Rapid LC/MS Analysis of Reduced and Intact Monoclonal Antibodies

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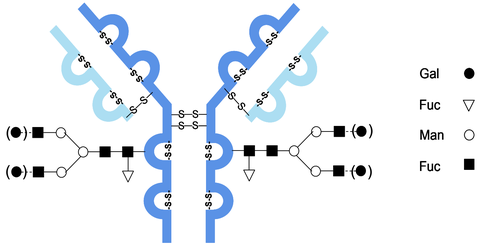
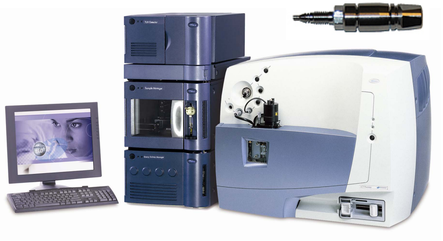
OVERVIEW

- Monoclonal antibodies comprise a significant proportion of biotechnology-derived molecules used for diagnostic and therapeutic applications.
- The inherent heterogeneity of such products has dictated the need for thorough analytical characterization methodologies so that safe, effective, and reproducible products can be produced.
- Most antibodies are stored in a matrix of biological buffers, non-volatile salts and stabilizers. Their removal (desalting) is one of the challenges encountered during mass analysis.
- In this study, we have developed two rapid, sensitive, and efficient desalting/cleanup LC/ESI-TOF MS methods that can be used for:
  - ⇒ Characterization of an intact antibody and its variants
  - ⇒ Characterization of constituent heavy and light chain structures of a reduced antibody

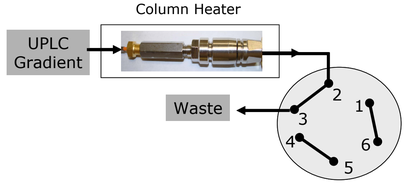


Structure of a monoclonal IgG1 antibody: The overall molecule (MW ~150 kD) comprises two identical heavy chains (HC, dark blue) linked together through two disulfide bonds, and two light chains (LC, light blue), each linked to heavy chain by single disulfide bond.

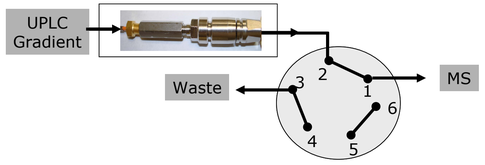
SYSTEM CONFIGURATION



- An LC/MS system was configured with a Waters ACQUITY UPLC™ chromatography system and an LCT Premier™ orthogonal time-of-flight mass spectrometer (Waters).
- LC separations were accomplished on a 2.1 x 5 mm MassPREP™ Micro Desalting column (Waters).
- A system controlled post-column multiport 2-position valve was used for diversion of buffers and salts present in the sample.
- Mass spectra were acquired in positive ion V mode. MassLynx 4.1 software was used for instrument control and data processing.



Position 1: Sample loading and salt divert to Waste



Position 2: Sample elution to MS

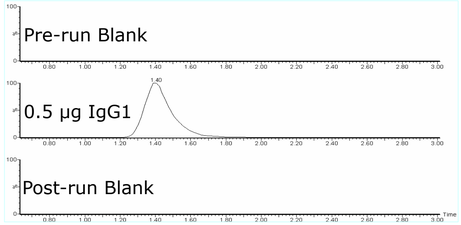
Desalting plus MS analysis fluidic configurations.

