Characterization of Biotherapeutics with High-Resolution Ion Mobility-Mass Spectrometry

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Abstract

- Ensuring the efficacy and safety of biotherapeutics is essential and often requires multiple workflows (intact, subunit, peptide, and released glycan) that rely on LC-MS for full characterization.
- Here we demonstrate the use of an LC-HRIM-MS system combined with Protein Metrics Byos for HRIM data processing module as a single platform solution for in-depth biotherapeutic characterization for all workflows.

Methods

Instrumentation and Software:

Sample Introduction – Agilent 1290 Infinity II UHPLC

Intact/Subunit:

PLRP-S 1000A 50mm 5µm

Peptide Mapping:

Waters BEH C18 150 x 2.1mm 1.7µm

Released Glycan:

Zorbax HILIC Plus 2.1 x 5mm 1.8µm

Data Acquisition – MOBIE™ HRIM system coupled to Agilent 6545XT

Software – Acquisition: *EyeOn, MassHunter* ***Analysis:*** *Protein Metrics, IM-MS Browser*

Sample Preparation – All samples were prepared according to respective care and use manuals unless otherwise noted

Intact: Waters Humanized mAb Mass Check Standard

Subunit: Waters mAb Subunit Standard

Peptide Mapping: NIST mAb RM 8671 was denatured using guanidine HCl, reduced using DTT, alkylated using IAA, desalted using 10kDa Amicon filters, and digested with trypsin for 4 hours at 37°C

Released Glycan: Waters Rapifluor Released MS-Glycan Standard

Intact Protein Analysis

- Protein Metrics Byos for HRIM Intact workflow filters data based on mobility through trapezoidal region selection for intact protein analysis.

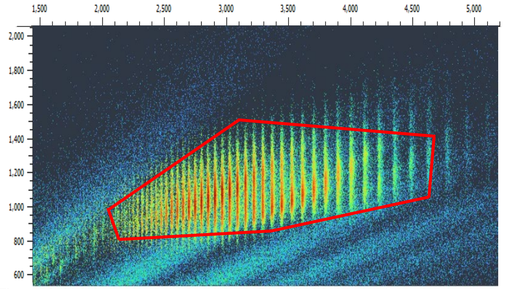

Figure 1. Heat-map plot as shown in Protein Metrics software showing charge distribution for intact NIST mAb. Trapezoidal extraction region outlined in red.

Table 1. Relative glycoform abundance and mass accuracy.

Glycoforms	% Relative Abundance	Mass Accuracy (ppm)
G2F/G2F	7.64	-5.18
G1F/G2F	13.76	-7.46
G1F/G1F	22.18	13.90
G0F/G1F	22.79	3.08
G0F/G0F	13.06	17.63
G0F/G1F+Lys	10.47	-17.58
G1F/G1F+Lys	9.43	5.21
G1F/G2F+Lys	6.67	-10.08

Subunit Analysis

- MOBIE HRIM separates the mAb LC and HC subunits in the mobility dimension. Protein Metrics Byos for HRIM Intact workflow filters data based on mobility for targeted deconvolution.

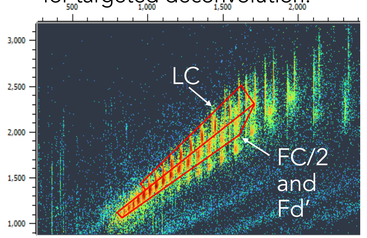

Figure 2. Heat-map plot as shown in Protein Metrics software mobility separation of the LC and FC/2 and Fd' subunits.

Table 2. Mass accuracy of subunits.

Subunit	Mass Accuracy (ppm)
Light Chain	2.2
Heavy Chain Fd'	1.4
Heavy Chain Fc, G2F	10.6
Heavy Chain Fc, G1F	1.7
Heavy Chain Fc, G0F	8.6

Peptide Mapping – Deamidation and Isomerization

- HRIM increases confidence in identification of deamidation and isomerization by providing unique arrival times for modified and unmodified peptides.

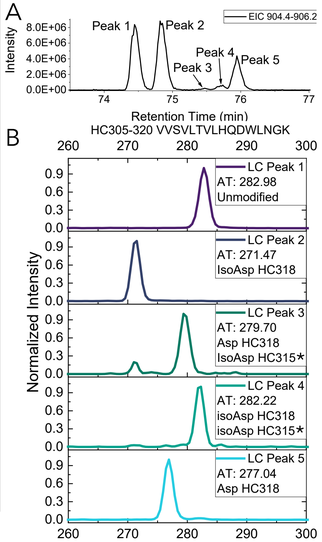

Figure 3. (A) Extracted ion chromatogram for the 2+ charge state of the VVSVLTVLHQDWLNGK peptide. (B) Extracted ion mobiligrams for each LC peak showing arrival time distributions for each. NOTE: * indicates tentative ID

Table 3. Average percent modification of deamidation and isomerization for 6 replicate injections

Chain	Residue	%Modified
L	Asn136	1.0%
L	Asn137	0.16%
H	Asp283	0.15%
H	Asn289	0.58%
H	Asp315	0.82%
H	Asn318	6.7%
H	Asn364	0.39%
H	Asn387	3.4%
H	Asn392/Asn393	1.1%
H	Asn424/436	1.2%

Peptide Mapping – Oxidation and Glycation

- Protein Metrics Byos for HRIM Peptide workflow identifies common PTMs and calculates % modification.

