Monitoring Critical Quality Attributes Supporting Development of Biologicals

Sean McCarthy[1]; Kerstin Pohl[2]; Annu Uppal[3]; Sibylle Heidelberger[4]

[1]AB SCIEX, 500 Old Connecticut Path, Framingham, MA, USA; [2]AB SCIEX, Darmstadt , Germany ; [3]AB SCIEX, 121 Udyog Vihar, Phase IV, Gurgaon Haryana, India; [4]AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

INTRODUCTION

A rapidly emerging trend is the application of mass spectrometry as a key application for identification and tracking of biopharmaceutical critical quality attributes. These approaches are finding increased use as they allow a greater ability to interrogate specific sites susceptible to post translational modification, whether this modification is desired or non-desired. A particular challenge in implementing these approaches has been the complexity of mass spectrometry and limited informatics solutions to address these needs. Complicating this further is the need for implementable solutions which can be used effectively by non-expert users.

The X500B QTOF system is an easy-to-use platform for the analysis of biopharmaceuticals. For this study, NIST monoclonal antibody (mAb) reference standard was subjected to oxidative stress conditions to investigate the impact on protein post-translational modifications and verify the ability of the X500B QTOF to detect such changes. Additionally, the limit of detection was assessed by spiking in known peptides into the NIST mAb. In all cases, samples were separated using reversed phase UHPLC separation and detected with the X500B QTOF instrument. Data was processed using dedicated biopharmaceutical software BioPharmaView™.

MATERIALS AND METHODS

Materials:
NIST reference standard was purchased from National Institute of Standards (#RM8671). Tris-HCl, iodoacetamide, DTT, methionine, hydrogen peroxide and formic acid were purchased from Sigma (St. Louis, MI, USA). ProteaseMax™ and trypsin were purchased from Promega (Madison, WI, USA). Premixed mobile phases were purchased from VWR (Radnor, PA, USA). PepCalMix was purchased from SCIEX (Framingham, MA, USA).

Sample Preparation:
NIST reference standard was incubated with hydrogen peroxide (0%, 0.003125%, 0.00625%, 0.0125%, 0.025% and 0.05%) at 37˚ C for four hours to induce oxidative stress. The reaction was quenched with the addition of methionine (50 mM). NIST reference standard was denatured with 1% ProteaseMax followed by reduction with DTT and alkylation by 2-iodoacetamide before digestion with trypsin at a ratio of 1:30 overnight at 37˚ C. Control sample was divided and a portion was spiked with concentrations of heavy labeled PepCalMix at 0.01, 0.025, 0.05, 0.1 and 0.2 % final molar concentration of NIST.

HPLC Conditions:
An ExionLC™ system with a Waters Acquity UPLC® CSH C18, 1.7 µm 2.1 x 100 mm column at 40˚ C with a gradient of mobile phase A: water + 0.1 % formic acid and mobile phase B: acetonitrile + 0.1 % formic acid was used at a flow rate of 300 µl/min. A 60 minute gradient was run with an injection volume of 6 µl for the samples incubated with H_2O_2 and 4 µl for the NIST samples spiked with PepCalMix.

MS/MS Conditions:
AB SCIEX X500B QTOF system with IonDrive™ source and Electrospray Ionization (ESI) probe was used. SWATH® acquisition was acquired using variable windows across a mass range of 350 – 2000 and TOFMS accumulation of 200 ms. MSMS data was acquired over 100 to 2000 with an accumulation time of 50 ms.

Data Processing:
Data was processed using BioPharmaView™ software. NIST standard was set as the reference standard and the samples were processed in a batch for oxidation and deamidation attributes. Spiked in samples were processed in another set with NIST set as the reference standard. The peptides and their XIC areas were extracted and plotted from BioPharmaView software. Limit of quantitation (LOQ) and limit of detection (LOD) were calculated.

SCIEX X500B QTOF system

SCIEX ExionLC™ AC system SCIEX OS software

RESULTS

OXIDATION RESULTS

NIST mAb standard was incubated with varying concentrations of H_2O_2 to determine the susceptibility of oxidation of methionine containing peptides to oxidation. All stressed samples were processed with BioPharmaView software and compared across the concentrations of H_2O_2 used.

