COMPREHENSIVE PTM CHARACTERIZATION OF THE NIST mAb REFERENCE STANDARD USING A HRMS MASS SPECTROMETRY

Jing Fang, Nilini Ranadabe, Henry Shion, William Alley and Ying Qing Yu
Waters Corporation, Milford MA 01757

INTRODUCTION

The NIST mAb RM 8671 reference standard material can function as a common standard for the biopharmaceutical industry, analytical instrument/software companies, and regulators. RM 8671 can be used to evaluate and improve current analytical technologies and capabilities for determining the physicochemical and biophysical attributes of monoclonal antibodies.

An exhaustive set of characterization data has been collected from QTOF and Orbitrap mass spectrometers and published (ACS Book series: "State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization").

In this study, we demonstrate the use of a new bench top IMS QTOF MS controlled by a workflow driven software for common PTM characterization of the new NIST mAb reference standard (RM 8671).

RESULTS

The NIST mAb RM 8671 reference standard material can regulators. RM 8671 can be used to evaluate and improve current analytical technologies and capabilities for determining ppm the physicochemical and biophysical attributes of monoclonal

Antibodies. An exhaustive set of characterization data has been collected from QTOF and Orbitrap mass spectrometers and published (ACS Book series: "State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization").

4. Peptide Mapping (PTM Analysis)

C-terminal Lysine Truncation

Table 4. Peptide mapping data collected using an RP C4 column. Mass accuracy for the major glycoforms are listed in the table. High levels of lysine truncation were observed for high abundance glycoforms.

<table>
<thead>
<tr>
<th>Glycoform</th>
<th>Theoretical Mass (Da)</th>
<th>Observed Mass (Da)</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1/FA1</td>
<td>147630.8</td>
<td>147629.6</td>
<td>-8.1</td>
</tr>
<tr>
<td>FA2/FA1</td>
<td>147834</td>
<td>147834.4</td>
<td>2.7</td>
</tr>
<tr>
<td>FA2/FA1G1</td>
<td>147996.1</td>
<td>147996.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>FA2/FA2</td>
<td>148036.4</td>
<td>148037.4</td>
<td>3.9</td>
</tr>
<tr>
<td>FA2/FA2G1</td>
<td>148198.6</td>
<td>148199.2</td>
<td>-0.7</td>
</tr>
<tr>
<td>FA2G1/FA2G1+K</td>
<td>148327.5</td>
<td>148324.8</td>
<td>-18.2</td>
</tr>
<tr>
<td>FA2G1/FA2G2</td>
<td>148489.6</td>
<td>148487.2</td>
<td>-16.2</td>
</tr>
<tr>
<td>FA2G1/FA2G2+K</td>
<td>148651.8</td>
<td>148650.4</td>
<td>-9.4</td>
</tr>
<tr>
<td>FA2G2/FA2G2</td>
<td>148685.0</td>
<td>148685.6</td>
<td>5.4</td>
</tr>
</tbody>
</table>

5. Future Work: Utilizing Ion Mobility Separation

Reducing interfering ions for precursor and fragment ions

Automated CCS value generation for all peptide components

CONCLUSIONS

• NIST RM 8671 was characterized using the latest Vion/IMS QToF MS system controlled by UNIFI Scientific Informatics System.

• Established analytical workflows with UNIFI were used for the comprehensive analysis of this reference standard and included:
  - Accurate MW measurements for major glycoforms achieved at the intact protein level.
  - Glycation levels in the LC and Fd region by subunit analysis.
  - A thorough investigation of PTMs, including C-terminal lysine truncation, deamidation, oxidation, and glycation.
  - N-linked glycan profiling using RapFluor-MS tagging chemistry and an RMRMS Glycan OLI library built into UNIFI.

• Attributed from the characterization data can be incorporated in targeted monitoring for mAb development and QC (see P-131-W).

• Future work will be focused on utilizing CCS values (from IMS) and accurate mass information to monitor critical quality attributes for biologics.

METHODS

Instrumentation:

- ACQUITY UPLC H-Class Bio (low pH ESI source and BioAcq triple quad)
- BEH C18 2.1x150 mm column (polyclonal antibodies)
- Acquity BEH C4 2.1x100 mm column (monoclonal antibodies)
- ACQUITY UPLC FLR
- Vion IMS QTOF MS

Sample Preparation:

- Reduced Glycans: 
  - Glycan from NIST RM 8671 is released and labeled with RapiFluor-MS kit (Glycoworks RapiFluor-MS kit)

Peptide Mapping:

- Peptide mapping was performed using an RMRMS Glycan OLI library

CONCLUSIONS

- NIST RM 8671 was characterized using the latest Vion/IMS QToF MS system controlled by UNIFI Scientific Informatics System.

- Established analytical workflows with UNIFI were used for the comprehensive analysis of this reference standard and included:
  - Accurate MW measurements for major glycoforms achieved at the intact protein level.
  - Glycation levels in the LC and Fd region by subunit analysis.
  - A thorough investigation of PTMs, including C-terminal lysine truncation, deamidation, oxidation, and glycation.
  - N-linked glycan profiling using RapFluor-MS tagging chemistry and an RMRMS Glycan OLI library built into UNIFI.

- Attributed from the characterization data can be incorporated in targeted monitoring for mAb development and QC (see P-131-W).

- Future work will be focused on utilizing CCS values (from IMS) and accurate mass information to monitor critical quality attributes for biologics.

REFERENCES

1. Wang et al. Clinical Applications of Emerging Technologies for Therapeutic Monoclonal Antibody Characterization (Volume 1)


TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS