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Application News

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LC-MS

Determination of Molecular Mass and Quantification of Oligonucleotide Therapeutics Using Quadrupole Time-of-Flight Mass Spectrometer LCMS™-9030

Overview

Oligonucleotide therapeutics are synthetic oligonucleotides that demonstrate their medical efficacy through binding to target genes or target proteins that may be responsible for a range of diseases. To date, eight types of oligonucleotide therapeutics have been approved, many of which have a length of approximately 20 bases.

This article introduces an example of analysis using the Q-TOF mass spectrometer, LCMS-9030. As an oligonucleotide therapeutic, the 2'-MOE modified oligonucleotide having 20 bases was used. Accurate mass spectrometry determined the molecular mass of the therapeutic with an error of 3 mDa (0.05 ppm). When a calibration curve was prepared using the MRM mode on the LCMS-9030 mass spectrometer, linearity was observed within a range of 1 - 1000 ng/mL.

N. Kato

Method Used to Identify Oligonucleotide Therapeutics

Several methods are used to identify the structure of oligonucleotide therapeutics, such as mass spectrometry, NMR and UV spectrum. However, in recent years, the LC-MS/MS method, which has excellent specificity and versatility and can quantify unchanged drugs and metabolites simultaneously, has been attracting attention.

As oligonucleotide therapeutics have 20 bases, indicating that the molecular mass is 6000 or more, a high-performance mass spectrometer, such as a quadrupole time-of-flight (Q-TOF) mass spectrometer, needs to be used to determine the molecular mass. Meanwhile, a triple quadrupole mass spectrometer (TQ-MS), which typically has a wide dynamic range, will be used for quantitative analysis.

Q-TOF mass spectrometer LCMS™-9030

LC-QTOF-MS

LC-QTOF-MS is an LCMS combining a high-performance liquid chromatograph with a quadrupole mass spectrometer and a TOF mass spectrometer (TOF).

Shimadzu's first quadrupole time-of-flight mass spectrometer, the LCMS-9030, inherits the same high-performance and high ion convergence characteristics of the LCMS-8000 series (TQ-LCMS), but incorporates the newly developed TQF technology. It includes Shimadzu's proprietary technology, such as UFgrating™, a high strength fine lattice electrode, iRefTOF™, an ideal reflection, and a highly accurate temperature control system. Data characterized by both high sensitivity and high resolution can be acquired, while maintaining consistently stable mass accuracy.

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)
(*) 5-methylated derivatives of C and U
(d) 2'-deoxyribonucleoside
Monoisotopic mass：6431.7239

Analysis Conditions

The HPLC and MS analysis conditions are shown in Table 1. For reversed-phase separation of oligonucleotides, ion-pairing reagents are typically used, and as amine-type substances of those reagents, TEA*1, for example, is commonly used. In this analysis, HFIP*2 and DIPEA*3 were used as the mobile phase to allow highly sensitive measurement to be performed.

Table 1 1 Analysis Conditions

[HPLC conditions] (Nexera™)	
Column	: Shim-pack Scepter™ C18 (2.0 × 75, 1.9 µm)
Mobile phases	: A) 50 mmol/L HFIP and 10 mmol/L DIPEA B) Acetonitrile
Gradient Program	B 5 % (0-0.5 min) – 15 % (0.5-6 min)
Flow rate	: 0.2 mL/min
Column Temp.	: 50 °C
Injection volume	: 5 µL
[MS conditions] (LCMS-9030)	
Ionization	: ESI (Negative mode)
Probe Voltage	: -3 kV
Mode	: Full scan (m/z 500 – 3000) MRM（803.4626 > 94.9358）
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL Temp.	: 250 °C
Heat Block Temp.	: 400 °C
Interface Temp.	: 350 °C

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

Deconvolution Using Multi-Charged Ion Analysis Software

Figure 1 shows the mass spectra extracted from the scan mode data. In the mass spectra, multi-charged ions such as those at m/z 1071.6, 918.4 and 803.5 were detected. The spectra of these multi-charged ions were deconvoluted using the "ReSpect" algorithm, an option available in the LabSolutions Insight Explore™ software.

