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アプリケーションノート

Routine Profiling of Reduced Antibodies by LC/ESI Quadrupole MS

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Abstract

In this application note, we have demonstrated a quadrupolebased LC-MS methodology that is capable of rapidly resolving and profiling the light and heavy chain variants obtained from a reduced IgG1 monoclonal antibody.

Benefits

The combination of TOF and quadrupole UPLC-MS platforms can help laboratories achieve both analytical goals without over-extending their financial or human resources.

Introduction

Biopharmaceutical companies are requiring their analysts to screen increasingly larger quantities of monoclonal antibody samples to support clone selection, stability, and product formulation studies. Much of this analysis involves routine confirmation of reduced antibody (light/heavy chain) masses and rapid profiling of their structural variants.

Analysis of reduced antibody subunits enables the detection and subunit localization of large mass difference variants (e.g. proteolytic clipping, glycosylation, lysine processing) and lower mass modifications such as

oxidation and pyroglutamate formation. These smaller mass modifications are obscured under the isotopic envelope during intact antibody analysis.

Antibody light and heavy chains produce electrospray charge envelopes well within the ion transmission and detection capabilities of both high resolution time-of-flight (TOF) MS systems and lower resolution quadrupole-based mass detectors. For antibodies of typical complexity, heterogeneity of the reduced subunits can be profiled using both mass detection technologies.

While TOF instruments provide greater precision for mass determination and the additional resolution to discern more complex variant profiles, the utility of the Waters ACQUITY SQD and its single quadrupole mass detection for routine screening and mass confirmation also provides tangible benefits. In fact, the system's features – such as low start-up costs, easy-to-use automated tuning and calibration, and walk-up open access operation – enable organizations to provide their analytical groups with mass analysis capabilities for routine antibody screening using UPLC-MS.

Antibodies are typically 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 combined a quadrupole-based LC-MS system with robust methodology for rapid sample desalting and efficient variant profiling of reduced monoclonal antibodies.

Figure 1. The ACQUITY SQD LC-MS system.

Experimental

UPLC Conditions

LC system:	ACQUITY SQD
Column:	MassPREP Micro Desalting Column (2.1 x 5 mm)
Column temp.:	80 °C

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.

MS Conditions

MS system:	Waters SQ Detector

Ionization mode:	ESI Positive
Capillary voltage:	4500 V
Cone voltage:	30 V
Desolvation temp.:	450 °C
Source temp.:	150 °C
Desolvation gas:	800 L/Hr
LM 1/HM 1 resolution:	11.2/14.8
Acquisition range:	600 to 2000 *m/z*

Reduction of an IgG1 to heavy (HC) and light (LC) chain subunits

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 and Discussion

A rapid LC/ESI-MS method (Table 1) was used for resolution and mass analysis of IgG1 heavy and light chains. For efficient sample desalting, a system-controlled post-column valve (Figure 2) was used for waste diversion of sample buffers and salts prior to initiating the 7.5 min analysis gradient. Additional sawtooth (rapid) gradient cycles were applied following the analysis gradient to regenerate the column back to pre-injection conditions (Figure 3). To minimize run cycle times and maximize system performance, higher flow rates were applied for column regeneration.

