MONITORING CRITICAL QUALITY ATTRIBUTES OF MONOCLONAL ANTIBODIES AT SUBUNIT AND PEPTIDE LEVEL USING A SINGLE HIGH RESOLUTION LC/MS PLATFORM

Jing Fang, Nilini Ranbaduge, Henry Shion, Min Du, Ying Qing Yu, Scott Berger, and Weibin Chen
Waters Corporation, Milford, MA 01757

INTRODUCTION

The application of mass spectrometry techniques in late development and QC environments has shown significant advantages in monitoring critical quality attributes (CQAs) at the molecular level, which provides an in-depth understanding of the biotherapeutic production process and facilitates the implementation of the principles of Quality by Design (QbD) for the process, stability and formulation studies. Here, we demonstrate the applicability of QbD for attribute monitoring at both the peptide and subunit levels using an HRMS platform capable of operating under the compliance setting. The HRMS platform is able to implement domain-specific CQAs, whereas peptide mapping provides more precise site-specific information.

METHODS

Sample Preparation:
Transformed variants were treated with either oxidation levels or alkali stress. NIST mAbs were treated by H2O2, and then IdeS and oxidation stress, followed by denaturation, and Trastuzumab samples were treated with alkaline stress. Sample Preparation

Peptide analysis:

LC System: ACQUITY UPLC H-Class Bio System

LC/MS:
Desolvation Temperature: 250 ºC (pepmap) and 600 ºC (subunit)
Source Temperature: 100 ºC (pepmap) and 125 ºC (subunit)
Cone Voltage: 30 V (pepmap) and 70 (subunit)

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Total run Time: 14 min

C. 0.5%TFA in Water
B. 0.1% FA in Acetonitrile

Column: ACQUITY UPLC BEH C4, 1.7 µm, 2.1 x 50 mm

Subunit analysis:

B. 0.1% FA in Acetonitrile

Mobile phase: A. 0.1% FA in water,

Column temperature: 65 ºC
Gradient: 3-33 %B over 120 min

Column: ACQUITY UPLC CSH300 C18, 1.7 µm, 2.1 x 100 mm

NIST mAbs were treated by H2O2, and then IdeS and oxidation stress, followed by denaturation, and Trastuzumab samples were treated with alkaline stress.

RESULTS

Monitoring Product Attributes using Subunit Mass Workflow

Figure 3. LC HPLC chromatograms for control, 0.003% H2O2, and 0.01% H2O2 stressed NIST mAb subunits. UNIFI automatically generated trending plots for component ranges (C).

CONCLUSION

• Subunit and Peptide-based analysis both facilitate qualitative attribute monitoring assays
• The UNIFI based platform demonstrated all elements required of systems deployed for Characterization, GCP Development and GCP/Lot Release
• Common platform for characterization and attribute monitoring for efficient tech transfer
• Support both subunit and peptide MAM
• Automation of reporting, processing, and reporting of data
• Compliant architecture ensuring full data integrity

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