APPLICATION BRIEF 73556

Confident peptide mapping and disulfide bond analysis of an IgG2 monoclonal antibody

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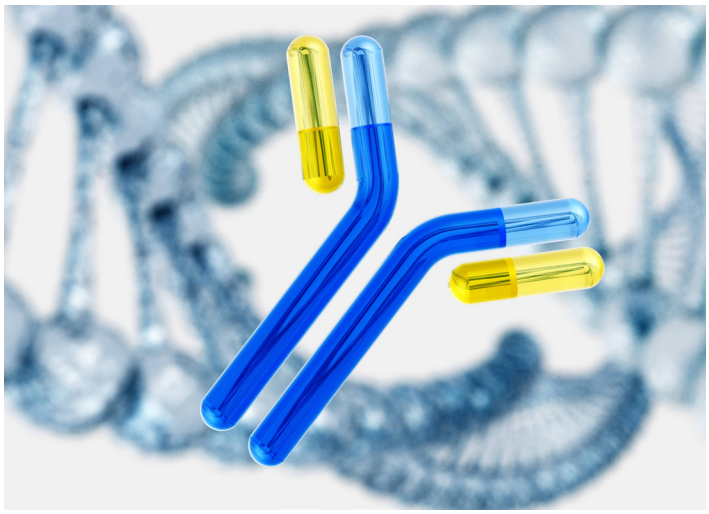


Key benefits

- Complete sequence coverage peptide map of monoclonal antibody
- Confident detection of inter- and intrachain disulfide bonds
- Sensitive detection of low-level post-translational modifications (PTMs) with excellent mass accuracy

Goal

Demonstrate the utility and capability of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer for the routine characterization of biotherapeutics through LC-MS peptide mapping. Provide the analyst with full sequence coverage, high confidence in low level PTM assignment through high-resolution accurate-mass (HRAM) MS/MS identification and overall ease in the interpretation of complex data.

Introduction

Due to variations in production processes and the intrinsic complexity of biotherapeutic proteins, such as monoclonal antibodies (mAbs), there are many process- and product-related critical quality attributes (CQAs) that must be monitored to ensure drug quality, efficacy and safety.

LC-MS peptide mapping is widely used in the biopharmaceutical industry to measure multiple CQAs of monoclonal antibodies. The results of this assay are used to confirm the primary sequence (amino acid sequence) and therefore infer genetic stability, as well as to evaluate a vast number of post-translational and chemical modifications.

The biopharmaceutical industry demands instruments and analytical methods that can be transferred and adopted routinely across organizations, with external collaborators, and contract laboratories. The Orbitrap Exploris 240 mass spectrometer provides operational simplicity and flexibility, enabling a wide range of characterization assays to be performed with high confidence in a small footprint.

In this application brief, we perform peptide mapping analysis using the Orbitrap Exploris 240 mass spectrometer to confirm 100% sequence coverage of an IgG2 mAb, denosumab, after both reducing and non-reducing sample preparation conditions. We provide examples of low-level PTM identification with confidence and demonstrate how the instrument can be used to investigate and confirm the location of disulfide bonds. Disulfide bonds are important structural features of protein therapeutics. They ensure correct protein folding which in turn provides stability and ensures proper function. Mapping the disulfide bond locations to confirm their arrangement is essential. Any variation in these bonds could lead to alteration in protein structure, which could negatively impact the function of the therapeutic.

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)

Column

- Thermo Scientific™ Acclaim™ VANQUISH™ C18 column (P/N 074812-V), 2.2 µm, 2.1 × 250 mm
  - A) Water with 0.1% formic acid (v/v), Optima™ LC/MS grade (P/N 10188164)
  - B) Acetonitrile with 0.1% formic acid (v/v), Optima™ LC/MS grade (P/N 10118464) 0.3 mL/min. 10 µL injected.