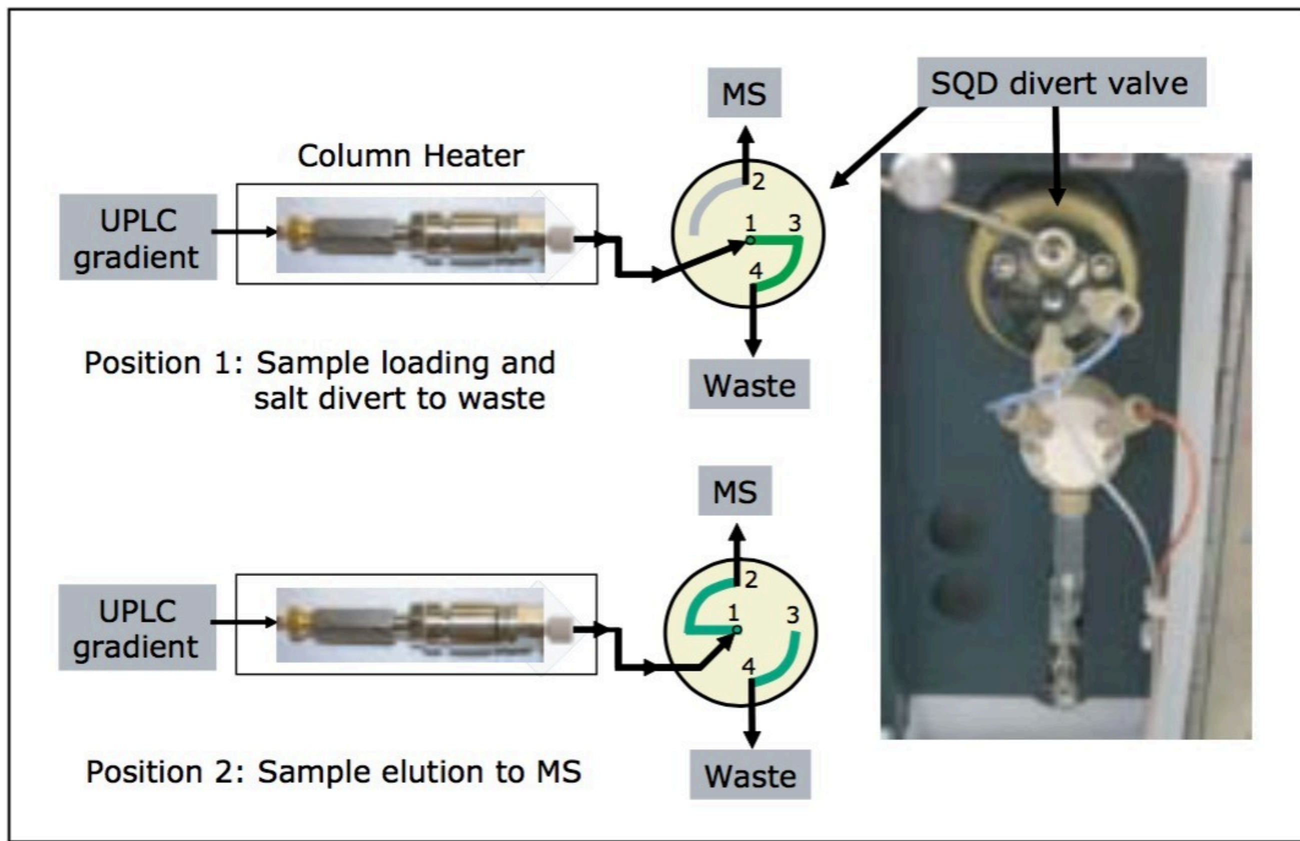

Figure 2. Fluidic configuration for LC-MS analysis. An integrated post-column valve used for salt diversion during sample loading is located behind the righthand door of the SQ mass detector. This valve is also utilized to divert LC flow and direct the AutoTune calibrant during automated system startup.

The total ion chromatogram (TIC) from a reduced antibody LC-MS analysis is displayed in Figure 3. The 10-minute LC-MS run largely resolved the light chain from the later-eluting heterogeneously glycosylated heavy chain. Comparison of pre-run and post-run TIC traces demonstrates the efficient regeneration of the LC system following the reduced antibody analysis.

