Bioanalytical Characterization of Therapeutic Proteins by Electrophoresis Techniques
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Introduction

Therapeutic proteins such as monoclonal antibodies (mAbs) are major biopharmaceutical products with diagnostic and clinical applications. Regulatory agencies demand comprehensive protein characterization data hence different analytical techniques are employed for the analysis. Complexity of sample cannot be covered by a single analytical technique but requires a suite of tools to provide necessary quality of data. In this work, we present a workflow based approach for the analysis of therapeutic proteins like purity, accurate mass, aggregation, peptide sequence, glycopeptide and glycans.

Initial screening for product purity, fragments of mAb, protein PEGylation products were analyzed using microfluidic electrophoresis as a QC tool. Capillary electrophoresis (CE), liquid chromatography (LC), and combinations of these with mass spectrometry (MS) are applied for the analysis of intact protein (mAb), tryptic digest, peptide mapping, glycopeptide/glycan and accurate mass measurement. Further, mAb charge heterogeneity was separated using liquid-phase isoelectric focusing (IEF) technique followed by microfluidic CE analysis. The result presented here shows the utility of multiple analytical platforms for in-depth protein characterization.

Results and Discussion

Analytical performance and quality control

Figure 1A. Bioanalyzer analysis of IgG2 preparation under reducing conditions

Microfluidic CE

Figure 1B. Bioanalyzer analysis of IgG2 preparation under non-reducing conditions

Characterization of PEGylated proteins

• The Bioanalyzer P230 Assay for Protein PEGylation
  ✓ Easy-to-use tool that provides high level of resolution
  ✓ Allows efficient optimization of PEGylation reaction conditions
  ✓ Fast and quantitative monitoring of production batches

PEGylation reagent (pNPA)

Figure 3. Bioanalyzer analysis of protein PEGylation. PEGlyating reagents: Methoxy PEG p-nitrophenyl carbonate (mPEG pNPA, MW 5000)

Results and Discussion

CE-QTOF MS analysis of glycopeptide and glycans of monoclonal antibodies

Figure 4. Base peak electropherogram (BPE) of a trypsin-digested mAb and CE-MS/MS analysis

Analysis of antibody charge heterogeneity

Figure 5. Extracted ion electropherogram (EIE) and the representative MS trace from CE-MS analysis of APTS labeled neutral (A) and neutral/sialylated(B) glycans

Results and Discussion

CE-QTOF MS analysis of released glycans from a glycoprotein

Figure 6. CE-MS analysis of released glycans from a glycoprotein

LC-MS analysis of mAb

Figure 7. LC-MS analysis of mAb

Conclusions

• Initial characterization of therapeutic protein/mAb is achieved using the electrophoretic techniques such as OFFGEL and microfluidic based electrophoresis. This sets further stage for detail analysis of mAb by advanced mass spectrometric techniques (CE-MS, LC-MS).

• The combination of CE with Q-TOF MS is a valuable tool for peptide mapping of small quantity biopharmaceuticals, especially in analysis of glycoproteins/peptides.

• Highly sialylated glycans was more suited when CE-MS was used as analysis tool while LC-MS seems to be better adapted for analysis of neutral glycans.

• Combination of various electrophoretic and LC techniques with mass spectrometry techniques was demonstrated for comprehensive protein characterization.

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