Monoclonal antibody mass confirmation using intact protein deconvolution for a compliance-ready manufacturing environment

Authors: Kristina Srzentić¹, Kai Scheffler², Royston Quintyn³, Artem Akhmetov⁴, Jennifer Sutton¹

Thermo Fisher Scientific,
¹Cambridge, Massachusetts, US
²Germering, Germany
³West Palm Beach, Florida, US
⁴Bannockburn, Illinois, US

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Key benefits
- Simple, compliance-ready chromatography data system (CDS) software with Intact Protein Deconvolution (IPD) capabilities provides a seamless workflow from data acquisition to data processing and reporting, allowing the platform to be implemented in a QC laboratory for compliant intact protein mass confirmation tests with automation capabilities
- Streamlined data acquisition of large sample batches, data analysis, and reporting based on raw data with exceptional spectral clarity and confident mass accuracy
- Operational simplicity for mass spectrometer setup and acquisition under denaturing and/or native conditions using standardized tune conditions
- Exceptional sensitivity and mass accuracy for intact mass analysis under denaturing and native conditions providing high-confidence results

Goal
- Demonstrate the benefits of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer for biopharmaceutical applications involving intact protein mass analysis with target mass confirmation of large sample batches
- Demonstrate the entire workflow from sample to results with data acquisition and data analysis, all performed under Thermo Scientific™ Chromeleon™ CDS using the import option for the Intact Protein Workbook generated in Thermo Scientific™ BioPharma Finder™ software
Introduction

Mass spectrometry (MS) of intact proteins is increasingly applied in biopharmaceutical analysis for rapidly providing structural insights based on intact masses of various proteoforms, without laborious sample preparation potentially interfering with endogenous post-translational modifications (PTMs) affecting the drug substance. Whereas analysis has traditionally been performed under denaturing conditions, the analysis under native conditions has gained significant interest in the past few years.¹⁻⁵

Spectra collected from analysis under denaturing versus native condition differ in the number of charge states and the actual charge state range detected, consequently resulting in different detected m/z ranges. This requires parameter settings in the deconvolution method to be adapted to the applied analysis conditions. Moreover, a deconvolution algorithm needs to be selected based on the resolution achieved in the mass spectra. For small proteins acquired with a high-resolution setting, an isotopic resolution is achieved and requires the use of the Xtract deconvolution algorithm. In contrast, larger proteins, such as monoclonal antibodies, are typically not detected with an isotopic resolution, and thus require the use of the Thermo Scientific™ ReSpect™ deconvolution algorithm. Both algorithms can be selected and applied in BioPharma Finder software and Chromeleon CDS.

Depending on the information available for the analyzed sample, the deconvolution step can be performed as a targeted or untargeted approach. The untargeted approach is suitable when an unknown sample has been analyzed and no expected mass or mass list is available. However, it may be possible to narrow the retention time window to the area where a chromatographic peak is expected or has been observed. The targeted approach is suitable when the scope of the analysis is to confirm an expected mass or even a list of masses, e.g., representing a selection of abundant mAb glycoforms. Figure 1 outlines both data analysis approaches for deconvolution in mass spectra obtained from intact protein analysis in BioPharma Finder software and Chromeleon CDS. The targeted approach always starts in the BioPharma Finder Sequence Manager that allows import of a FASTA protein sequence file and definition of the entire list of modifications including disulfide bonds, glycosylation, N- and C-terminal truncations, etc. The next step is the deconvolution in BioPharma Finder software, which aims to match the masses obtained upon deconvolution with the masses of the selected protein sequence considering the selected fixed or variable modifications. BioPharma Finder software lists all matched components by name and associated modifications in a result table that allows exporting of all or a subset of the components into a protein workbook (Figure 1C), which includes the list of selected components with masses, retention times, and modifications. In addition, the workbook contains information on the experimental data and protein sequence, as well as all processing parameters applied in the analysis. The targeted protein deconvolution in Chromeleon CDS starts with the import of an Intact Protein Workbook that is the basis for generating a processing method. As the workbook contains all the required parameters, no further input is required to complete the processing method.

In this study we present the streamlined analysis of a series of 50 sequential mAb injections from data acquisition performed on the new Orbitrap Exploris 240 mass spectrometer with data acquisition and subsequent data processing all performed under Chromeleon software. A targeted deconvolution approach was applied based on Intact Protein Workbook imported from BioPharma Finder software, resulting in a report that includes a comprehensive result summary.
**Experimental**

**Liquid chromatography**
Thermo Scientific™ Vanquish™ Horizon UHPLC system consisting of:

- Thermo Scientific System Base Vanquish Flex/Horizon (P/N VF-S01-A-02)
- Thermo Scientific Vanquish Binary Pump H (P/N VH-P10-A-02)
- Thermo Scientific Vanquish Split Sampler HT (P/N VH-A10-A-02)
- Thermo Scientific Vanquish Column Compartment H (P/N VH-C10-A-02)
- MS Connection Kit Vanquish (P/N 6720.0405)

