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MALDImini™-1, MALDI Digital Ion Trap Mass Spectrometer
LabSolutions Insight™ Biologics, Software for Oligonucleotide Sequence Characterization

Application News

Oligonucleotide Sequence Analysis Using MALDImini-1 and LabSolutions Insight Biologics Software

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User Benefits

- The compact space-saving MALDImini-1 MALDI-DIT-MS system enables operation in facilities with limited space.
- LabSolutions Insight Biologics software rapidly analyzes complex fragment spectra of oligonucleotides to reduce data analysis time.
- The combination of the MALDImini-1 spectrometer and LabSolutions Insight Biologics software enables molecular weight and sequence analysis in approximately 10 minutes, from measurement to final results.

■ Introduction

With rapid advancements in the practical application of oligonucleotide therapeutics, there is an increasing need for effective analytical techniques for such applications. With mass spectrometry (MS) now being applied to the analysis of oligonucleotide therapeutics, simpler and faster analytical and sequencing techniques are increasingly desired.

This Application News article describes an example of an oligonucleotide sequence analysis using the MALDImini-1 matrix-assisted laser desorption/ionization digital ion trap mass spectrometer (MALDI-DIT-MS) system in combination with LabSolutions Insight Biologics software for oligonucleotide sequence characterization (Fig. 1).

Due to the compact and space-saving size of the MALDImini-1 MALDI-DIT-MS system it can operate on a 100 V power supply in facilities with limited space. In addition, because it only generates singly-charged ion species, the LabSolutions Insight Biologics software can rapidly estimate the base sequence of the oligonucleotides, including modification information. Because the sequence analysis is limited to singly-charged fragment ions, the estimated sequences displayed are simple and contribute to the reliability of analysis results.

Due to the compact space-saving design of the analytical system, the system offers rapid and simple molecular weight (MW) analysis and sequence analysis of oligonucleotides.

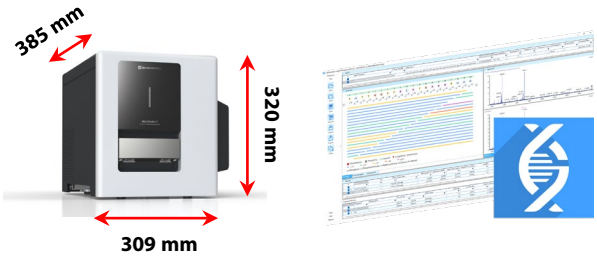


Fig. 1 MALDImini™-1 (Left) Spectrometer with LabSolutions Insight™ Biologics Software (Right)

■ Synthetic Oligonucleotide Sample

Table 1 shows the sequence of an oligonucleotide used as a model sample for oligonucleotide therapeutics. This sequence is the same as that of nusinersen.

Table 1 Sequence Information of the Synthetic Oligonucleotide in this Article*

MW	Sequence (18 bases)
7127	5'-mU-mC-mA-mC-mU-mU-mU-mC-mA-mU-mA-mA-mU-mG-mC-mU-mG-mG-3'

* m: 2'-O-(2-methoxyethyl) nucleoside; A: adenine, G: guanine; C: cytosine; U: uracil, where cytosine and uracil are substituted at position 5 with a methyl group and phosphodiester bonds between all nucleotides are substituted with phosphorothioate bonds.

■ Preprocessing and Analysis Conditions

● MW and Sequence Analysis by MALDI-DIT-MS

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

The measurement was performed using raster scanning in the MALDImini-1 system. Table 2 shows the instrument setting conditions for MW and sequence analysis. By using the setting conditions for sequence analysis, a large number of fragment ions derived from oligonucleotide samples can be detected, due to the specific cleavage of MALDI-DIT-MS.

1. Mix the matrix solution with the sample solution (1:1, v/v).

2. Drip (1 µL) onto a sample plate and dry.

Fig. 2 Analytical Sample Preparation Method of MALDI-DIT-MS

Table 2 Setting conditions of MALDImini-1**

Purpose	LP	DV-1 (V)	DV-2 (V)	RF Delay (*m*s)
MW Analysis	60 - 65	1300	7000	25
Sequence Analysis	65 – 75	1600	8000	15-17

** LP: laser power; DV-1: detector voltage; DV-2: dynode voltage. The same condition is available for scan range *m/z* 650 - 5000 and *m/z* 2000 - 18,000. The values in the table are examples and may vary depending on the instrument usage. As a guide, set the DV-1 value for sequence analysis to 300 V higher than the value for MW analysis.

● Data Analysis with LabSolutions Insight Biologics

Data for MW and sequence analysis obtained by MALDI-DIT-MS can be exported as an mzML file and opened in LabSolutions Insight Biologics. By entering the theoretical base sequence and clicking the "Identify" button, the analysis results are displayed immediately (see below for details). The sequence you enter can be easily created and saved in the Insight Biologics "Settings" window.

■ Results of MW Analysis

After opening the mzML file of the mass spectrum (Fig. 3), obtained using MALDImini-1 conditions for MW analysis, in Insight Biologics as an "Identification File," click the "Identify" button. The identification result will be displayed immediately as shown in Fig. 4.

