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MALDI-TOF Mass Spectrometer MALDI-8030
Software for Oligonucleotide Sequence Characterization LabSolutions Insight™ Biologics

Application News

# Sequence Analysis of Antisense Oligonucleotides Using a MALDI-8030 Benchtop MALDI-TOF Mass Spectrometer

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## User Benefits

- MALDI-ISD enables simple and reliable sequencing of antisense oligonucleotide.
- There is no need to interpret complicated MS/MS spectra, which saves labor and time.
- Oligonucleotide therapeutics can be analyzed using a simple and affordable benchtop instrument.

## ■ Introduction

Oligonucleotide therapeutics have recently attracted particular attention as a new pharmaceutical modality. Unlike biopharmaceuticals, oligonucleotide therapeutics can be produced by cost-effective chemical synthesis. The development and standardization of analytical methods for quality control are still in progress, with efforts being made in collaboration with industry and academia.

Mass spectrometry (MS) has now become an established analytical tool for analyzing biopolymers, such as oligonucleotides, proteins, and glycans, thanks to the development of various soft ionization methods and instrument performance improvements. Although tandem mass spectrometry (MS/MS) is generally used to sequence oligonucleotide, collision-induced dissociation (CID), the technique typically used for conventional ion cleavage, has been problematic because of the difficulty in producing fragments that suggest particularly internal sequences.

Matrix-assisted laser desorption/ionization (MALDI) is used as a technique for ionizing various biopolymers, including oligonucleotides. MALDI can softly ionize a sample without causing unwanted fragmentation. However, by using a specific MALDI matrix with increased laser irradiation, it is possible to cause ionization and fragmentation at the same time. Then the resulting in-source decay (ISD) fragment ions can be used for structural analysis. MALDI-ISD fragmentation of oligonucleotides provides successive fragment ions by simple cleavages and is therefore highly useful for simple sequencing of oligonucleotides.

This Application News describes an example in which synthetic antisense oligonucleotides were subjected to ISD fragmentation using the Shimadzu MALDI-8030 benchtop linear MALDI-TOF mass spectrometer, and the resulting mass spectra were analyzed for sequence assignment with LabSolutions Insight Biologics, a software designed for oligonucleotide sequence characterization.

## ■ Model Oligonucleotide Therapeutics Samples

In this study we analyzed three synthetic antisense oligonucleotides ("model therapeutics") which replicate the oligonucleotide therapeutics mipomersen, nusinersen and inotersen (Table 1). Each sample was dissolved in water to a concentration of 0.1 mg/mL (about 13 to 14 pmol/µL) and then 0.5 µL of the sample solution was measured with a MALDI-TOF MS system.

Table 1 3 Model Oligonucleotide Therapeutics Used in This Study

| Name | Chemical Formula | Mw | Note |
|---|---|---|---|
| **Mipomersen** | C230H324N67O122P19S19 | 7177.2 | ASO, PS (full), 2'-MOE, 20 mer |
| 5'-G*-mC*-mC*-mU*-mC*-dA-dG-dT-dmC-dT-dG-dmC-dT-dT-dmC-G*-mC*-A*-mC*-mC*-3' | | | |
| **Nusinersen** | C234H340N61O128P17S17 | 7127.2 | ASO, PS (full), 2'-MOE, 18 mer |
| 5'-T*-mC*-A*-mC*-T*-T*-T*-mC*-A*-T*-A*-A*-T*-G*-mC*-T*-G*-G*-3' | | | |
| **Inotersen** | C230H318N69O121P19S19 | 7183.1 | ASO, PS (full), 2'-MOE, 20 mer |
| 5'-T*-mC*-T*-T*-G*-dG-dT-dT-dA-dmC-dA-dT-dG-dA-dA-A*-T*-mC*-mC*-mC*-3' | | | |

* = 2'-O-(2-methoxyethyl) m = 5-methyl d = 2'-deoxy

## ■ Mass Spectrometry

0.5 µL of the sample solution was deposited on a MALDI target plate, mixed with a 0.5 µL matrix solution on the plate, and left to dry. Mass spectra were obtained using the MALDI-8030 system (Fig. 1).

| | |
|---|---|
| Matrix solution: | 40 mg/mL THAP or HPA in 50 % acetonitrile with 40 mM ammonium citrate dibasic |
| THAP: | 2',4',6'-Trihydroxyacetophenone monohydrate |
| HPA: | 2-Hydroxypicolinic acid |

Fig. 1 MALDI-8030 MALDI-TOF Mass Spectrometer (left)
LabSolutions Insight™ Biologics (right)

## ■ Mass Spectrum of Model oligonucleotide Therapeutics

HPA and THAP are typical MALDI matrices for ionizing oligonucleotides. These matrices successfully ionized model oligonucleotide therapeutics as deprotonated form $[M-H]^-$ in negative-ion mode. The formation of unwanted cation adducts was suppressed by adding ammonium citrate to the matrix solution (Fig. 2).

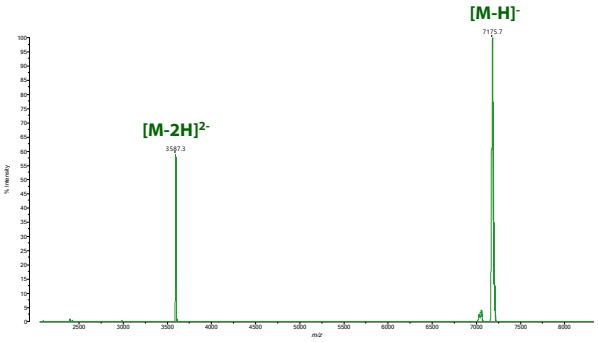


Fig. 2 Negative-Ion MALDI Mass Spectrum of Mipomersen Using a THAP as a Matrix

## ■ Characteristics of MALDI-ISD Fragments of Oligonucleotides

MALDI-ISD of oligonucleotides produces two specific types of consecutive fragment ions, either a-series ions from the 5'-terminus side or w-series ions from the 3'-terminus side, which are formed by simple cleavage at the phosphodiester bond (Fig. 3). This simplicity offers simpler sequencing than using electrospray ionization (ESI) followed by MS/MS using CID, which generally produces various types of cleavages that depend on the precursor charge-state ([M-nH]$^{n-}$).

