Multi-Attribute Method (MAM) for the Assessment of Forced Degradation Samples

Mercy Oyugi, Sarah Rogstad

Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration (Silver Spring, MD)

INTRODUCTION

Multi-attribute method (MAM) is a liquid chromatography-high resolution mass spectrometry (LC-HRMS) technique for monitoring product quality attributes (PQAs) in therapeutic proteins. MAM also has a new peak detection (NPD) feature for monitoring non-targeted peaks. MAM has been proposed as a replacement for conventional quality control (QC) tests for therapeutic proteins. The Office of Testing and Research (OTR) has developed an in-house MAM approach using rituximab as the model protein. Here, the in-house MAM approach was used to analyze stressed rituximab samples with the aim of assessing MAM's capability to monitor stress-induced changes in rituximab PQA levels and new peaks.

METHODS

Stress Conditions and LC-MS Experiments

- The stress conditions were: hydrogen peroxide (H_2O_2) (0.05% and 0.1%, 5 hours at 4 °C); pH 3.4 and pH 10.0 (room temperature for 24 and 72 hours); 50 °C (24 and 72 hours); and UV (480 and 1440 Whr/m^2) plus Vis light (1200 kluxhr)
- Tryptic rituximab digests were separated on an Agilent Zorbax C18 300-SB column using a Thermo Accela LC system coupled with a Thermo QE hybrid quadrupole-orbitrap mass spectrometer.
- Spectra were acquired in positive ion mode (300-1800 m/z), with a resolution of 140,000, AGC target of 3e6 and max IT of 200 ms.

Targeted PQA processing

- Raw LC-MS files were imported into Chromeleon for PQA identification and quantification.
- Genesis algorithm was used for AUC integration, and the sum of all observable *m/z* for the top four isotopes at each charge state were used to calculate areas.
- The 21 rituximab PQAs monitored include methionine oxidation, lysine clipping, deamidation, pyroglutamination, and N-glycosylation.

New Peak Detection (NPD)

- NPD was performed using non-stressed controls as reference.
- NPD parameters were: 300-1800 *m/z* range; 10 ppm *m/z* width; 0-70 min retention time; 1 min frame time width; 16,000 maximum frames; and 0.5% peak intensity threshold.
- NPD filter rules were: PR element = 0; PR size > 1; charge between +2 and +5; ratio > 10.0.
- Rituximab peptide mapping results from a previous Proteome Discoverer search were used to identify the new peaks.

RESULTS

The plots below represent select trends observed for the various stress conditions. In each plot, error bars represent standard deviation of triplicate measurements, while asterisks (*) denote significant variations based on > 20.0% deviation from the control, and ANOVA tests ($p \leq 0.05$).

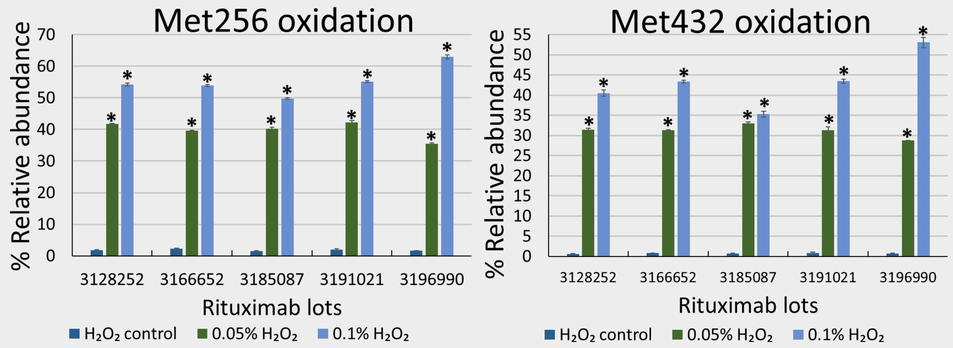

FIGURE 1: Effect of H_2O_2 treatment on oxidation levels. Heavy chain (HC) Met256 and Met432 increased by up to 60 % compared to control. Smaller increases of 0.5-2.0% in oxidation levels were observed for HC Met20, Met34, Met81, and light chain (LC) Met21 oxidation (data not shown).

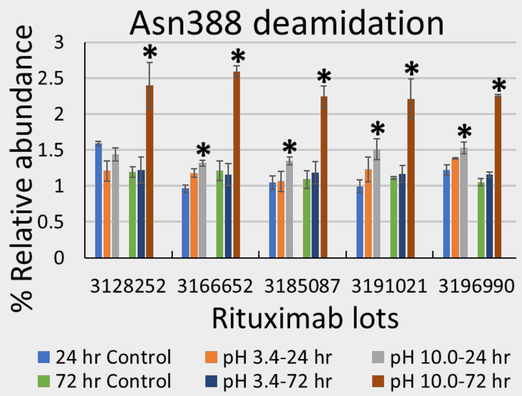

FIGURE 2: Effect of pH on deamidation. Increased Met256 and Met432 oxidation was also observed for both pH 3.4 and pH 10.0 at the 72 hour incubation period (data not shown).

