Characterization of Intact Monoclonal Antibodies under Native and Reverse Phase Conditions Using High Resolution Mass Spectrometry

Ryo Yokoyama[1], Sibylle Heidelberger[2] and Annu Uppal[3]

[1]SCIEX, Tokyo, Japan, [2]SCIEX Warrington, UK, [3] SCIEX, Gurugram, India.

INTRODUCTION

The first and foremost requirement in the development of biopharmaceuticals is the extensive characterization of the molecule at the intact level to ensure safety and efficacy. Mass spectrometric approaches have routinely been used for the determination of molecular weight as well as glycan heterogeneity. More recently characterization of monoclonal antibodies under native conditions is gaining importance over reverse phase conditions as it helps preserves the noncovalent interactions and retain the folded structure. The native like conditions simplifies the spectral resolution with reduced charge states and increased signal at higher m/z. Here, we demonstrated complete characterization of two monoclonal antibodies under both native conditions and reverse phase conditions using high resolution mass spectrometer.

MATERIALS AND METHODS

Sample Preparation:
The Trastuzumab and NIST reference standard (#RM8671) were studied under native and reverse phase conditions using the SCIEX X500B and TripleTOF® 6600 systems coupled to high-flow liquid chromatography (LC) setup using the workflow as described in Figure 1.

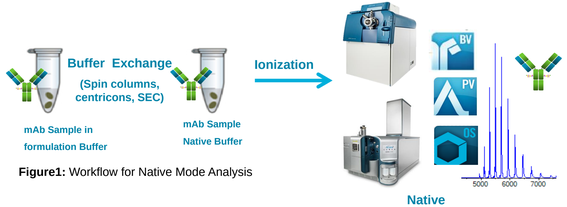

Figure1: Workflow for Native Mode Analysis

HPLC Conditions:
Sciex Exion LC System was used for both Reverse phase chromatography and Size exclusion chromatography (SEC) connected with both X500B and TripleTOF 6600 systems. The LC conditions for both reverse phase chromatography and SEC-LC are given in Table 1 A & B.

MS Detection:

MS detection was carried out in replicates using High resolution TOF-MS mode in both the X500B and TripleTOF 6600 systems. The mass range selected was from 1000-4000m/z and 1000-8000 m/z for reverse phase and native mode respectively. The source parameters were optimized for both the modes to get the best quality accurate mass reproducible data. The complete data processing was performed with BioPharmaView™ software.

Table 1A: Intact Native Mode

Column	1.0 x 300mm TSKgel SuperSW3000 SEC Column
Column Temp.	25OC
Flow Rate	20ul/min
Mobile Phase A	Water
Mobile Phase B	50mM Ammonium Acetate
Isocratic	Time(min) %B
	0.1 50
	18 50

Table 1B: Intact Reverse Phase

Column	Phenomenex Aeris 3.6um WIDEPORE C4 200, 50x2.1mm
Column Temp.	80°C
Flow Rate	500ul/min
Mobile Phase A	Water containing 0.1% formic acid
Mobile Phase B	Acetonitrile containing 0.1% formic acid
Gradient	Time(min) %B
	0.1 5
	2 5
	4 35
	5 90
	6 90
	6.5 5
	10 5

RESULTS

The analysis of intact antibodies under traditional reversed phase chromatography involves separation based on organic solvent gradient containing acidic pH. These denaturing conditions result in broad distribution of highly charged species at lower m/z ranges ranging from ~2000 to 4000 m/z (Figure 2-4 A &B).

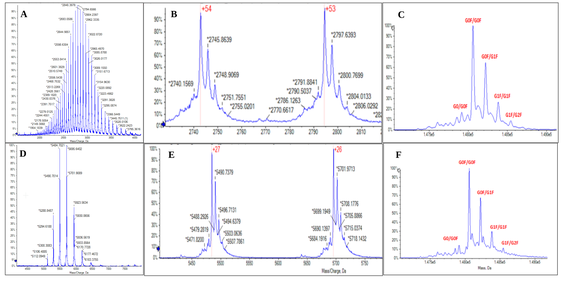

Figure 2. Analysis of intact trastuzumab under acidic reverse phase conditions and native conditions on the X500B system. A & D: TOFMS; B & E: Zoomed TOS-MS spectra showing the charge state distribution and highly resolved glycoforms heterogeneity; C &F: Reconstructed chromatogram under reverse phase conditions and native conditions respectively resulting highly similar results.

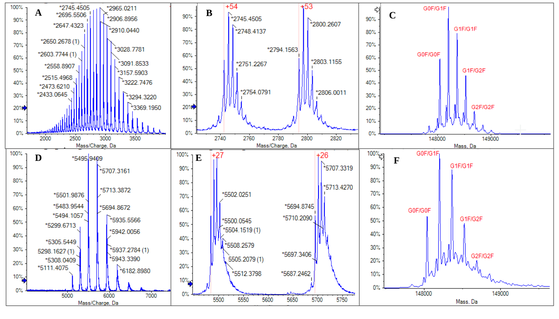

Figure 3. Analysis of Intact NIST standard under acidic reverse phase conditions and native conditions on the X500B System. A & D: TOFMS; B & E: Zoomed TOS-MS spectra showing the charge state distribution and highly resolved glycoforms heterogeneity; C &F: Reconstructed chromatogram under reverse phase conditions and native conditions respectively resulting highly similar results.

