Comprehensive Characterization on Monoclonal Antibody using a newly developed LC/Q-TOF Instrument

David Wong¹, Chris Klein¹ and Jing Chen²
1. Agilent Technologies, Inc., Santa Clara, CA, USA
2. Agilent Technologies, Inc., Madison, WI, USA
Overview

This work describes the utilization of the Agilent’s 6545XT AdvanceBio LC/Q-TOF system for rapid characterization of Monoclonal Antibody (mAb) samples.

Introduction

Monoclonal antibodies and their derivatives comprise a very important class of biopharmaceutical molecules with a wide range of therapeutic and diagnostic applications. The comprehensive characterization provides not only the complete amino acid sequences of mAbs and their variants, but also the information on their post-translational modifications (PTMs) and locations. However, the lack of automatic workflow in the sample preparation, data processing and result interpretation has been the rate-limiting step for most biopharmaceutical laboratories. In this study, we demonstrate a high throughput workflow that utilizes an automated liquid-handling robot (AssayMAP), an Agilent Infinity II UHPLC system, a newly developed Agilent 6545XT AdvanceBio LC/Q-TOF system, and automated data processing using BioConfirm software for the intact mAb, the mAb subunits and its complete sequence mapping analysis. This system can also be used for the native protein complex analysis (over 800 kDa).

Figure 1. Agilent 6545XT AdvanceBio LC/Q-TOF system.

Experimental

Sample Preparation:

Monoclonal antibody (mAb) standard RM 8571 was purchased from National Institute of Standards and Technology (NIST).

The Agilent AssayMAP Bravo liquid handling system was used to dilute, digest, and desalt the NIST mAb sample. Enzymatic digestions were done using the IcleS protease (Genovis, Inc.) and the Rapid PNGase F (NEB). The digested samples were dried down and resuspended with 0.1 % TFA in DI water.

Approximately 0.5 µg of intact mAb, mAb subunits and mAb digested samples were injected for each LC/MS analysis.

Native GroEL sample was dissolved with 200 mM NH₄OAc into 1 pmol/µL.

LC/MS Analysis:

LC/MS analyses were conducted on an Agilent 1290 Infinity II UHPLC system coupled with an Agilent 6545XT AdvanceBio LC/Q-TOF system equipped with an Agilent Dual Jet Stream ESI source. LC separation for the intact and subunits samples was obtained with Agilent’s PLRP-S column (2.1 X 50 mm, 5 µm, 1000Å), while the digested mAb sample was separated by an Agilent AdvanceBio Peptide Mapping column (2.1 X 150 mm, 2.7 µm).

Native protein samples (NIST mAb and GroEL) were infused into the Q-TOF at a flow rate of 200 – 500 nL/min using the 8 µm SilicaTip (PicoTip, New Objective).

Data Analysis:

All MS data of the mAbs were analyzed using the Protein Deconvolution feature of MassHunter BioConfirm B.08.00 software that uses the Maximum Entropy algorithm for accurate molecular mass calculation. Raw data acquired from LC/MS/MS were also processed using the BioConfirm B.08.00 software. This powerful algorithm simplifies downstream data analysis, enabling the automatic identification of peptides and PTMs when compared to a theoretically digested NIST mAb sequence.
Results and Discussion

Workflow 1: Intact mAb Analysis (Agilent App Note: 5991-7813EN)

Figure 2. Intact NIST mAb Analysis (0.5 μg injection).

Figure 3. MS Deconvolution of Intact NIST mAb. Mass accuracies for all major glycoforms are: < 5 ppm.

Workflow 2: mAb Subunits Analysis

Scheme 1: Sample Preparation Workflow. Enzymatic digestions (IdeS and PNGaseF) and reduction on mAb.

Figure 4. Light and heavy chain of NIST mAb.

Figure 5. Light chain, Fc and Fd' of heavy chain of NIST mAb. MS resolution: > 66,000 at 2300 m/z.
Results and Discussion

Workflow 3: Peptide Sequence Mapping and PTM Identification: (App Note: 5991-7815EN)

Figure 6. Peptide Mapping and PTM Identification of NIST mAb.

Workflow 4: Native Protein Analysis:

Figure 8. Native MS analysis of intact NIST mAb

Workflow 5: mAb Sensitivity Analysis
(App Note: 5991-7814EN)(ASMS Poster: WP 637)
Workflow 6: mAb Glycan Quantitative Analysis
(ASMS Poster: TP 316)
Workflow 7: ADC DAR Analysis
(ASMS Poster: TP 082)
Workflow 8: Host Cell Proteins Analysis
(ASMS Poster: WP 694)
Workflow 9: mAb Disulfide Bonds Analysis
(App Note: 5991-6951EN)

Figure 7. Agilent MassHunter BioConfirm software with representative peptide mapping results and protein sequence coverage.

Figure 9. Native MS analysis of GroEL Tridecamer. (Mass range of the 6545XT m/z: 30,000 Da)(see Poster ThP 161 for more details)

Conclusions

We have demonstrated comprehensive high-throughput mAb analysis workflow solutions integrating automated liquid-handling robot (AssayMAP Bravo), high-performance chromatography technologies, the Agilent 6545XT AdvanceBio LC/Q-TOF, and Agilent MassHunter BioConfirm software for automatic data processing.

For Research Use Only. Not for use in diagnostic procedures.