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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-9030

Multi-Charged Ion Analysis of Intact Antibodies Using a Quadrupole Time-of-Flight Mass Spectrometer

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User Benefits

- Analysis of intact antibodies can be performed using the LCMS-9030 capable of acquiring accurate mass.
- The ReSpect algorithm installed in LabSolutions Insight Explore™ CSD enables multi-charged ion analysis (charge deconvolution) of macromolecular compounds such as proteins.
- The reconstructed zero charge spectrum of glycoform patterns of monoclonal antibodies can be detected with this workflow.

■ Introduction

In recent years, antibody drugs such as monoclonal antibodies and antibody drug conjugates have been actively researched and developed because of their high specificity for target molecules and fewer side effects. Unlike low molecular drugs, which can be mass-produced by chemical synthesis, antibody drugs are produced using animal cells. Therefore, it is difficult to prevent structural heterogeneity, and characterization is indispensable in the research and development of antibody drugs. Characterization items by mass spectrometry include molecular weight confirmation and glycosylation analysis of intact antibodies.

In this article, the analysis of trastuzumab, a monoclonal antibody drug, using the quadrupole time-of-flight mass spectrometer LCMS-9030 (Fig. 1) is introduced as an example. Multi-charged ion analysis (charge deconvolution) was performed on the obtained mass spectrum using the analysis software LabSolutions Insight Explore CSD.

Fig. 1 Exterior of LCMS-9030

■ Sample Preparation

Trastuzumab purified in a protein A column was used for this analysis. After column purification, the sample was diluted with 0.1 % formic acid-water to a concentration of 0.5 µg/µL.

■ Analysis of Trastuzumab

The HPLC and MS conditions are shown in Table 1. Using a diverter valve, the sample was introduced to the waste side at the measurement time of 0 to 4.9 minutes, and to the mass spectrometer at the measurement time of 5 to 15 minutes.

A mass chromatogram of trastuzumab is shown in Fig. 2. A peak was detected at a retention time of approximately 8.2 minutes.

Table 1 Analytical Conditions

UHPLC (Nexera™ X3 system)	
Column:	Triart Bio C4 (2.1 mm I.D. x 150 mm L., S-3 µm, 300 Å, YMC)
Mobile Phase A: Mobile Phase B:	0.1 % Formic acid - Water 0.1 % Formic acid - Acetonitrile
Gradient Program:	B Conc. 0 % (0-5 min) – 70 % (9 min) – 95 % (9.5-10.5 min) – 0 % (10.51 -15 min)
Flowrate:	0.4 mL/min
Column Temp.:	50 °C
Injection Volume:	1 µL

MS (LCMS-9030)	
Ionization:	ESI positive
Nebulizing Gas Flow:	3.0 L/min
Drying gas Flow:	10.0 L/min
Heating gas Flow:	10.0 L/min
Interface Temp.:	300 °C
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
MS Scan Range:	*m/z* 1000-4000

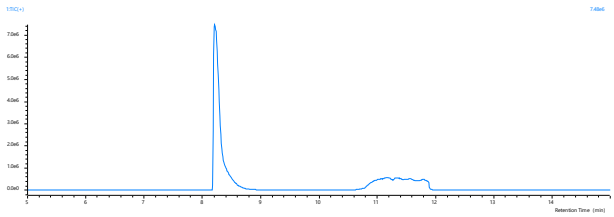

Fig. 2 Total Ion Current Chromatogram of Trastuzumab

■ Multi-Charged Ion Analysis

Multi-charged ions are distributed in the mass spectrum of macromolecular compounds such as proteins. The ReSpect algorithm (Positive Probability Ltd.) installed in LabSolutions Insight Explore CSD can analyze the mass of each molecule from the mass spectrum in which multi-charged ions are distributed.

Fig. 3 shows the result of multi-charged ion analysis of trastuzumab (molecular weight: approximately 148,000). In the result view, the raw data of the mass spectrum to be analyzed is displayed at the top (Fig. 3-A), and the reconstructed zero charge spectrum obtained as a result is displayed below it (Fig. 3-B). The obtained peaks in the reconstructed zero charge spectrum are listed in the lower left (Fig. 3-C), and the mass spectrum peak information corresponding to the row selected in this list is displayed in the lower right (Fig. 3-D) as a list.

In this result, five peaks were obtained in the reconstructed zero charge spectrum (Fig. 3-B), corresponding to the pattern of peaks in the mass spectrum before analysis (Fig. 3-E, enlarged view of Fig. 3-A). The numbers in blue (Fig. 3-B) indicate the mass difference between the peaks, which are considered to be the peaks of fucose (average molecular weight: 146) and galactose (average molecular weight: 162).