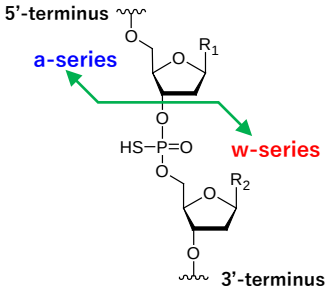


Fig. 3 Ion Fragmentation Nomenclature of Oligonucleotides Observed from MALDI-ISD

## ■ Characteristics of MALDI-ISD Fragments of Model Oligonucleotide Therapeutics

Fig. 4 shows negative-ion MALDI-ISD mass spectra of model oligonucleotide therapeutics (mipomersen, nusinersen, and inotersen) obtained using HPA as a matrix. These results indicate that HPA is more suitable than THAP for ISD ion measurements (Fig. 2 and 4). The presence of simple and consecutive ISD ions allows for the confirmation of the sequences of model oligonucleotide therapeutics, even those with various types of modifications such as phosphorothioation. These mass spectra, composed of singly charged ions, is simple enough to allow for manual assignment, but it can also be assigned automatically with LabSolutions Insight Biologics. By exporting the mass spectrum in the mzML format and entering the sequence information in LabSolutions Insight Biologics before running the analysis, the software quickly displays the assignment results.

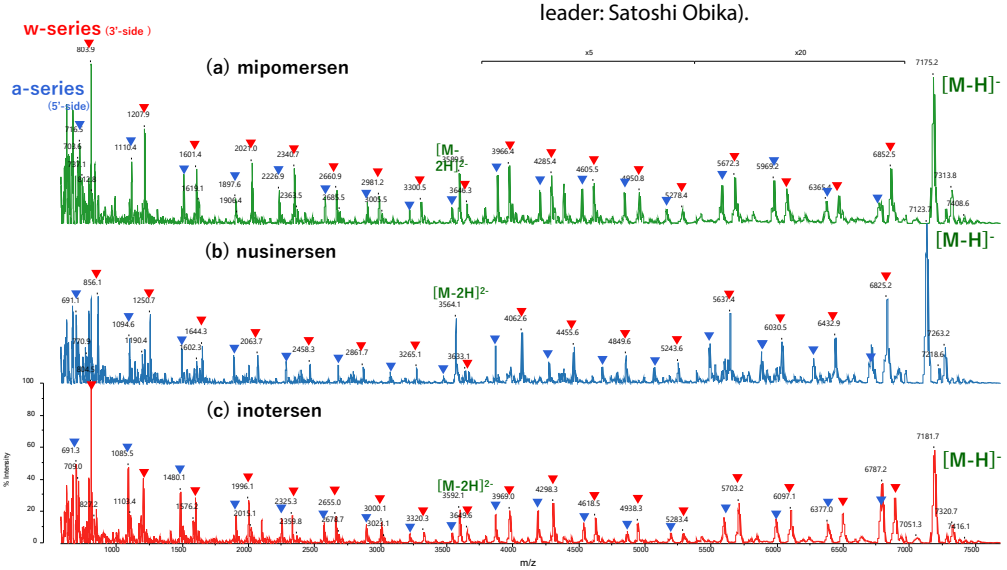


Fig. 4 Negative-Ion MALDI-ISD Mass Spectra of Model Oligonucleotide Therapeutics Using HPA as a Matrix
(a) mipomersen, (b) nusinersen, and (c) inotersen

Fig. 5 shows an example of the results for a model oligonucleotide therapeutics analyzed using LabSolutions Insight Biologics.

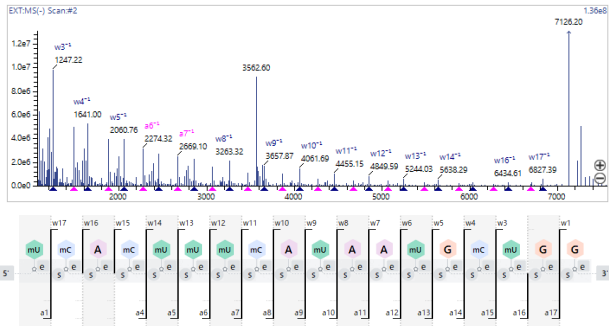

Fig. 5 Example of the result of a model oligonucleotide therapeutic analysis using LabSolutions Insight Biologics.

Because the MALDI-8030 is a linear TOF mass spectrometer, it does not support MS/MS measurements. Nevertheless, nucleic acid sequencing is possible by using MALDI-ISD measurements. This technique would be applicable not only for synthetic oligonucleotides but also for their unknown impurities isolated by HPLC.

## ■ Conclusion

Because MALDI preferentially produces singly-charged ions, mass confirmation of synthetic oligonucleotides can be achieved easily without interpreting complicated mass spectra. Furthermore, the ability to perform MALDI-ISD measurements with an instrument lacking MS/MS capability means the internal sequence of oligonucleotides can be confirmed using a simple benchtop-type linear MALDI-TOF MS system. That also enables the sequencing of model oligonucleotide therapeutics that contain various chemical modifications based on the successive ISD fragment ions originating from a simple cleavage.

## ■ Acknowledgements

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