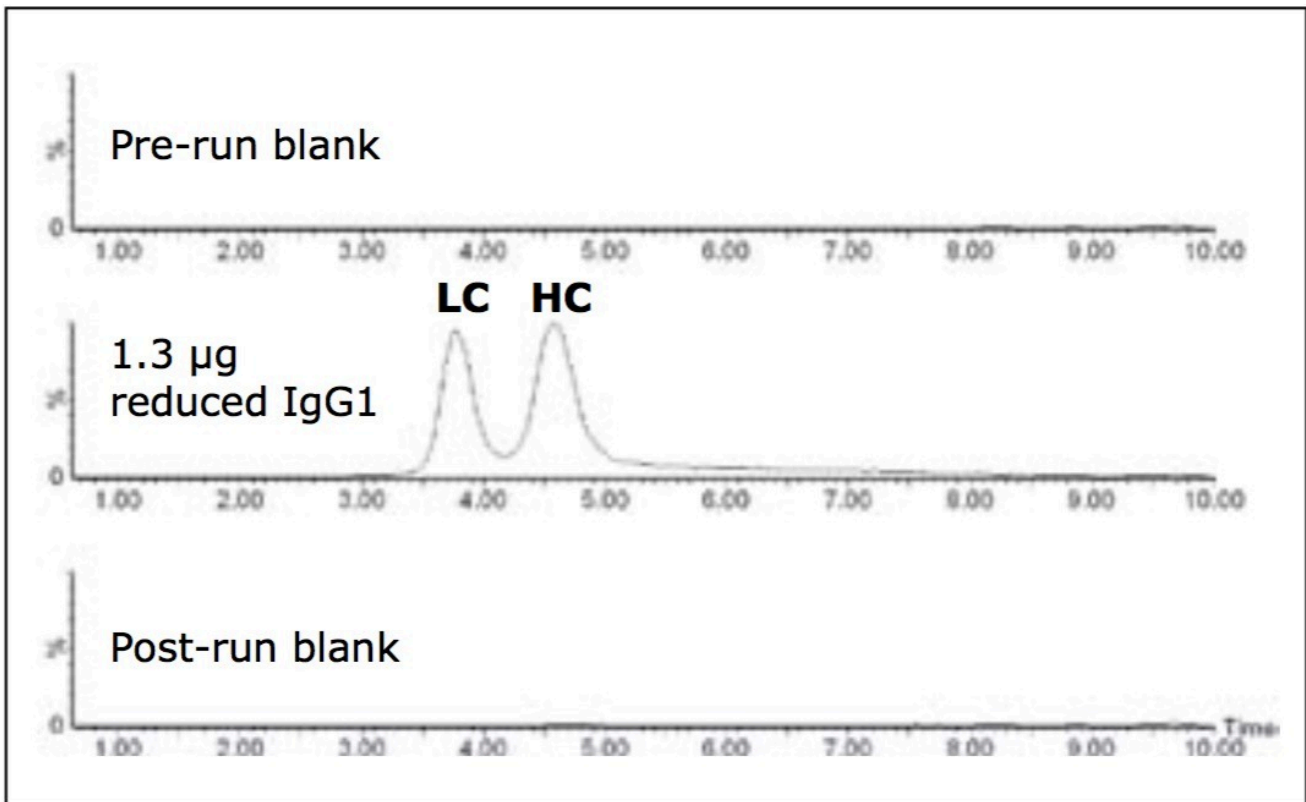

Figure 3. Total ion chromatograms (TICs) from LC-MS analysis of light and heavy chains from a reduced antibody.

Figure 4 displays the summed mass spectrum (inset) and MaxEnt1 deconvoluted spectrum of the light chain, which reveals a single major peak at 24,200 Da. Minor peaks (corresponding to the sodium adduct and loss of water) were also visible in the deconvoluted spectrum.