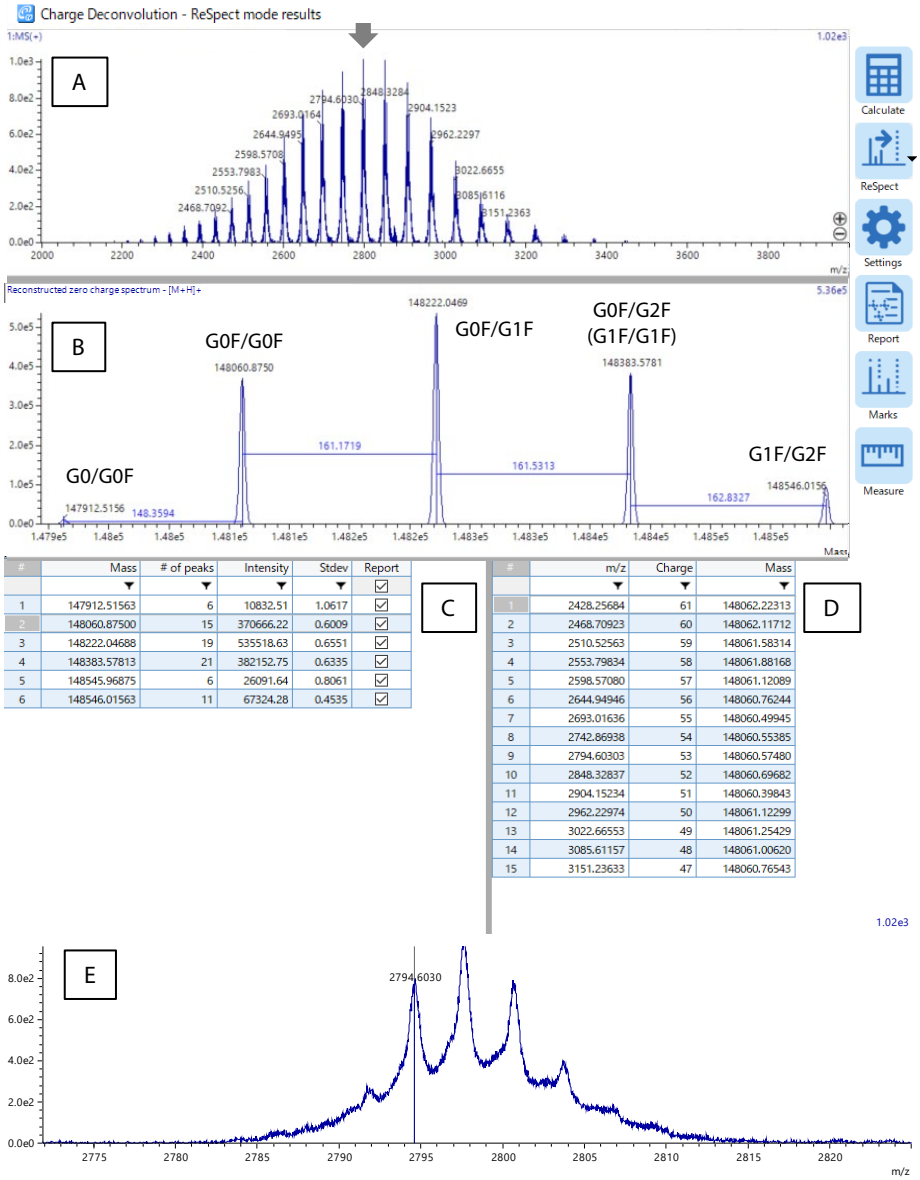

Table 3 Result of Multi-charged Ion Analysis for Trastuzumab
A: raw mass spectrum before analysis, B: reconstructed zero charge spectrum, C: list of the multivalent ion analysis spectrum,
D: list of the mass spectrum peaks corresponding to the row selected in the list (C), E: enlargement of the most abundant peak, indicated with the gray arrow in (A)

■ Conclusion

Intact analysis of trastuzumab was performed by the quadrupole time-of-flight mass spectrometer LCMS-9030. The reconstructed zero charge spectrum of glycoform patterns can be detected as a result of multivalent ion analysis by the ReSpect algorithm installed in LabSolutions Insight Explore CSD. This method is applicable for the intact analysis of monoclonal antibodies and antibody drug conjugates.

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