industry has continued its focus on the development of biotherapeutic monoclonal antibody (mAb) drugs. This requires full characterization of these complex molecules, including any modifications that may occur. In this study, a Thermo Scientific™ Q Exactive Plus™ mass spectrometer was used for the characterization of Herceptin® (Roche) and T-DM1® (Trastuzumab Emtansine, Roche). Intact molecule weights were determined for the mAb/ADC and the light and heavy chains of ADC were measured independently. Complete peptide mapping experiments were performed yielding 100% sequence coverage, site specific information of the drug-conjugate as well as expected and unexpected modifications.

Methods

Sample Preparation

The T-DM1 sample was reduced by DTT for light/heavy chain molecular weight measurement. For peptide mapping, the ADC sample were reduced by DTT, following IAA treatment and then digested with chymotrypsin.

Liquid Chromatography

The peptide samples were separated on a Thermo Scientific™ Accela™ HPLC system (A: 0.1% formic acid in water,B: 0.1% formic acid in acetonitrile). Different gradients and LC conditions were used for the intact analysis and for the peptide mapping analysis. For intact mass analysis a Thermo Scientific ™ BioBasic™ C4 column (5μm, 10×2.1mm) and a BioBasic™ C8 column (5μm, 50×2.1mm) were employed; for peptide mapping a Thermo Scientific™ Accucore™-150-C18 column (100×2.1mm) was employed.

Mass Spectrometry

A Q Exactive Plus mass spectrometer was used for intact molecular weight, heavy and light chain molecular weight, and peptide mapping analysis.

Data Analysis

Data analysis was performed using Thermo Scientific™ PepFinder™ software(v1.0) for peptide mapping and characterization of modifications; Full MS spectra of intact mAbs and ADCs were analyzed using Thermo Scientific™ Protein Deconvolution software (v 3.0) that utilizes the ReSpect algorithm for molecular mass determination.

TABLE 1. HPLC conditions of Herceptin and T-DM1 analysis.

Intact mass			reduced		
Time	B%	Flow(ml/min)	Time	B%	Flow(ml/min)
0.00	5	0.5	0.00	5	0.3
0.50	5	0.5	3.00	5	0.3
0.51	5	0.2	11.00	95	0.3
2.00	90	0.2	13.00	95	0.3
2.10	5	0.5	13.10	5	0.3
2.70	90	0.5	15.00	5	0.3
2.80	5	0.5			
3.40	90	0.5			
3.50	5	0.5			
4.00	5	0.5			

Column temp.:75℃

Results

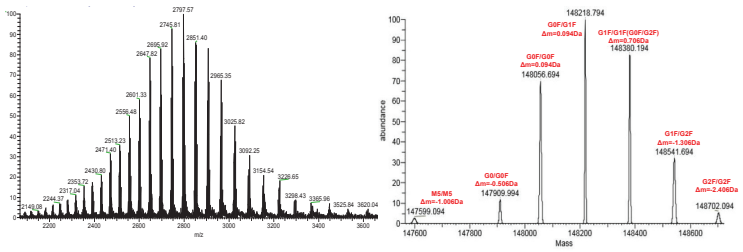
Molecule weight determination of intact mAb

The HPLC conditions of intact Herceptin analysis were described in table1, and the MS conditions as below:

In-source CID: 80.0 eV, Microscans: 10, Resolution: 17,500, AGC target: 3e6, Maximum IT: 200 ms, Scan range : 2000 to 4000 m/z (intact mass), 1000 to 4000 m/z (LC/HC mass)

For intact mAb, 7 major glycoforms could be found; FIGURE 1 shows the results of molecule weight measurement.

FIGURE 1. molecule weight determination of intact Herceptin.



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Molecule weight determination of intact ADC and its light and heavy chains

Next we employed T-DM1 sample for analysis. The HPLC conditions of T-DM1 and its LC and HC analysis were described in table1, and the MS conditions as below:

In-source CID: 70.0 eV, Microscans: 10, Resolution: 35,00, AGC target: 3e6, Maximum IT: 200 ms, Scan range： 2000 to 6000 m/z (intact mass), 1000 to 4000 m/z (LC/HC mass)

In intact mass result, mAb conjugated different drugs could be detected, with typical glycoform distribution. The DAR is 3.34 calculated on the result, which matched the description of this commercial product (3~4 in the datasheet, FIGURE 2).

When reviewing the light chain and heavy chain results, it was clear that partial T-DM1 carried different number of drugs and different glycoforms could be detected (FIGURE 3).

FIGURE 2. molecule weight determination of intact T-DM1.