On the other hand, native mode analysis involves analysis of antibody in aqueous solution at near neutral pH, containing volatile salts such as ammonium acetate. Under these conditions, the protein's three dimensional structure remains preserved and results in TOF-MS envelope with relatively lower charge states ranging from 5000-8000m/z (Figure 2-4 D&E). This results in higher spatial resolution and improved base resolved peaks due to the detection at higher m/z as evident in the zoomed view of the most abundant charge states in Figure 2-4 (B&E). The high quality spectra obtained with X500B and TripleTOF 6600 system along with the deconvolution using BioPharmaView™ software enabled the accurate molecular weight determination of all the possible glycoforms with excellent reproducibility and mass accuracies. (Figure 2-4).

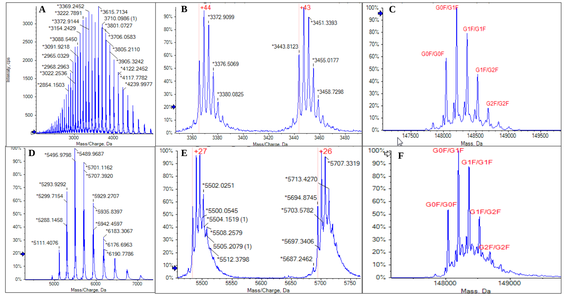

Figure 4: Analysis of Intact NIST antibody standard under acidic reverse phase conditions and native conditions on the TripleTOF 6600 System. A & D: TOF-MS; B & E: Zoomed TOS-MS spectra showing the charge state distribution and highly resolved glycoforms heterogeneity; C &F: Reconstructed chromatogram under reverse phase conditions and native conditions respectively resulting highly similar results.

One of the challenges with the native mode analysis is performing electrospray ionization from the aqueous buffer solutions used to preserve the native conditions of the antibodies. The source conditions need to be optimized for the efficient desolvation in the source in order to get the robust and sensitive performance in the higher mass range while performing analysis under native conditions. The source temperature was found to play an important role on the resolution of the glycoforms heterogeneity (Figure 6). Likewise other source parameters were also optimized to get the best quality data (data not shown).

The SCIEX DuoSpray™ source in both the platforms X500B and TripleTOF 6600 system provides highly efficient desolvation and ionization thus providing high quality data under native conditions.

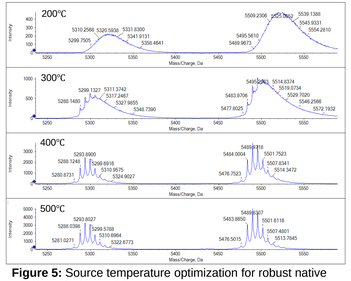

Figure 5: Source temperature optimization for robust native mode analysis conditions.

It has been reported that the native monomer in solution has a tendency to form reversible native conformation aggregates which are the result of association and disassociation of the protein molecules (Philo & Arakawa 2009). The reversible native conformation dimers are innate within the formulation and are present in protein solutions from the time of production to administration. The high sensitivity of the SCIEX accurate mass platform along with SEC-LCMS resulted in the detection of these non-covalent interactions (Figure 6). This can potentially be used for the fast screening of formation of any non-covalent dimers under stress conditions during long term storage, manufacturing and formulation.

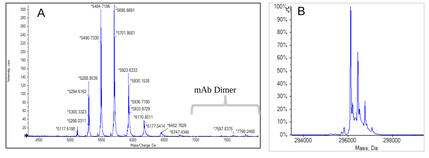

Figure 6. Detection of non covalent dimer formation in trastuzumab sample during native mode analysis. A: TOF-MS spectra; B: Reconstructed spectra of dimer.

CONCLUSIONS

- SCIEX accurate mass platforms TripleTOF 6600 and X500B systems, provide robust, fast, and reproducible data for both reverse phase and native mass spectrometric analysis of intact mass of monoclonal antibodies.
- Native mass spectrometry yields accurate mass measurements of the molecules, glycoforms identification, and assessment of higher-order structures (dimer, trimer, tetramer).
- SCIEX DuoSpray™ source with simplistic source architecture and orthogonal spray design with no complex spray path provides uniform temperature distribution with efficient desolvation under native conditions with improved robustness and ruggedness.

REFERENCES

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2. S. Heidelberger and S. McCarthy. Routine workflow for comparability assessment of protein biopharmaceuticals Trastuzumab. *SCIEX Technical Note: RUO-MKT-02-5590-A., 2017.*
3. Z. Zoe and S. Heidelberger. Routine and Enhanced Intact Mass Analysis without Compromise on TripleTOF® Mass Spectrometry Platforms. *SCIEX Technical Note:* RUO-MKT-02-6928-B , *2018.*

TRADEMARKS/LICENSING

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