BUILDING RAPID AND REPRODUCIBLE METHODS FOR LC/MS ANALYSIS OF INTACT AND REDUCED MONOCLONAL ANTIBODIES

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OVERVIEW

◊ A significant amount of antibody characterization can be accomplished by intact LC/MS analysis of the antibody and its reduced subunits.

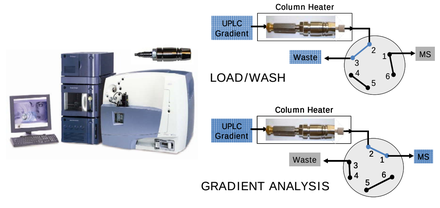
◊ Protein-level analyses provide a "holistic view" of the molecule, including verification of primary structure, and profiling of common glycosylation and processing variants.

◊ While such analyses are straightforward, many labs struggle to generate robust and reproducible methodologies for routine antibody characterization.

◊ Our group has identified an optimized chromatographic configuration, and focused on developing methods for generating reproducible high-quality antibody LC/MS data.

◊ In this poster, we illustrate two rapid, efficient, and reproducible methodologies for LC/MS profiling of the intact and reduced structures of an $IgG1_k$ monoclonal antibody.

SYSTEM CONFIGURATION



UPLC/LCT Premier XE Desalting LC/MS Configuration

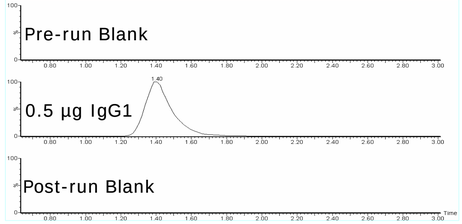
- LC/MS data were acquired using an ACQUITY UPLC™ system coupled with an LCT Premier™ orthogonal time-of-flight MS (Waters) operating in ESI+ V-mode.
- Desalting and reversed phase separations were accomplished on a 2.1 x 5 mm MassPREP™ Micro Desalting column (Waters).
- A system controlled post-column 2-position valve was used for diversion of buffers and salts present in the sample.
- MassLynx 4.1 and the included OpenLynx application manager were used for instrument control, data acquisition, and data processing.

INTACT IgG1 ANALYSIS (4 min)

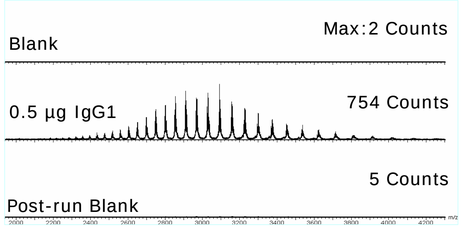
Time (min)	%B	Flow (ml/min)	Curve	
0.00	5	0.5	Initial	Load/Wash -Divert Flow-
0.50	5	0.5	6	
0.51	5	0.2	6	Gradient
2.0	90	0.2	6	
2.1	5	0.5	6	
2.7	90	0.5	6	
2.8	5	0.5	6	Column Washing and Regeneration
3.4	90	0.5	6	
3.5	5	0.5	6	
4.0	5	0.5	6	

A= 0.1%Formic Acid (Water) B= 0.1% Formic Acid (ACN)

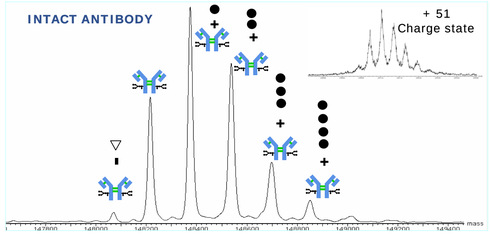
Gradient profile used for intact IgG1 analysis.



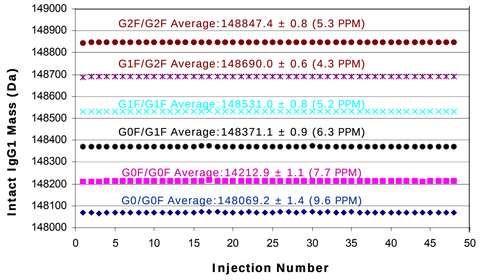
Total ion chromatograms (TIC) from LC/MS analyses of an intact IgG1, and pre and post run blank injections.



