Application News

LCMS-2050 Liquid Chromatograph Mass Spectrometer

Qualitative Analysis of Synthetic DNA Using a Single Quadrupole Mass Spectrometer

Junna Nakazono

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

LIDIC	Conditions	(NIamara	VC incrt)

Column: Shim-pack Scepter Claris C18-120*

 $(100 \text{ mm} \times 2.1 \text{ mm I.D., } 1.9 \text{ }\mu\text{m})$

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) \rightarrow 35 % B (15 min) \rightarrow 80 % B (16 to 17 min) \rightarrow 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

■ 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.

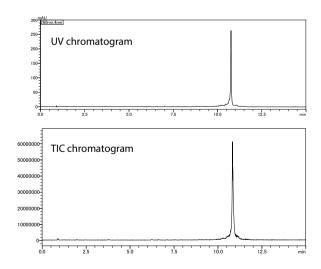


Fig. 2 UV and TIC Chromatograms of the Synthetic DNA

^{*1} P/N: 227-31210-02

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

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

Acknowledgments

We are grateful to Dr. Yuuya Kasahara (National Institutes of Biomedical Innovation, Health and Nutrition, Japan) for generously providing the sample.

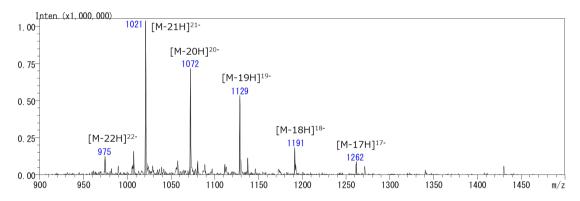


Fig. 3 Mass Spectrum of the Synthetic DNA

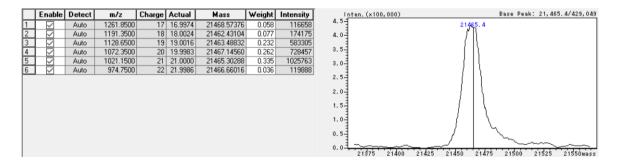


Fig. 4 Deconvoluted Mass Spectrum

Nexera, Shim-pack Scepter, and DUIS are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.



Shimadzu Corporation

www.shimadzu.com/an/

For Research Use Only, Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these

products in your country.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
See http://www.shimadzu.com/about/trademarks/index.html for details.
Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not

they are used with trademark symbol "TM" or "@". Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

First Edition: Dec. 2023

01-00662-EN