Chromatographic and Mass Spectrometry Approaches to Supporting Biologics Development

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Overview

- Accurate characterization of biologics is vital to the development of biotherapeutics.
- It is essential to know the properties of biotherapeutic products to optimize bioprocess production, product formulation, and dose.
- The latest innovations from Shimadzu include LC, LC/MS, mini and conventional MALDI, and in-house as well as collaborator informatics to support the complex analysis of biologics.

1. Introduction

Biopharmaceuticals are being developed using biotechnology techniques such as genome editing and cell fusion. These drugs are getting attention worldwide as they are expected to be effective in treating various chronic diseases including cancers. Antibody drugs, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), are known to offer high therapeutic efficacy and reduced side effects due to their specificity and affinity for target molecules. However, because these biopharmaceuticals are manufactured using animal cells, the challenge is to ensure their structural homogeneity is not encountered in small molecule pharmaceuticals manufactured by chemical synthesis. Accordingly, biopharmaceuticals require appropriate quality controls at every production step. For example, ICH-Q6B (1), (2), which proposes specifications and test procedures for biological products, stipulates that product-related impurities should be separated and/or their percentage levels determined in the manufacturing process. In most cases, these analyses are performed using liquid chromatography

2. Methods

A variety of instruments have been used for analysis, including UHPLC, LCMS triple quad, LCMS QTOF, and MALDI. A brief summary of the methods is given below:

- **LC Method**

Adsorption of an analyte by a wetted surface of a UHPLC instrument poses some critical challenges when analyzing biomolecules. In part of the study, the Nexera XS inert system was used to separate biomolecules by combining the elevated pressure tolerance of a UHPLC system with a flow path that was completely inert, as there were no wetted metal surfaces in the sample flow path, assuring ultra-high corrosion resistance.

- **LCMS-9030 QTOF Method**

Innovative TOF architecture allows ultra-fast, precise pulsing of ions into the flight tube and back to the detector. Thus, high-speed data acquisition is compatible with high-throughput laboratories. Utilizing the Nexera Mikros, the Micro-ESI 9030 interface allows microflow analysis to be performed on the Q-TOF LCMS-9030. Using the system, recombinant human IgG1* NIST mAbs, Trastuzumab, and Bevacizumab were evaluated as model biotherapeutics.

- **LCMS-8060 (Triple Quad) Method**

LCMS-8060NX redefines LC-MS/MS detection limits and quantitative capabilities. A new ion focusing technique in the 8060NX transforms trace quantitative detection by redefining "sensitivity". In addition, the fastest polarity switching available in the industry enables switching from positive to negative modes in 5 msec. With data acquisition occurring so quickly, it is possible to perform triggered MS/MS scans and MRM quantification using the same acquisition method.

- **MALDI Method**

Shimadzu's MALDI-8030 is part of a long line of MALDI-TOF instrumentation. This dual-polarity, benchtop MALDI-TOF mass spectrometer provides outstanding performance in a compact footprint, making it the ideal choice for today's high-quality laboratories. It can deliver the performance required for analytical method development and quality control applications.

- **Data processing**

Chromatographic data were collected and analyzed by LabSolutions software. LC-MS data were taken by LabSolutions and analyzed by Protein Metrics and Skyline. The MALDI-MS data were collected by MALDI Solutions and analyzed with Protein Metrics software

2. Results

Analytical Performances by Nexera XS inert

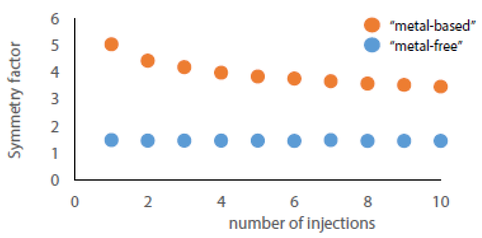


Figure 1: Transitions in symmetry factor of ATP by the number of injections.

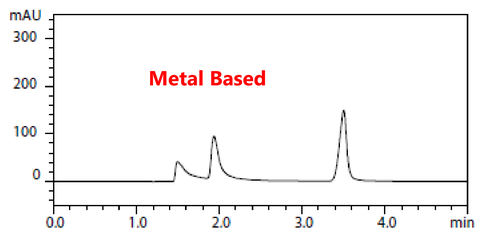


Figure 3: Mixed standard solutions of AMP, ADP, and ATP (50 µg/mL) measured in the "metal-based".

