

## Rapid Profiling of Monoclonal Intact Antibodies by LC/ESI-TOF MS

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### Abstract

In this application note, we have devised a methodology using UPLC-MS technology for rapid desalting and efficient mass profiling of an intact antibody and its associated variants.

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### Introduction

The pipeline of therapeutic antibodies is growing, as the pharmaceutical industry increasingly focuses on development of biotechnology-derived drugs and diagnostic agents. This dynamic has put pressure on bioanalytical groups to produce more generic methodologies for antibody characterization, with higher throughput and faster sample turnaround times.

While antibody selectivity varies appreciably, overall antibody structures are highly conserved. Standard analytical methods can often be employed as the basis for optimizing analysis of individual molecules and their variants. High resolution mass spectrometry provides a powerful holistic approach for profiling batch-to-batch variations, and studying the structural changes associated with drug production, formulation, and storage.

Most antibodies are stored in a matrix of nonvolatile buffers, salts, and stabilizers, and their removal (desalting) is a common challenge encountered for antibody mass analysis.

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In this study, we have devised a methodology using UPLC-MS technology for rapid desalting and efficient mass profiling of an intact antibody and its associated variants.



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

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## 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

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MS system:	Waters LCT Premier 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:	100 V
Acquisition range:	600 to 5000 <i>m/z</i>

## System Configuration

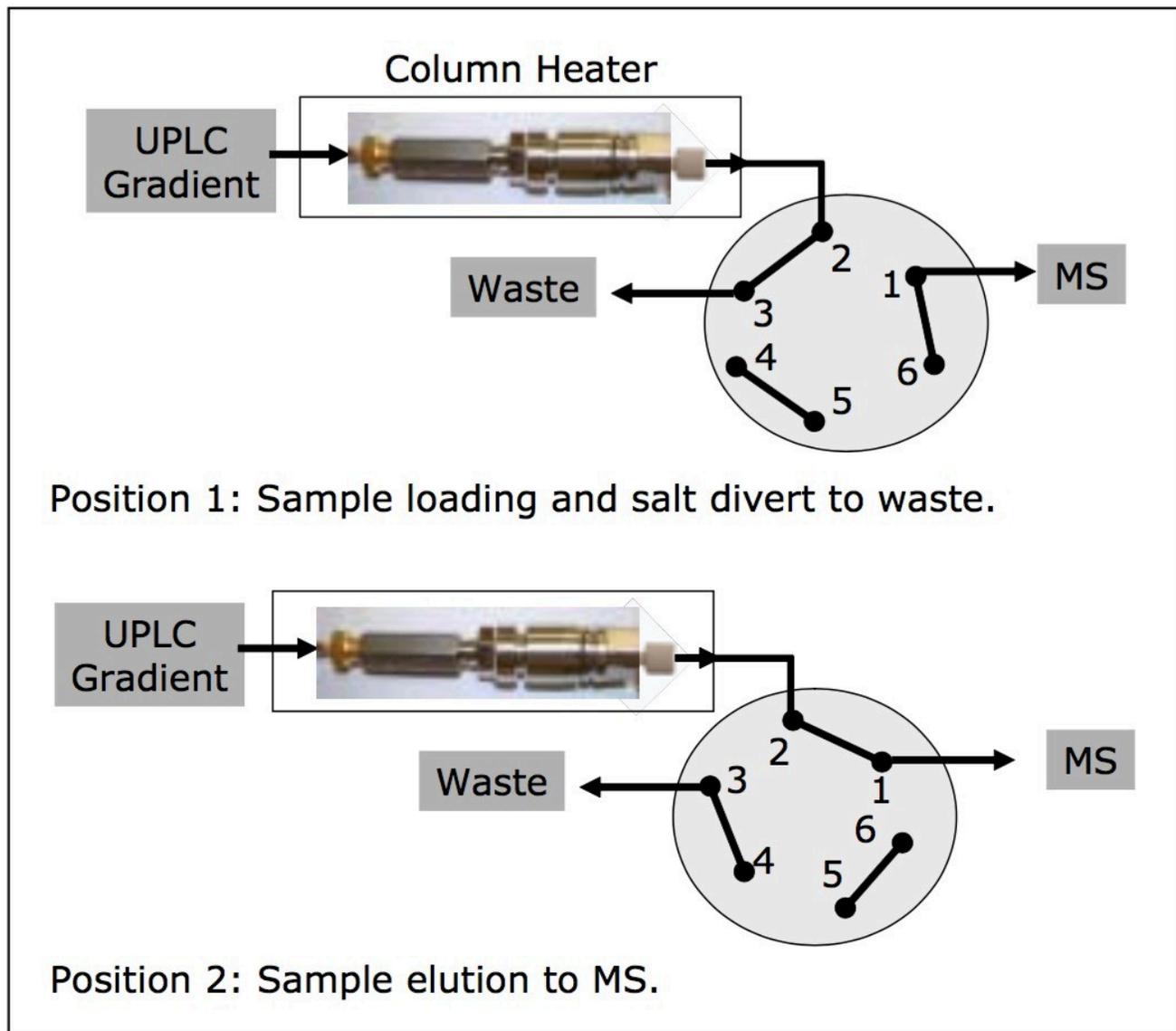


Figure 2. Fluidic configuration for LC-MS analysis. A post-column salt diversion valve (top-left corner of the LCT Premier XE) was utilized to divert buffers and nonvolatile salts to waste during the sample loading step.

Time (min)	%B	Flow (ml/min)	Curve
0.00	5	0.5	Initial
0.50	5	0.5	6
0.51	5	0.2	6
2.00	90	0.2	6
2.10	5	0.5	6
2.70	90	0.5	6
2.80	5	0.5	6
3.40	90	0.5	6
3.50	5	0.5	6
4.00	5	0.5	6

Load/Wash  
-Divert Flow-

Gradient

Column  
Washing  
and  
Regeneration

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

Table 1. Gradient profile used for antibody analysis.

Preparation of intact IgG1 A monoclonal murine IgG1 (11.3  $\mu\text{g}/\mu\text{L}$ , 0.1 M  $\text{NaHCO}_3$ /0.5 M NaCl, pH 8.3) was diluted with 50 mM  $\text{NH}_4\text{HCO}_3$  to 0.1  $\mu\text{g}/\mu\text{L}$  for analysis. Following microcentrifugation, LC/MS analyses were performed on 5  $\mu\text{L}$  of the diluted sample.

## Results and Discussion

A fast (4 min cycle time) and efficient LC/ESI-MS method was used to profile multiple structural variants of an

IgG. To minimize cycle time and maximize system performance, higher flow rates were used for loading, desalting, and column regeneration. A system controlled post-column valve was used for waste diversion of sample buffers and salts.

Additional sawtooth (rapid) gradient cycles were applied to regenerate the column to pre-injection conditions as part of each analysis (Figure 3). This avoided the potential need to separate difficult samples with inter-run blank injections.

