

Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - IdeZ Digested Monoclonal Antibody

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Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called “Native MS” is especially useful for monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the analysis of an IdeZ digested mAb. IdeZ is a cysteine protease which cleaves IgGs below the hinge region, yielding a Fab'2 and two Fc/2 fragments. This allows for specific identification of any post-translational modifications (PTMs) specific to the hypervariable region, as well as higher fidelity for Fc PTMs, including glycosylation.

Figure 1 shows the TIC for IdeZ digested trastuzumab. Good chromatographic separation was achieved for both Fab'2 and Fc/2, allowing for easier data interpretation for each respective fragment. **Figure 2** shows the Fab'2, which shows a glycosylation associated to the Fab region of the antibody.

LC Conditions

Column: Biozen™ 1.8 μm dSEC-2, 200 Å
Dimensions: 150 x 2.1 mm
Part No.: [00F-4787-AN](#)
Mobile Phase: 100 mM Ammonium Acetate
Flow Rate: 90 μL/min
Injection Volume: 2.5 μL
Temperature: 25 °C
Detection: HRMS
Sample: Trastuzumab, IdeZ digested, 10 mg/mL

Figure 3 shows the deconvoluted spectrum for Fc/2, identifying glycoforms commonly associated with trastuzumab; namely core-fucosylated, biantennary neutral glycans. Lysine variants are also observed.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data (including confirming primary sequence and glycoforms) and, when coupled with site specific proteases, can identify many post-translational modifications. This also shows a good proof of concept that smaller mAb formats are amenable to this workflow.

