Development of a generic LC/MS methodology for protein-level analysis of IgG1 monoclonal antibodies and their related substructures

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Scott J. Berger, Asish B. Chakraborty, and John C. Gebler
Life Sciences R&D, Waters Corporation, Milford, MA

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.
- Intact protein LC/MS has become a powerful tool as part of the standard analytical package used to characterize these important biomolecules.
- Most antibodies are stored in a matrix of biological buffers and non-volatile salts and stabilizers and their removal (desalting) is one of the challenges encountered during mass analysis.
- In this study, we have developed two rapid, sensitive, and efficient generic 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
  - ⇒ Characterization of the common antibody fragments from papain cleavage.

SAMPLE PREPARATION

Materials: Protein A affinity purified mouse monoclonal antibody (IgG1,κ) was obtained from VICAM Inc. Papain was purchased from Boehringer Mannheim. Dithiothreitol (DTT) and cysteine-HCl were obtained from Pierce. Peptide N-glycosidase F (PNGase F) was purchased from New England BioLabs.

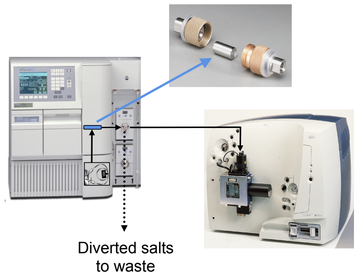
Preparation of Intact IgG1: Intact IgG1 stock (11.3 µg/µl, 0.1 M $NaHCO_3$/0.5 M NaCl, pH 8.3) was diluted with 50 mM ammonium bicarbonate to achieve 1.0 µg/µl IgG1. LC/MS analyses were performed on 10 µl of diluted IgG1 samples.

Preparation of Reduced IgG1 (to form heavy and light chains): Reduction of disulfides in the IgG1 (0.5 µg/µl) was accomplished using 20 mM DTT at 80°C for 15 min. The reduced sample was injected onto the column for LC/MS analysis (10 µl).

Papain digestion (no cysteine): Stock IgG1 was buffer exchanged against cysteine free papain digestion buffer (1 mM EDTA, 50 mM sodium phosphate buffer, pH 6.3) by centrifugal ultrafiltration (VIVASPIN, 5000 MWCO, 11,000 x g, 5 °C). Papain was activated by adding one part papain suspension (10 mg/ml) to nine parts freshly prepared activation buffer (1 mM EDTA, 10 mM cysteine, 50 mM sodium phosphate buffer, pH 7.0), and incubating for 15 min at 37 °C. The excess cysteine was removed by buffer exchange (against 6 vol. cysteine-free digestion buffer) of using centrifugal ultrafiltration. The activated papain was then diluted in cysteine free digestion buffer (1 µg/µl), added to the IgG1 solution at an enzyme: antibody ratio of 1% (w/w), and incubated at 37 °C for 2 h. The papain digest was diluted with 5% acetonitrile in 0.1% formic acid to 0.5 µg/µl, and used for LC/MS analysis (10 µl).

Papain Digestion (addition of cysteine): Stock IgG1 was buffer exchanged against papain digestion buffer plus cysteine (10 mM cysteine, 1 mM EDTA, 50 mM sodium phosphate buffer, pH 7.0) by centrifugal ultrafiltration (VIVASPIN, 5000 MWCO, 11,000 x g, 5 °C).Papain was activated by adding one part papain suspension (10 mg/ml) to nine parts freshly prepared activation buffer (1 mM EDTA, 10 mM cysteine, 50 mM sodium phosphate buffer, pH 7.0), and incubating for 15 min at 37 °C. The excess cysteine was removed by buffer exchange (against 6 vol. cysteine-free digestion buffer) of using centrifugal ultrafiltration. Papain digestion was carried out in digestion buffer plus cysteine at 37°C overnight at an enzyme: antibody ratio of 1% w/w. The papain digest was diluted with 5% acetonitrile in 0.1% formic acid to 0.5 µg/µl, and used for LC/MS analysis (10 µl).

METHODS



LC SYSTEM: An Alliance® 2796 Bioseparations system (Waters)

COLUMN: Reversed Phase MassPREP™ Desalting Cartridge (2.1 x 10 mm)

CONDITIONS: 0.4 ml/min, 30 °C

ELUENTS: (A) 0.1% formic acid in water, (B) 0.1% formic acid in acetonitrile

MS SYSTEM: LCT Premier™ ESI-Tof MS (Waters)

MODE: W-optics ESI+ mode, 1 Hz data acquisition

SOURCE: Temp: 150 °C, Desolvation Temp: 350 °C, Desolvation gas 800 L/hr, Cone voltage: 40 V, capillary voltage: 3.2 kV, Ion guide 1: 100 V.

CALIBRATION: External multi-point calibration using CsI ions (2 mg/ml CsI dissolved in 50% isopropanol). Mass spectra were acquired in the m/z range of 600-5000.

