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RAPID SCREENING OF REDUCED MONOCLONAL ANTIBODIES BY LC/ESI-TOF MS

Asish B. Chakraborty, Scott J. Berger, and John C. Gebler
Waters Corporation, Milford, MA, U.S.

INTRODUCTION

Biopharmaceutical R&D groups are asking their analysts to screen increasingly larger numbers of monoclonal antibody samples to support clone selection, stability, and product formulation studies. While various antibodies can exhibit unique selectivity, their overall structures are conserved within an antibody class, and standard analytical methods can often be employed for their routine analysis.

While intact antibody LC/MS analysis is useful for profiling glycovariants and C-terminal Lys processing, even more information can be gleamed from analysis of the light and heavy chain subunits from the disulfide-reduced antibody. LC/MS analysis of reduced antibodies offers the ability to detect the lower mass modifications such as oxidation (+16 Da) and pyroglutamate formation (-17 Da) that are obscured under the isotopic envelope of an intact antibody. Additionally, larger mass modifications such as nonenzymatic glycations (+162 Da) can be more clearly revealed with analysis of individual subunits.

Most antibodies are stored in a nonvolatile matrix of buffers, salts, and stabilizers. Their removal (desalting) is one of the challenges encountered during routine mass analysis.

In this study, we have devised an LC/MS methodology, using UPLC®/MS technology, for rapid sample desalting and efficient characterization of the heavy and light chain structures from a reduced antibody.

Figure 1. The ACQUITY UPLC System with LCT Premier XE Mass Spectrometer.

EXPERIMENTAL

UPLC conditions

LC system:	Waters ACQUITY UPLC® System
Column:	Waters MassPREP™ Micro Desalting Column (2.1 x 5 mm)
Column temp.:	80 °C

MS conditions

MS System:	Waters LCT Premier™ XE ESI-TOF MS
Ionization mode:	ESI Positive, V mode
Capillary voltage:	3200 V
Cone voltage:	40 V
Desolvation temp.:	350 °C
Source temp.:	150 °C
Desolvation gas:	800 L/Hr
Ion guide 1:	5 V
Acquisition range:	600 to 2500 m/z

System configuration

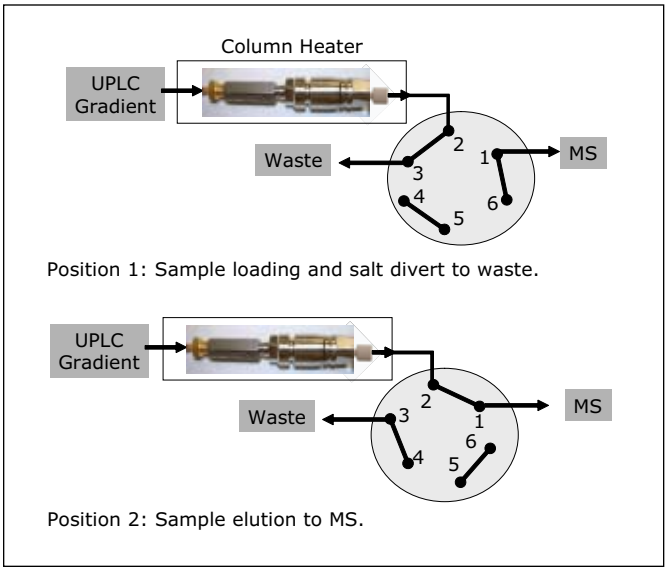

Figure 2: Fluidic configuration for LC/MS analysis. A post-column salt diversion valve (top-left corner of the LCT Premier XE) was utilized to divert buffers and nonvolatile salts to waste during the sample loading step.

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	
8.00	90	0.5	6	
8.10	5	0.5	6	Column Washing and Regeneration
8.60	90	0.5	6	
8.70	5	0.5	6	
9.20	90	0.5	6	
9.30	5	0.5	6	
9.80	5	0.5	6	

A = 0.1% formic acid (water) B = 0.1% formic acid (ACN)

Table 1: Gradient profile used for reduced IgG1 analysis.

Preparation of reduced IgG1 (to form HC and LC)

Reduction of disulfides in an IgG1 (0.5 µg/µL) was achieved using 20 mM DTT at 80 °C for 15 min. The reduced sample was acidified with formic acid (to 1%), microcentrifuged, and injected onto the column for LC/MS analysis (2.5 µL).

RESULTS

A rapid LC/ESI-MS method was used to resolve and characterize IgG heavy and light chains. For efficient sample desalting, a system-controlled post-column valve was used for waste diversion of sample buffers and salts prior to initiating the analysis gradient.

