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IM Q-TOF MS Applications of Monoclonal Antibody and its Derivatives

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Overview

This work demonstrates the use of Ion Mobility Q-TOF mass spectrometer for the characterization and structural comparison of the native mAbs and its derivatives, such as: IgG-1, IgG-2, Herceptin, Therapeutic ADC and Rituximab (Innovator vs. Biosimilar).

Introduction

Monoclonal antibodies (mAbs) and their derivative products such as Antibody-Drug Conjugate (ADC) and Biosimilars comprise a very important class of biopharmaceutical molecules with a wide range of therapeutic and diagnostic applications. It has also become increasingly important to monitor the proper structures (folding) of the mAbs during the development and production so that the maximum activity can be achieved. However, the characterization of these types of molecules by traditional mass spectrometer posed challenges due to their large sizes, many disulfide bonds (hydrophobicity) and the variation in glycan structures. The recent development in ion mobility mass spectrometry demonstrated the additional separation power in analyzing many biological molecules, such as the isobaric glycans, glycopeptides and the native proteins.

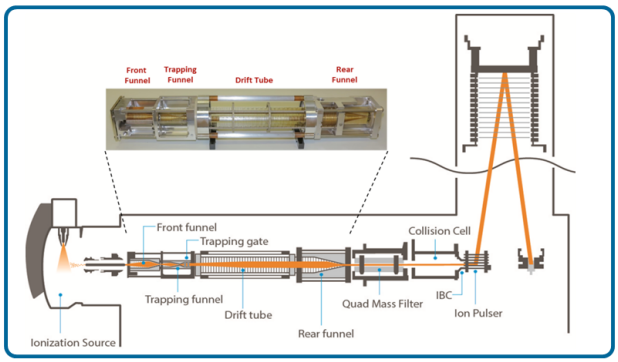


Figure 1. Schematic diagram of Agilent 6560 IM Q-TOF system.

Experimental

In order to obtain the information about the correct folding structure (collision cross section) on the mAbs, it is critical to preserve and maintain the native protein conformation throughout the entire analytical experiments. Therefore, a special workflow has been developed, including sample preparation (mAb deglycosylation and desalting), LC/MS analysis and IM data analysis (Bioconfirm SW).

Samples: IgG-1 vs. IgG-2, IgG-1 vs. Herceptin, Herceptin vs. Therapeutic ADC, Rituximab (Innovator vs. Biosimilar).

Sample Preparation: Deglycosylation of the antibodies and its derivatives were achieved by incubating with the Rapid PNGase F (NEB) in the Rapid buffer at 37°C for 20 min. Samples were then desalted using the Micro Bio-spin columns (Bio-Red) against 100 mM ammonium acetate (NH_4OAc) buffer (pH 7.8).

HPLC System: Agilent 1290 Infinity LC with Cap Pump was used. Flow injection was done at the micro flow rate of 7 uL/min with 100 mM NH_4OAc as the running solvent.

Dual AJS ESI Source Settings: 6560 IM Q-TOF MS

Parameter	Setting
Source	Dual Agilent Jet Stream
Acquisition Mode	Positive, Extended (10000 m/z) Mass Range (2 GHz)
Gas Temp	250 ºC
Gas Flow	5 L/min
Nebulizer	20 psig
Sheath Gas Temp	275 ºC
Sheath Gas Flow	12 L/min
VCap	4000 V
Nozzle Voltage	2000V
Fragmentor	400 V
Mass Range	300-10000 *m/z*
Scan Rate	0.9 frames/s
IM Trap Fill Time	50,000 us
IM Trap Release Time	300 us

Data Analysis: The data obtained from LC/MS were analyzed using Agilent MassHunter IM-MS Analysis software and Agilent MassHunter BioConfirm software. Maximum Entropy deconvolution algorithm was used for obtaining zero-charge spectrum of mAb.

