Development of an Analytical Toolbox for the Detection, Confirmation and Characterisation of Partially Reduced Species in Monoclonal Antibodies

Analytical Technologies Europe
Vanessa Wong
Analytical Sciences, BioPharmaceuticals Development, R&D, AstraZeneca, Cambridge, UK

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Overview

➢ Introduction

➢ Detection:
  ▪ Fragmentation monitoring
  ▪ Anomalies observed during routine testing

➢ Confirmation:
  ▪ PA800+ (gold standard technique)
  ▪ Possible cause of fragmentation

➢ Characterisation
  ▪ Heavy Chain or Light Chain dimer?
  ▪ LC-ESI-QTOF-MS

➢ Summary and questions
**Fragmentation**

A major degradation pathway ubiquitous to all monoclonal antibodies

**Fragmentation:**
Peptide hydrolysis
Reduction of disulphide bonds

Important to monitor due to safety & efficacy concerns!

Figures obtained from BioRender
A (brief) introduction to CE-SDS

- Capillary Electrophoresis – Sodium Dodecyl Sulphate
- Proteins are denatured using SDS -> gives proteins similar mass to charge ratio
- Protein is separated in capillary based on their hydrodynamic size
Detection:
• HPSEC
• MCE

HPSEC = High Performance Size Exclusion Chromatography
MCE = Microchip Capillary Electrophoresis
Purity Monitoring

Lab Chip GX II Touch by Perkin Elmer
- Microchip CE-SDS
- High Throughput capabilities
- Used for screening high numbers of in process samples

PA800 plus by SCIEX
- Industry gold standard for CE-SDS – highly resolved fragments
- Lower throughput capabilities

Size Exclusion Chromatography
- Better suited for aggregate detection than fragments
- Underestimates fragmentation
Anomalies observed during routine testing

• Anomalous levels of fragmentation was observed in a sample using MCE.
• Lot A showed increased fragmentation when compared to control Lot B

LMWS = Low molecular weight species
High Performance Size Exclusion Chromatography

- Run in tandem to MCE
- HPSEC also shows a small LMWS peak but this is significantly smaller than the peaks observed in MCE
## Comparing Fragmentation Levels

<table>
<thead>
<tr>
<th>Sample</th>
<th>HPSEC</th>
<th>Non-reduced MCE</th>
<th>Non-reduced CE-SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Major Product peak (%)</td>
<td>Fragment (%)</td>
<td>Major Product peak (%)</td>
</tr>
<tr>
<td>Lot A</td>
<td>97.1*</td>
<td>0.4</td>
<td>96.4</td>
</tr>
<tr>
<td>Lot B</td>
<td>99.4</td>
<td>0.0</td>
<td>99.1</td>
</tr>
</tbody>
</table>

* % High molecular weight species not shown in table.

- HPSEC shows significantly less % fragmentation compared to nrMCE
- HPSEC not suitable for fragmentation monitoring
- Significant difference between MCE and CE-SDS % fragmentation
- MCE still able to distinguish between pure and impure samples
MCE vs CE-SDS

- Five equally resolved species observed in both techniques.
- MCE unable to resolve ng-IgG shoulder from the main peak which is well resolved in CE-SDS.
- Able to detect all major LMWs and increased fragmentation in both techniques.

MCE remains a good option for use in HT screening assays in place of conventional CE-SDS.
Confirmation

Detection:
• HPSEC
• MCE

Confirmation:
• CE-SDS
Lot A and B run on conventional CE-SDS confirmed presence of all LMWs detected in MCE
Possible Fragmentation Pathway Determined via CE-SDS

Fragments = partially reduced species!
Characterisation

Detection:
• HPSEC
• MCE

Confirmation:
• CE-SDS

Characterisation
Initial Characterisation
Further Peak Characterisation

Non-reduced CE-SDS

Reduced CE-SDS
## Heavy Chain or Light Chain Dimer?

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Theoretical Mass (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Chain Dimer</td>
<td>48294</td>
</tr>
<tr>
<td>Heavy Chain</td>
<td>49952</td>
</tr>
</tbody>
</table>
## LC-ESI-QTOF-MS Analysis
(Liquid chromatography electrospray ionisation quadrupole time-of-flight mass spectrometry)

<table>
<thead>
<tr>
<th>Component</th>
<th>Theoretical Mass (Da)</th>
<th>Observed Mass (Da)</th>
<th>ppm error (absolute value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact deglycosylated mAb</td>
<td>148196</td>
<td>148202</td>
<td>37</td>
</tr>
<tr>
<td>HHL</td>
<td>124053</td>
<td>124057</td>
<td>32</td>
</tr>
<tr>
<td>HH</td>
<td>99906</td>
<td>99911</td>
<td>40</td>
</tr>
<tr>
<td>HL</td>
<td>74100</td>
<td>74102</td>
<td>28</td>
</tr>
<tr>
<td>HC</td>
<td>49952</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>LL</td>
<td>48294</td>
<td>48295</td>
<td>26</td>
</tr>
<tr>
<td>LC</td>
<td>24147</td>
<td>24147</td>
<td>0</td>
</tr>
</tbody>
</table>
Fully characterised fragments!

LC-MS was able to characterise all fragment peaks
Summary: Analytical Workflow

Detection:
- HPSEC
- MCE

Confirmation:
- CE-SDS

Characterisation:
- MS
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