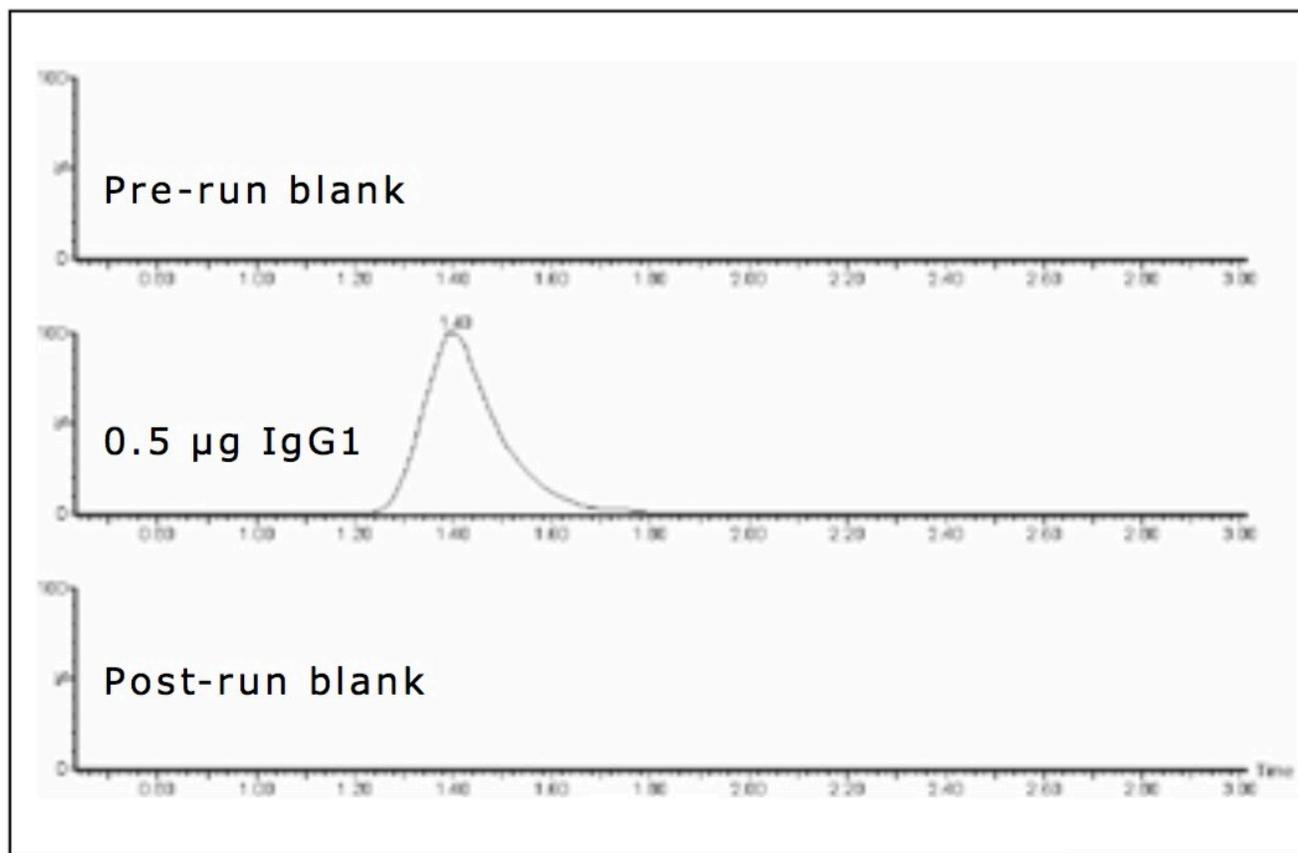


Figure 3. Total ion chromatograms (TICs) resulting from LC/MS analyses of an intact antibody.

Overlaid TICs (y-axis linked) for this experiment and the associated summed mass spectra are shown as Figures 3 and 4, respectively. The results reveal no detectable carryover following a 0.5 µg injection of the antibody.

