Rapid monitoring of mAb aggregates during the purification process development of therapeutic mAbs using a modified Orbitrap hybrid MS

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Abstract

Purpose: Develop an LC-UV-MS platform method to determine the molecular weights of aggregates and monitor their relative quantitative changes in mAb samples.

Methods: The main and high-mass species from an in-house mAb sample were separated using size exclusion chromatography and detected by UV and intact MS on a new Thermo Scientific™ Orbitrap™ Excedion™ Pro BioPharma mass spectrometer, coupled with a Thermo Scientific™ Vanquish™ Horizon UHPLC system connected to a UV detector.

Results: High-mass aggregates, including dimers in the in-house mAb sample, were measured directly using the extended high mass range (up to *m/z* 12,000) of the Orbitrap Excedion Pro BioPharma mass spectrometer. The aggregates from the in-house mAb sample were efficiently removed after applying Thermo Scientific™ POROS™ Caprylate mixed-mode cation exchange chromatography resin (POROS Caprylate mixed-mode resin) for polish purification.

Introduction

In monoclonal antibody (mAb) development and manufacturing, protein aggregation is a common challenge that can lead to serious efficacy and safety issues. Aggregate levels in drug substances and final drug products are key factors in assessing the quality attributes of the molecules and need to be monitored and controlled during downstream mAb purification processes. The native intact mass method allows for the direct measurement of the molecular weights of aggregates and can be used for monitoring aggregates during mAb manufacturing.

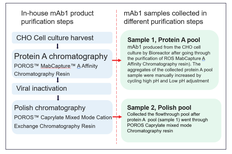
The goal of this study is to develop an LC-UV-MS platform method that utilizes size exclusion chromatography (SEC) for separating high-mass aggregates from monomers, along with UV detection and the Orbitrap Excedion Pro BioPharma mass spectrometer for monitoring their molecular weights and relative quantification. The developed method was applied to an in-house mAb (mAb1) sample to evaluate the efficiency of aggregate removal using POROS Caprylate Mixed-Mode resin for polishing purification.

Materials and methods

Sample Preparation

Commercially available NISTmAb (RM8671) and tetrameric β-galactosidase from E. coli (G3153, Sigma-Aldrich) were used for sensitivity and mass accuracy evaluation of extended high mass range (up to *m/z* 12,000). For aggregates monitoring study, an in-house mAb (mAb1) was used for method development and evaluation. Sample 1 was collected from Protein A pool with manually increased aggregate concentrations by pH adjustment. Sample 2 was collected from the polish purification pool of the sample 1 after POROS Caprylate polish purification (Table 1).

Table 1. Information on two samples from different purification pools of mAb1



HPLC _UV conditions

SEC was performed on the Vanquish Horizon UHPLC system using a Thermo Scientific™ MAbPac™ SEC-1 Size Exclusion Chromatography HPLC column (300 Å, 5 µm, 4 x 300 mm) that operated at 30 ºC and a flow rate of 250 µL/min using 50 mM ammonium acetate buffer. UV data was collected using 280 nm wavelength.

MS Conditions

All the data was collected on a Orbitrap Excedion Pro BioPharma mass spectrometer using a full MS scan mode. The ESI and mass spectrometer set ups are shown in Table 2 and 3, respectively.

Table 2. ESI source parameter set ups

ESI source settings	
Sheath gas (a.u.)	40
Aux. gas (a.u.)	10
Sweep gas (a. u.)	0
Spray voltage (+V)	3400
Capillary Temp. (ºC)	275
Vaporizer Temp. (ºC)	175

Table 3. MS parameter set ups

MS conditions	
Method type	Full MS
Scan range (m/z)	4000-12,000
Application mode	Intact
Pressure mode	High
Resolution	60,000 at m/z 200
	7,500 at m/z 200
RF lens (%)	200
AGC target Value	300
Max inject time (ms)	200
Microscans	10
Source fragmentation (V)	135

Data Analysis

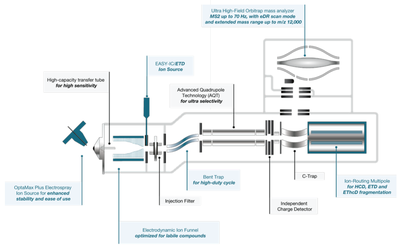
All data were processed using Thermo Scientific™ BioPharma Finder™ 5.3 software.