INTACT IgG1 LC/MS ANALYSIS (4 min)

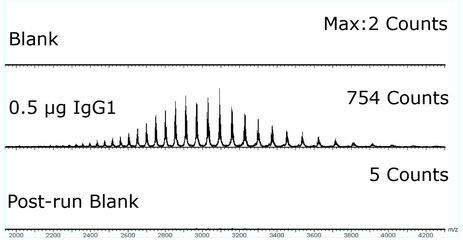
Time (min)	%B	Flow (ml/min)	Curve	
0.00	5	0.5	Initial	Load/Wash -Divert Flow-
0.50	5	0.5	6	
0.51	5	0.2	6	Gradient
2.0	90	0.2	6	
2.1	5	0.5	6	
2.7	90	0.5	6	
2.8	5	0.5	6	Column Washing and Regeneration
3.4	90	0.5	6	
3.5	5	0.5	6	
4.0	5	0.5	6	

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

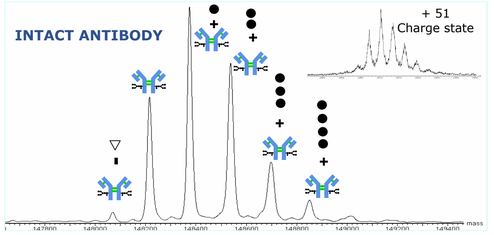
Gradient profile used for intact IgG1 analysis.



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



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.

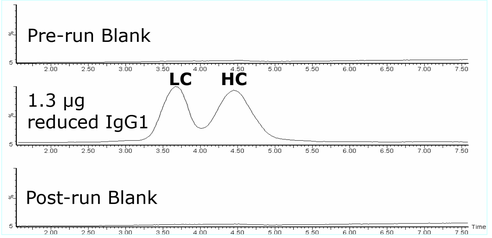


MaxEnt 1 deconvoluted mass spectrum of the intact IgG1. Major variants observed were due to carbohydrate heterogeneity.

Reduced IgG1 LC/MS ANALYSIS (10 min)

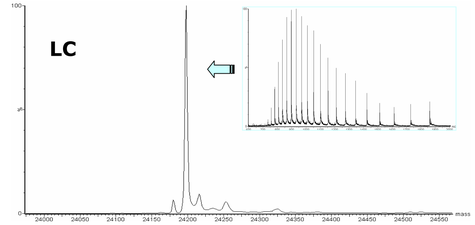
Time (min)	%B	Flow (ml/min)	Curve	
0.00	5	0.2	Initial	Load/Wash -Divert Flow-
0.50	5	0.2	6	
0.51	10	0.2	6	Gradient
7.61	50	0.2	6	
8.0	90	0.5	6	
8.1	5	0.5	6	
8.6	90	0.5	6	Column Washing and Regeneration
8.7	5	0.5	6	
9.2	90	0.5	6	
9.3	5	0.5	6	
9.8	5	0.5	6	

Gradient profile used for reduced IgG1 analysis.

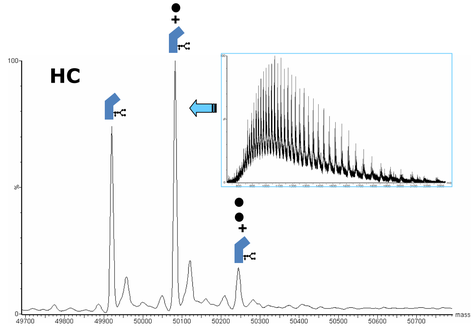


TIC chromatograms from LC/MS analyses of an reduced IgG1, and pre and post blank runs.

REDUCED ANTIBODY



Combined mass spectrum (inset) and deconvoluted mass spectrum of the light chain derived from the IgG1.



Combined mass spectrum (inset) and deconvoluted mass spectrum of the glycosylated heavy chain derived from the IgG1. The major peaks differing by ~162 Da corresponded to the HC with no, one, and two terminal galactose residues.

CONCLUSIONS

- A single LC/MS configuration has been demonstrated to permit rapid desalting and LC/MS analysis of an intact IgG1 antibody, and the reduced antibody.
- Overall, a four minute LC/MS method for the analysis of intact antibody, and a ten minute LC/MS analysis capable of analyzing resolved heavy and light chain subunits were obtained from our efforts.
- Heavy chain glycoform patterns were maintained through all analyses, and resulting masses of IgG1 fragments correlated with those of the intact antibody.

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