Table 4. Average percent modification of glycation for 6 replicate injections.

Chain	Residue	%Modified
L	Lys52	0.26%
L	Lys148	1.5%
L	Lys182	0.40%
L	Lys187	1.8%
L	Lys189	0.037%
H	Lys58	0.12%
H	Lys329	15.4%

Table 5. Average percent modification of oxidation for 6 replicate injections.

Chain	Residue	%Modified
L	M4	1.1%
H	M255	3.3%
H	M261	1.1%
H	Trp280	1.8%

Peptide Mapping – Glycopeptides, C-terminal Lysine Loss, and N-terminal *pyro*Glutamine

- The LC-HRIM-MS workflow can mobility separate glycopeptide isomers and calculate relative abundance.

Table 6. Average relative percent area of *N*-glycopeptides for 6 replicate injections.

N-Glycan	Relative % Area
FA1	28%
FA2G1	23%
FA2	15%
FA2G1	13%
FA1G1	11%
FA2G2	3%
FA2G2Ga1	2%
FA2G2	2%
FA2G2Ga2	1%
FA3G1	1%

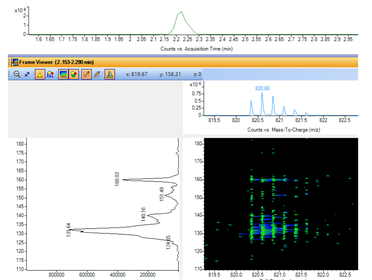

Figure 4. IM-MS Browser data visualization tool shows possible HRIM separation of G1F glycopeptide isomers, with only partial LC separation.

Table 7. Average relative percent area of heavy chain N-terminal pyroglutamate and C-terminal lysine clipping.

N/C Terminal Modifications	Relative % Area
N-term Pyroglutamate	100%
C-term Lysine Clipping	95%

Released Glycan Analysis

- The LC-HRIM-MS workflow can mobility separate released glycan isomers and calculate relative abundance.

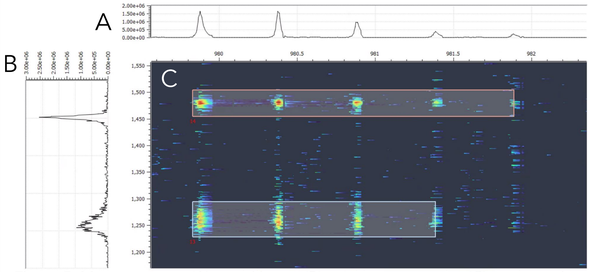

Figure 5. Mass spectrum (A), mobiligram (B), and heat-map plot (C) as shown in Protein Metrics software demonstrating separation of G1F isomers in the mobility dimension.

Table 8. Relative quantitation and reproducibility for released glycans identified across 6 replicate injections.

RapiFluor Labelled Glycan	Average %	RSD %	RapiFluor Labelled Glycan	Average %	RSD %
G1F	24.2	0.6	G1	1.3	3.6
G2F	20.3	1.1	G0 (CATION: NA)	1.2	4.4
G0F	16.9	2.2	G2	1.1	2.2
G2F+SA	10.4	2.2	G2+SA	0.7	3.5
G1F+GN	6.2	1.7	G2F+SA (CATION: NA)	0.7	5
G0F+GN	4.2	1.5	G0	0.6	7.7
G1F+SA	2.6	2.6	G2+2SA	0.3	9.6
G2F+GN+SA	1.9	8.1	G0F+GN (CATION: NA)	0.2	4.4
G2F+GN+2SA	1.8	7.1	G1F+GN (CATION: NA)	0.2	7
G2F+GN	1.6	2.9	G1F+SA (CATION: NA)	0.2	9.4
G1F (CATION: NA)	1.5	4.5	G2F+2SA (CATION: NA)	0.1	9.6
G2F+2SA	1.5	5.1			

Conclusions

- ***Comprehensive biotherapeutic analysis was performed on a single platform*** including, intact, subunit, peptide mapping, and released glycan LC-HRIM-MS workflows.
- Incorporation of MOBIE HRIM into traditional characterization workflows ***increases confidence in results*** by measuring unique and consistent arrival times.
- The Protein Metrics Byos for HRIM workflows provide an ***efficient data processing solution for all characterization workflows.***