Results and Discussion

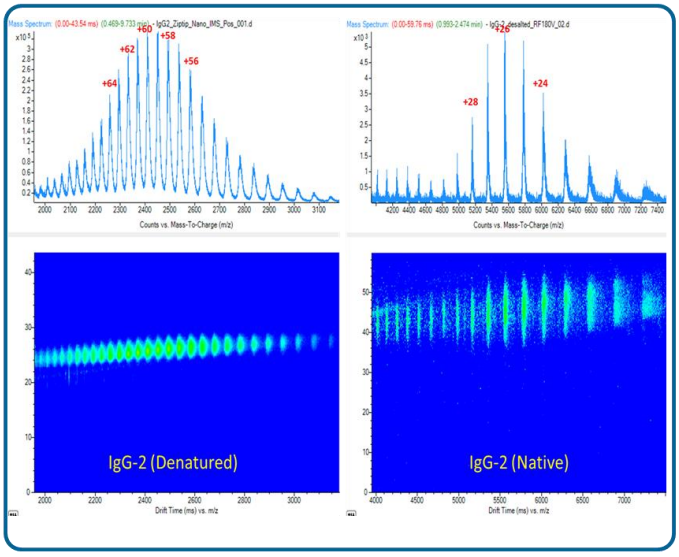


Figure 2: IM Q-TOF/MS analysis of IgG-2 under the denatured and native conditions. Top: Mass spectrum (Raw) of IgG-2. Bottom: Ion mobility abundance map (Drift time vs. m/z) of IgG-2. All charge ions of IgG-2 under denatured condition (+45 to +70) posed the much smaller drift times than the charge ions (+20 to +35) of native IgG-2.

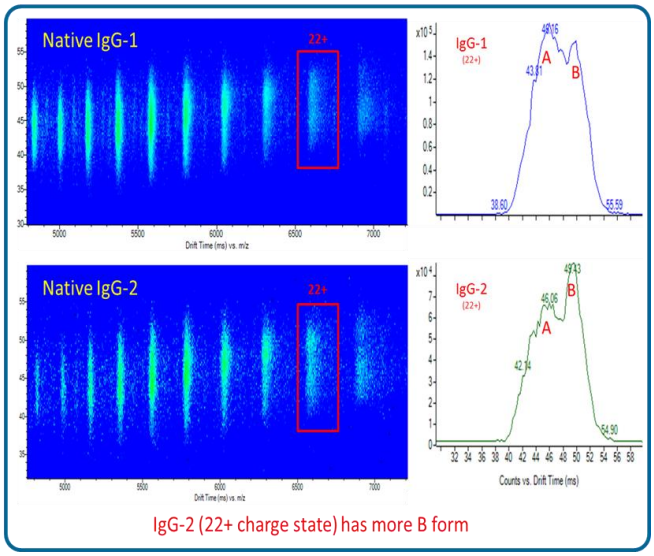


Figure 3: IM Q-TOF Comparison of IgG-1 and IgG-2 under native condition. IgG-1 posted slightly higher % of isoform A (native) than B at its 22+ charge state molecule. On the other hand, higher % of isoform B was detected in the IgG-2 sample.

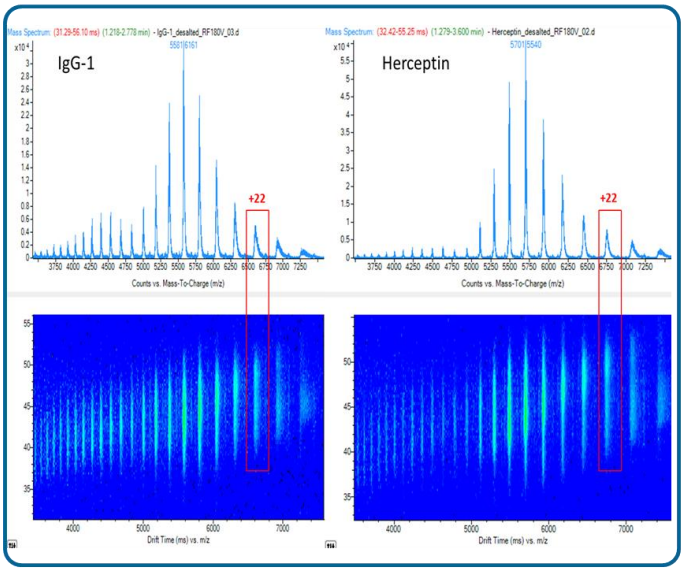
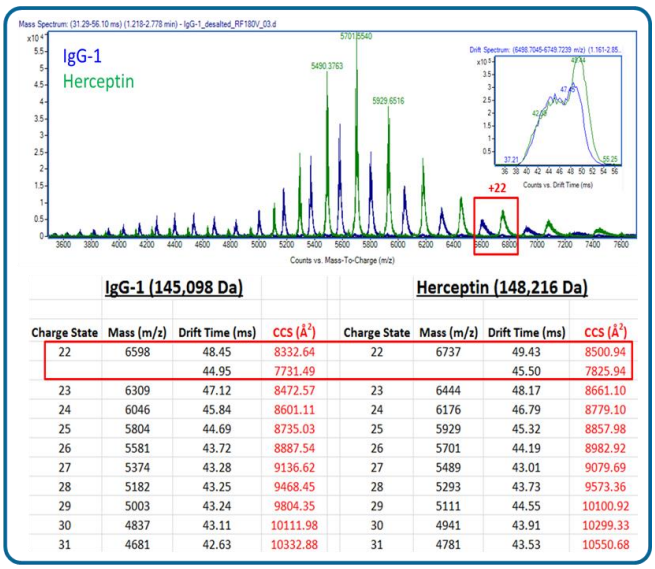