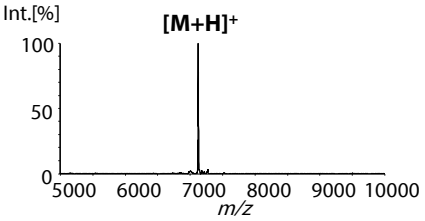


Fig. 3 Mass spectrum by MALDImini-1

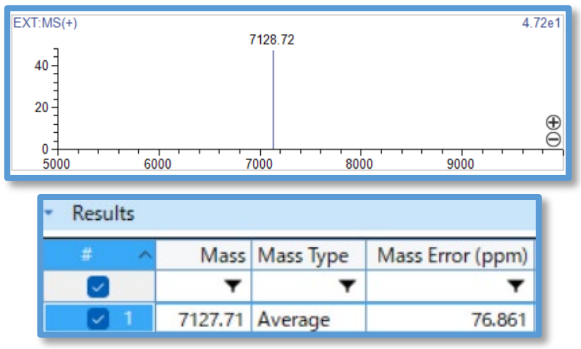


Fig. 4 Results of MALDImini-1 data analysis by LabSolutions Insight Biologics
Example of a Window Display Showing Mass Spectrum (Top) and Identification Results (Bottom)

■ Results of Sequence Analysis

Next, open the mzML file of the spectrum (Fig. 5), obtained using MALDImini-1 conditions for sequence analysis, in Insight Biologics as a "Coverage Assignment File" and click the "Identify" button. The software automatically merges the two spectra and immediately displays the fragment spectrum, sequence coverage, and identification results (Fig. 6).

Because the fragments that occur preferentially in MALDImini-1 results are limited to the set of fragment ion species based on the cleavage of a specific bond and are detected primarily as singly-charged ions, the inferred sequences displayed in Insight Biologics are simple and the ion species to explore can be selected. The fragment characteristics of MALDI-MS make the identification results easy to understand and contribute to the reliability of the analysis.

■ Conclusion

Using the compact MALDImini-1 mass spectrometer in combination with LabSolutions Insight Biologics software for oligonucleotides sequence analysis enables highly reliable sequence analysis based on a limited set of singly-charged ion species. Therefore, it is offered as a compact space-saving analysis system for rapid and simple sequence analysis.

Related Applications

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

Application News No. 01-00594-EN

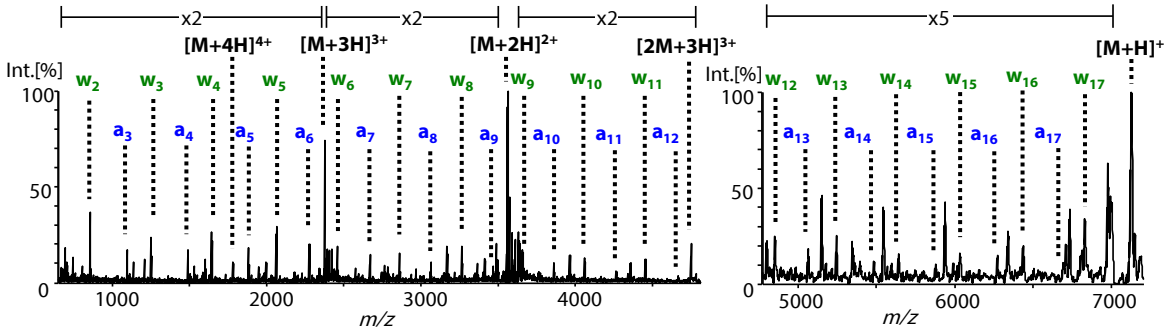
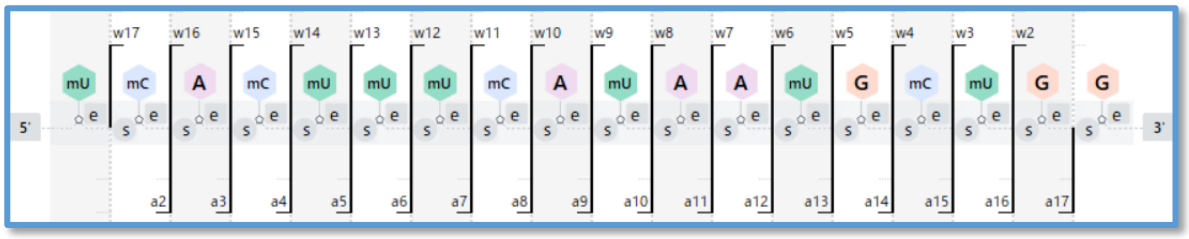


Fig. 5 Fragment Spectra Obtained by MALDImini-1



#	Fragment	Modification	Found Mz	m/z	Mass Type	Intensity	% Intensity
1	a2	----	692.71	692.65	Average	71	8.68
2	w17	----	6830.74	6829.88	Average	51	6.67
3	a3	----	1095.98	1096.00	Average	67	8.22
4	w16	----	6436.32	6436.53	Average	29	3.86
5	a4	----	1489.48	1489.35	Average	71	8.71

Fig. 6 Results of MALDImini-1 Data Analysis by LabSolutions Insight Biologics
Example of a Window Display Showing Sequence Coverage (Top) and Identification Results (Bottom) , where s: thiophosphate, e: methoxyethoxy, and m: methyl

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