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LCMS-2050 Liquid Chromatograph Mass Spectrometer

Application News

Qualitative Analysis of Synthetic DNA Using a Single Quadrupole Mass Spectrometer

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User Benefits

- Oligonucleotides can be easily analyzed using a Nexera™ XS inert UHPLC system and an LCMS-2050 single quadrupole mass spectrometer.
- The molecular weight of oligonucleotides can be estimated by deconvoluting the obtained mass spectra.

■ Introduction

Oligonucleotide therapeutics have attracted attention in recent years as a new modality for drug discovery. Typically, they are produced by chemical synthesis, so to ensure product quality it is important to confirm that the synthesized oligonucleotides have the expected base sequence. Mass spectrometry, which provides molecular weight information, is a valuable analytical tool in such cases. This article describes, using an inert UHPLC system and an LCMS-2050 single quadrupole mass spectrometer (Fig. 1) with user-friendly operability similar to LC systems to analyze the mass of oligonucleotides within a 1 Da margin of error from theoretical values.

Fig. 1 Nexera™ XS inert and LCMS-2050 Systems

■ Samples

10 pmol of a 70-mer synthetic single-stranded DNA (sequence: GGTGTCAGGCTCACGGACCACTGCACAACAATCCCACGACGTCGC CATTTTCTGCGATCCGGCAAGGCGA) was analyzed.

■ Analysis Conditions

The analysis conditions are shown in Table 1. A Nexera XS inert UHPLC system with a Shim-pack Scepter™ Claris C18-120 inert column was used to reduce sample adsorption. The Shim-pack Scepter Claris column is an inert column with a Scepter series stationary phase packed in a newly developed column body with a bio-inert coating. For mass spectrometry, an LCMS-2050 single quadrupole mass spectrometer was used. The LCMS-2050 is equipped with a heated DUIS™ ion source for ionization, which combines the advantages of both ESI and APCI sources. It covers a mass range of *m/z* 2 to 2,000, making it suitable for analyzing oligonucleotide therapeutics with a high molecular weight (MW).

Table 1 Analysis Conditions

HPLC Conditions (Nexera XS inert)	
Column:	Shim-pack Scepter Claris C18-120*1 (100 mm × 2.1 mm I.D., 1.9 µm)
Flow Rate:	0.3 mL/min
Mobile Phase A:	95.4 mM HFIP and 7.1 mM TEA in water
Mobile Phase B:	95.4 mM HFIP and 7.1 mM TEA in methanol
Time Program:	5 % B (0 to 2 min) → 35 % B (15 min) → 80 % B (16 to 17 min) → 5 % B (18 to 25 min)
Column Temp.:	50 °C
Detection:	PDA at 200 to 400 nm
Injection Volume:	2.33 µL (10 pmol)
MS Conditions (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), negative mode
Interface Voltage:	-2.0 kV
Mode:	Scan (*m/z* 600 to 2000)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	450 °C
DL Temp.:	200 °C

*1 P/N: 227-31210-02

■ Results

Fig. 2 shows the UV (260 nm) and TIC chromatograms of the synthetic DNA. A peak was detected around the retention time of 11 minutes.

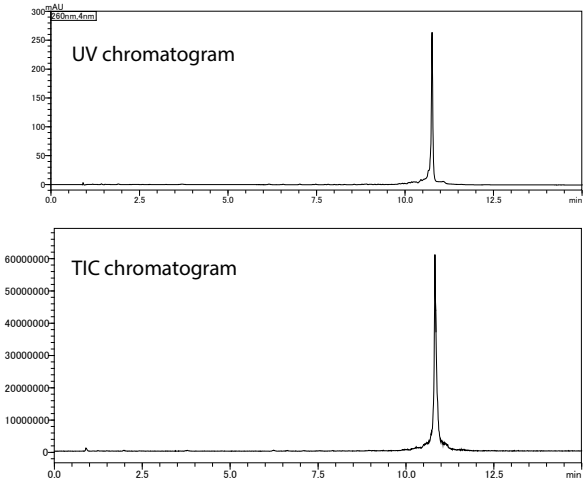

Fig. 2 UV and TIC Chromatograms of the Synthetic DNA

Fig. 3 shows the mass spectrum for the peak detected around the retention time of 11 minutes. Multiply-charged ions with charges from 17 to 22 were detected. The mass spectrum was deconvoluted to estimate the molecular weight (Fig. 4). That resulted in an estimated molecular weight of 21465.4, within a 1 Da margin of error with respect to the theoretical molecular weight (21465.9).

