Structural Characterizations of Intact Monoclonal Antibodies by Native MS

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ABSTRACT

Purpose: Structural characterizations of intact monoclonal antibodies by native MS

Methods: Size exclusion chromatography and charge variant chromatography were coupled with a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.

Results: The two methods provided greater depth of analysis of mAbs heterogeneity, showing different benefits respectively.

INTRODUCTION

In the production of therapeutic monoclonal antibodies (mAbs), intact mass spectrometry analysis has become a key step. In particular, the characterization of PTMs is a fundamental regulatory requirement as these modifications may affect quality, efficacy and safety of these biopharmaceuticals. These MS analyses can be performed under denaturing or native conditions. The main advantage of native MS is to avoid acid and organic solvents. The protein, which then can maintain a 3D structure, will accept fewer charges during the ionization process with an improvement in terms of spectral resolution. Most commonly, these analyses are performed by size exclusion chromatography (SEC) coupled with MS. Alternatively, charge variant (CV) analysis by cation exchange chromatography has shown is potential to reveal mAb heterogeneity[1,2].

MATERIALS AND METHODS

Sample Preparation

Trastuzumab 21µg/µL (formulation buffer) was diluted 6 times in a concentration range between 0.33 and 21 µg/µL. 1µL of each solution was injected and analyzed as described below.

LC-MS Method

All experiments were performed using a Thermo Scientific™ Vanquish™ UHPLC system. For size exclusion chromatography, Trastuzumab was desalted online on a Thermo Scientific™ MAbPac™ SEC-1 column by isocratic elution with 50 ammonium acetate (pH7.4). The system was operated at 25° C and a flow rate of 250 µL/min. Charge variant separation by cation exchange chromatography was executed on 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 were used. Separation was performed using the gradient conditions shown in Table 1A.

ESI–MS analyses were performed in positive ion mode on a Orbitrap Exploris 480 mass spectrometer using the Intact mAb Native system template (Figure 2 and Table 1).

Data Analysis

Data analyses were performed with Thermo Scientific™ BioPharma Finder™ 3.1 software using the ReSpect™ algorithm.

Figure 1. Vanquish UHPLC system coupled to new Orbitrap Exploris 480 mass spectrometer.

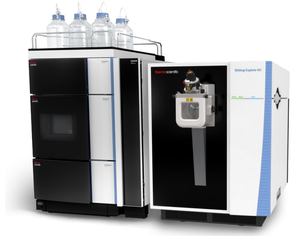


Figure 2. Method Editor Orbitrap Exploris 480 mass spectrometer and Intact mAb native system template.

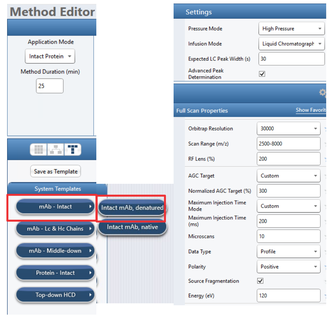


Table 1. LC (A) and MS (B) conditions used for analysis of Trastuzumab

(A)

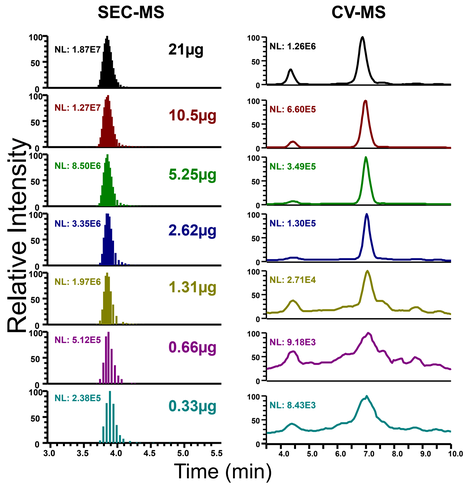
Time	B%
-12	40
0	40
10	100
14	100
14.1	0
17	0

(B)

HESI Source
Sheath gas 25 (au);
Aux gas 10 (au)
Spray voltage 3800 (V)
Ion transfer Tube Temp 250 ° C)
Vaporizer Temp 225 (° C)

RESULTS

Figure 3. MS base peak chromatograms of trastuzumab 0.33-21 µg resulting from SEC and CV separations.



The MS base peak chromatograms for the two separation techniques are significantly different. For size exclusion chromatography the protein elutes in a single sharp peak (about 20 seconds at the baseline), while the charge variant separation is able to separate different proteoforms of Trastuzumab. This translates to different absolute intensities of the main peak; in general SEC-MS shows higher intensity than CV-MS.

Figure 4. Trend lines demonstrating SEC (A) and CV (B) methods linearity. Data points are based on measurements of the peak areas of the trastuzumab main peak. Total amounts injected varied from 0.33 to 21 µg.

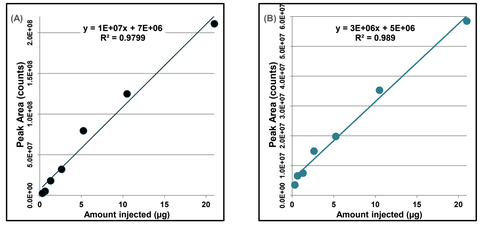


Figure 5. Full MS spectra of trastuzumab for different injection amount and relative magnification of the most prominent charge state (z=26).