Figure 4: IM Q-TOF/MS analysis of IgG-1 (left) and Herceptin (right) under the native condition. Top: Mass spectrum (Raw) of the antibodies. Bottom: Ion mobility abundance map (Drift time vs. m/z) of the antibodies. Various charge states (+22 to +31) of both samples were selected for their CCS values comparison.



IgG-1 (145,098 Da)				Herceptin (148,216 Da)			
Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å²)	Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å²)
22	6598	48.45	8332.64	22	6737	49.43	8500.94
		44.95	7731.49			45.50	7825.94
23	6309	47.12	8472.57	23	6444	48.17	8661.10
24	6046	45.84	8601.11	24	6176	46.79	8779.10
25	5804	44.69	8735.03	25	5929	45.32	8857.98
26	5581	43.72	8887.54	26	5701	44.19	8982.92
27	5374	43.28	9136.62	27	5489	43.01	9079.69
28	5182	43.25	9468.45	28	5293	43.73	9573.36
29	5003	43.24	9804.35	29	5111	44.55	10100.92
30	4837	43.11	10111.98	30	4941	43.91	10299.33
31	4681	42.63	10332.88	31	4781	43.53	10550.68

Figure 5: Collision Cross Section (CCS) Comparison of IgG-1 and Herceptin. IgG-1 posed slightly lower % of isoform B at its 22+ charge state (top insert). Overall, Herceptin has slightly larger CCS values than IgG-1 with the same charge states.

Results and Discussion

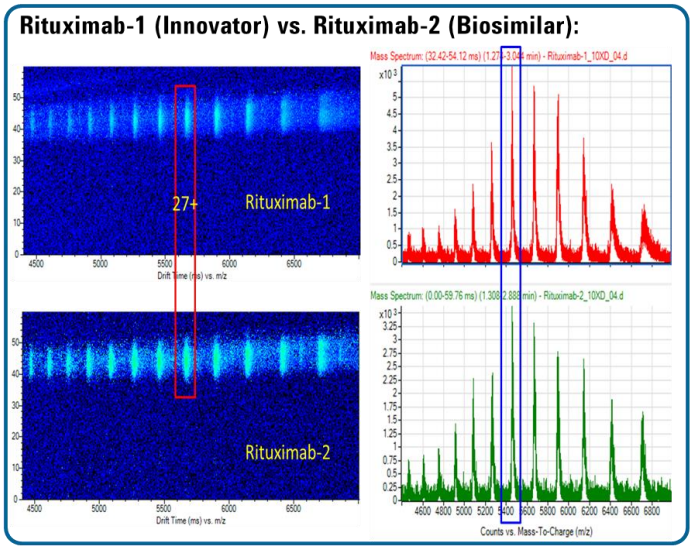
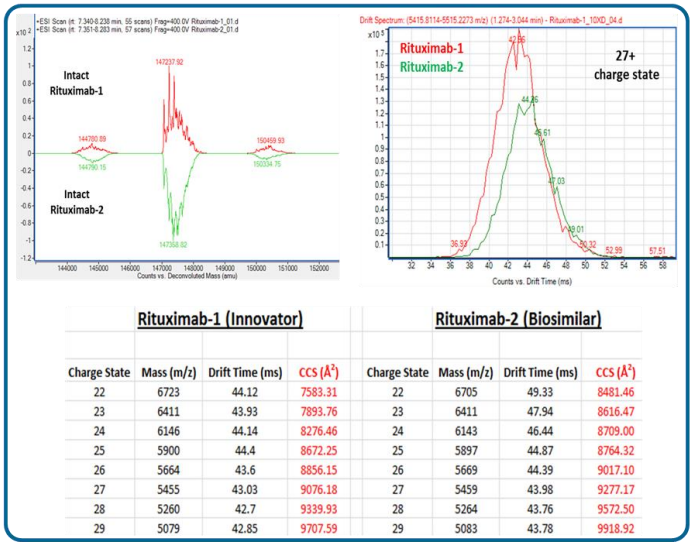