Results

Evaluating signal sensitivity and mass accuracy using extended high mass range for intact protein analysis

The Orbitrap Excedion Pro BioPharma mass spectrometer is the latest addition to the hybrid Orbitrap mass spectrometer family. Figure 1 presents the schematic of this new instrument. One of the new features implemented in this instrument is the extended high mass range capability, reaching up to *m/z* 12,000. This allows for the direct detection of *m/z* ion distributions for large molecules and high mass aggregates, such as dimers.

Figure 1. Schematic of Orbitrap Excedion Pro BioPharma mass spectrometer



To evaluate the intact mass accuracy of therapeutic proteins using a high mass range (up to *m/z* 120,000) for native intact mass analysis, the NISTmAb standard was employed. Figure 2 shows the average mass measurement of NISTmAb using native intact mass analysis with a resolution of 60,000 at *m/z* 200. Excellent quality of full MS spectra was observed with the high resolution (60,000 at *m/z* 200) and the extended high mass range (*m/z* 4,000 – 12,000). The deconvoluted results of the raw MS spectra demonstrated less than 10 ppm mass accuracy for the major detected glycoforms of NISTmAb.

Figure 2. Average mass measurement of NISTmAb using native intact mass analysis with resolution of 60,000 at *m/z* 200

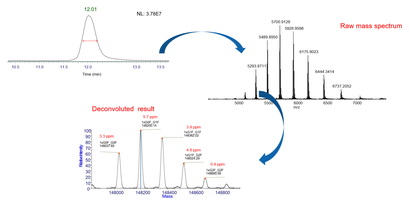
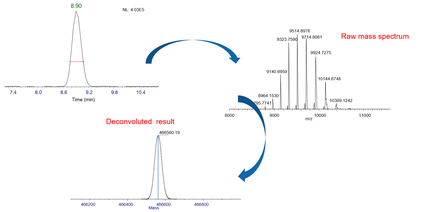


Figure 3. Average mass measurement of tetrameric β-galactosidase from E. coli using native intact mass analysis with resolution of 7,500 at *m/z* 200

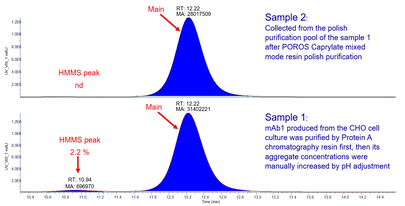


To evaluate the signal sensitivities of the detected ion distributions in the extended high mass ranges, we used a tetrameric β-galactosidase from E. coli (approximately 466,310 Da molecular weight)[1]. Figure 3 shows the average mass measurement of the tetrameric β-galactosidase from E. coli using native intact mass analysis with a resolution of 7,500 at *m/z* 200. The ion distribution pattern across the high mass range (*m/z* 8,500 – 10,500) was observed clearly with good sensitivity. The determined average mass after deconvolution was 466,560 Da, which is very close to the expected molecular weight of the tetrameric β-galactosidase from E. coli, proving that the extended high mass range allows sufficient sensitivity and mass accuracy to measure the average mass of high molecular weight species, such as dimers.

Applying the native intact mass method for mAb dimer monitoring across polish purification process

As a proof of concept, two samples (mAb1 collected at different purification steps) were analyzed using the developed SEC-UV-native intact mass analysis method to monitor high mass aggregates before and after the polish purification step. Figure 4 shows a comparison of the UV profiles of sample 1 and sample 2. A LC peak indicating high molecular weight species (HMMS) was clearly detected at a 2.2% ratio prior to the elution of the monomer mAb (main) peak in sample 1. This HMMS peak was not detected in sample 2, demonstrating that the high molecular weight species were efficiently removed through polish purification using the POROS Caprylate mixed-mode resin.

