

Application News

No. C115

Liquid Chromatography Mass Spectrometry

Determination of Molecular Weight of Synthetic Peptides by LC/MS Using Multi-Charged Ion Analysis Software

Many natural poisons of animal origin contain peptides as their principle component, and the structural information of these peptides is utilized not only for investigating the mechanism of toxicity and addiction from the pharmacological point of view, but in basic research over a wide range of life sciences, including neuroscience and psychiatry. And, depending on their pharmacological possibilities, they may be used in the development of new drugs.

A sufficient amount of sample is required to conduct activity measurement and functional analysis for peptide research, and for this, samples may be obtained via extraction from natural products, or depending on the case, they may be synthesized based on structural information. At this point, verification of sample

synthesis and impurity analysis are required to ensure high-purity peptide synthesis. LC/MS, with its excellent sensitivity and qualitative performance, is widely used for peptide synthesis verification and impurity analysis.

Here, Asp-His-Pro-Asn-Pro-Arg (DHPNPR), a synthetic product consisting of the N-terminal partial fragment of the C-type natriuretic peptide (OvCNP) that is present in platypus venom, was measured using the Nexera-i and LMCS-2020. Multi-Charged ion analysis software was then used to conduct molecular weight confirmation and impurity analysis. This Application News introduces the results of the analysis.

Note: The sample used for this analysis was provided by Associate Professor Masaki Kita at the Graduate School of Pure and Applied Sciences, University of Tsukuba.

■ Analysis of the Synthetic Peptide Using LCMS-2020 with Nexera-i

Fig. 1 shows the total ion current chromatogram (TICC), the mass chromatogram, and the mass spectrum of the synthetic peptide (DHPNPR, M.W. 734). The peak attributed to DHPNPR was detected at 1.5 minutes. In the mass spectrum, the ions at m/z 246, 368, and

735 were detected, and attributed to the trivalent, divalent, and monovalent protonated molecules, respectively. In addition, an impurity peak was detected at 0.8 minutes, and in the mass spectrum, the m/z 311 ion was detected.

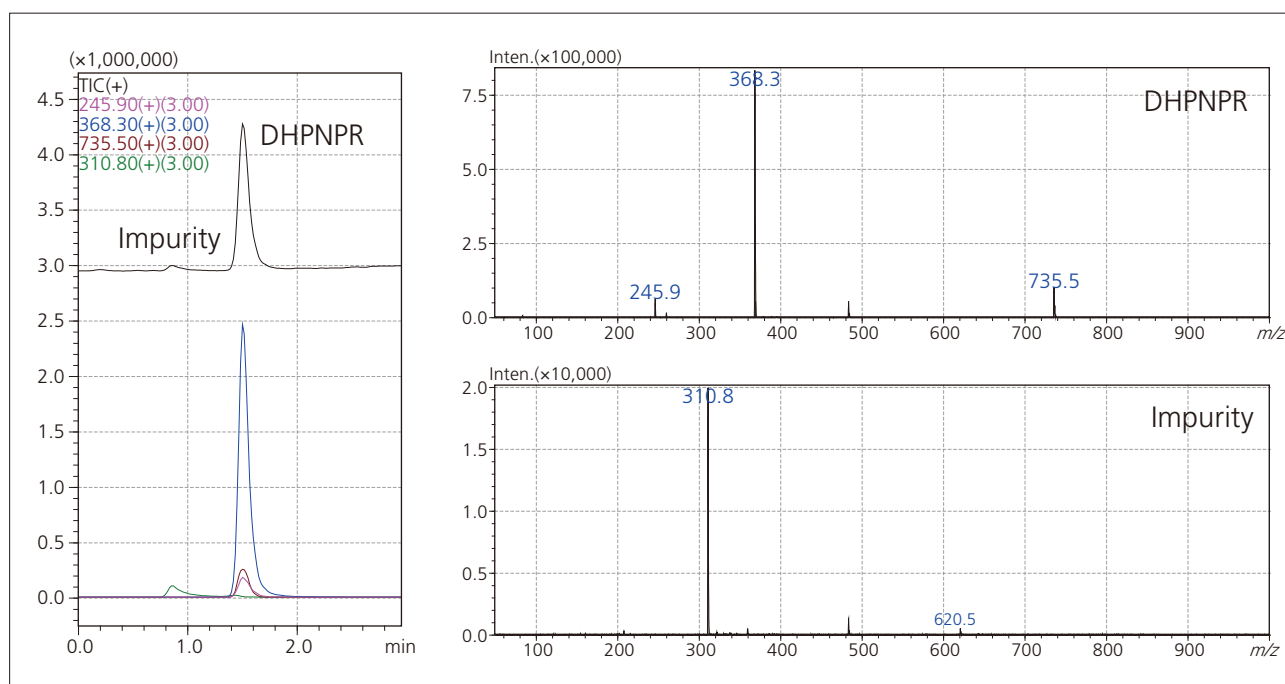


Fig. 1 Total Ion Current Chromatogram (TICC), Mass Chromatograms (left) and Mass Spectra (right) of Synthetic Peptide DHPNPR and Its Principal Impurity