Figure 6: IM Q-TOF/MS comparison of Rituximab-1 (top) and Rituximab-2 (bottom) under the native condition. Left: Ion mobility abundance map (Drift time vs. m/z) of the antibodies. Right: Mass Spectrum (Raw) of the antibodies. The charge states (+27) of both samples were selected for their CCS values comparison (see below).



Rituximab-1 (Innovator)

Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å²)
22	6723	44.12	7583.31
23	6411	43.93	7893.76
24	6146	44.14	8276.46
25	5900	44.4	8672.25
26	5664	43.6	8856.15
27	5455	43.03	9076.18
28	5260	42.7	9339.93
29	5079	42.85	9707.59

Rituximab-2 (Biosimilar)

Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å²)
22	6705	49.33	8481.46
23	6411	47.94	8616.47
24	6143	46.44	8709.00
25	5897	44.87	8764.32
26	5669	44.39	9017.10
27	5459	43.98	9277.17
28	5264	43.76	9572.50
29	5083	43.78	9918.92

Figure 7: Collision Cross Section (CCS) Comparison of Rituximab-1 and Rituximab-2. The average size of glycans on the Rituximab-1 were slightly smaller than those on the Rituximab-2 (top left, Mirror Plot). The CCS of the 27+ molecule was larger for the Rituximab-2 (top right). Ion mobility can provide not only the size but also the molecule structural information in the Biosimilar study.

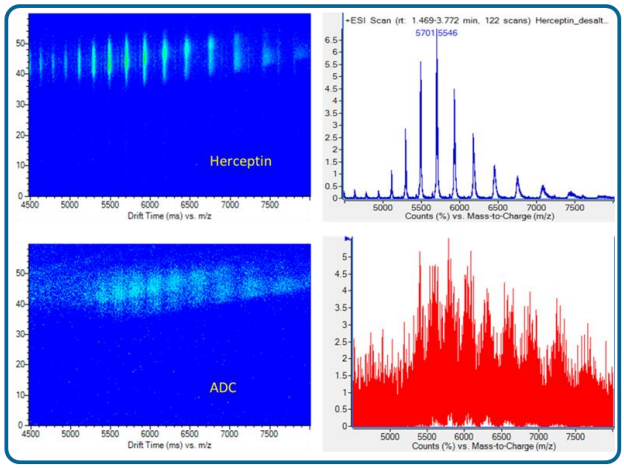


Figure 8: IM Q-TOF Comparison of Herceptin and ADC.

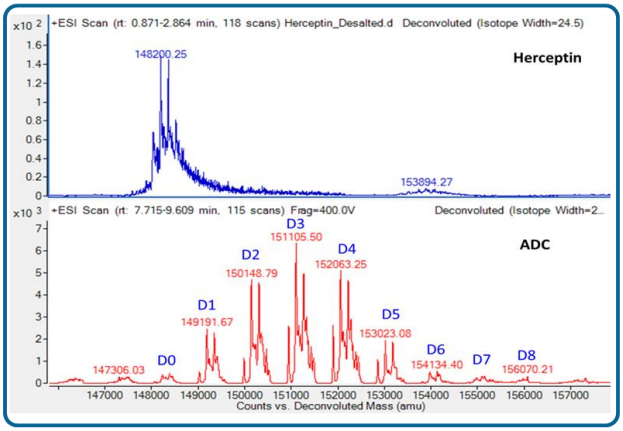


Figure 9: Mass Spectrometric Comparison of Herceptin and ADC. The de-convoluted spectrum showed 8 major drug attachments and the calculated drug antibody ratio (DAR) was ~3.4. For more details, please see ASMS Poster WP 674.

Conclusions

- **Ion Mobility Q-TOF MS system provided a new dimension of separation power for the characterization and structural comparison of the native mAbs and its derivatives.**
- **The accurate CCS values from biomolecules can be used as a great fingerprinting tool in the characterization and identification of various mAbs and their derivatives.**