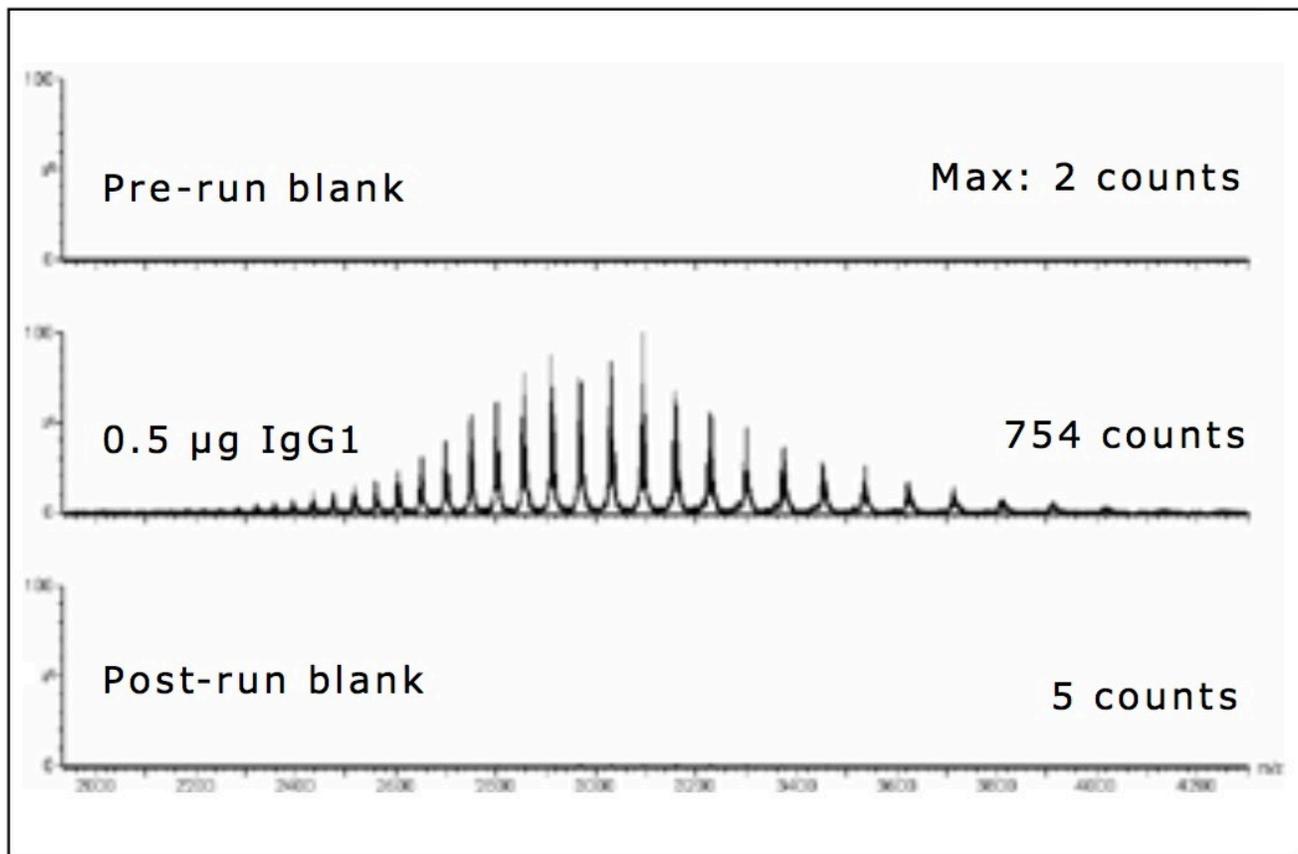


Figure 4. Combined ESI-TOF mass spectra (1.2 to 1.7 min, Figure 3) demonstrating system regeneration post-injection.

The summed ESI-TOF mass spectrum for the antibody revealed a charge state envelope over 2300 to 4000  $m/z$  (Figure 4). The +51 charge state region has been enlarged (Figure 5, inset) to show greater detail, indicating at least six significant antibody variants.

