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Comprehensive Characterization of Monoclonal Antibody and Antibody Drug Conjugate on a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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ABSTRACT

Purpose: Evaluate the performance of the new Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer equipped with BioPharma option for complete characterization of monoclonal antibodies and antibody-drug conjugates

Methods: (1) Intact mass analysis under native and denaturing conditions using SEC LC-MS and RP LC-MS, (2) Top-down and middle-down analysis using RP LC-MS/MS, and (3) Peptide mapping using RP LC-MS/MS

Results: Demonstrated excellent performance of the new Orbitrap Exploris 480 mass spectrometer for complete characterization of antibodies

INTRODUCTION

The complexity of modern therapeutic proteins, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) presents a great analytical challenge. MS approaches such as peptide mapping, top-down/middle-down and intact mass analysis are critical during development and production as those can provide complementary information needed for in-depth insight into the structure and composition of biopharmaceuticals. Native MS analysis allows probing molecules while preserving critical structural features, such as cysteine-linked ADCs and produces decreased charge states thus improves spatial resolution. Here we demonstrate the capabilities of the new mass spectrometer to fully characterize therapeutic proteins across a wide range of analysis, from peptide mapping to native intact mass analysis on a single platform.

MATERIALS AND METHODS

Sample Preparation

Herceptin® (trastuzumab) IgG mAb (Genentech), Sigma SILu™ mAb universal standard and SigmaMAb Antibody Drug Conjugate Mimic (Lot # SLCC0520) were used as standard samples. For native intact mass analysis using SEC LC-MS, the antibodies and ADC samples were injected without any further dilution. For subunit analysis, samples were either reduced in 4M GgHCl/50mM TCEP or first digested with FabRICATOR (Genovis) enzyme according to the manufacturer's protocol and then reduced. For peptide analysis, antibodies were proteolytically digested using the SMART Digest kit following reduction with 10mM DTT.

Test Method(s)

Intact denatured and native mass measurements of the intact antibodies and ADC were performed using either SEC LC-MS or RP LC-MS. The top-down, middle-down, and peptide mapping experiments were performed using RP LC-MS/MS. All measurements were carried out on an Orbitrap Exploris 480 mass spectrometer with BioPharma option.

Data Analysis

Data analysis was performed using Thermo Scientific™ BioPharma Finder™ 3.2 software.

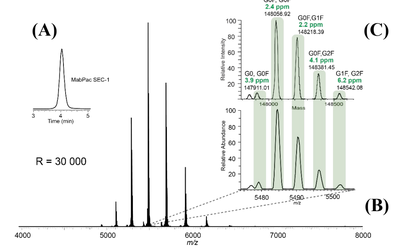
Figure 1. TNG templates and method editor. The Thermo Scientific Orbitrap Exploris 480 MS BioPharma method editor is intuitive and features a drag-and-drop user-friendly interface with a library of pre-defined templates for standard methods and easy instrument setup, enabling the analyst to focus on the science rather than the instrument setup.

RESULTS

Intact Mass Analysis of Herceptin Under Native Conditions

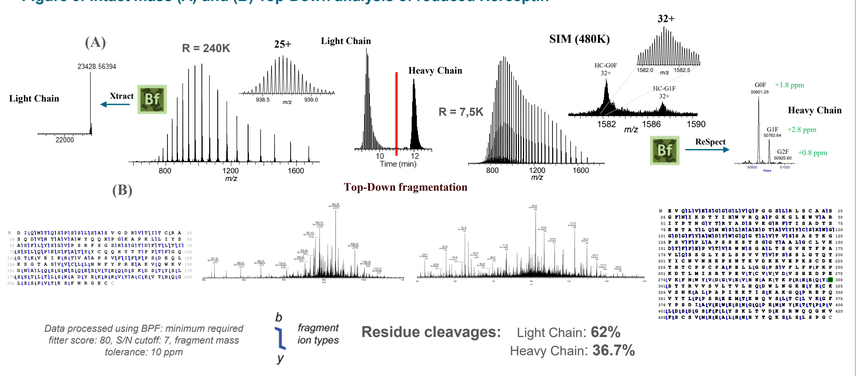
Full MS spectra of intact Herceptin monoclonal antibody were acquired using SEC LC-MS see Figure 2). Orbitrap Exploris 480 provides accurate intact mAb mass analysis under native conditions using ReSpect deconvolution.

Figure 2. (A) Single scan (10 microscans) FTMS broadband mass spectrum of intact monoclonal antibody trastuzumab (IgG1) under native conditions, acquired at 30,000 resolving power (at *m/z* 200). (B) Expanded view of most abundant charge state (z=26) with baseline resolved glycoform pattern. (C) Deconvolution spectrum obtained from BioPharma Finder 4.0 using Sliding Window ReSpect algorithm providing average mass accuracy below 7 ppm for all trastuzumab variants.



Top-down Analysis of Reduced Herceptin

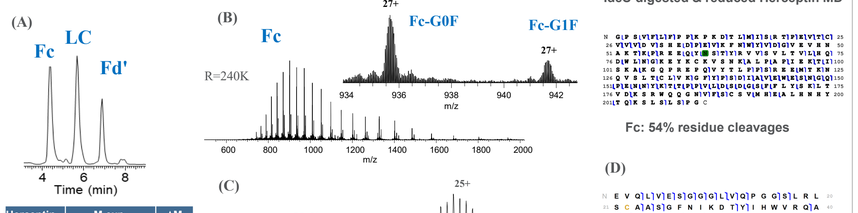
Figure 3: Intact mass (A) and (B) Top-Down analysis of reduced Herceptin



Middle-down Analysis of Ides-digested Reduced Herceptin

Herceptin monoclonal antibody was analyzed after FabRICATOR® digestion followed by reduction. The resulted 23-25kDa subunits were analyzed to produce baseline resolved MS spectra with a resolution setting of 240,000 (at *m/z* 200). For subunit fragmentation experiments the 100 Th isolation window was centered at *m/z* 900.

