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Sensitive profiling of IgG1 monoclonal antibody variants under native conditions

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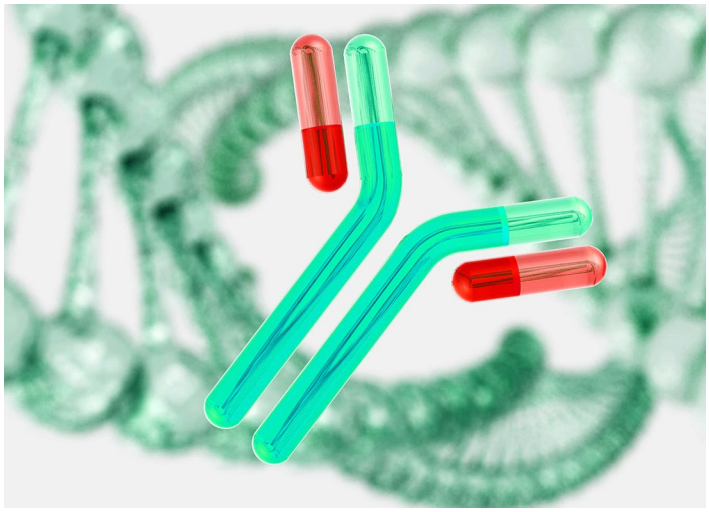
Key benefits

- Operational simplicity for mass spectrometer setup and acquisition under native conditions using standardized tune conditions
- Simplified data interpretation from exceptional spectral clarity and confident mass accuracy
- Exceptional sensitivity and mass accuracy for intact mass analysis under native conditions allowing confident analysis from low sample loading



Goal

Demonstrate the benefits of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer for biopharmaceutical applications involving separation under native conditions and showcase excellent performance for intact high-resolution accurate-mass (HRAM) mass analysis using size exclusion chromatography (SEC) and cation exchange chromatography (CEX).

Show excellent sensitivity for the detection of low abundant species, as well as wide dynamic range, for the analysis of monoclonal antibody (mAb) glycoforms and charge variants.

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Introduction

Mass spectrometry (MS) of intact proteins is increasingly applied in biopharmaceutical analysis as it is both rapid and provides significant structural insights, without laborious sample preparation steps that may interfere with endogenous post-translational modifications (PTMs) present on the drug substance. Significant advances in the use of chromatography under native conditions hyphenated with MS have been made, including optimized electrospray ionization (ESI) source conditions and the introduction of volatile mobile phases.[1]

Under native conditions the charge envelope of a mAb is typically detected at values ≥ *m/z* 4,000. Native conditions provide spectra with greater spatial separation and less overlap of charge states compared to spectra obtained under denaturing conditions. Liquid chromatography enhances structural characterization by separating microheterogeneous proteoforms prior to MS detection, providing additional insights to mAb structure.[1]

Ion exchange chromatography can be performed using volatile buffers with low salt concentrations to provide compatibility with MS, enabling detailed characterization of low abundant modifications that may be more difficult to obtain with SEC.[2,3]

In this study we evaluated the performance of the new Orbitrap Exploris 240 mass spectrometer for native MS analysis of two commercially available IgG1 therapeutic mAbs; trastuzumab and mAb #2. The SEC and pH gradient elution cation exchange chromatography (CEX) were employed to explore the sensitivity and dynamic range of the instrument.

Experimental

Liquid chromatography

- Thermo Scientific™ Vanquish™ Duo UHPLC system consisting of:
  - Two Vanquish Flex Binary Pumps (P/N VF-P10-A01) for tandem LC-MS operation
  - Split Sampler (P/N VF-A10-A-02)
  - Column Compartment (P/N VH-C10-A-02))

Columns

- Thermo Scientific™ MAbPac™ SEC-1 column (P/N 075592), 5 µm, 4.0 × 150 mm
  - Isocratic, 50 mM ammonium acetate, 0.3 mL/min, 30 °C. 10 µL injection.
- Thermo Scientific™ MAbPac™ SCX-10 RS column (P/N 082675), 2.1 × 50 mm, 5 µm
  - A) 25 mM ammonium bicarbonate, 30 mM acetic acid (pH 5.3)
  - B) 10 mM ammonium hydroxide (pH 10.9), 0.4 mL/min, 25 °C. 10 µL injection.

Mass spectrometer

- Orbitrap Exploris 240 mass spectrometer (P/N BRE725535) with Thermo Scientific™ BioPharma Option (P/N BRE725539)

MS acquisition

- Resolution: 30,000 FWHM @ *m/z* 200
- Application-specific MS tune and acquisition settings are templated and provided within the software
- High pressure source conditions for intact and native mass analysis are directly transferable from instrument to instrument, enabling easy method transfer and operational simplicity.

Software

- Thermo Scientific™ Xcalibur™ 4.2 software
- Thermo Scientific™ BioPharma Finder™ 4.0 software (P/N OPTON-30985) incorporating Sliding Window and ReSpect™ algorithms

Results and discussion

Improvements in the sensitivity of native MS approaches for biopharmaceutical applications using standard HPLC flow rates are highly desirable to allow identity testing and monitoring of PTMs even at an early stage of product development when sample availability is limited.