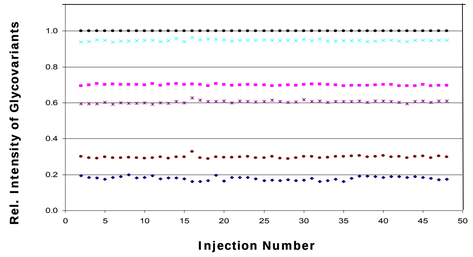
Combined ESI-TOF mass spectra of an intact IgG1 demonstrating regeneration to pre-injection conditions w/o the need of an inter-sample blank run.



MaxEnt 1 deconvoluted mass spectrum of an intact IgG1. Major variants observed were due to carbohydrate heterogeneity (Triangle=Fucose , Circles=Galactose).



The stability of modern TOF-MS systems produces consistent results over large sample sets. 48 vials of an IgG1 swere analyzed and the average mass precision (~5-10 ppm) was determined for each glycoform. Data collection and processing was automated using OpenLynx.



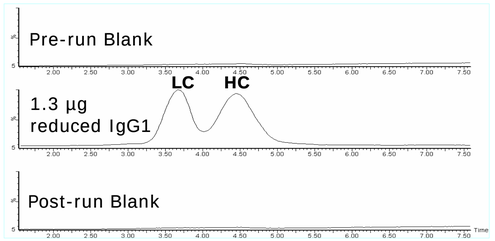
An average relative intensity CV of ~5% was achieved for all major IgG1 glycovariants over the 48 injection series.

REDUCED IgG1 ANALYSIS (10 min)

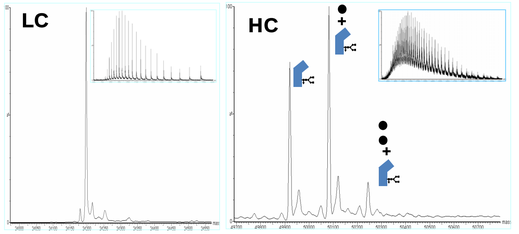
Time (min)	%B	Flow (ml/min)	Curve	
0.00	5	0.2	Initial	Load/Wash -Divert Flow-
0.50	5	0.2	6	
0.51	10	0.2	6	
7.61	50	0.2	6	Gradient
8.0	90	0.5	6	
8.1	5	0.5	6	
8.6	90	0.5	6	
8.7	5	0.5	6	Column Washing and Regeneration
9.2	90	0.5	6	
9.3	5	0.5	6	
9.8	5	0.5	6	

A= 0.1%Formic Acid (Water) B= 0.1% Formic Acid (ACN)

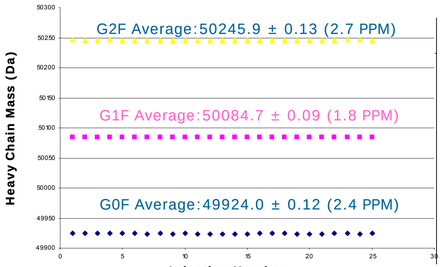
Gradient profile used for reduced IgG1 analysis.



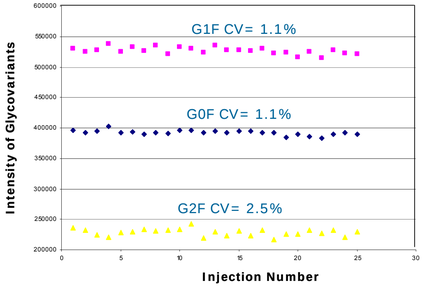
TIC from LC/MS analyses of a reduced IgG1, and the pre and post run blank injections.



Combined mass spectra (insets) and deconvoluted mass spectra of the light (LEFT) and heavy (RIGHT) chain subunits of an IgG1.



25 reduced IgG1 samples were analyzed using an OpenLynx automated acquisition and data processing method. The average mass measurement precision for the light chain (not shown) and three major heavy chain glycovariants were ~ 3 ppm.



An average intensity CV of 1.6% for all heavy chain glycovariants (0.5% for light chain) was achieved over the 25 injection sample series.

CONCLUSIONS

- A generic LC/MS configuration and methodology for intact (and reduced) antibodies has generated robust qualitatative and quantitative results with run cycle times of only four (or ten) minutes, no intersample blank injections, and automated data processing.
- This improved throughput and performance of this methodology should permit laboratories to better handle the increasing pipelines and assay demands now common to the biopharmaceutical industry.

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