Drug-to-Antibody Ratio by Nexera XS inert

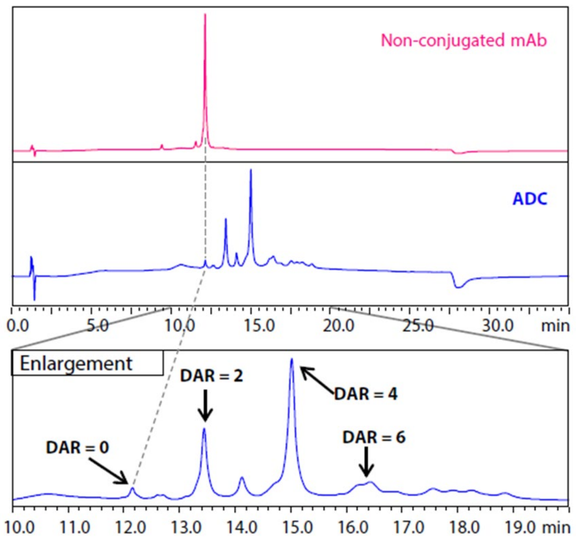


Figure 5: Result of drug-to-antibody ratio (DAR) analysis.

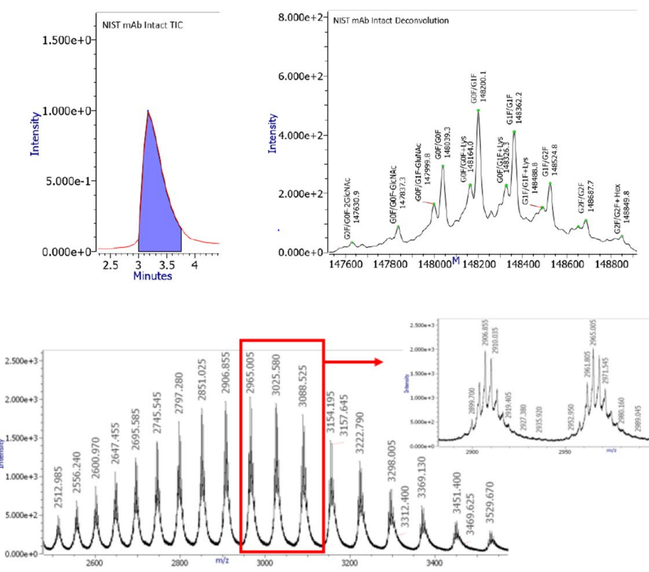


Figure 6: NIST mAb Intact TIC chromatogram (top, left), deconvoluted spectrum (top right), and mass spectrum (bottom).

Accurate characterization of monoclonal antibodies is essential to development of biotherapeutics. Thorough understanding of biotherapeutic properties aids in the optimization of bioprocess production, product formulation, and product dosage. LCMS-9030 QTOF and Nexera XS inert have been used to characterize the recombinant human NIST mAb and Bevacizumab

Nexera XS Inert

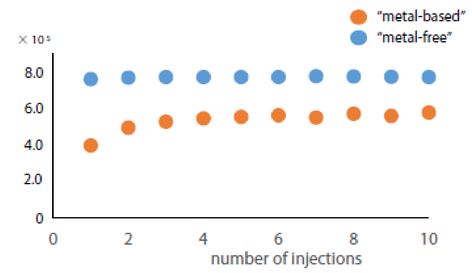


Figure 2: Transitions in peak area of ATP by number of injections.

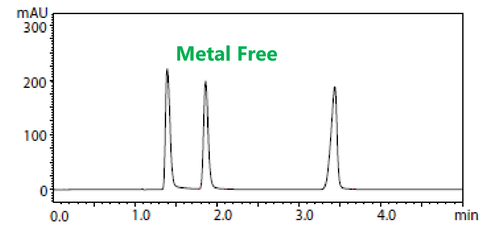


Figure 4: Mixed standard solutions of AMP, ADP, and ATP (50 µg/mL) measured in the "metal-free".

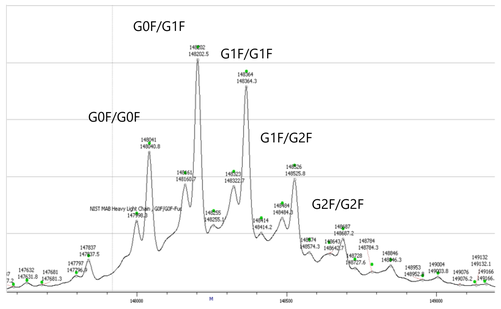


Figure 7: Deconvoluted mass spectrum of NIST mAb.

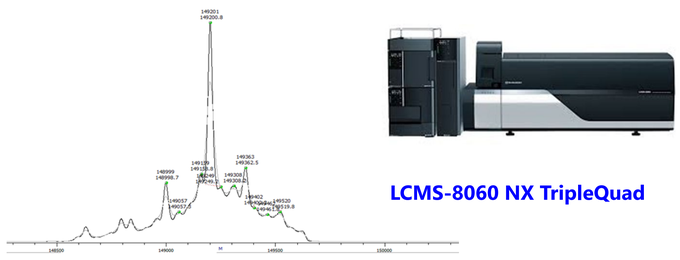


Figure 8: Deconvoluted mass spectrum of Bevacizumab.