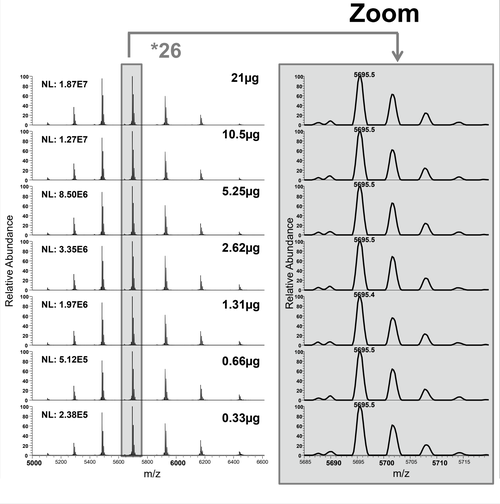
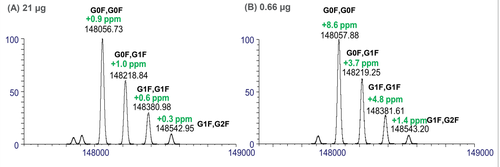
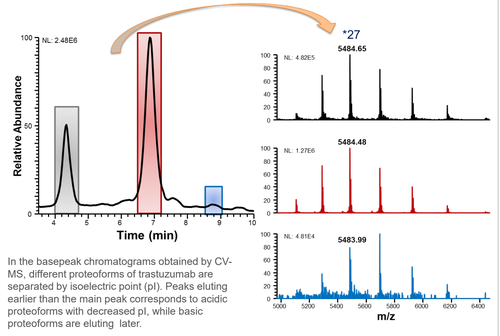


Figure 6. SEC-MS: deconvoluted spectra for 21µg (A) and 0.66µg (B) injection of Trastuzumab.



Full MS average spectra, for all the different amount of Trastuzumab injected, show nicely distributed envelope with charge state between 23 and 29. Zooming inside the most prominent charge state (z=26) five major baseline separated glycoforms are revealed. Noticeably, high quality spectra and high mass accuracy can be achieved by the Orbitrap Exploris 480 mass spectrometer regardless of the amount of the sample analyzed, as shown in Figures 5 and 6.

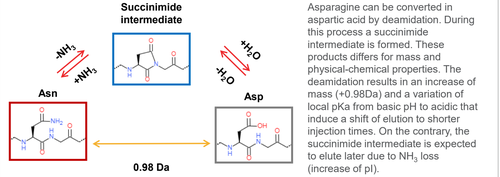
Figure 7. Base peak chromatograms of Trastuzumab (21µg injection) resulting from CVMS method. The full charge envelopes of the three main peaks (labelled in grey, red and blue in the BPC's) are shown with the corresponding m/z for generally predominant charge state.



In the basepeak chromatograms obtained by CV-MS, different proteoforms of trastuzumab are separated by isoelectric point (pI). Peaks eluting earlier than the main peak corresponds to acidic proteoforms with decreased pI, while basic proteoforms are eluting later.

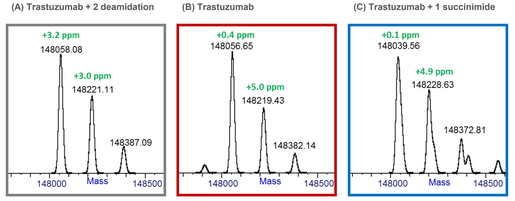
In the mass spectra relative to the three main peaks are evident variations of the protein charge envelope (especially for the blue highlighted peak) and of the relative m/z (Figure 7).

Figure 8. Pathway for asparagine deamidation



Asparagine can be converted in aspartic acid by deamidation. During this process a succinimide intermediate is formed. These products differs for mass and physical-chemical properties. The deamidation results in an increase of mass (+0.98Da) and a variation of local pKa from basic pH to acidic that induce a shift of elution to shorter injection times. On the contrary, the succinimide intermediate is expected to elute later due to NH_3 loss (increase of pI).

Figure 9. Deconvoluted spectra, obtained with the Respect alghorim, of three main peak of trastuzumab (A,B,C).



Deconvoluted spectra of the three main chromatographic peaks (Figure 7) allowed the identification of the deamidated form and the succinimide intermediate. As expected, the deamidated spectral peaks elute earlier relative to the main spectral peaks, followed by the succinimide intermediate spectral peaks in the latest peak. The most abundant glycoform maintained a mass standard deviation of less than 5 ppm even for low abundant proteoforms, allowing confident identification of these products.

CONCLUSIONS

Here, we present the successful implementation of two different LC-MS methods for native analysis of monoclonal antibodies on the new Orbitrap Exploris 480 mass spectrometer.

Size exclusion chromatography showed a single sharp peak. The average spectrum over the elution time shows a nicely distributed envelope and the deconvoluted observed masses matched very well to the average theoretical masses for the known amino acid sequence with various combinations of glycoforms (delta ppm < 10) even for low sample amounts injected.

In charge variant separation the retention time is influenced by the chemical nature of the PTM. This method allowed us to separate proteoforms with very similar molecular weight, as deamidation derivatives. The combination of this approach with the high resolution, accurate-mass (HRAM) of the Orbitrap Exploris 480 mass spectrometer allowed confident identification of proteoforms which could not be detected by SEC-MS.

REFERENCES

1. Bailey et al. MABS 2018 doi:10.1080/19420862.2018.1521131
2. Fuessl et al. MABS 2018 doi:10.1016/j.jchromb.2018.07.037

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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