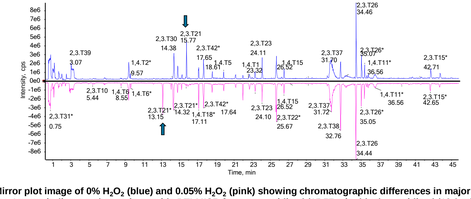

Figure 1. Mirror plot image of 0% H_2O_2 (blue) and 0.05% H_2O_2 (pink) showing chromatographic differences in major peaks across the gradient. Arrows indicate a change in peptide DTLMISR from non oxidized (15.77 min, blue) to oxidized (13.2 min, pink)

Peptide DTLMISR was one of the peptides that changed dramatically with increasing H_2O_2 concentration.

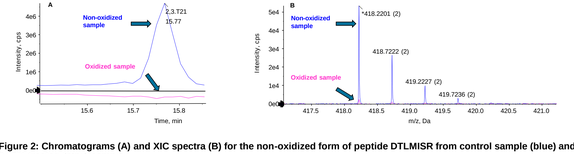

Figure 2: Chromatograms (A) and XIC spectra (B) for the non-oxidized form of peptide DTLMISR from control sample (blue) and 0.05% treated (pink) sample.

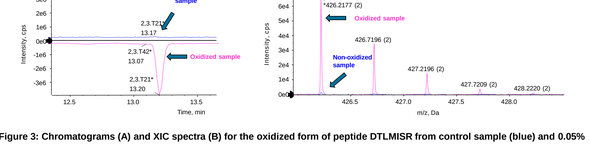

Figure 3: Chromatograms (A) and XIC spectra (B) for the oxidized form of peptide DTLMISR from control sample (blue) and 0.05% treated (pink) sample.

The level of methionine oxidation was dependent on the peptide as seen by the plot of % oxidation vs % H_2O_2 used.

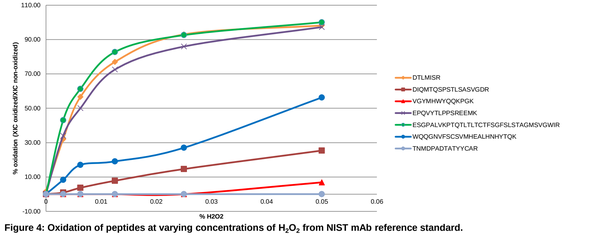

Figure 4: Oxidation of peptides at varying concentrations of H_2O_2 from NIST mAb reference standard.

Three methionine containing peptides were highly susceptible to methionine oxidation the other methionine containing peptides only showed partial or little susceptibility to oxidation. One peptide showed no susceptibility to methionine oxidation using H_2O_2.

DEAMIDATION

While oxidation was expected to increase with increasing concentration of H_2O_2, other post-translational modifications were interrogated to see if the levels of H_2O_2 would also affect them. Deamidation data was extracted from BioPharmaview™ software. Of the peptides containing asparagine, only one peptide, GFYPSDIAVEWESNGQPENNYK, showed a significant increase in deamidation.

Table 1: Comparison of Control (0%) and Treated (0.05%) Areas of NIST mAb Reference Standard

Peptide Results | Matched | Unmatched | Filter

	Filename	Sequence	Modifications	Charge	Observed Mono m/z	Theoretical Mono m/z	Error (PPM)	Score	RT	XIC Area
1	16_NIST_digest_SWATH_60min_0%.wiff2	GFYPSDIAVEWESNGQPENNYK		2	1272.5729	1272.5693	2.8	5.880	31.74	8.07e4
2	16_NIST_digest_SWATH_60min_0%.wiff2	GFYPSDIAVEWESNGQPENNYK		3	848.7173	848.7153	2.3	12.210	31.74	1.67e5
3	56_NIST_digest_SWATH_60min_0.05%.wiff2	GFYPSDIAVEWESNGQPENNYK		2	1272.5717	1272.5693	1.8	11.761	31.67	4.85e5
4	56_NIST_digest_SWATH_60min_0.05%.wiff2	GFYPSDIAVEWESNGQPENNYK	Deamidated@*	2	1273.0633	1273.0613	1.5	10.606	32.01	4.40e4
5	56_NIST_digest_SWATH_60min_0.05%.wiff2	GFYPSDIAVEWESNGQPENNYK	Deamidated@*	2	1273.0630	1273.0613	1.3	8.292	32.24	5.31e4
6	56_NIST_digest_SWATH_60min_0.05%.wiff2	GFYPSDIAVEWESNGQPENNYK		3	848.7168	848.7153	1.7	14.718	31.67	7.95e5
7	56_NIST_digest_SWATH_60min_0.05%.wiff2	GFYPSDIAVEWESNGQPENNYK		4	636.7893	636.7883	1.6	8.675	31.67	1.80e4