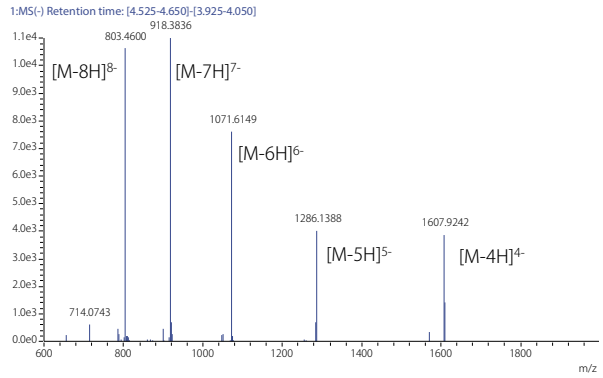

Fig. 1 Mass Spectra of Oligonucleotide Therapeutics

Figure 2 shows the results of molecular mass calculation. As shown in the deconvolution spectra, the monoisotopic mass was 6431.72 with a mass error of 3 mDa (0.05 ppm).

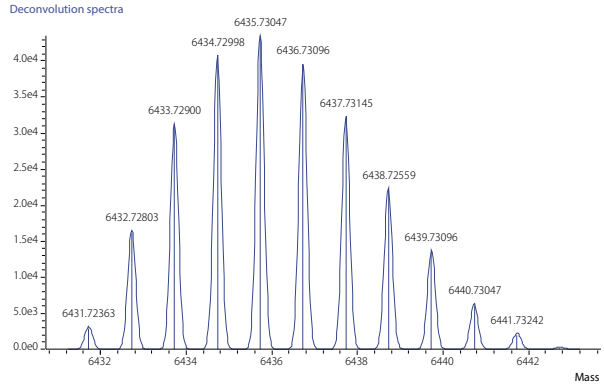

Fig. 2 Deconvolution spectra

Analysis of Reference Standards

As is the case with triple quadrupole devices, the LCMS-9030 mass spectrometer can perform highly sensitive quantification by using the multiple reaction monitoring (MRM) mode. The octavalent ion at m/z 803.4626 was selected as a precursor ion. The product ion at m/z 94.9358 (PSO2-) was used as a monitor ion. Figure 3 shows representative chromatograms obtained using the MRM mode.

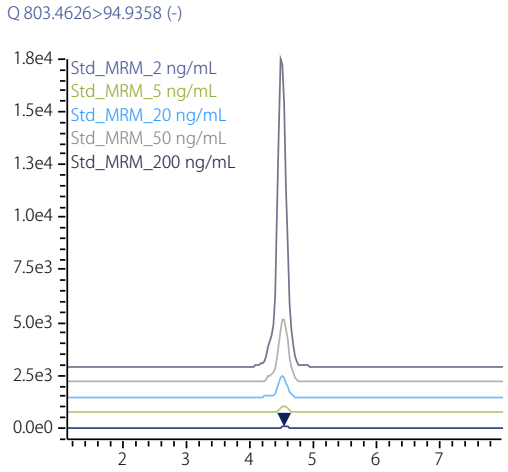

Fig. 3 MRM Chromatograms of Oligonucleotide Therapeutics

Calibration curve

The calibration curve is shown in Figure 4. The calibration curve was prepared within a range of 1 - 1000 ng/mL. The contribution ratio (R^2) was 0.996.

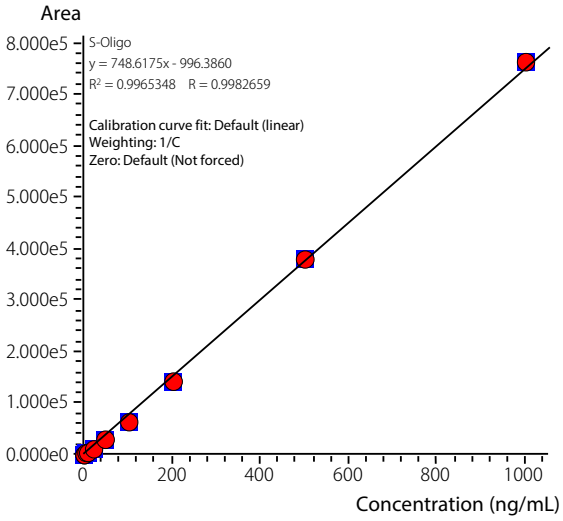

Fig. 4 Calibration Curve

Conclusion

Accurate mass spectrometry using the Q-TOF mass spectrometer LCMS-9030 determined the molecular mass with an error of 0.05 ppm. Additionally, linearity was observed within a range of 1 - 1000 ng/mL.

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