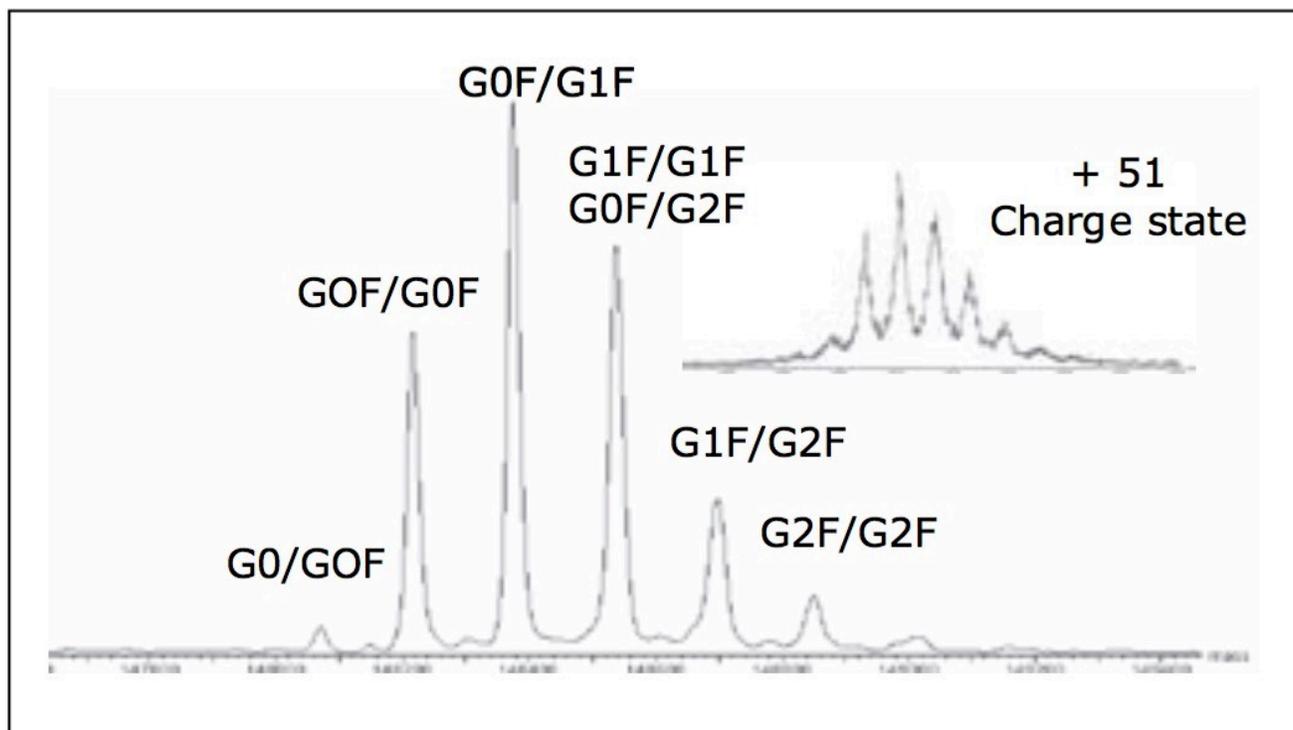


Figure 5. MaxEnt1 deconvoluted mass spectrum of the intact IgG1. Major variants observed were due to carbohydrate heterogeneity.

The MaxEnt1 deconvoluted spectrum (Figure 5) clearly revealed carbohydrate-related heterogeneity. Five peaks demonstrate characteristic sequential mass differences of ~162 Da consistent with extension of the two core glycan structures by up to four hexose (galactose) residues. The earliest peak pair shows a mass difference of 146 Da corresponding to incomplete occupancy of a fucosylation site on the core glycans.

The MaxEnt1 algorithm produces a result that is quantitatively conserved with the raw spectral data, and is often used for profiling and monitoring this glycoform pattern. The analysis also confirmed that other potential variants (e.g. incomplete heavy chain lysine processing) were absent from this molecule.

## Conclusion

A simple LC-MS configuration has been demonstrated to permit efficient desalting and rapid (4 min) LC-MS analysis of an intact murine IgG1 monoclonal antibody. This methodology yielded a simple holistic view of the

molecule by providing intact mass information that can be used to confirm primary structure, and profiles of macro-heterogeneity (glycosylation, Lys processing) that can be used to assess consistency of IgG production.

Overall, the adoption of rapid characterization methodologies using UPLC-MS should permit analytical groups to make maximal use of their personnel and resources, and adapt to the demand of R and D organizations for greater sample capacity, and decreased analysis turnaround times.

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ACQUITY UPLC System <<https://www.waters.com/514207>>

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