■ Conclusion

A Nexera XS inert UHPLC system and an LCMS-2050 single quadrupole mass spectrometer were used to analyze the molecular weight of synthesized single-stranded DNA. Generally, when estimating molecular weight using a deconvolution function, the more multiply-charged ions that can be obtained, the more reliable the molecular weight estimation will be. As a result of deconvolution of the mass spectrum for the detected peaks, it was possible to estimate the molecular weight of the principal components of the oligonucleotide therapeutic within a 1 Da margin of error from the theoretical value.

The LCMS-2050 enables fast and highly sensitive analysis across a wide mass range, while maintaining user-friendly operability similar to LC systems. The system described above provides a useful analytical tool for quality control of oligonucleotide therapeutics.

Related Applications

1. Simple Analysis of Impurities in Oligonucleotide Therapeutics Using a Single Quadrupole Mass Spectrometer, Application News No.01-00656-EN
2. An Oligonucleotide Impurity Analysis Workflow Using LabSolutions Insight™ Biologics Software, Application News No.01-00595A-EN

Acknowledgments

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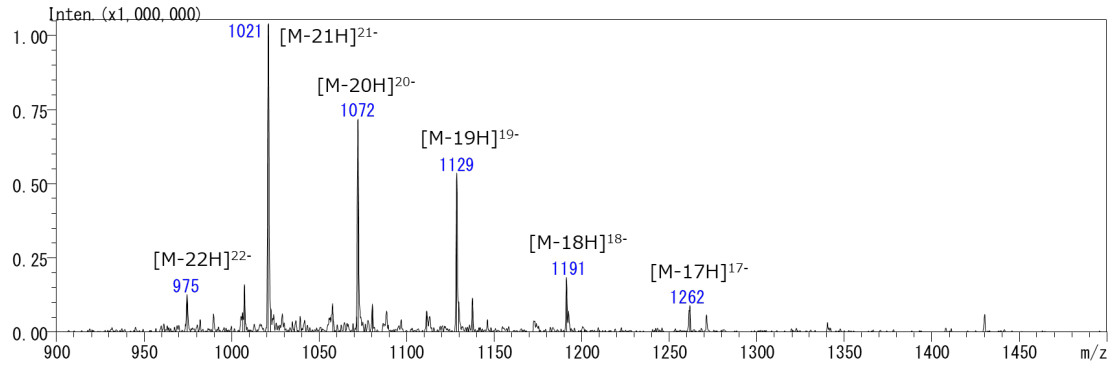
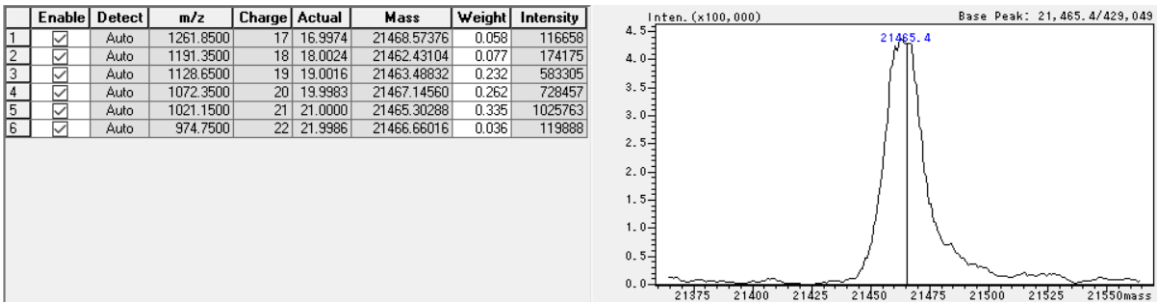


Fig. 3 Mass Spectrum of the Synthetic DNA



	Enable	Detect	m/z	Charge	Actual	Mass	Weight	Intensity
1	☑	Auto	1261.8500	17	16.9974	21468.57376	0.058	116658
2	☑	Auto	1191.3500	18	18.0024	21462.43104	0.077	174175
3	☑	Auto	1128.6500	19	19.0016	21463.48832	0.232	583305
4	☑	Auto	1072.3500	20	19.9983	21467.14560	0.262	728457
5	☑	Auto	1021.1500	21	21.0000	21465.30288	0.335	1025763
6	☑	Auto	974.7500	22	21.9986	21466.66016	0.036	119888

Fig. 4 Deconvoluted Mass Spectrum

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