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Application News

No. C231

Liquid Chromatography Mass Spectrometry

Verification of Quantitativity and Confirmation of Molecular Weight of Oligonucleotide Therapeutics Using LCMS™-8060

Overview

Oligonucleotide therapeutics are synthetic oligonucleotides which demonstrate medical efficacy by bonding with target genes or target proteins that cause various diseases.

This article introduces an example of an analysis of a 20 base 2'-MOE modified oligonucleotide using an LCMS-8060 triple quadrupole mass spectrometer. In preparation of the calibration curve in the MRM mode, linearity was confirmed in the range of 1 to 300 ng/mL, and the molecular weight was confirmed by deconvolution from the multivalent ion mass spectrum obtained in the scan mode.

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Analysis Conditions

Table 1 shows the HPLC and MS analysis conditions. An ion-pair reagent is generally used in reversed phase separation of oligonucleotides, and TEA*1 is often used as an amine-type reagent. Here, HFIP*2 and DIPEA*3 were used in the mobile phase, as these substances enable measurement with higher sensitivity.

Table 1 Analysis Conditions

[HPLC conditions] (Nexera™)	
Column	: C18 ODS Column (50 mm × 2.1 mm I.D., 2.5 µm)
Mobile phases	: A) 50 mmol/L HFIP and 10 mmol/L DIPEA B) Acetonitrile
Gradient program	: B 5 % (0-0.5 min) – 15 % (0.5-3 min)
Flow rate	: 0.2 mL/min
Column temp.	: 60 °C
Injection volume	: 2 µL
[MS conditions] (LCMS-8060)	
Ionization	: ESI (Negative mode)
Probe voltage	: -4 kV
Mode	: Full scan (*m/z* 500 - 2000) MRM (*m/z* 803.5 > 95.0)
CID gas	: 330 kPa
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 8.0 L/min
Heating gas flow	: 12.0 L/min
DL temp.	: 300 °C
Heat block temp.	: 450 °C
Interface temp.	: 250 °C

*1 Triethylamine
*2 1,1,1,3,3,3-Hexafluoro-2-propanol
*3 N,N-diisopropylethylamine

Appearance of LCMS™-8060

Sample

Sequence: 5'-mG-mC*-mC*-mU*-mC*-dA-dG-dT-dC*-dT-dG-dC*-dT-dT-dC*-mG-mC*-mA-mC*-mC*-3'

(m) 2'-O-(2-methoxyethyl) nucleoside (2'-MOE)
(*) Indicates 5-C or 5-U methylation
(d) 2'-deoxynucleoside

Average mass: 6436.39

Calibration Curve

Fig. 1 shows a representative chromatogram acquired in the MRM mode, and Fig. 2 shows the calibration curve. The calibration curve was prepared for the range of 1 to 300 ng/mL. The coefficient of determination (R^2) was 0.998.

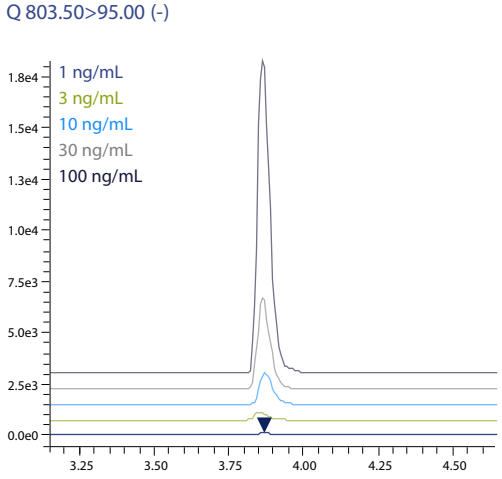

Fig. 1 MRM Chromatogram of Oligonucleotide Therapeutics

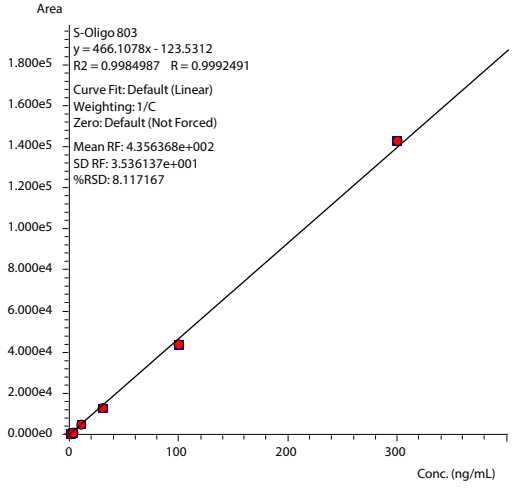

Fig. 2 Calibration Curve

Scan Mode Chromatogram and Mass Spectrum

Fig. 3 shows the mass chromatogram measured in the scan mode. In the mass chromatogram, hexavalent, heptavalent, and octavalent ions were selected.