Additional sawtooth (rapid) gradient cycles were applied following the analysis gradient to regenerate the column back to pre-injection conditions (Figure 3). This avoids the need to separate difficult samples with inter-run blank injections. To minimize run cycle time, and maximize system performance, higher flow rates were applied for column regeneration.

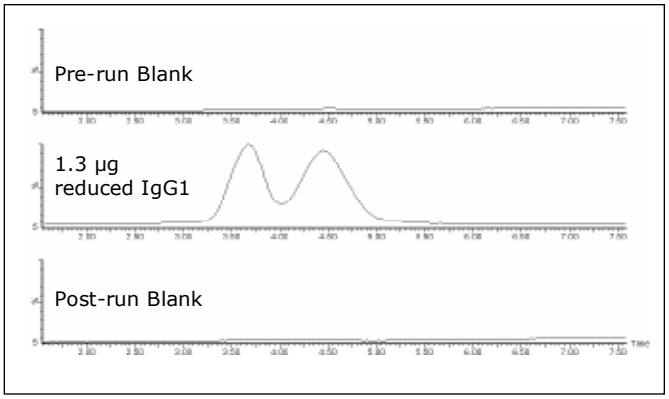

Figure 3: Total ion chromatograms (TICs) from LC/MS analyses of light and heavy chains from a reduced antibody.

The TIC of the reduced antibody analysis is displayed in Figure 3. The 10-minute LC/MS run largely resolved the earlier eluting light chain from the later eluting glycosylated heavy chains.

Figure 4 displays the summed mass spectrum (inset) and MaxEnt1 deconvoluted spectrum of the light chain, which reveals a single major peak at 24,199 Da. Minor peaks (sodium adduct and loss of water) are also visible in the deconvoluted spectrum.

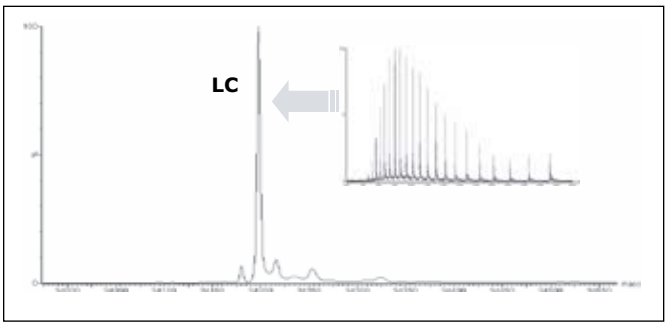

Figure 4: Combined ESI-TOF mass spectrum (inset, 2.3 to 3.9 min, Figure 3) and resulting MaxEnt1 deconvoluted mass spectrum of the light chain.

Figure 5 depicts the summed mass spectrum (inset) and resulting MaxEnt1 deconvoluted mass spectrum of the glycosylated heavy chain. Three major peaks with mass differentials of ~162 Da correspond to the heavy chain containing the core fucosylated glycan, and variants extended by one or two terminal galactose residues.

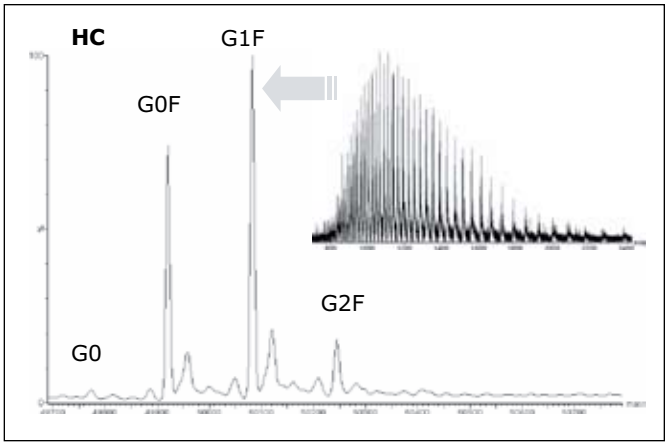

Figure 5: Combined mass spectrum (inset, 4.1 to 5.0 min, Figure 2) and resulting MaxEnt1 deconvoluted mass spectrum of the heavy chain. The major peaks differing by 162 Da corresponded to zero (49,922 Da), one, and two terminal galactose on the bound core N-glycan structure.

CONCLUSION

We have demonstrated an LC/MS methodology that is capable of rapidly resolving and efficiently characterizing light and heavy chains obtained from a reduced monoclonal antibody. The inclusion of additional sawtooth gradient cycles in the method eliminated the need for blank injections between samples, further increasing the utility of this approach for routine antibody screening.

Overall, sample throughput gains achieved with rapid characterization methods using UPLC/MS should provide bioanalytical groups the flexibility and capacity to adapt to the ever expanding demands of their parent organizations.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com