LCMS-9030 QTOF

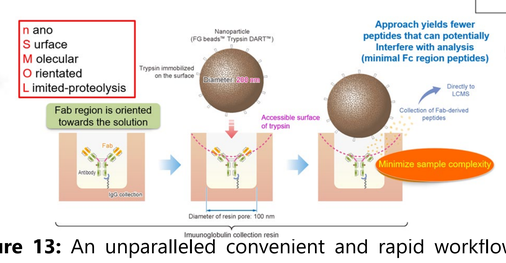


Figure 13: An unparalleled convenient and rapid workflow provided by the nSMOL Antibody BA Kit dramatically improves the productivity and robustness of LCMS mAb bioanalysis.

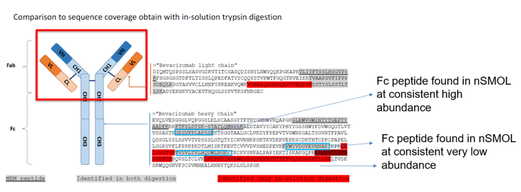


Figure 14: nSMOL coupled with LC-MS bioanalysis for monitoring the Fc-fusion biopharmaceuticals Etanercept and Abatacept in human serum.

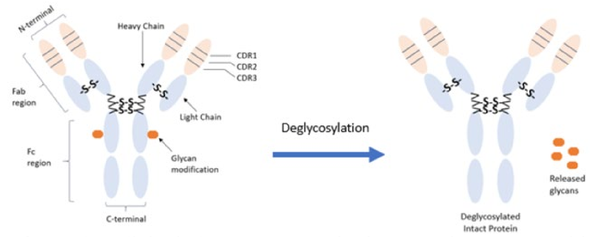
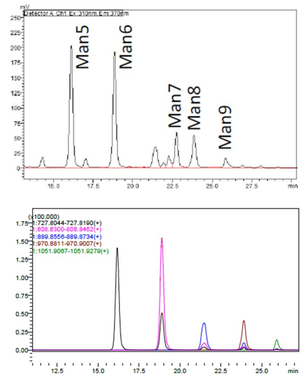


Figure 9: N-linked glycans are attached to proteins at Asn residues on the Fc region.



MALDI-8030

Figure 10: Fluorescence chromatogram (upper) and extracted ion chromatogram (lower) for procainamide labeled RNaseB Glycans .

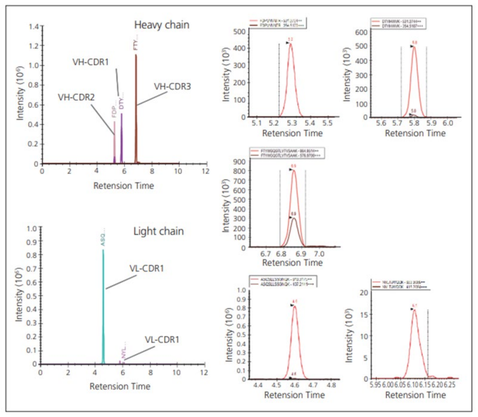


Figure 11: Detection of trypsin fragments containing CDR1-3 using standard monoclonal antibodies subjected to trypsin digestion (Data Analysis by Skyline).

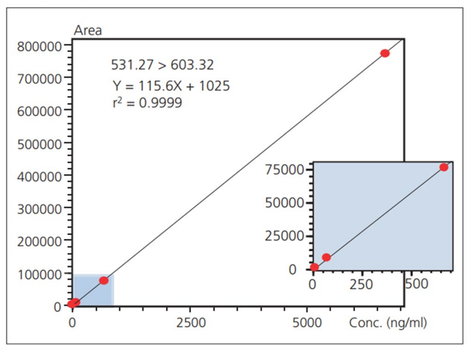


Figure 12: Calibration Data for the Transition 531.27 > 603.32 on a Linear Scaling .

4. Conclusions

- The topics comprise innovator biopharmaceuticals, biosimilars, antibody-drug conjugates, and keeping up with changes in the regulatory environment for development of biologics.
- In addition to full protein, subunit, peptide fragment, and N-Glycan analysis, these proven workflows can analyze mAbs, antibody drug conjugates (ADC), oligonucleotides, proteins, and peptides.
- A newly developed bioinert UHPLC system effectively eliminates metal ion interaction with targets such as proteins and nucleic acids, preventing peak tailing and low recovery of target components. As a result, data can be obtained with high sensitivity and sharp peaks even with low concentrations of sample.