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MALDI-DIT-MS

Application News

# Oligonucleotide Analysis Using the Compact MALDImini™-1 MALDI Digital Ion Trap Mass Spectrometer

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## User Benefits

- Enables molecular weight analysis and sequence analysis of oligonucleotides, including modification sites.
- $MS^n$ enables reliable end sequence analysis.
- The compact benchtop design does not take up much space.

## ■ Introduction

Due to rapid progress in practical applications of oligonucleotide therapeutics in recent years, there is a growing need for corresponding analytical technologies. The application of mass spectrometry (MS) to oligonucleotide therapeutics analysis has already begun, but more accurate and rapid analytical techniques are required.

Most approved oligonucleotide therapeutics are based on single- or double-stranded synthetic oligonucleotides that are several tens of bases in length (molecular weight of about 6000 to 15000 Da). Such oligonucleotides contain a variety of chemical modifications depending on the purpose.

The MALDImini-1 matrix-assisted laser desorption/ionization-digital ion trap-mass spectrometer (MALDI-DIT-MS) (Fig. 1) is a compact benchtop instrument that can perform MS analysis for *m/z* values up to 70,000 and $MS^n$ analysis for *m/z* values below 5,000 in positive ion mode.

This article presents an example of using the MALDImini-1 system to analyze the molecular weight and sequence of a synthetic oligonucleotide with the same sequence as mipomersen, an oligonucleotide therapeutic.

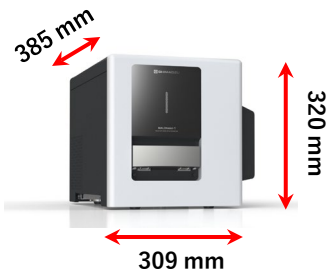


Fig. 1 MALDImini™-1 (MALDI-DIT-MS)

## ■ Synthetic Oligonucleotide Sample

Table 1 shows the sequence information of the synthetic oligonucleotide used as a model for the oligonucleotide therapeutic. This sequence is the same as mipomersen.

Table 1 Sequence Information of Synthetic Oligonucleotide Used in this Application News*1

| MW | Sequence (20 bases) |
|---|---|
| 7177 | 5'-MG-MC-MC-MU-MC-dA-dG-dT-dC-dT-dG-dC-dT-dT-dC-MG-MC-MA-MC-MC-3' |

*1 M: 2'-O-(2-methoxyethyl) nucleoside; d: 2'-deoxynucleoside; The 5-positions of cytosine and uracil are substituted with methyl groups. Phosphodiester bonds between all nucleotides are replaced by phosphorothioate bonds.

## ■ Sample Preparation and Measurement Conditions

A 20 pmol/µL solution of synthetic oligonucleotide was prepared as a sample solution. As matrix solutions, 3-hydroxypicolic acid (3-HPA) and 2,4-dihydroxyacetophenone (2,4-DHAP) were each dissolved in acetonitrile/water (50/50, v/v) solution containing 70 mM diammonium hydrogen citrate to make a 40 mg/mL solution of each. A 3-HPA/2,4-DHAP matrix mixture solution was prepared by mixing the above 3-HPA and 2,4-DHAP solutions at a 1:1 (v/v) ratio. This matrix mixture improves sensitivity and mass spectral quality in the MALDImini-1 system compared to conventional methods (patent pending). After mixing the sample and matrix solutions at 1:1 (v/v), 1 µL of the mixture was dropped onto the sample plate, dried, and measured.

Measurements were performed by raster-scanning with the MALDImini-1 system. Table 2 shows the condition settings used for molecular weight analysis and sequence analysis. Using those settings for sequence analysis, a large number of fragment ions derived from the oligonucleotide can be detected by MALDI-DIT-MS specific degradation (detailed below).

