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Automated Workflow for Proteoforms 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 (BPF 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 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[1]. 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/µL (formulation buffer) was diluted 6 times in a concentration range between 0.25 and 10 µg/µL. 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 bicarbonate and 30 mM acetic acid in water (pH 5.3) as buffer A and 10 mM ammonium hydroxide in water (pH 10.9) as buffer B.

ESI–MS analyses were performed in positive ion mode on a Q Exactive Plus mass spectrometer.

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

Data Analysis

The obtained data were then processed using sliding windows and ReSpect™ 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).

Table 1. LC (A) and MS (B) condition used for analysis of Adalimumab

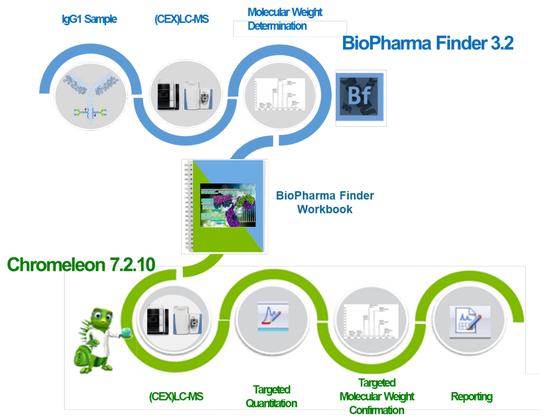
(A)

LC Conditions	
Time	**B%**
0	40
10	100
14	100
14.1	0
16	0
16.1	40
25	40

(B)

MS Conditions	
Method Type	HMR - Full MS
Scan range (*m/z*)	2500-8000
Resolution	35,000
AGC target (counts)	3e6
Max inject time	200 ms
Microscans	10
Source Settings	
Spray voltage (+ V)	3600
Ion transfer tube temperature (° C)	275
Sheath gas (a.u.)	25
Aux gas (a.u.)	10
Sweep gas (a.u.)	0
Vaporizer temp (° C)	275
S-lens RF Level (%)	200

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



BioPharmaFinder 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.

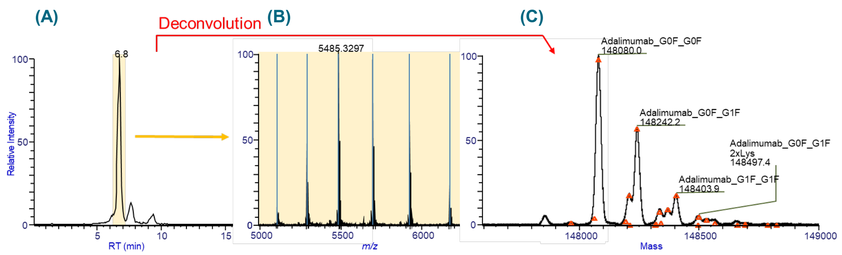
RESULTS

BioPharma Finder 3.2 Results

Samples analyzed by LC-MS were processed with in BPF 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 2. Base peak chromatograms of Adalimumab (A), source average spectrum for the main component (B) and deconvoluted spectrum (C) obtained by processing in BPF 3.2.



Chromeleon Results

The generated workbook was imported into Chromeleon 7.2.10 CDS for data analysis. All the injections in the sequence were processed using the same BPF 3.2 parameters.

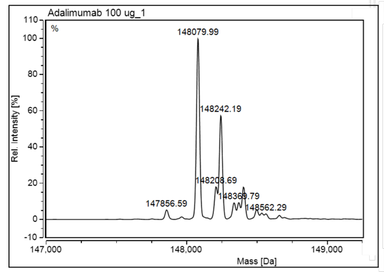
After generation of the customized report, it was possible to automatically identify and quantify all the target proteoforms for all the injections in the sequence.

Figure 3 shows an example of results. In this case, a vast majority of proteoforms showed mass deviation lower than 15ppm; higher mass errors are probably imputable to a low signal abundance or near co-eluting substances.

Concerning the quantification, it was possible to detect all the targeted species. Noticeably, it was possible to quantify low abundant species that are nearly co-eluting with other proteoforms (Figure 3B). This clearly shows the high potential of this method, being superior to CEX-UV methods where it's not possible to distinguish them.

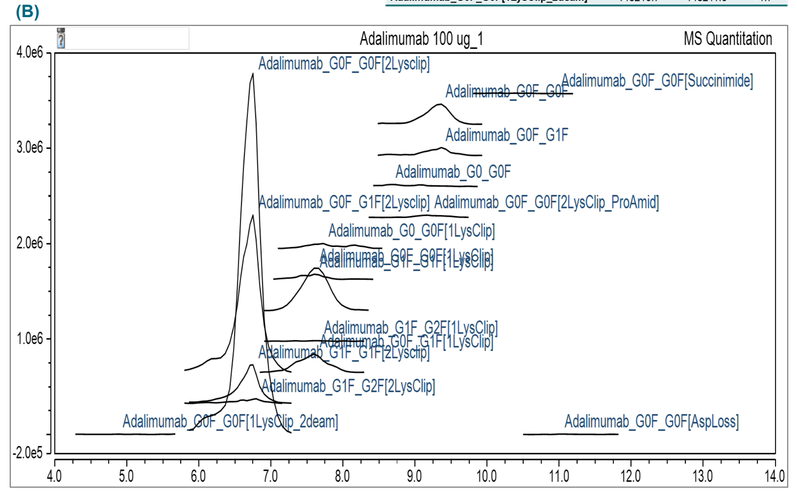
Figure 3. Chromeleon 7.2.10 CDS intact protein deconvolution results(A) and integration of target components (B).

(A)



Peak Name	Theoretical Mass (Da)	Measured Mass (Da)	Delta Mass (ppm)
Adalimumab_G0F_G0F[2Lysclip]	148080.6	148080.0	3.8
Adalimumab_G0F_G1F[2Lysclip]	148242.7	148242.2	3.6
Adalimumab_G1F_G1F[2Lysclip]	148404.8	148403.9	6.5
Adalimumab_G0F_G0F[1LysClip]	148209.7	148208.7	6.6
Adalimumab_G0F_G1F[1LysClip]	148370.9	148369.8	7.2
Adalimumab_G0F_G0F	148336.9	148336.0	6.1
Adalimumab_G0F_G1F	148499.0	148497.4	11.1
Adalimumab_G0_G0F[1LysClip]	148062.6	148063.0	-2.6
Adalimumab_G1F_G1F[1LysClip]	148533.0	148531.8	8.3
Adalimumab_G0_G0F	148190.8	148192.7	-13.2
Adalimumab_G0F_G0F[2LysClip_ProAmid]	147964.5	147963.6	5.8
Adalimumab_G1F_G2F[2LysClip]	148567.0	148564.9	14.1
Adalimumab_G1F_G2F[1LysClip]	148695.2	148692.7	16.6
Adalimumab_G0F_G0F[Succinimide]	148318.9	148320.3	-9.3
Adalimumab_G0F_G0F[AspLoss]	148221.8	148226.2	-29.6
Adalimumab_G0F_G0F[1LysClip_2deam]	148210.7	148211.0	-1.7

(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. Füssl et al. mAbs, 2018. doi:10.1016/j.jchromb.2018.07.037

TRADEMARKS/LICENSING

PO65764-EN 0422S

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