Figure 4: RP-LC separation (A) Intact mass analysis (B-C) and Middle-Down analysis of Herceptin Subunits
LC separation **(A)** and Full MS spectra **(B, C)** at 240k resolution for the Herceptin Fc, and Fd' subunits. Very accurate intact masses for the Fc, LC and Fd' subunits are obtained (see table) and middle-down (MD) analysis thereof provides very good sequence coverage for all subunits (MD cleavage map for Fc and Fd' **(D)**).



Herceptin subunits	M exp. (monoisot.)	ΔM (ppm)
Fc - G0F	25,220.50382	+1.6
Fc - G1F	25,382.53286	+0.7
LC	23,428.56981	+2.0
Fd'	25,367.54613	+1.1

Herceptin Characterization at peptide level

Herceptin peptide mixture obtained after performing a Thermo Scientific™ SMART Digest™ was analyzed using RP LC-MS/MS and resulted in 100% sequence coverage as shown in Figure 5.

Figure 5: Herceptin peptide mapping results showing base peak chromatograms and sequence coverage

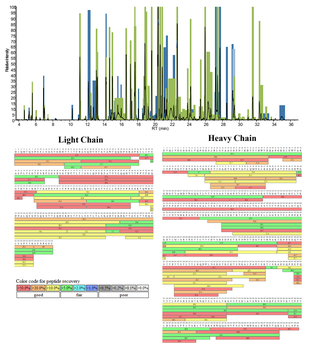
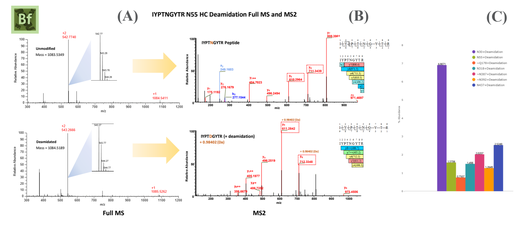


Figure 6 : MS and MS/MS results for IYPTNGYTR N55 Heavy chain peptide of Trastuzumab with and without deamidation. MS and zoomed main mass peak **(A)** and fragmentation for the IYPTNGYTR peptide in the heavy chain with and without deamidation **(B)**. Deamidation assessment in Biopharma Finder modification plot **(C)** showing the % abundance for asparagine (N) and glutamine (Q) deamidation observed in the light and heavy chains of trastuzumab.



Native Intact Mass Analysis of SIGMA Silu mAb And SIGMA ADC Mimic

Figure 7: Analysis of Silu mAb and ADC under native conditions allowing DAR determination
Native size exclusion chromatography (SEC) coupled to mass spectrometry for the analysis of the SigmaMAb Antibody (1) and its Drug Conjugate Mimic (2). **(A1-A2)** Corresponding base peak chromatograms. **(B1-B2)** Orbitrap full MS spectra acquired on Orbitrap Exploris 480 with BioPharma Option at R=60,000 (at *m/z* 200). **(C)** A detailed view of the 27+ charge state for ADC mimic. **(D1-D2)** ReSpect deconvolution results for mAb **(D1),** and more complex results for ADC **(D2)** showing a distribution of 0-8 dansyl-cadaverine SMCC linker-drug attachments.

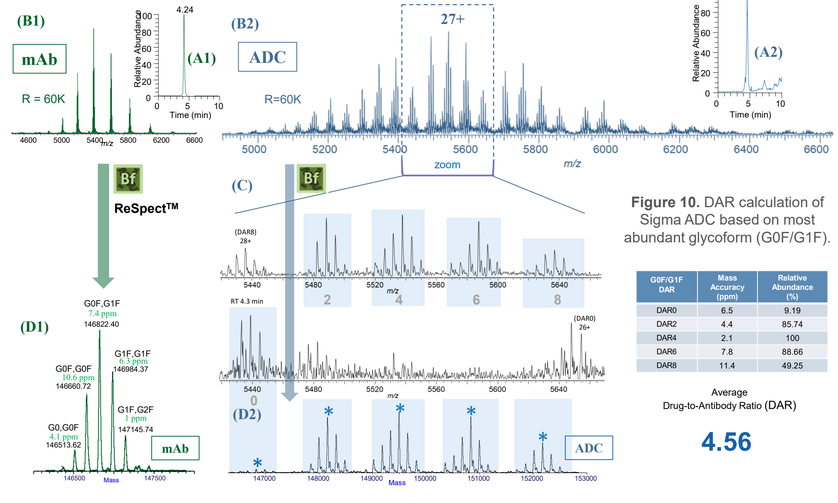


Figure 10. DAR calculation of Sigma ADC based on most abundant glycoform (G0F/G1F).

G0F/G1F DAR	Mass Accuracy (ppm)	Relative Abundance (%)
DAR0	6.5	9.19
DAR2	4.4	85.74
DAR4	2.1	100
DAR6	7.8	88.66
DAR8	11.4	49.25

Average Drug-to-Antibody Ratio (DAR)

4.56

CONCLUSIONS

- In this work we demonstrated outstanding performance of the Orbitrap Exploris 480 mass spectrometer for complete antibody characterization from intact and subunit to peptide level.

TRADEMARKS/LICENSING

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