**Column**
Thermo Scientific™ MAbPac™ RP column, (P/N 088648), 4 μm, 2.1 × 50 mm

**Solvents and sample**
- Fisher Scientific™, Water, Optima™ LC/MS grade (P/N W81)
- Fisher Scientific™, Acetonitrile, Optima™ LC/MS grade (P/N A955-1)
- Fisher Scientific™, Formic acid, Optima™ LC/MS grade (P/N A11710X1AMP)
- 1 µL of NISTmAb sample (10 mg/mL original concentration diluted to 1 µg/µL in water with 0.1% formic acid) was injected for each analysis

**Separation conditions**
- Mobile phase A - Water with 0.1% Formic acid (V/V)
- Mobile phase B - Acetonitrile 0.1% Formic acid (V/V)
- 8 min linear gradient 25–80% B at a flow rate of 0.25 mL/min
**Mass spectrometer**

An Orbitrap Exploris 240 mass spectrometer (P/N BRE725535), equipped with Thermo Scientific™ BioPharma Option (P/N BRE725539), and controlled with Exploris Series Tune 2.0 software was used.

Data were acquired in Full MS mode with the mass range set to m/z 2000–5000, a resolution setting of 30,000 (FWHM) at m/z 200, an AGC target of 3E6, maximum injection time of 200 ms, and source fragmentation of 110 V.

**Software**

- Chromeleon 7.2.10 CDS incorporating the Intact Protein Deconvolution option
- BioPharma Finder 4.0 software incorporating Sliding Window, ReSpect, and Xtract algorithms

**Deconvolution parameter settings**

Algorithm: Respect (isotopically unresolved), peak model: intact protein, model mass range: 10,000–160,000 Da, charge state range 10–100, target mass 160,000 Da, left/right peak shape 2, target mass matching filter criteria: 10 ppm

**Results and discussion**

The LC-MS performance is illustrated in Figure 2, representing 50 sequential injections with highly reproducible chromatograms (RSD = 0.61%) (A), representing the acquired mass spectra with excellent reproducibility of the overall charge envelopes spanning over the m/z range from 2200 to 4000 (B), as well as consistent relative abundances of the glycoform distribution across detected charge states as highlighted with charge states +46 and +47 (C).

Targeted intact protein deconvolution was performed upon import of an Intact Protein Workbook generated in BioPharma Finder software as a basis for a new processing method (Figure 3). The new method does not require further entering of processing parameters or selection of the deconvolution method or algorithm. Only the target mass matching criteria must be selected. Mass accuracy can be set as a single pass/fail criterion, which is applied to all entries on the target list. In case of a large number of entries on the target mass list, one option is to request to match only a subset of these masses. Here a pass criterion of mass accuracy within a 10 ppm window was applied for all five target mass entries on the component list.

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**Figure 2. Chromatograms and raw spectra obtained from long sequence analysis.**

A) Stacked plot of chromatograms obtained from 50 consecutive injections of a NIST mAb sample analyzed under denaturing chromatographic conditions with mass detection performed on the Orbitrap Exploris 240 mass spectrometer. B) Stack plot of every 10th injection in the acquired series, showing the entire charge envelopes obtained upon averaging 4 scans each acquired at 10 μscans, highlighting the reproducibility of results on a raw data level. C) Zoom into two adjacent charge states z = +46 and z = +47 representing the same data files and charge envelopes shown in panel B, demonstrating the reproducibility in relative abundances across charge states and different analytical runs.
Figure 3. Target component mass matching and reporting in Chromeleon software. A) Example of results view in the Chromatography Studio, showcasing a customizable display of panels representing the injection sequence (1), the list of target components (2), deconvolution parameter settings (3), chromatogram view (4), Full MS (5), deconvoluted spectrum (6), source spectrum (7) and result component table (8). B) Sequence analysis results captured in an example report generated based on the report template for intact protein deconvolution, available in Chromeleon CDS. The panel in the back shows the injection list with the five target components with pass/fail results for each injection. The panel in the front represents XICs for the target components for each injection.
Conclusions

• The Orbitrap Exploris 240 mass spectrometer provides excellent sensitivity and reproducibility in spectral quality for mass analysis of intact biopharmaceuticals under native and denaturing conditions.

• The acquisition of long sample sequences is supported by operating the LC-MS instrument under Chromeleon CDS.

• The targeted intact protein deconvolution approach available for data processing in Chromeleon software is enabled via the import of an Intact Protein Workbook generated in BioPharma Finder software, eliminating the need for further processing parameter settings.

• Chromeleon CDS provides customizable visualization for target mass matching results.

• Results from long sequence analyses can easily be summarized in a report based on an intact protein deconvolution report template provided within Chromeleon software.

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References


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