Application News

High-Performance Liquid Chromatograph Mass Spectrometer

Confirmation of Intact Protein Molecular Weight Using a Single Quadrupole Mass Spectrometer

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User Benefits

- The molecular weight of intact proteins can be confirmed by using the simple handling LCMS-2050 single quadrupole mass spectrometer.
- By using LabSolutions Insight™ Biologics, the results of multiply-charged ion analyses can be understood easily by a simple visual comparison.

■ Introduction

Biopharmaceuticals are a class of pharmaceuticals produced using biotechnologies such as genetic modification and cell culture. The proteins that serve as the active components of biopharmaceuticals are macromolecular compounds with multiple ionization sites. When multiply-charged ions are detected, their molecular weights can be estimated by analysis processing (deconvolution) on an appropriate software program. More accurate estimation of molecular weight is possible by using a high-resolution mass spectrometer. However, when the main components can be assumed in advance, for example, in quality control applications, the molecular weight can be confirmed by using a simple handling single quadrupole mass spectrometer.

This article introduces an example of analysis of proteins using a single quadrupole mass spectrometer for synthesis confirmation of the macromolecular compounds.

Fig. 1 Nexera™, LCMS-2050 System

■ Samples

Table 1 shows the proteins that were measured as model samples. These commercially-available standard samples were dissolved and diluted with ultrapure water for use as the analysis samples.

Table 1 List of Measured Compounds

#	Compounds
1	Myoglobin from equine skeletal muscle
2	Carbonic anhydrase from bovine red blood cells
3	*β*-casein from bovine milk

■ Analysis Conditions

The measuring instruments used in this experiment were a Nexera X3 high-performance liquid chromatograph and an LCMS-2050 single quadrupole mass spectrometer. Table 2 and Table 3 show the analysis conditions used with the two instruments, respectively.

Table 2 Liquid Chromatograph (LC) Analysis Conditions

System	: Nexera X3
Column	: Shim-pack Scepter™ C4-300 *1 (50 mm × 2.1 mm I.D., 3.0 µm)
Temperature	: 40 °C
Injection volume	: 5 µL
Mobile phases	: 0.1 % formic acid in Water 0.1 % formic acid in Acetonitrile
Flow rate	: 0.4 ml/min
Time program (%B)	: 5 % (0.00 min)→60 % (5.00-15.00 min)→ 5 % (15.01-18.00 min)

*1 P/N: 227-31177-03

Table 3 Mass Spectrometer (MS) Analysis Conditions

System	: LCMS-2050
Ionization	: ESI/APCI (DUIS), Positive mode
Interface voltage	: 3.0 kV
Mode	: Scan *m/z* 500 - 2000
Nebulizing gas flow	: 2 L/min
Drying gas flow	: 5 L/min
Heating gas flow	: 7 L/min
Desolvation temp.	: 450 °C
DL temp.	: 200 °C

■ Deconvolution Results

Fig. 2 and Fig. 3 show the mass spectra of the detected peaks and the multiply-charged ion analysis mass spectra obtained by deconvolution of the myoglobin and bovine carbonic anhydrase samples, respectively. LabSolutions Insight Biologics includes the multiply-charged ion analysis algorithm "ReSpect" (Positive Probability Limited), which is useful when estimating the molecular weights of proteins with large molecular weights by multiply-charged ion analysis.

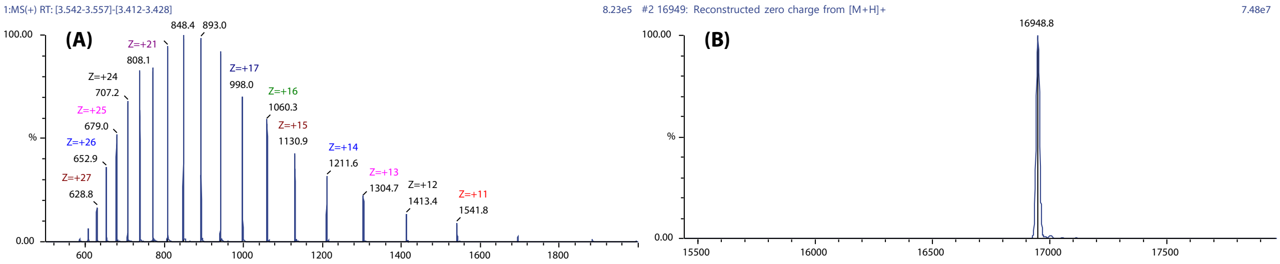


Fig. 2 (A) Mass Spectrum and (B) Multiply-Charged Ion Analysis Mass Spectrum of Myoglobin