In the present study, SEC-MS analysis of trastuzumab was performed using a dilution series to explore limits of detection (LOD) and limits of quantitation (LOQ). The Orbitrap Exploris 240 mass spectrometer demonstrated excellent performance that facilitated accurate analysis of mAb microheterogeneity with superb sensitivity.

Nine data points were obtained, injecting a dilution series in triplicate over the range 0.05–50.0 µg on column loading (Figure 1A). The charge envelope between m/z 5,000 and 7,000 is clearly detected for all data points (Figure 1B). By plotting normalized intensities of abundant charge states against the amount of sample analyzed, it was possible to calculate the limit of detection and quantitation of the method, LOD = 0.03 µg and LOQ = 0.08 µg, respectively (Figure 1C). Moreover, analyzing low amounts of protein did not compromise spectral quality and detection even of the low abundant glycoforms (Figure 1D).

Charge variant analysis also demands high MS sensitivity to ensure confident identification of low abundant species. CEX-MS analysis of mAb #2 showed an abundant acidic variant corresponding to a deamidated species eluting earlier than the unmodified variant. Other microvariants were also observed in the base peak chromatogram (BPC), all with abundance levels ≤3% (Figure 2).

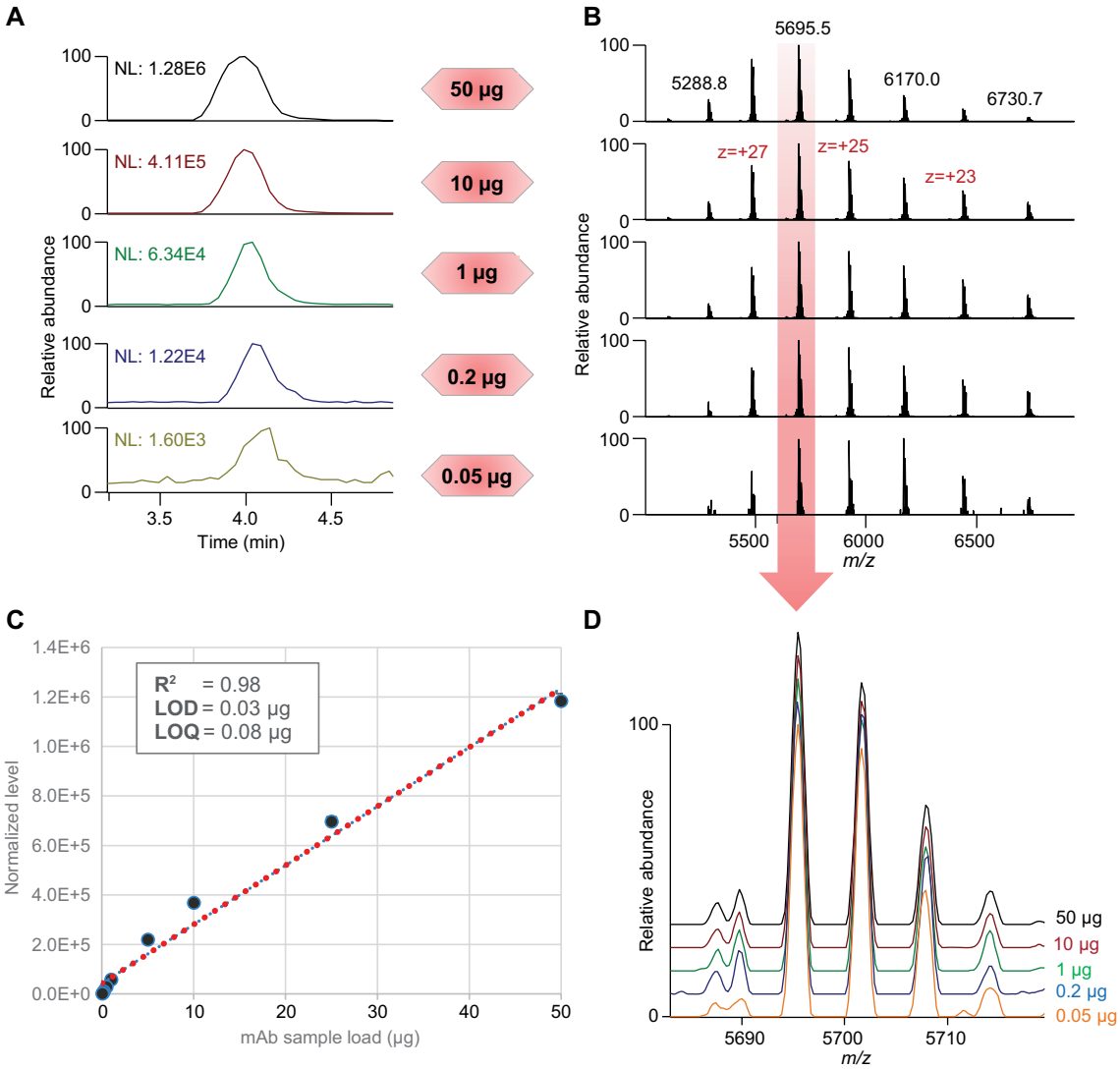

Figure 1. Sensitivity under native conditions.
A) SEC-MS analysis. BPCs of sample load from 0.05 µg to 50 µg of trastuzumab