DATA ANALYIS: MassLynx Software, MaxEnt1 deconvolution

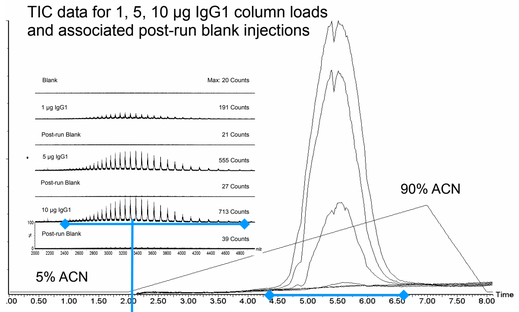
LC Method for an Intact Antibody

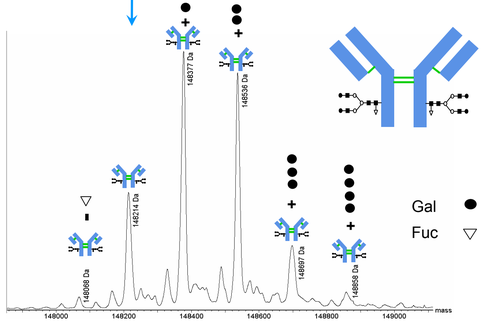
Time (min)	%A	%B
0.00 (Inject)	95	5
2.00 (Hold)	95	5
7.00 (Gradient)	10	90
8.00 (Regeneration)	95	5
10.00 (End)	95	5
Divert valve to MS	2:00- 8:10 min	

LC Method for Reduced Antibody (HC/LC) or Papain Fragments

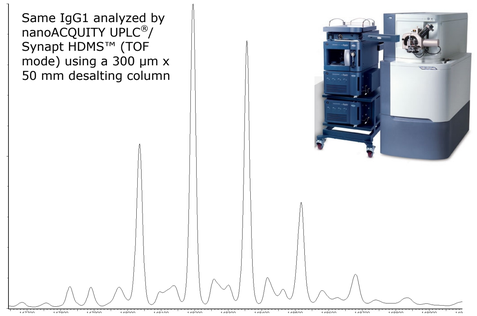
Time (min)	%A	%B
0.00 (Inject)	95	5
2.00 (Hold)	95	5
17.00 (Gradient)	10	90
18.00 (Regeneration)	95	5
20.00 (End)	95	5
Divert valve to MS	2:00- 18:10 min	

INTACT ANTIBODY (IgG1)

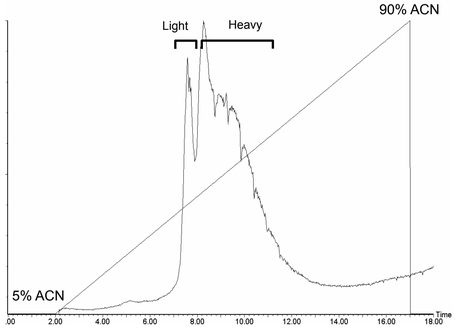


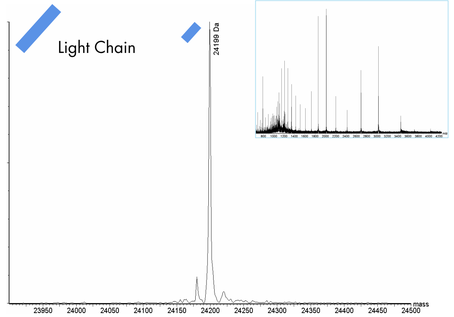


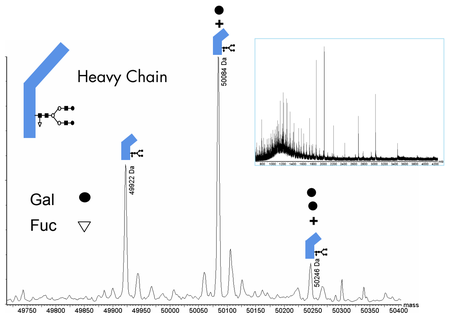
Same IgG1 analyzed by nanoACQUITY UPLC®/ Synapt HDMS™ (TOF mode) using a 300 µm x 50 mm desalting column



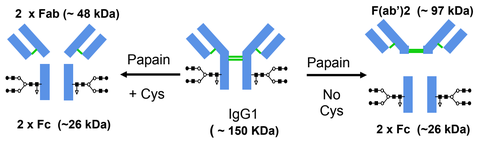
REDUCED ANTIBODY (HC/LC)

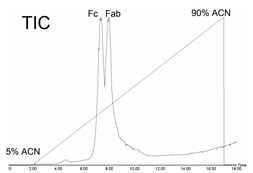


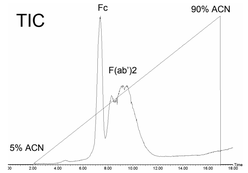


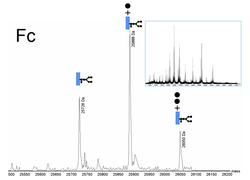


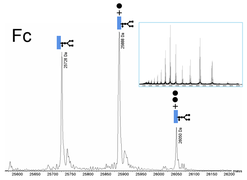
PAPAIN FRAGMENTS

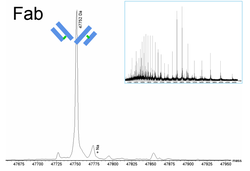


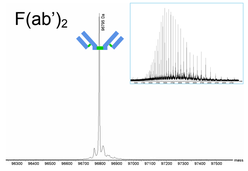












CONCLUSIONS

- A single LC/MS configuration has been demonstrated to permit desalting and LC/MS analysis of an intact IgG1 antibody, the reduced antibody, and the major antibody papain digest fragments.
- Heavy chain glycoform patterns were maintained through all analyses, and resulting masses of IgG1 fragments correlate with that of the intact antibody.
- Not shown: This methodology also extends to the deglycosylated intact antibody and it's related substructures, without modification.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720002103EN