BiopharmaView™ Software for Fast and Efficient monitoring of pH induced Deamidation

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ABSTRACT

Deamidation is one of the most common modifications which can be induced in monoclonal antibodies during production and prolonged storage. It is critical to evaluate and monitor of these biologically relevant product quality attributes (PQAs) during the various stages of product life cycle as these changes can cause structural changes thus reducing the biological activity and efficacy of the biotherapeutic. Here, we monitored the degree and site of deamidation induced in pH stressed antibody digest using Multi Attribute Monitoring (MAM) workflow in BiopharmaView™ software. This enables the automatic and accurate quantification of all the asparagine sites undergoing deamidation due to pH stress thus resulting in high throughput assessment of product stability and determination of appropriate control strategy for therapeutic monoclonal antibodies.

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INTRODUCTION

Monoclonal antibodies are susceptible to various chemical modifications during production and prolonged storage. Deamidation is one of the most common modifications that may cause structural changes in the biotherapeutics resulting in decrease in its biological activity and stability. Thus, it is necessary to routinely monitor the deamidation level and sites for the assessment of product stability and efficacy.

Here, we demonstrated a workflow for fast and efficient monitoring of pH induced deamidation levels using SCIEX benchtop high resolution QTOF and BiopharmaView™ software. The trastuzumab antibody was digested in digestion buffer with increasing pH to induce higher deamidation levels. The accurate identification and quantification of deamidation by BiopharmaView™ software enabled the high throughput assessment of product stability and would help in determining the appropriate control strategy for therapeutic monoclonal antibodies.

MATERIALS AND METHODS

Sample Preparation:

Trastuzumab was obtained from Myoderm (Norristown, PA, USA). The samples were digested in 25mM Tris-HCl, buffer with different pH values (6.8, 7.6, 8.0 and 8.8) to enhance the deamidation levels. The samples were then denatured, reduced and alkylated using *DL*-dithiothreitol and 2-iodoacetamide (Sigma Aldrich). Trypsin (Promega) was added in a ratio of 1:30 (w:w; Trypsin:mAb) followed by an incubation at 37° C for 4hr. Digestion was stopped by adding formic acid and supernatant was subsequently measured using LC-MS.

Chromatography:
The trastuzumab tryptic digests were separated using ExionLC™ system. The Solvent A consisted of water containing 0.1 % formic acid and Solvent B consisted of acetonitrile containing 0.1 % formic acid. A gradient of was used. A gradient of 2 to 55 % B over 35 min was used at a flow rate of 300 µl/min. The injection volume was set to 4µl.

Mass Spectrometry:
All measurements were carried out in replicates on X500B Q-TOF (Fig. 1) coupled to a Duospray™ source using a data dependent (DDA) acquisition strategy. High resolution MS/MS data of eight candidate ions per cycle with a total cycle time of 1s was acquired.

Data Processing:
The complete data processing was performed with BiopharmaView™ software. The experimental peptide data was matched to the in-silico generated list of peptide masses with deamidation as variable modification. The maximum error tolerance of 5 ppm was used for peptide matching. All the possible deamidation sites were monitored using MAM workflow and reference ranges were set for pass/fail criteria.

RESULTS

The monoclonal antibody digest was digested in increasing pH level to induce higher deamidation levels. The deamidation was induced by pH stress keeping rest of the parameters like digestion time, temperature and buffer type constant so that method induced variations can be minimized All the Asparagine (Asn) sites were monitored and defined for quantification using MAM workflow in BiopharmaView™ software. The lowest levels of deamidation were observed with the neutral or nearly acidic pH (6.8) and were found to increase with increasing levels of pH.

The MAM workflow in the BiopharmaView™ software enabled the accurate and automated quantitation of all the feasible deamidation sites within the defined criteria's of pass/fail using neutral pH values as reference points. It's been always challenging with LCMS data to define the site of deamidation especially for peptides containing more than one probable site of modifications. The feature assignment function in the BiopharmaView™ software allows to accurately assign the site of modification based on multiple defined criteria's like charge state, RT and MSMS validated evidences (Figure 1).

Figure 1. BiopharmaView™ processing of Trastuzumab Digest showing correct assignment of site of deamidation in peptide FNWYVDGVEVHNAK with two possible Asparagine sites of modification. A; Extracted Ion chromatogram of peptide FNWYVDGVEVHNAK showing presence of Native and modified peaks. B: Assignment of site of modification based on MSMS scoring. C: The MS and MSMS of deamidated peptide showing b- and y- ion matching for the correct identification of modification site.

The list of Quality Attributes was created for all the possible Asn deamidation sites in the trastuzumab digest. The modification calculations were created as relative percentage based on the unmodified form. In case, where multiple peaks for the modified peptide are present due to the presence of more than one site of modification or due to isoasp conversion, sum total of all XIC areas were considered for relative percent calculation. Pass/Fail criteria's were set based on the results obtained from the near neutral pH sample.