Table 2 MALDImini-1 Condition Settings for Molecular Weight and Sequence Analysis*2

| | LP | DV-1 (V) | DV-2 (V) | RF delay (*ms*) |
|---|---|---|---|---|
| M.W. Analysis | 60-65 | 1300 | 7000 | 25 |
| Sequence Analysis | 65-75 | 1600 | 8000 | 15-17 |

*2 LP: laser power; DV-1: detector voltage; DV-2: dynode voltage. The same conditions can be used for scan ranges *m/z* 650 to 5000 and *m/z* 2000 to 18000. The values in the table are examples only and will vary depending on the system used. The DV-1 value for sequence analysis should be approximately 300 V higher than the value for molecular weight analysis.

## ■ Molecular Weight Analysis of Synthetic Oligonucleotide

Fig. 2 shows the mass spectrum of the synthetic oligonucleotide obtained by MALDImini-1 using a 3-HPA/2,4-DHAP matrix mixture and the settings for molecular weight analysis in Table 2.

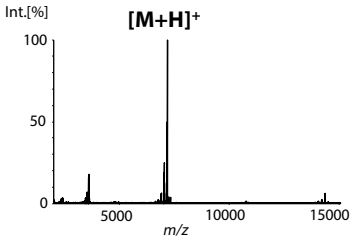


Fig. 2 Mass Spectrum of Synthetic Oligonucleotide

## ■ Sequence Analysis of Synthetic Oligonucleotide

Fig. 3 shows the definition of nucleic acid fragment ions[1]. Fig. 4 shows the mass spectra of the synthesized oligonucleotide obtained using the MALDImini-1 system and the condition settings for sequence analysis indicated in Table 2. Under those conditions, many fragment ions, mainly *a*/*w*-ions, were detected. The *a*/*w*-ions are obtained in the form of a ladder, allowing easy sequence analysis.

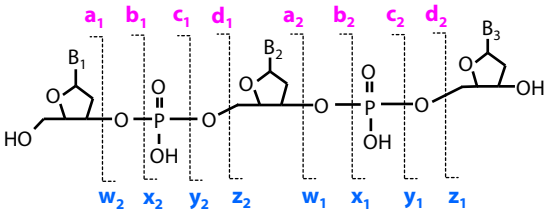


Fig. 3 Definition of Fragment Ions of Nucleic Acids[1]