This increase of deamidation of the PENNYK peptide only occurred that the highest concentration of H_2O_2 (0.05%) and specifically at GFYPSDIAVEWESNGQPENNYK sites.

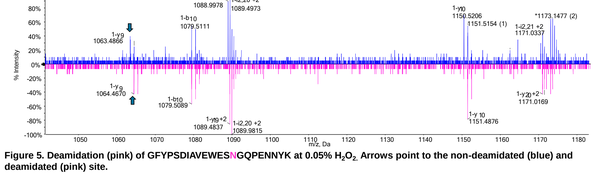

Figure 5. Deamidation (pink) of GFYPSDIAVEWESNGQPENNYK at 0.05% H_2O_2. Arrows point to the non-deamidated (blue) and deamidated (pink) site.

LIMIT OF DETECTION

In order to estimate the limits of detection, heavy labelled peptides were spiked at concentrations of 0.2 – 0.01% relative to the NIST mAb reference concentration. Data was batch processed in BioPharmaView™ software.

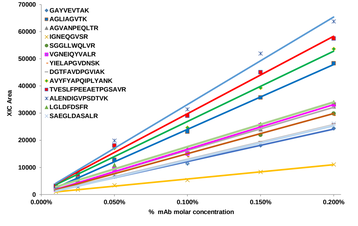

Table 2: LOQ and LOD for spiked peptides expressed as % mAb concentration

Peptide	LOQ	LOD
GAYVEVTAK	0.00116	0.00035
AGLIAGVTK	0.00081	0.00024
AVGANPEQLTR	0.00075	0.00023
SGGLLWQLVR	0.00215	0.00065
VGNEIQYVALR	0.00090	0.00027
YIELAPGVDNSK	0.00036	0.00011
AVYFYAPQIPLYANK	0.00158	0.00047
TVESLFPEEAETPGSAVR	0.00083	0.00025
ALENDIGVPSDTVK	0.00053	0.00016
LGLDFDSFR	0.00043	0.00013
SAEGLDASALR	0.00403	0.00121
LDSTSIPVAK	0.00241	0.00072

Figure 6: Plotted concentration vs XIC for 13 heavy labelled peptides.

LOQ and LOD extrapolated from S/N values of lowest concentration analyzed and are shown in Table 2. As per pharmaceutical industry standards, LOQ is defined as the concentration at which S/N = 10:1, and LOQ is defined as the concentration where S/N = 3:1. All S/N values calculated on raw, unsmoothed data using 1SD of noise, subtracting influence from blank injections if present. LOQ values for the peptides analyzed range from $3.6x10^{-4}$ to $4.0x10^{-3}$, expressed as a percentage of mAb molar concentration (or 3.6 to 40 ppm), and LOD values for the peptides analyzed range from $1.1x10^{-4}$ to $1.2x10^{-3}$, again expressed as a percentage of mAb molar concentration (or 1.1 to 12 ppm).

CONCLUSIONS

X500B QTOF system was tested for its ability to identify post translational modifications and to determine a limit of detection to ensure that low abundance attributes are identified and quantified. In this poster, we show

- Using oxidative stress, susceptibility of methionines to oxidation can be identified and confirmed using the unique SWATH acquisition capabilities of the X500B.
- In addition to the above, the SWATH acquisition capabilities of the X500B were also able to confirm that deamidation of GFYPSDIAVEWESNGQPENNYK occurs with increasing oxidative stress
- Limits of quantitation and detection, based on signal-to-noise values calculated from low concentration standards, are shown to be easily in the low ppm (parts per million) range, demonstrating how the sensitivity of the X500B platform can be exploited in the identification of low abundant attributes of biologics

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

Document number: RUO-MKT-10-5683