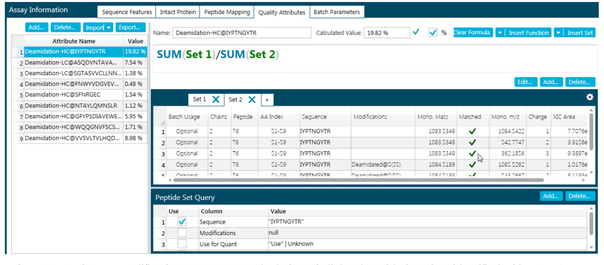
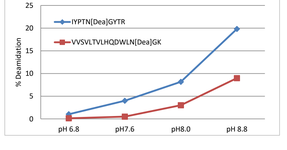


Figure 2: Setting up Modification percentage calculation of all the deamidation sites identified with MSMS validation for trastuzumab digest in BiopharmaView™ software.

The MAM workflow resulted in the fast and accurate monitoring of the Asparagine deamidation under pH stress conditions (Figure 3A). Only those modification sites were selected which were auto-validated by the software with MSMS evidence. Some of the Asn amino acid residues were found to be more susceptible to deamidation under pH stress as compared to that of others (Figure 3B). The Asn55 which is located in the CDR2 region of the heavy chain was found to be the most susceptible to pH changes. This is of particular interest as deamidation at this site greatly reduces the binding activity of the antibody.

	View	Quality Attributes	pH 6.8	pH 7.6	pH 8.0	pH 8.8
1		Deamidation-HC@FNWYVDGVEVHNAK	0.02 %	0.10 %	0.21 %	0.48 %
2		Deamidation-HC@GFYPSDIAVEWESNGQPENNYK	0.80 %	2.61 %	2.83 %	5.95 %
3		Deamidation-HC@IYPTNGYTR	1.03 %	4.00 %	8.17 %	19.82 %
4		Deamidation-HC@NTAYLQMNSLR	0.14 %	0.50 %	0.79 %	1.12 %
5		Deamidation-HC@SFNRGEC	0.00 %	0.00 %	0.00 %	1.54 %
6		Deamidation-HC@VVSVLTVLHQDWLNGK	0.14 %	1.25 %	3.01 %	8.98 %
7		Deamidation-HC@WQQGNVFSCSVMHEALHNHYTQK	0.82 %	1.23 %	1.62 %	1.71 %
8		Deamidation-LC@ASQDVNTAVAWYQQKPGK	7.17 %	6.84 %	7.27 %	7.54 %
9		Deamidation-LC@SGTASVVCLLNNFYPR	0.02 %	0.19 %	0.39 %	1.38 %

Figure 3: Relative Modification percentages of the Deamidation sites monitored under pH stress conditions. **A**: BiopharmaView™ results showing deamidation percentages observed with different pH levels. **B**: Two peptides IYPTNGYTR and VVSVLTVLHQDWLNGK showing greater degree of deamidation percentages are plotted as a function of pH.



Further, accuracy of the method was assessed by spiking the forced deamidated samples into the control sample at increasing levels. The sample with the lowest deamidation levels (pH 6.8) was used as control due to lack of samples with no deamidation. When the forced deamidated sample was spiked into the control sample, the deamidation amount measured in the spiked samples increased correspondingly with the amount of the spike as expected thus exhibiting excellent linearity for two peptides monitored.

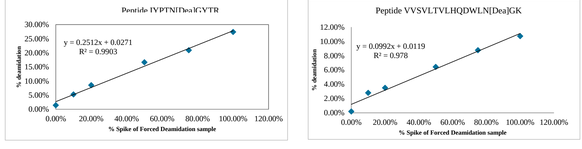


Figure 4. The linearity plots of two deamidated peptides IYPTN[Dea]GYTR (A) and VVSVLTVLHQDWLN[Dea]GK (B) of the trastuzumab digest as a function of the percentage of spiked forced deamidation sample.

CONCLUSIONS

- Using BiopharmaView™ software with the attribute calculator for the MAM workflow, levels of deamidation were calculated for all possible deamidation sites within trastuzumab. The data was automatically processed and showed the susceptibility of some residues to pH whereas others are far less sensitive. The results can be used to refine monitoring of attributes to those that are more susceptible to environmental changes.
- The spiking of the forced deamidated sample in various levels demonstrated the accuracy of the method as well as accurate & reliable relative quantitation of deamidation by BiopharmaView™ .
- The high quality accurate mass MSMS data obtained by SCIEX X500B enables confident identification of the site of modification in peptides containing more than one modification site or for peptides undergoing cyclization forming iso-asp.

REFERENCES

1. K. Pohl; A. Boudreau and A. Uppal. BiopharmaView™ Software as a Robust Tool for Automated Quantitation of Oxidation Sites in Monoclonal Antibody Characterization. *SCIEX Technical Note: RUO-MKT-02-6379-A*.
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TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

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