B) SEC-MS charge envelopes detected between *m/z* 5,000 and 7,000 (three scans, acquired with 10 µscans each, were averaged)
C) Normalized intensities plotted to estimate LOD and LOQ.
D) Zoom into the MS spectra showing +26 charge state of the SEC-MS analysis for the different sample amounts of mAb analyzed, demonstrating exceptional spectral quality reproducibility independent of sample loading

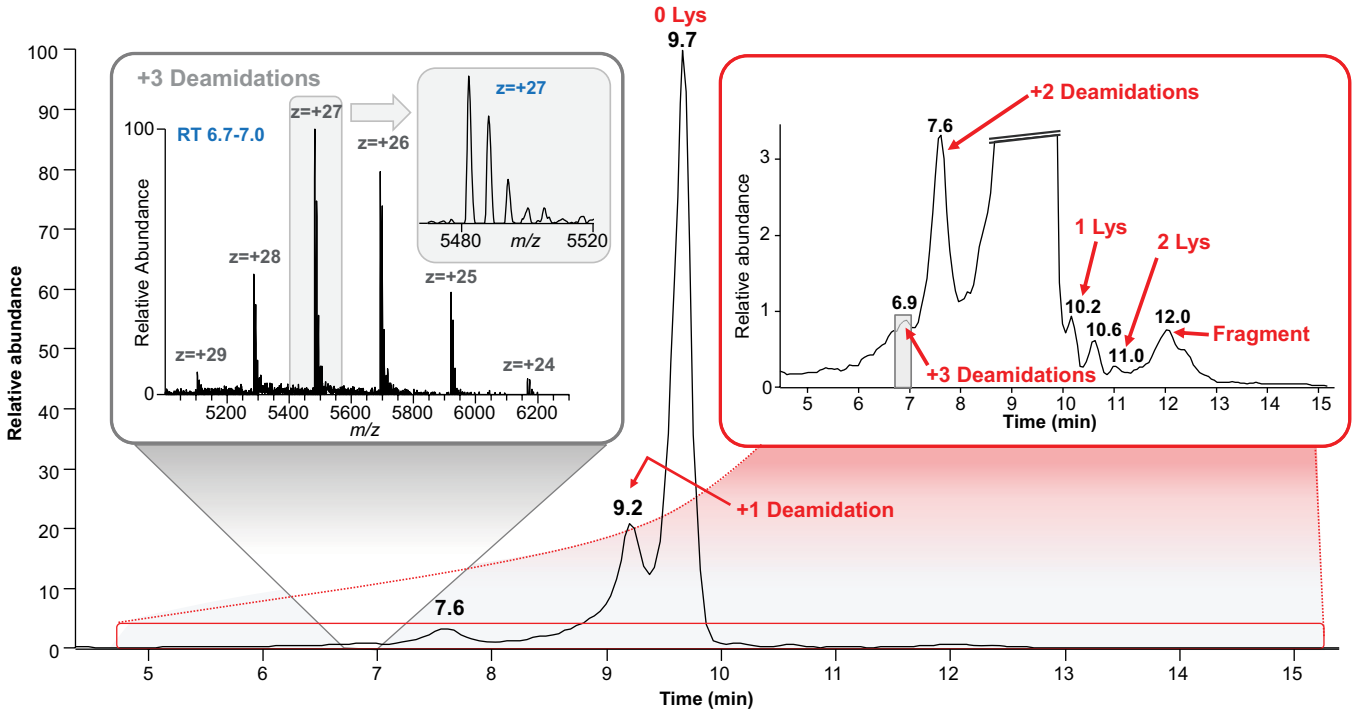

Figure 2. BPC of CEX-MS analysis of mAb #2. Left: Full scan spectrum (six scans consisting of 10 µscans each, averaged across RT 6.7–7.0 min) of the variant identified as the triply deamidated species, demonstrating the excellent sensitivity of this instrument. Right: Zoom showcases the complexity of the sample at very low abundance, further highlighting sensitivity and selectivity of acquisition of CEX-MS under native conditions.

Conclusions

The Orbitrap Exploris 240 mass spectrometer is an ideal instrument platform for performing MS analysis of intact biopharmaceuticals under native conditions. Sensitivity and dynamic range using SEC-MS, as highlighted with trastuzumab, provides exceptionally low limits of detection, enabling native MS to be used for studies where sample is limited, such as at the clone selection stage.

Charge variant analysis on the Orbitrap Exploris 240 mass spectrometer enables in-depth characterization and confident identification of mAb microheterogeneity due to excellent sensitivity and spectral quality.

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