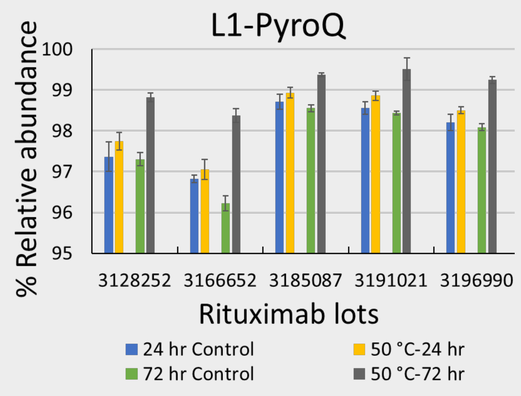

FIGURE 3: Effect of 50 ° C temperature on light chain pyroglutamination. Similar trends were observed for HC pyroglutamination and all six methionine oxidation sites (data not shown).

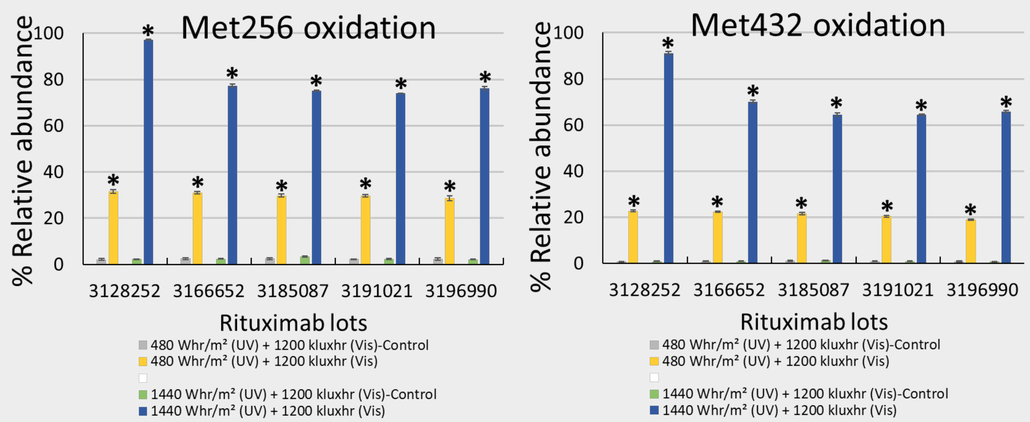

FIGURE 4: Effect of UV and Vis light exposure on oxidation. Similar trends were observed for HC Met20, Met34, and Met81 oxidation, as well as for LC Met21 oxidation (data not shown).

RESULTS (CONT'D)

Peptide sequence	Chain	Missed cleavages	*m/z*	Charge	Observed monoisotopic mass (Da)	Retention time (min)	Modification	Possible source of new peak
VVSVLTVLHQDWLNGK	Heavy	0	603.3384	3	1806.9933	52.58	N319 deamidation	Alkaline pH of the trypsin digestion buffer
VSNKALPAPIEK	Heavy	1	423.2583	3	1266.7532	21.77	N329 deamidation	
VEAEDAATYYCQQWTSNPPTFGGGTKLEIK	Light	1	1121.8483	3	3362.5230	43.5	C87 carboxymethyl	Reduction and alkylation during sample preparation
SSSTAYMQLSSLTSEDSAVYYCAR	Heavy	0	1339.5750	2	2678.1336	41.17	C96 carboxymethyl	
ADYEKHKVYACEVTHQGLSSPVTK	Light	2	550.4710	5	2748.3206	18.17	C194 carboxymethyl	
VVSVLTVLHQDWLNGKEYKCK	Heavy	2	630.0823	4	2517.3046	47.38	C325 carboxymethyl	
EPQVYTLPPSRDELTK	Heavy	1	624.9932	3	1871.95785	24.49	None	Missed cleavage
QTPGRGLEWIGAIYPGNGDTSYNQK	Heavy	1	908.1063	3	2722.29745	41.87	None	

TABLE 1: New peaks detected by NPD analysis and identified using Proteome Discoverer peptide mapping. Modified amino acid residues are highlighted in red. A total of 34 distinct peaks were detected using NPD analysis. Peaks not shown here could not be identified using Proteome Discover.

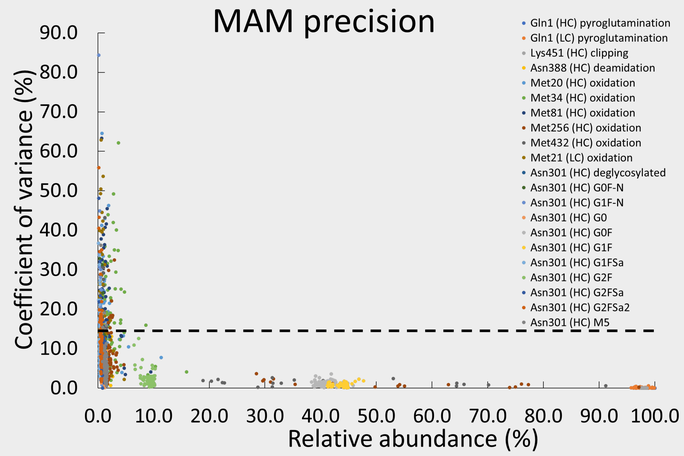

FIGURE 5: MAM precision for the stress study. The higher CV > 15.0% observed for methionine oxidation can be mitigated by adding 10 – 20 mM methionine to the sample processing buffers.

CONCLUSIONS

- The results show that MAM can detect stress-induced changes and variations in PQA levels.
- Additionally, the assessment of the NPD feature has shown that MAM can also reliably track new peaks in stressed samples.
- Overall, these results demonstrate MAM's potential as a quality control method for therapeutic proteins.

DISCLAIMER