Mass spectrometer

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

MS acquisition

- MS^1 Resolution: 120,000 FWHM @ *m/z* 200
  MS^2 Resolution: 15,000 FWHM @ *m/z* 200
  ddTop5 MS^2
- Application specific MS tune and acquisition settings are templated and provided within the software. Settings 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)

Automated proteolytic digestion

- Thermo Scientific™ SMART Digest™ Trypsin kits (P/N 60109-101-MB)
- Thermo Scientific™ KingFisher™ Duo Prime system (P/N 5400100)

Results and discussion

Sequence coverage

Peptide mapping assays must provide a high level of sequence coverage, including the product-specific complementarity-determining regions (CDRs), to give the user confidence that no critical regions of the molecule go undetected. The high selectivity, acquisition rate and sensitivity of the Orbitrap Exploris 240 mass spectrometer delivered 100% sequence coverage for both light and heavy chains of denosumab (Figure 1). Protein digests were performed using the magnetic resin based SMART Digest kits with automated sample handling on the KingFisher Duo Prime purification system.

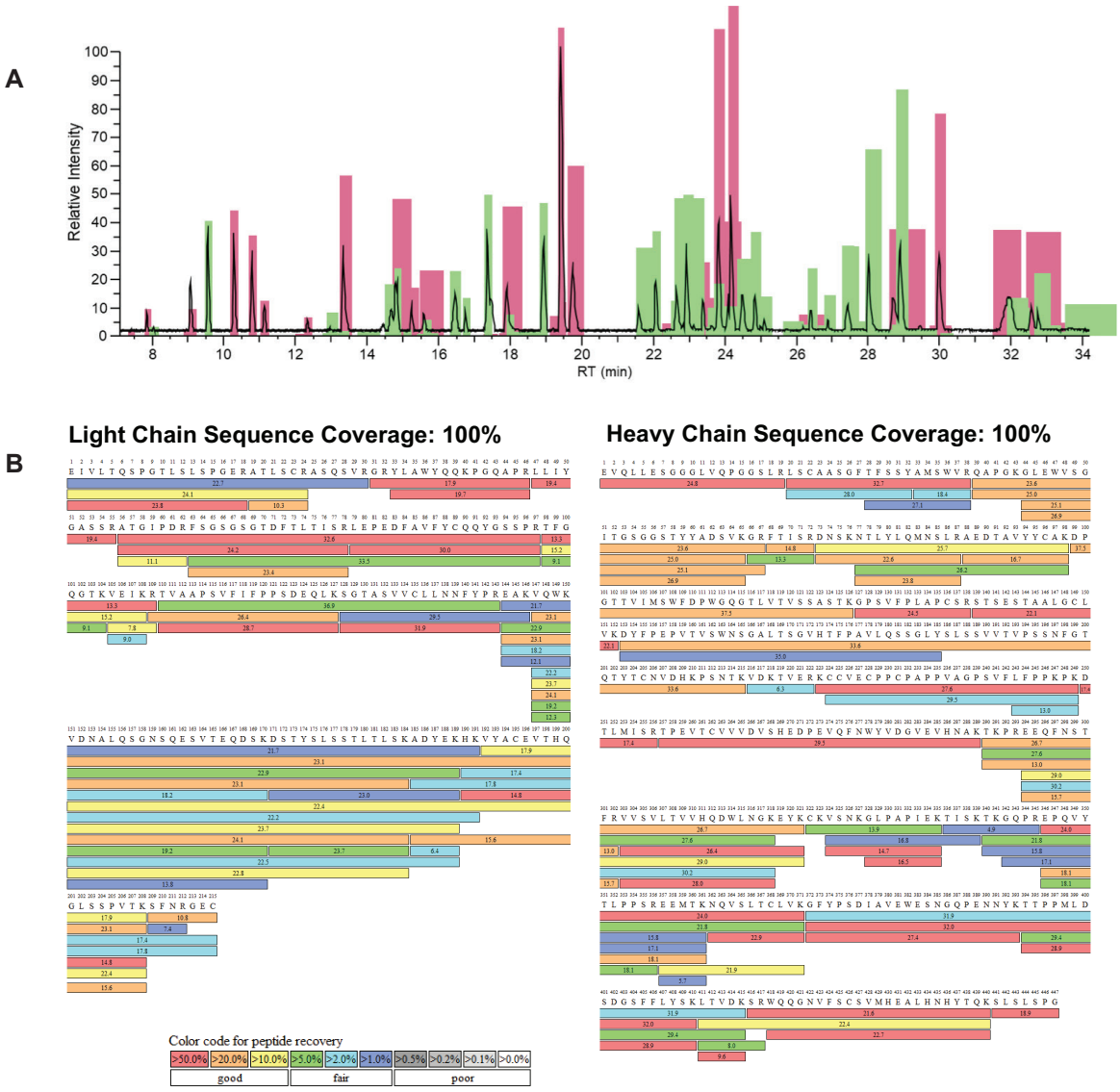


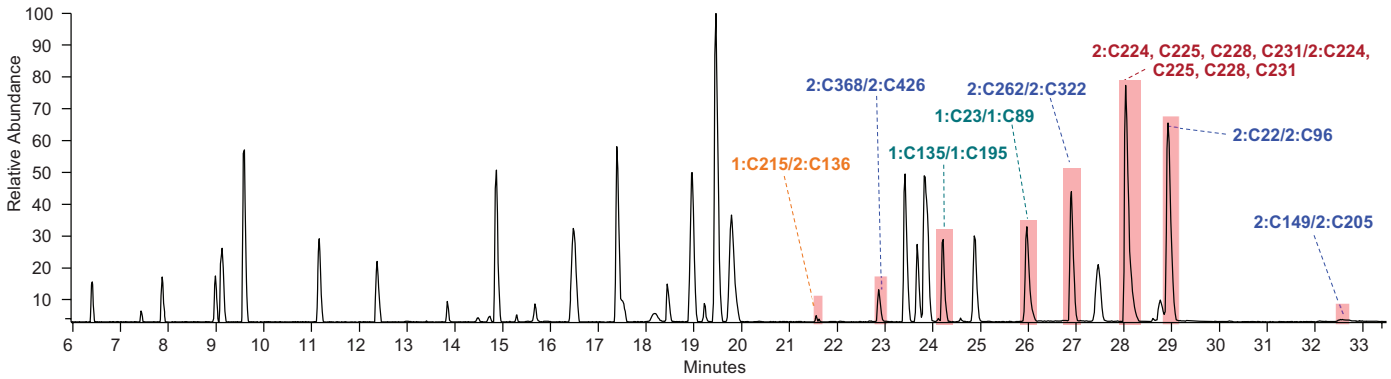
Figure 1. Chromatogram and sequence coverage maps. A) Base peak chromatogram (BPC) of denosumab indicating the peptide origin to light chain (red shading) or heavy chain (green shading). B) Colored bars represent identified peptides with colors indicating peptide intensities based on full MS spectra. 100% sequence coverage was obtained for denosumab light and heavy chains.

Disulfide bond mapping