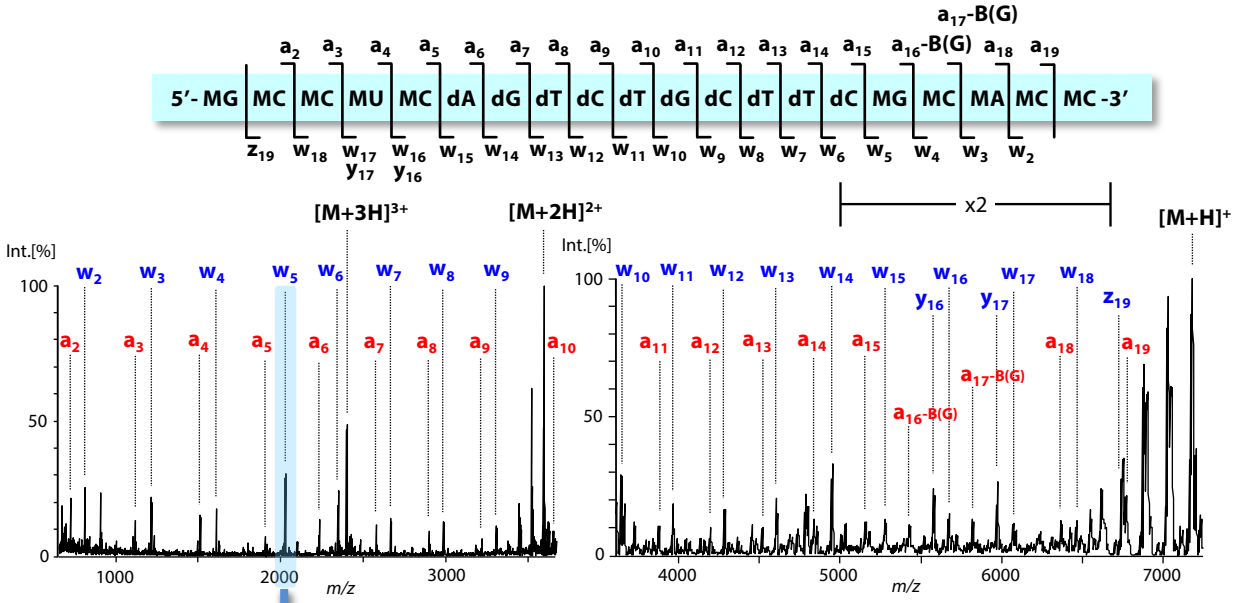


Fig. 4 Mass Spectra of Synthetic Oligonucleotide Obtained Using Condition Settings for Sequence Analysis

In Fig. 4, multiple fragment ions overlap in the vicinity of $[M+H]^+$, making it difficult to attribute the fragment ions used for sequence analysis of the terminal portion of the oligonucleotide. Fig. 5 shows the $MS^2$ spectrum (pseudo-$MS^3$ spectrum) obtained using one of the fragment ions shown in Fig. 4, the $w_5$-ion, as the precursor ion. The $MS^2$ spectrum enabled the fragment ions containing terminal sequence information to be easily attributed. By using the $MS^2$ spectrum in Fig. 5 in combination with the mass spectrum in Fig. 4, enabled more accurate sequence analysis.

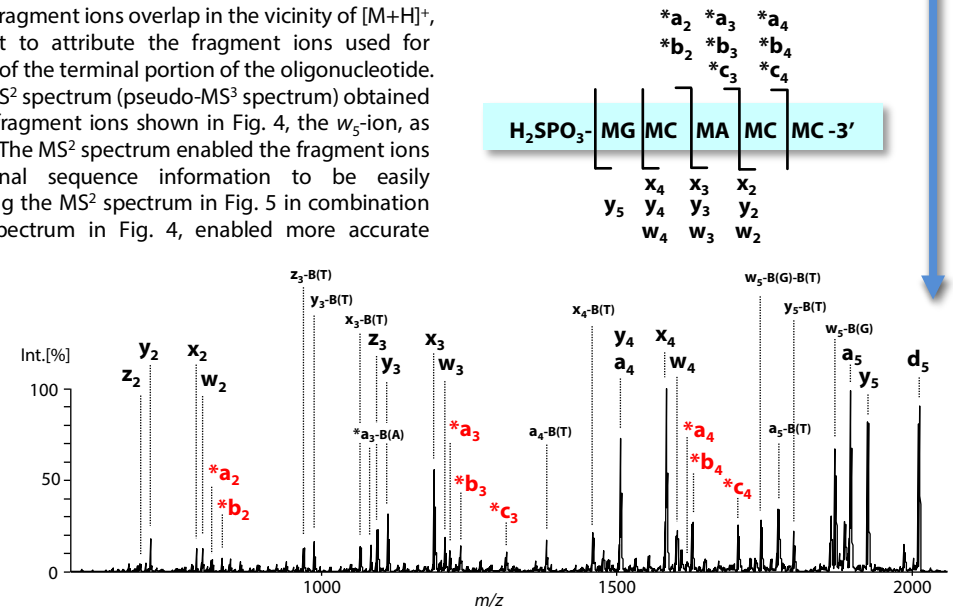


Fig. 5 $MS^2$ Spectrum of the $w_5$-ion in Fig. 4

## ■ Conclusion

This example confirmed that molecular weight information and base sequence information for entire synthetic oligonucleotides sequences, including modification sites, can be obtained easily and quickly by using a MALDI-DIT-MS system and adjusting the condition settings.

Consequently, the system is expected to be used in the field of nucleic acid analysis as a simple analytical device for research sites, while also offering the advantage of a compact benchtop design.

<Reference>

1) McLuckey SA, J. Am. Soc. Mass. Spectrom., 1992, 3, 60-70.

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