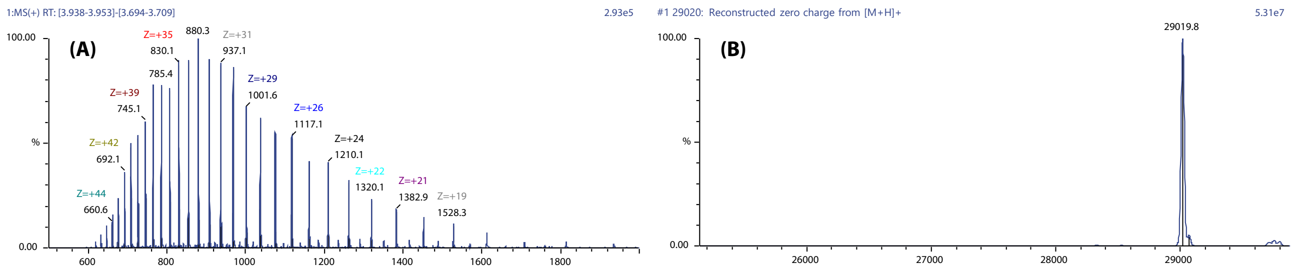
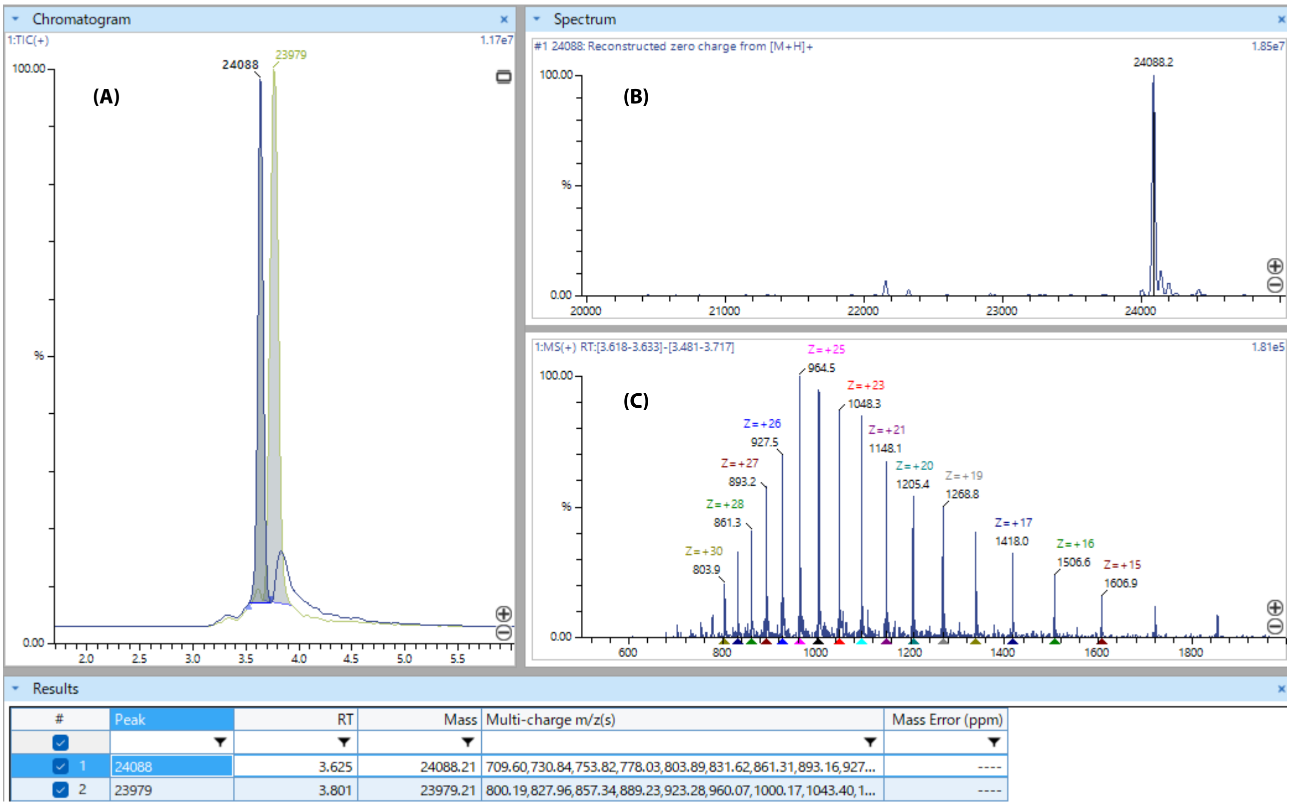


Fig. 3 (A) Mass Spectrum and (B) Multiply-Charged Ion Analysis Mass Spectrum of Bovine Carbonic Anhydrase



#	Peak	RT	Mass	Multi-charge m/z(s)	Mass Error (ppm)
☑					
☑ 1	24088	3.625	24088.21	709.60,730.84,753.82,778.03,803.89,831.62,861.31,893.16,927...	----
☑ 2	23979	3.801	23979.21	800.19,827.96,857.34,889.23,923.28,960.07,1000.17,1043.40,1...	----

Fig. 4 Results of Multiply-Charged Ion Analysis
(A) Overlay of Component Chromatograms, (B) Multiply-Charged Ion Analysis Mass Spectrum, (C) Mass Spectrum

Fig. 4 shows the analysis screen of β-casein by Insight Explore Biologics. The peaks detected from the chromatogram, the mass spectrum of the obtained peaks, and the mass spectrum after multiply-charged ion analysis can be observed on a single screen.

LabSolutions Insight Biologics can generate component chromatograms combining all different valences and isotope spectra. Since it is possible to overlay the component chromatograms and display the molecular weights calculated by deconvolution as peak tops, the analysis results can be understood easily by visual comparison when multiple components are assumed to exist or impurities are detected.

For example, in this experiment, two peaks were detected from β-casein, and from the overlay of the component chromatograms, it can be understood that the estimated molecular weights of the respective peaks are 24088 and 23979.

■ Conclusion

Simple conformation of the molecular weights of three intact protein samples was possible by using the LCMS-2050 single quadrupole mass spectrometer and LabSolutions Insight Biologics analysis software.

By using LabSolutions Insight Biologics, analysis results can be understood easily by visual comparison when multiple components are assumed to exist or impurities are detected.

<Related Application News article>

1. Analysis of Oligonucleotide Impurities Using Single Quadrupole Mass Spectrometer, Application News No. 01-00974-EN

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01-01023A-EN
First Edition: Jan. 2026
Revision A: Mar. 2026

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