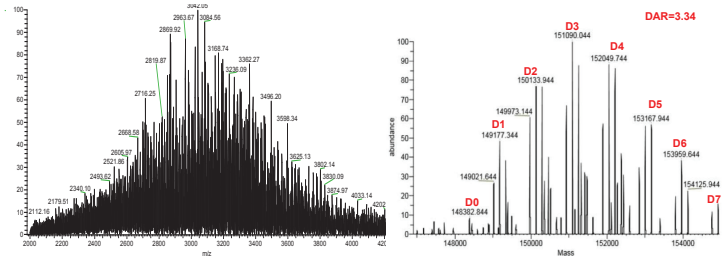
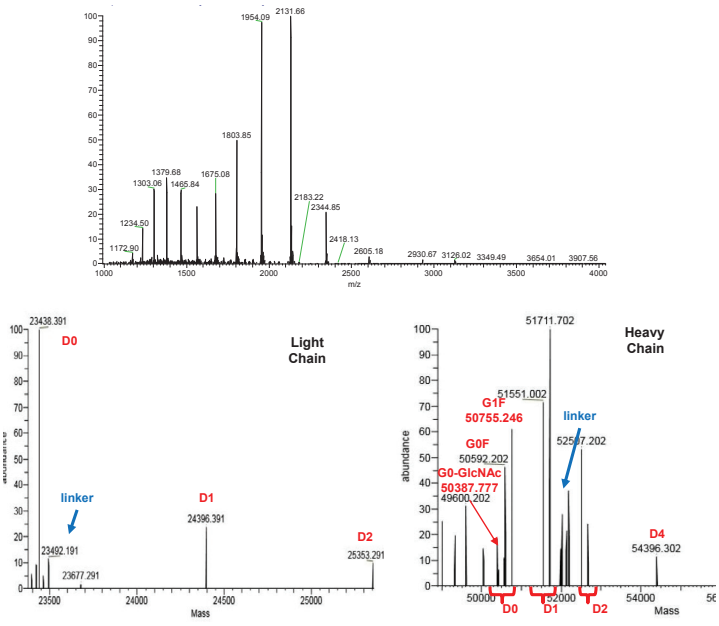


FIGURE 3. molecule weight determination of the light and heavy chains of T-DM1.



Peptide mapping results of T-DM1

The HPLC conditions are described in table2, and MS conditions as below:

Source settings:

spray voltage of 3500 V and capillary temperature of 275 °C.

Full MS settings:

Resolution=70,000; AGC target=3e6; Maximum IT=20 ms; scan range: 350 to 1200 m/z;

dd-MS² / dd-SIM settings:

Resolution=35,000; AGC target=5e5; Maximum IT=250 ms; TopN=8; NCE= 27.0

In this part, we used the software PepFinder to analyze the bottom up data of T-DM1. 100% sequence coverage of the ADC sample was achieved, and the modifications, including the drug-conjugated site information were determined (FIGURE 6).

TABLE 2. HPLC conditions of peptide mapping analysis.

Time	B%	Flow(ml/min)
0.00	4.00	0.2
4.00	20.00	0.2
15.00	50.00	0.2
17.00	90.00	0.2
18.00	90.00	0.2
18.10	4.00	0.2
20.00	4.00	0.2

Column temp.:55℃

FIGURE 4. The base peak chromatogram of chymotrypsin digested T-DM1, 0-3.8 min: to waste, labeled with the identified peptide sequences from PepFinder results.

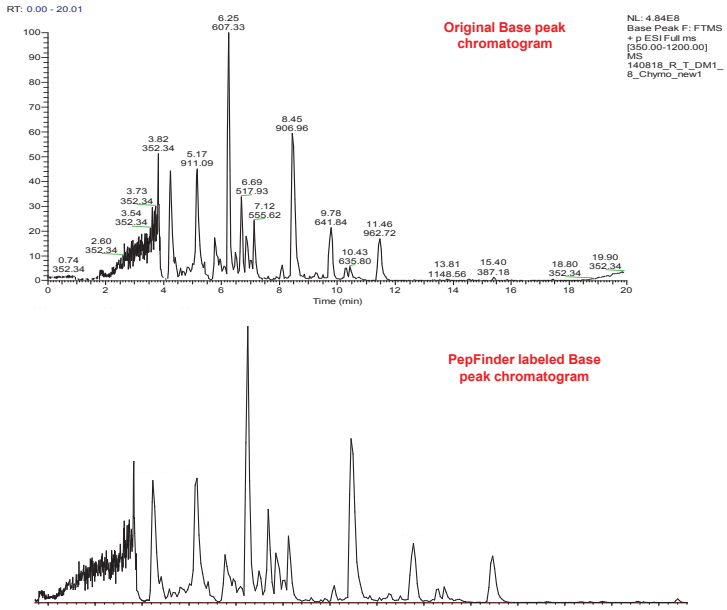


FIGURE 5. The sequence coverage result of chymotrypsin digested T-DM1(2 technical replicates) and sequence coverage map(partially).

Data File = 140818_R_T_DM1_8_Chymo_new1.raw
Protease = Chymotrypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:LC	164	28.0%	100.0%	62.27%
2:HC	350	32.3%	100.0%	37.73%

Data File = 140818_R_T_DM1_8_Chymo_new2.raw
Protease = Chymotrypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:LC	161	26.9%	100.0%	62.91%
2:HC	368	36.3%	100.0%	37.09%

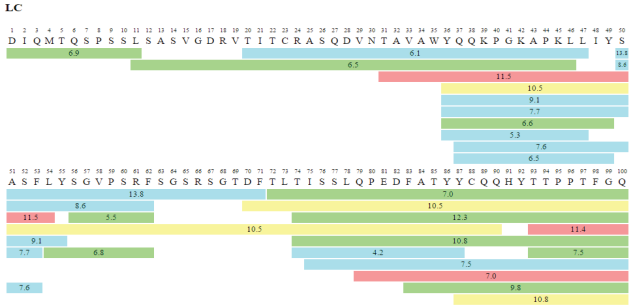


FIGURE 6. The Modification summary of digested T-DM1.