Deconvolution Analysis of Multi-Charged Ions Using Dedicated Software

Fig. 2 shows the molecular weights calculated for the peaks of DHPNPR using Multi-Charged Ion Analysis software. Calculations from the monovalent and

polyvalent ions shown in Fig. 1 confirmed that the peptide molecular weight is 734. The deconvolution spectrum is shown at the lower right in Fig. 2.

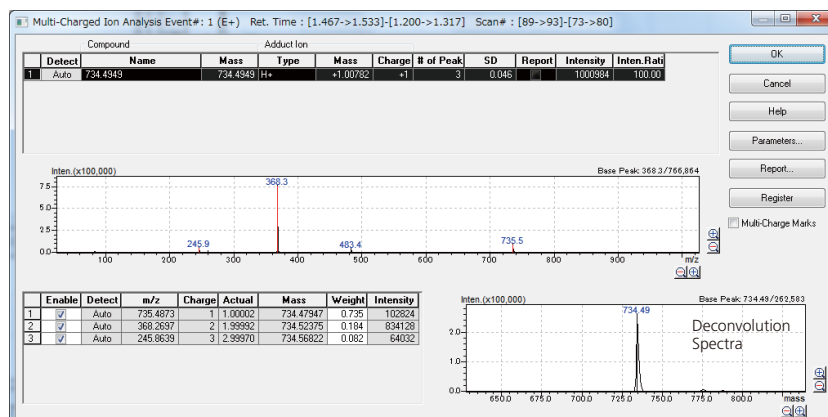


Fig. 2 Result of Deconvolution for Peptide DHPNPR

The molecular weight of the impurity was also calculated, as shown in Fig. 3. In addition to the *m/z* 310 ion, the *m/z* 620 ion was also detected, and these were attributed to divalent and monovalent protonated molecules, respectively, indicating a molecular weight of 619. From the mass difference of 115 with respect to the principal component, the impurity was presumed to be a peptide (His-Pro-Asn-

Pro-Arg) from which the N-terminus ASP had been removed.

Thus, by conducting analysis using the Multi-Charged Ion Analysis software with respect to the data obtained from measurement using the Nexera-i and LCMS-2020, it is possible to obtain useful information for structural verification of synthetic peptides and structural assumptions regarding impurities.

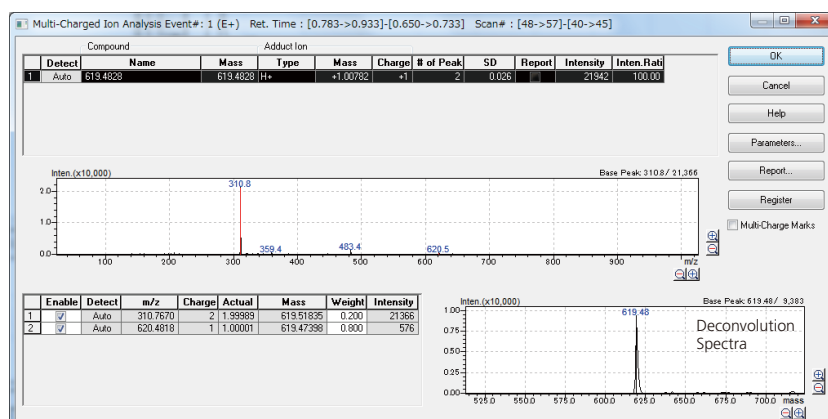


Fig. 3 Result of Deconvolution with Respect to Major Impurity

Table 1 Analytical Conditions

LCMS-2020 with Nexera-i system	
Column	: Shim-pack XR-ODS (30 mm L. × 2.0 mm I.D.)
Mobile Phase A	: Water containing 0.1 % Formic Acid
Mobile Phase B	: Acetonitrile
Gradient Program	: 0 %B (0 min) → 20 %B (2 min) → 0 %B (2.01-3 min)
Flowrate	: 0.5 mL/min
Injection Volume	: 1 µL
Probe Voltage	: 4.5 kV (ESI-Positive mode)
DL Temperature	: 250 °C
BH Temperature	: 400 °C
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Flow	: 15 L/min
Analysis Mode	: Profile
Scan Range	: <i>m/z</i> 50-1000