Figure 4. Comparison of UV profiles of sample 1 vs sample 2



The intact mass data collected with resolution of 60,000 at *m/z* 200 was processed using BioPharma Finder 5.3 software for average mass determination of the eluted HMMS and main peaks in sample 1. Figure 5 shows the average mass of the main peak (intact mAb1) was 147,077 Da, determined through the deconvolution of the raw intact mass spectra. Figure 6 shows the deconvoluted results for the HMMS peak, where two major high molecular weight species were detected. The determined average mass was 192,605 Da for HMMS 1 and 294,159 Da for HMMS 2. Based on these intact mass measurements, HMMS 1 can be assigned as intact mAb1 + 2LC, and HMMS 2 can be assigned as a dimer of intact mAb1 (Figure 7).

Figure 5. Measured average mass of main LC peak (intact mAb1)

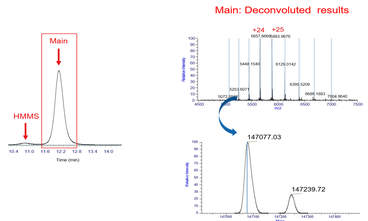


Figure 6. Measured average masses of HMMS LC peak (containing multiple HMMS species)

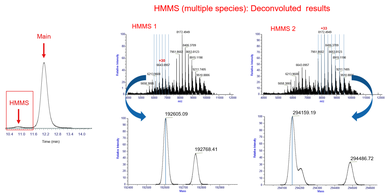
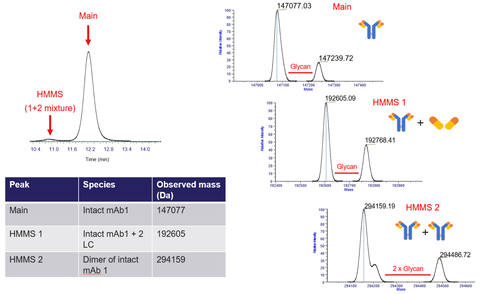


Figure 7. Assignment of detected LC peaks based on the observed average masses



Peak	Species	Observed mass (Da)
Main	Intact mAb1	147077
HMMS 1	Intact mAb1 + 2 LC	192605
HMMS 2	Dimer of intact mAb 1	294159

Conclusions

- An LC-UV-native intact mass method has been developed to separate the main monoclonal antibody (mAb) and its high-mass species using size-exclusion chromatography (SEC), followed by direct measurement of the average mass of mAb dimers and other high-mass species with the Orbitrap Excedion Pro BioPharma mass spectrometer. The extended high mass range of up to 12,000 *m/z* on the Orbitrap Excedion Pro mass spectrometer allowed the detection of ion distribution profiles of high-mass species with high sensitivity, enabling direct molecular weight determination of high-mass species, such as tetrameric β-galactosidase from E. coli (approximately 466 kDa).
- This method successfully detected and quantified high-mass aggregates in an mAb (mAb1) sample. Before polish purification, 2.2% high-mass aggregates (dimer and monomer + 2 LC) were detected in mAb1, while no high-mass aggregates were detected in mAb1 after using the POROS Caprylate mixed-mode resin for purification. This demonstrates the high effectiveness of the POROS Caprylate mixed-mode resin in removing high-mass aggregates.

References

1. McGee, J. P. et.al., Isotopic Resolution of Protein Complexes up to 466 kDa Using Individual Ion Mass Spectrometry. Analytical Chemistry, 2021, 93(5), 2723–2727. https://doi.org/10.1021/acs.analchem.0c03282Reference 2.

Conflict of interest disclosure

The authors declare no competing financial interest.

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