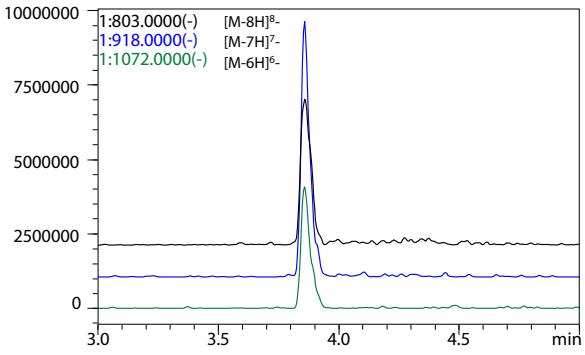

Fig. 3 Mass Chromatogram of Oligonucleotide Therapeutics

Fig. 4 shows the mass spectrum. Pentavalent to octavalent ions were detected.

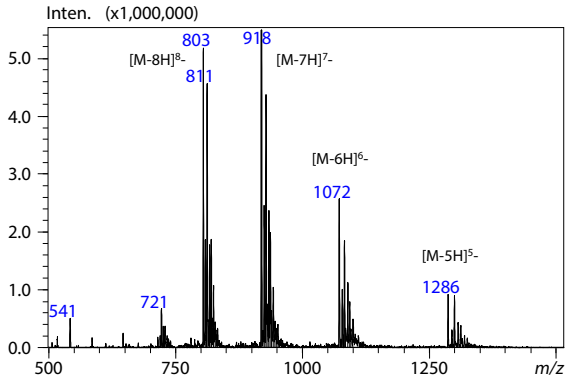

Fig. 4 Mass Spectrum of Oligonucleotide Therapeutics

Deconvolution by Multivalent Ion Analysis Software

Fig. 5 shows the results of a molecular weight calculation using the deconvolution function of the LabSolutions™ LCMS software. The target mass spectrum is displayed on LabSolutions LCMS, and multivalent ion analysis can be conducted simply by selecting the deconvolution menu.

As shown in the deconvolution results, the estimated molecular weight was confirmed to be 6436.37 (theoretical value: 6436.39). Thus, the molecular weight could be confirmed in a small error range.

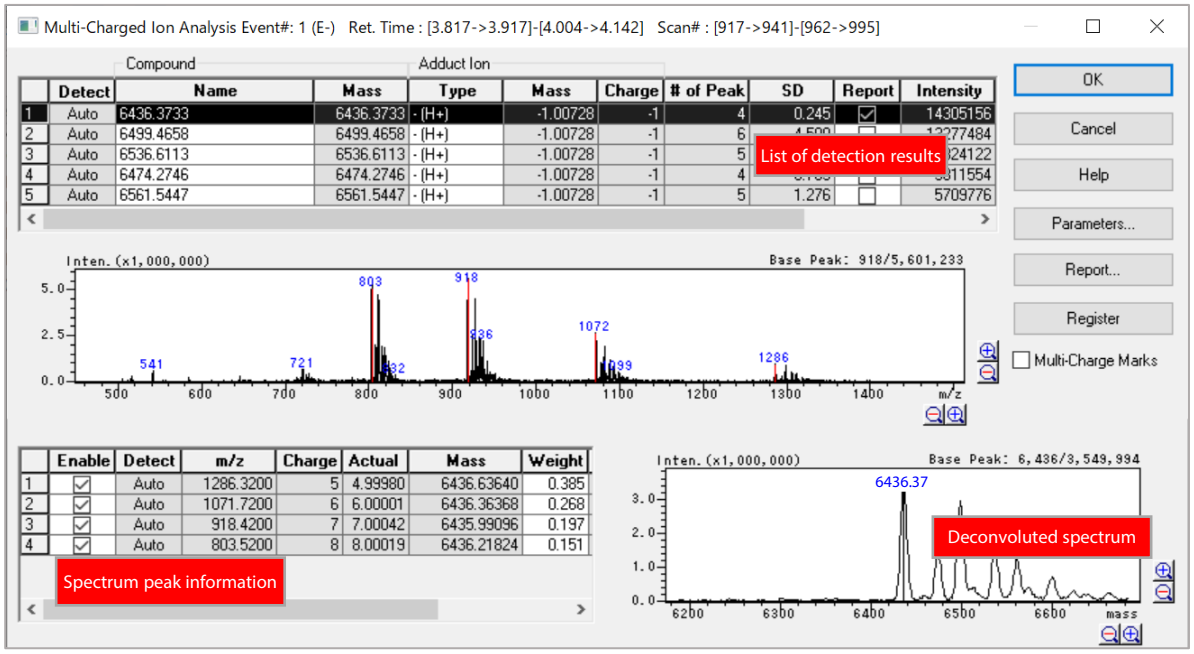

Fig. 5 Results of Multivalent Ion Analysis

Conclusion

An example of an analysis of a 2'-MOE modified oligonucleotide using an LCMS-8060 triple quadrupole mass spectrometer was introduced. A calibration curve for the range of 1 to 300 ng/mL could be obtained, and the molecular weight could be confirmed in a small error range by deconvolution function from the scan data.

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