A comparative analysis of reduced and non-reduced denosumab was used to characterize disulfide linkages. Using BioPharma Finder software, peptides were identified as either free cysteine-containing (reduced sample) or disulfide-linked peptides (non-reduced sample), illustrated as shaded peaks in the chromatogram (Figure 2). Most abundant expected intra- and interchain disulfide bonds were detected and confirmed based on MS/MS spectra with excellent (<3 ppm) precursor and fragment ion mass accuracies.

Low-level PTM identification

The ability to selectively resolve and measure trace PTMs is critical for methods used in manufacturing control. Here we demonstrate the identification of a trace level deamidation with high mass accuracy, providing additional confidence to the identification (Figure 3).



S-S bond type		Peptide sequence	Position	Δ ppm	RT
Intrachain	LC1	ATLSCR / LEPEDFAVFYCQQYGSSPR	1:C23/1:C89	-1.2	25.95
	LC2	SGTASVVCLLNNFYPR / HKVYACEVTHQGLSSPVTK	1:C135/1:C195	-2.1	24.15
	HC1	LSCAASGFTFSSYAMSWVR / AEDTAVYYCAK	2:C22/2:C96	-2.7	28.95
	HC2	STSESTAALGCLVK / DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTK	2:C149/2:C205	-0.9	32.54
	HC3	TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK / CK	2:C262/2:C322	-0.6	26.76
	HC4	NQVSLTCLVK / WQQGNVFSCSVMHEALHNHYTQK	2:C368/2:C426	0.4	22.87
Interchain	LC-HC	GEC / GPSVFPLAPCSR	1:C215/2:C136	-2.5	21.86
	Hinge	KCCVECPPCPAPPVAGPSVFLFPPKPK / KCCVECPPCPAPPVAGPSVFLFPPKPK	2:C224, C225, C228, C231/ 2:C224, C225, C228, C231	-0.02	28.05

Figure 2. BPC of a non-reduced digest of denosumab. Colored shading highlights identified cysteine-containing peptides connected via disulfide bonds. The table lists most abundant expected intra- and interchain disulfide bonds that were identified and confirmed.

