Automated Workflow for Proteoform Characterization and Quantification of Intact Monoclonal Antibodies by CEX-MS

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ABSTRACT

Purpose: Automated proteoform characterization and quantification of intact monoclonal antibodies.

Methods: Charge variant chromatography was coupled to a Thermo Scientific™ Q Exactive™ Plus mass spectrometer and data were processed with Thermo Scientific™ BioPharma Finder™ 3.2 (BPP 3.2) and Thermo Scientific™ Chromeleon™ 7.2.10 Chromatography Data System (CDS).

Results: The implementation of this CEX-MS method into Chromeleon 7.2.10 CDS allowed to identify and quantify different proteoforms of Adalimumab in an automated and simple way.

INTRODUCTION

Since 1985, the number of monoclonal antibodies (mAbs) commercially available rapidly increased. They are employed in the treatment of numerous diseases such as cancer. Complete characterization is a critical regulatory requirement, as post-translational modifications (PTMs) which may occur during the manufacturing process can affect quality, efficacy and safety of these molecules. A routinely employed method is charge variant analysis by cation exchange chromatography (CEX), which can separate antibody charge variants. Recently, successful hyphenation with mass spectrometry was reported demonstrating the capability of CEX-MS to reveal and monitor mAb heterogeneity. These workflows allow multi-attribute characterization and are particularly useful to identify and quantify proteoforms with low-mass PTMs. Furthermore, they show analytical improvements for both dynamic range and mass accuracy.

MATERIALS AND METHODS

Sample Preparation
Adalimumab 100 µg/mL (formulation buffer) was diluted 6 times in a concentration range between 0.25 and 10 µg/mL. 10 µL of each solution was injected and analyzed as described below.

LC-MS method
The IgG1 was separated by CEX using a Thermo Scientific™ MAbPac™ SCX-10 RS column; 25 mM ammonium hydroxide in water (pH 10.9) as buffer B. The proteoforms were then included and exported as a BioPharma Finder workbook and finally imported BioPharma Finder 3.2 software in order to identify the different proteoforms. The identified molecules were then included and exported as a BioPharma Finder workbook and evaluated later in Chromeleon 7.2.10 CDS.

All the samples were analyzed in triplicates using the LC-MS conditions described below (Table 1(A) LC conditions & 1(B) MS conditions).

Data Analysis
The obtained data were then processed using sliding windows and RefSpect™ algorithm in BioPharma Finder 3.2 software in order to identify the different proteoforms. The identified proteoforms were then included and exported as a BioPharma Finder workbook and finally imported into Chromeleon 7.2.10 CDS (Figure 1).

RESULTS

BioPharma Finder 3.2 Results
Samples analyzed by LC-MS were processed in BPP 3.2 (Figure 2). A second algorithm, sliding window, was used in order to increase the confidence of identification and to obtain proteoform specific retention time windows. In total more than 250 components were detected and more than 30 were identified, we selected sixteen different proteoforms to be included in the BioPharma Finder workbook and evaluated later in Chromeleon 7.2.10 CDS.

The selected components were different lysine clipping derivatives for the main three glycoforms, aspartate loss, succinimide formation, proline amidation and deamidation PTMs of the most abundant proteoform.

Figure 1. Automated Workflow for Proteoform Characterization and Quantification of Intact Monoclonal Antibodies by CEX-MS

Figure 2. Base peak chromatograms of Adalimumab (A), source average spectrum for the main component (B) and deconvoluted spectrum (C) obtained by processing in BPP 3.2.

BioPharma Finder 3.2 was used to:
- identify the components;
- select the proteoforms of interest;
- create target workbook. Chromeleon 7.2.10 CDS was used to:
- set up injection sequence;
- build LC and MS methods;
- confirm and integrate target proteoforms;
- automatically generate reports with custom outputs/formulas.

Figure 3. Chromeleon 7.2.10 CDS intact protein deconvolution results (A) and integration of target components (B).

CONCLUSIONS

Here, an automated and simple workflow was generated for proteoform characterization and quantification of intact monoclonal antibodies by CEX-MS. It allowed easy and fast data evaluation of the injection sequence, with customized outputs and reports.

REFERENCES

1. Füssli et al. mAbs, 2018. doi:10.1016/j.mab.2018.07.037

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PO65764-EN 0422S