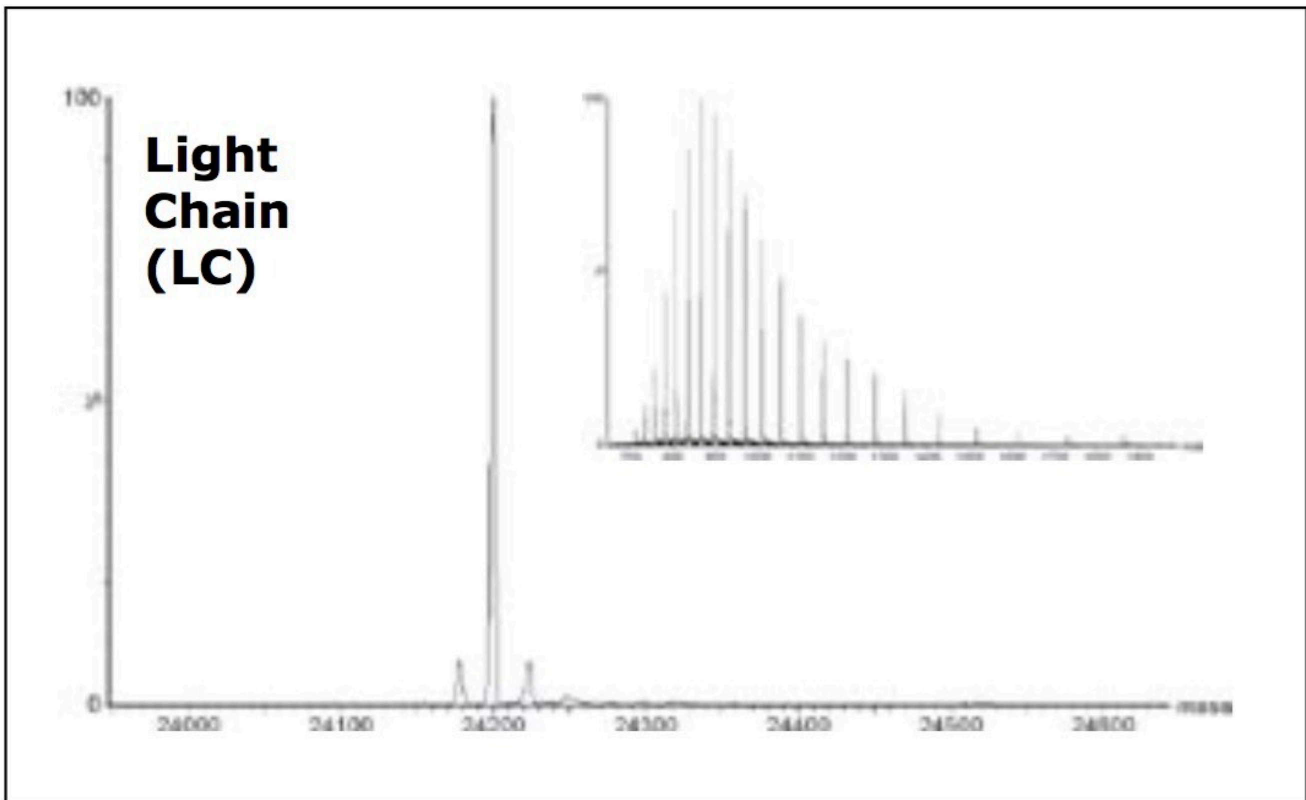

Figure 4. Combined ACQUITY SQD-ESI mass spectrum (inset, 3.5 to 4.0 min, Figure 3) and resulting MaxEnt1 deconvoluted mass spectrum of the antibody light chain.

Figure 5 depicts the summed mass spectrum (inset) and resulting MaxEnt1 deconvoluted mass spectrum of the glycosylated heavy chain. The major peaks correspond to the heavy chain containing the core fucosylated glycan (G0F 49,924 Da), a minor nonfucosylated form (G0), and core glycan variants extended by one or two terminal galactose residues (G1F, G2F). These results are fully consistent with studies of this antibody by LC/ESI-TOF MS shown in earlier application notes.

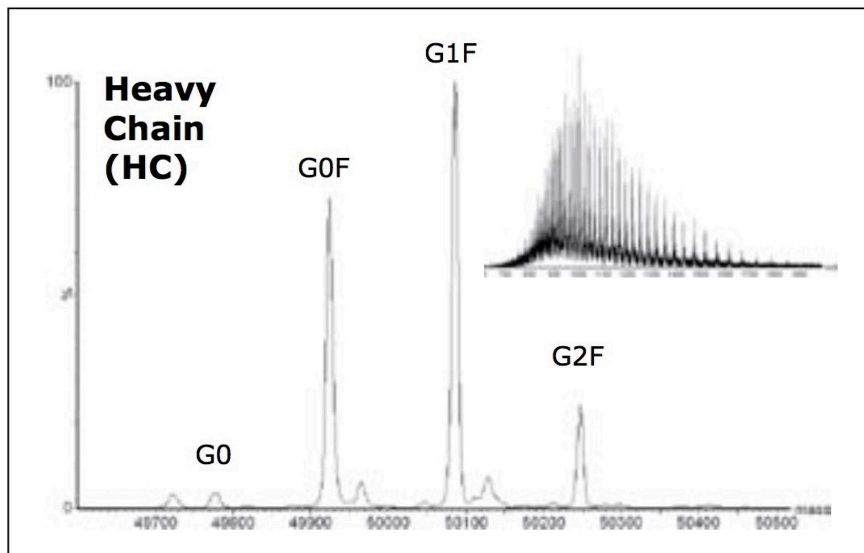

Figure 5: Combined mass spectrum (inset, 4.3 to 5.0 min, Figure 3) and resulting MaxEnt1 deconvoluted mass spectrum of the heavy chain.

Conclusion

In this application note, we have demonstrated a quadrupolebased LC-MS methodology that is capable of rapidly resolving and profiling the light and heavy chain variants obtained from a reduced IgG1 monoclonal antibody.

Many bioanalytical groups are looking to balance high-end protein characterization capabilities with lower-cost solutions for routine confirmation of protein structures. The combination of TOF and quadrupole UPLC-MS platforms can help laboratories achieve both analytical goals without over-extending their financial or human resources.

The utility of quadrupole mass detection for reduced antibody mass profiling, combined with the throughput, robustness, and ease-of-use of an integrated LC-MS system, presents biopharmaceutical organizations with flexible capabilities to respond to their ever-expanding demands for routine antibody mass analysis.

References

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