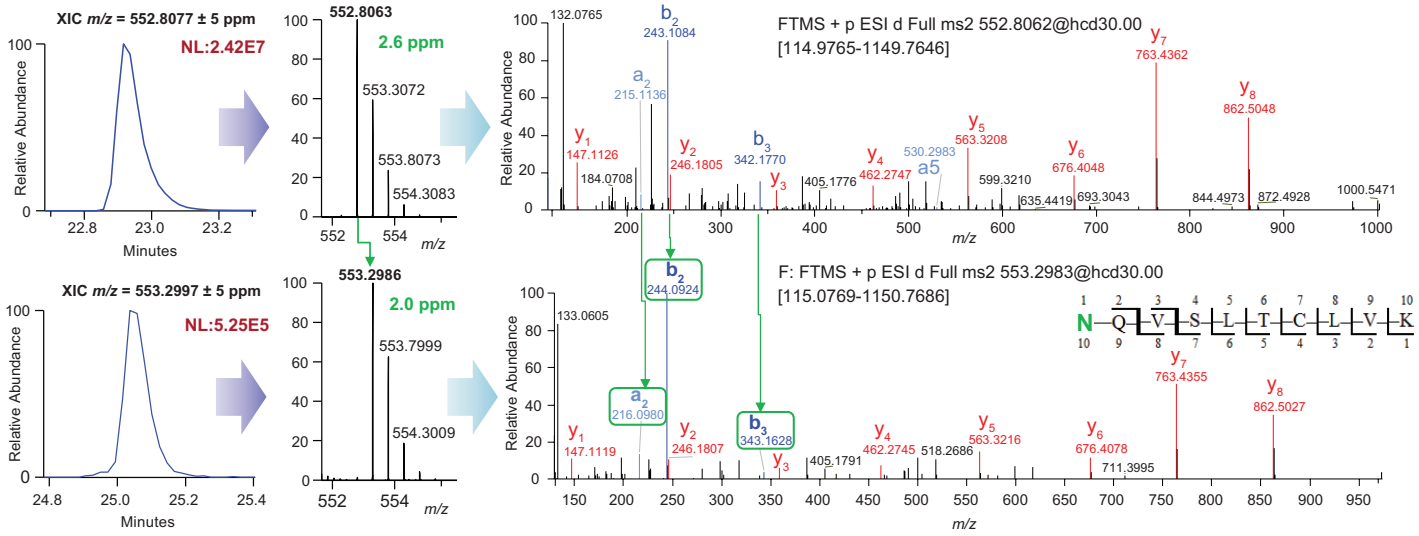


Figure 3. Identification of low level deamidation of N362. Left: Extracted ion chromatograms of *m/z* 552.8077 and 553.2997 represent the doubly charged ions for peptide NQVSLTCLVK in the native (upper) and deamidated (lower) form. Intensities represented by the NL (normalized largest) levels highlight the ~2.5% relative abundance for the peptide containing the deamidation. Middle: Zoom into the full MS spectra highlights the mass difference for the intact precursor ions and their isotope patterns, demonstrating the excellent mass accuracy. Right: MS/MS spectra for both forms of the peptide support the unambiguous identification.

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Conclusion

The Orbitrap Exploris 240 mass spectrometer delivers confident tracking of low-level PTMs together with the detection and identification of intra- and interchain disulfide bonds in mAbs. The system delivers operational simplicity and walk-up performance without the requirement for complex setup and tuning procedures.

Acknowledgements

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Find out more at **thermofisher.com/OrbitrapExploris240**

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