Figure 1. Total ion chromatogram (TIC) for IdeZ Digested Trastuzumab

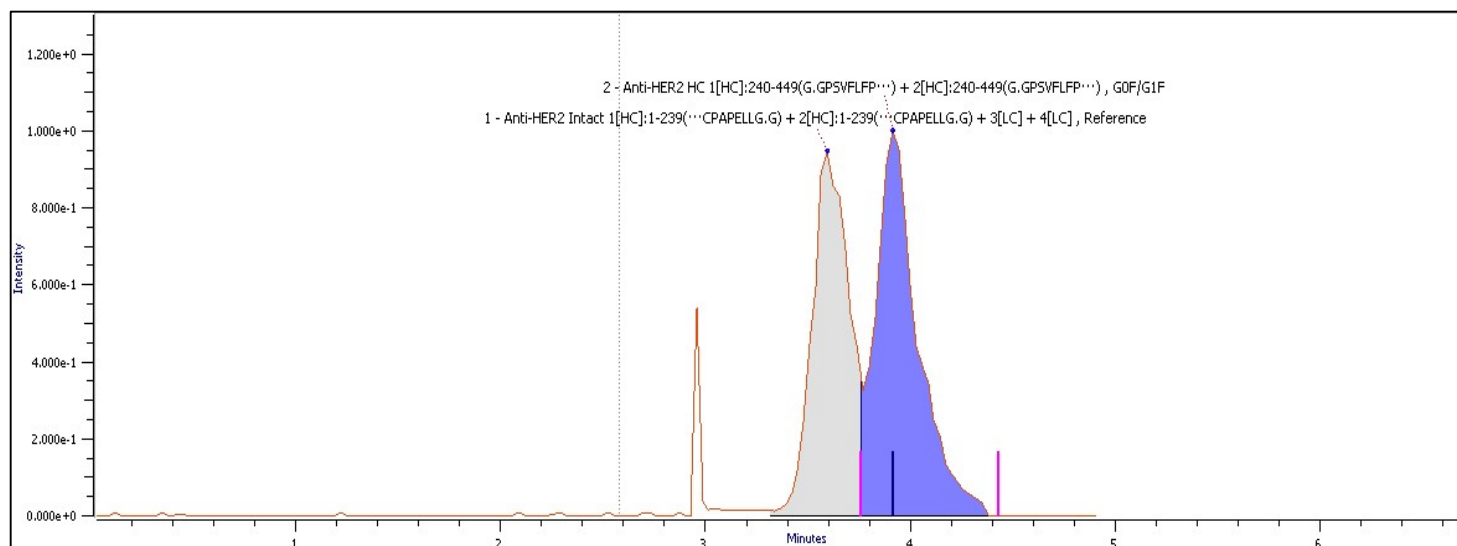


Figure 2. Deconvoluted Spectrum, Fab'2- Trastuzumab

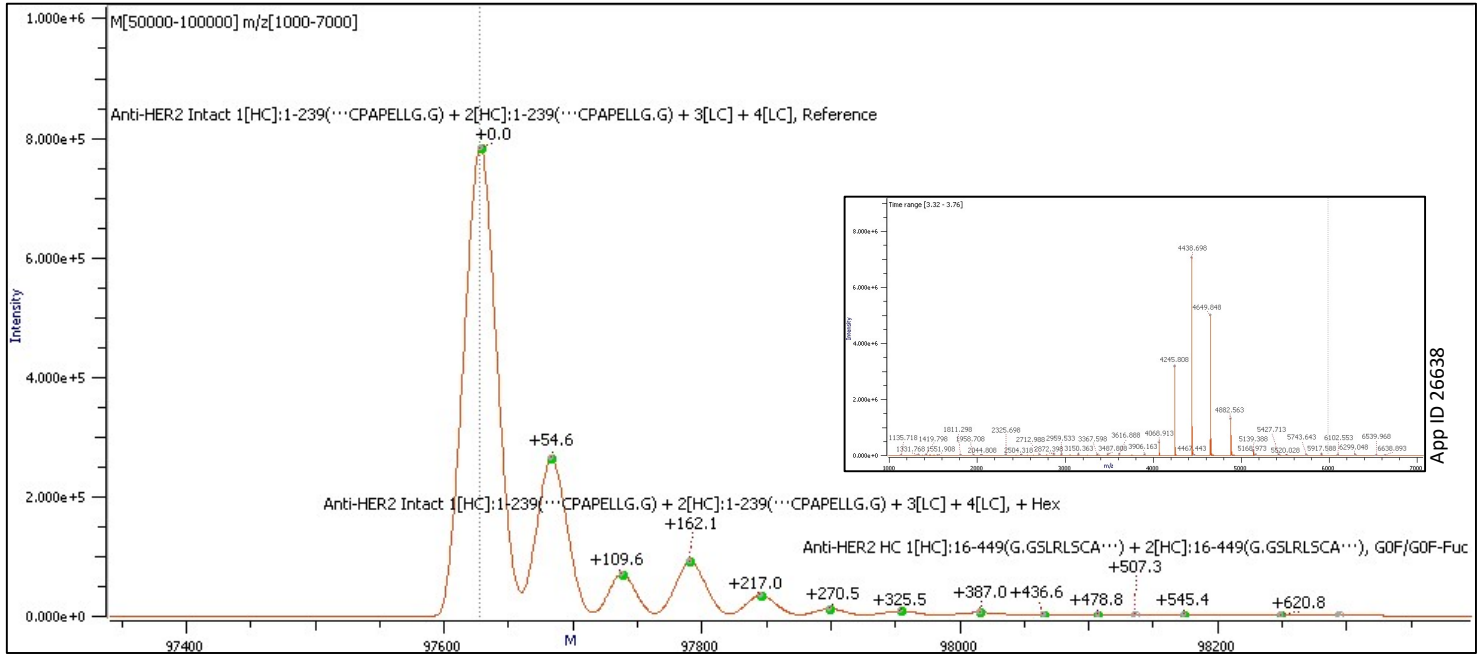
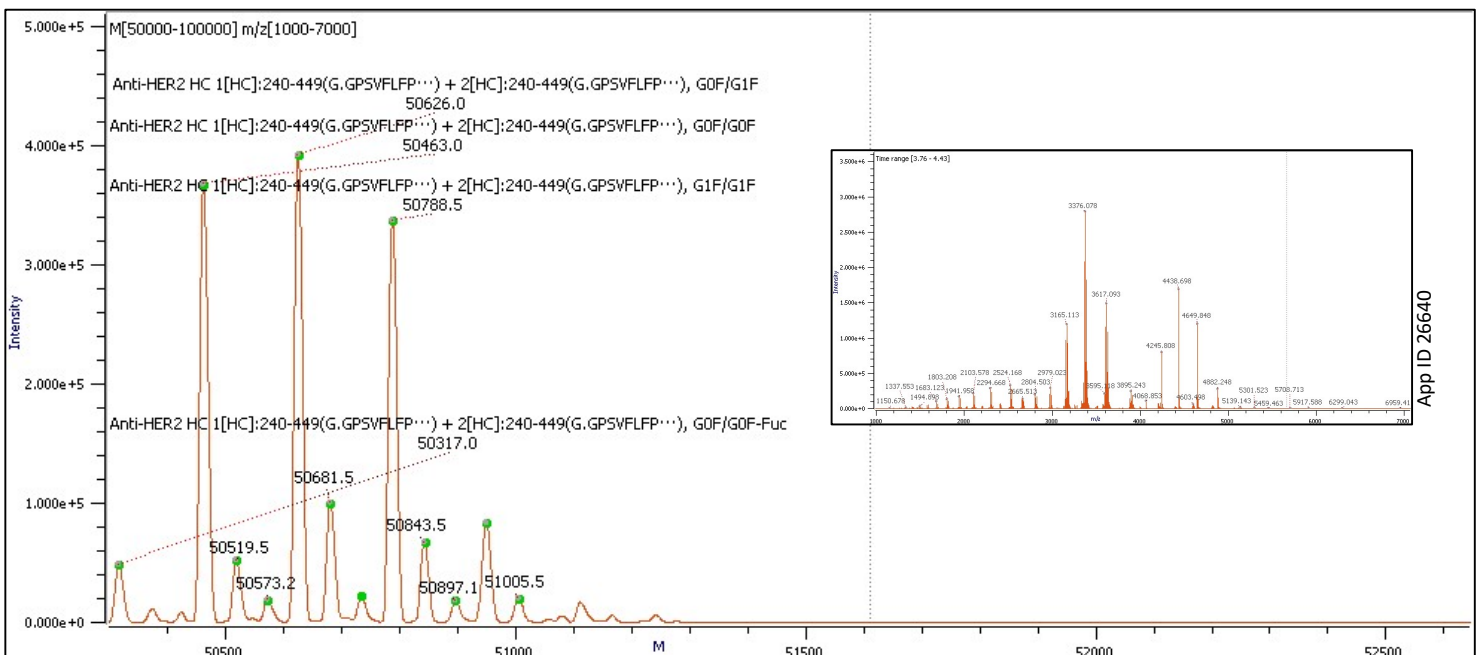


Figure 3. Deconvoluted Spectrum, Fc/2- Trastuzumab



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