Data File = 140818_R_T_DM1_8_Chymo_new1.raw
Protease = Chymotrypsin

Protein	Modification	Recovery	Abundance
LC	N30+Deamidation	Poor	100.0000%
LC	~K42+DM1	Good	100.0000%
LC	~K42+Glycation	Fair	100.0000%
LC	~K103+DM1	Good	100.0000%
LC	~K107+DM1	Good	100.0000%
LC	Q124+Deamidation	Fair	100.0000%
LC	~N137+Deamidation	Good	1.8777%
LC	K149+Glycation	Good	0.3443%
LC	~K188+Glycation	Good	0.9233%
LC	~K190+DM1	Good	12.7532%
HC	~Q3+Deamidation	Fair	100.0000%
HC	~N55+Deamidation	Good	100.0000%
HC	~K76+Glycation	Good	100.0000%
HC	~N77+Glycan A2G0F	Good	100.0000%
HC	~K124+Glycation	Fair	100.0000%
HC	W161+Oxidation	Good	99.6907%
HC	~K251+DM1	Good	1.8659%
HC	~K251+Glycation	Fair	100.0000%
HC	M255+Oxidation	Good	7.4486%
HC	N300+A1G0	Good	38.1873%
HC	N300+A1G0M4	Good	55.5388%
HC	N300+A1G0M5	Good	9.4837%
HC	N300+A1S1	Fair	1.1079%
HC	N300+A1S1M4	Good	3.0445%
HC	N300+A2G0M4	Fair	50.1340%
HC	N300+A2G0M5	Fair	62.5256%
HC	N300+A2G1F	Fair	91.1237%
HC	N300+A2G2M5	Fair	26.2452%
HC	N300+M3	Good	2.0603%
HC	N300+Unglycosylated	Fair	8.8763%
HC	Q365+Deamidation	Good	100.0000%
HC	~K417+Glycation	Good	100.0000%

Data File = 140818_R_T_DM1_8_Chymo_new2.raw
Protease = Chymotrypsin

Protein	Modification	Recovery	Abundance
LC	~K42+DM1	Good	100.0000%
LC	~K103+DM1	Good	100.0000%
LC	Q124+Deamidation	Fair	100.0000%
LC	~N137+Deamidation	Good	2.0347%
LC	~K149+Glycation	Good	0.3331%
LC	~K188+Glycation	Good	0.8505%
LC	~K190+DM1	Good	11.8754%
HC	~Q13+Deamidation	Fair	100.0000%
HC	~N55+Deamidation	Good	100.0000%
HC	~N55+Glycan A2G0F	Good	100.0000%
HC	~K76+Glycation	Good	100.0000%
HC	~K136+DM1	Good	100.0000%
HC	W161+Oxidation	Good	100.0000%
HC	N162+Deamidation	Good	0.6571%
HC	~K249+Glycation	Fair	37.4916%
HC	~K251+DM1	Good	1.9683%
HC	~K251+Glycation	Fair	62.5084%
HC	M255+Oxidation	Good	7.1572%
HC	N300+A1G0	Fair	35.7612%
HC	N300+A1G0M4	Fair	49.6199%
HC	N300+A1G0M5	Fair	11.4647%
HC	N300+A1S1M4	Fair	3.1522%
HC	N300+A2G0M5	Fair	39.2572%
HC	N300+A2G1M5	Fair	62.2606%
HC	N300+A3S1G0	Fair	100.0000%
HC	N318+Deamidation	Good	0.8287%
HC	K320+DM1	Good	0.3226%
HC	Q365+Deamidation	Good	100.0000%
HC	~N387+Deamidation	Good	100.0000%
HC	K442+DM1	Good	100.0000%

FIGURE 6 shows the modification summary of T-DM1. It is easy to find some Lysines were modified by DM1. For K42 and K 103, they were 100% modified, K 190 was modified ~12% and the modified ratio of K251 was ~2%. All of these sites and good modification recovery, indicates high confidence results. Although it is difficult to identify low abundant glycoforms of T-DM1 on intact mass level, we could find them in the peptide mapping result.

Conclusion

In this study we measured the molecular weight of Herceptin and T-DM1, also their light and heavy chains.

- We can get accurate results and the DAR of T-DM1. Using the powerful software PepFinder, which was designed for biopharmaceutical industry, we can achieve 100% sequence coverage of the ADC sample and all of the modifications, including the drug-conjugated site information.
- In consideration of the significance of modification analysis in biopharmaceutical industry, this method can provide high throughput and reproducible MS and MS/MS information, which means high confident results.
- PepFinder software, which was designed for biopharmaceutical industry was used for data analysis.

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