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Liquid Chromatograph Mass Spectrometer

Application News

Ion-Pair-Free Reversed-Phase LC/MS Analysis of siRNA Using a Single Quadrupole Mass Spectrometer

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User Benefits

- The LCMS-2050 single quadrupole mass spectrometer and the LabSolutions Insight™ Biologics analysis software can be used to confirm the molecular weight of siRNA.
- The ion-pair-free method does not use ion-pair reagents, so it decreases the risk of instrument contamination and does not need an instrument dedicated to ion-pair regents.
- LabSolutions Insight Biologics enables analysis of multiple oligonucleotide sequences at the same time.

■ Introduction

Among oligonucleotide therapeutics, antisense and siRNA therapeutics are actively studied as new modalities for treating genetic and intractable diseases. Unlike single-stranded antisense, siRNA is double-stranded RNA known to separate into single-stranded RNAs under heated or alkaline conditions.

LC/MS analysis of oligonucleotides commonly involves using reversed-phase chromatography with mobile phases containing an alkylamine ion-pair reagent, such as hexylamine or triethylamine. However, the use of ion-pair reagents raises concerns that instrument contamination may affect other analyses. This Application News article describes an example of ion-pair-free reversed-phase LC/MS analysis of siRNA using a single quadrupole mass spectrometer.

■ Samples

Double-stranded siRNA based on the sequence of Patisiran were used.

Sense:
G-Um-A-A-Cm-Cm-A-A-G-A-G-Um-A-Um-Um-Cm-Cm-A-Um-dT-dT

Antisense:
A-U-G-G-A-A-Um-A-C-U-C-U-U-G-G-U-Um-A-C-dT-dT

(d: 2'-deoxy and m: 5-methyl)

■ Analytical Conditions

Analysis was performed with Nexera™ XS inert UHPLC and LCMS-2050 single quadrupole spectrometer systems. The analytical conditions are shown in Table 1. The LCMS-2050 is equipped with a heated DUIS™ ion source for ionization, which offers the advantages of both ESI and APCI methods.

Table 1 Analytical Conditions

UHPLC (Nexera XS inert)	
Column:	Shim-pack Scepter Claris C18-300*1 (50 mm x 2.1 mm I.D., 1.9 µm)
Mobile Phase A:	10 mM Ammonium bicarbonate – water
Mobile Phase B:	Methanol
Gradient Program:	B Conc. 2 % (0 min) – 40 % (5 min) – 90 % (5.1-6 min) – 2 % (6.1-12 min)
Flowrate:	0.4 mL/min
Column Temp.:	25 or 60 °C
Injection Volume:	1 µL

*1: P/N 227-31209-01

MS (LCMS-2050)	
Ionization:	ESI/APCI (DUIS) positive
Mode:	MS *m/z* 800-2000
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

■ Configuring the Data Analysis Parameters

First, the user configures an oligonucleotide sequence in the parameter configuration window using the nucleobases, linkers, ribose and modifications provided by the software (Fig. 1). Nucleobases, linkers, ribose, and base modifications can be added and removed on each tab page as required. Once an oligonucleotide sequence is entered, the software displays the molecular formula, monoisotopic mass (to the left side), and structural formula (to the right side) of that oligonucleotide.

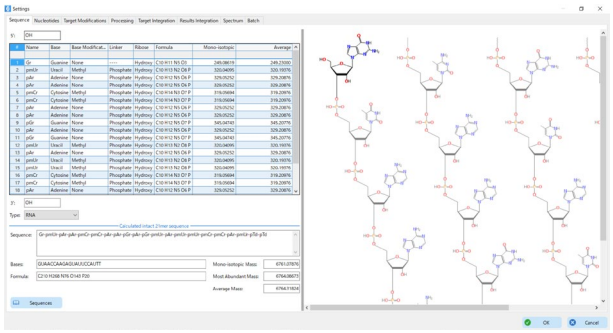

Fig. 1 Parameter Configuration Window

Insight Biologics can analyze multiple sequences. As an example, in Fig. 2, the sequences of sense and antisense strands were set as analysis targets. The specified sequences and optionally added information about nucleobases, linkers, riboses, and base modifications can be saved as an analysis settings file.

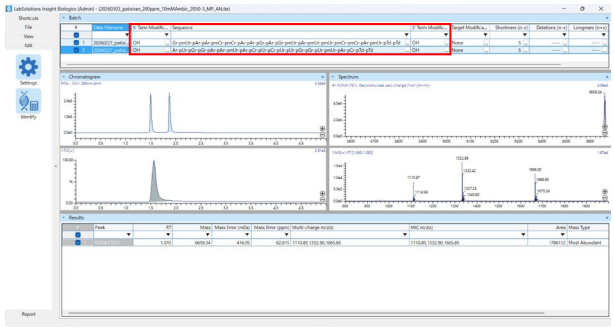

Fig. 2 Example of Multiple Sequence Settings

■ Results

The UV chromatograms of the siRNA are shown in Fig. 3. Regardless of column oven temperatures, the siRNA was eluted as two peaks. That suggests the siRNA was dissociated into sense and antisense strands.

Insight Biologics displays the identified oligonucleotide sequence as a component chromatogram based on the MS1 spectrum combined with different valences and isotopes. Fig. 4 shows the component chromatograms obtained by LC/MS analysis of siRNA with a 60 °C column oven temperature. The sense strand and antisense strand sequences were identified in the order eluted, which confirmed that the two peaks detected in the UV chromatogram (Fig. 3) eluted in the single-stranded state.

The sense strand and antisense strand sequences were identified with a mass error within 1 Da of their theoretical molecular weights. As an example, Fig. 5 shows the identification results for the sense strand with a column temperature of 60 °C. Under those conditions and using a mobile phase spiked with ammonium bicarbonate, multiply-charged ions (4 to 6) were detected, demonstrating that the molecular weight of the oligonucleotides could be confirmed even without using an ion-pair reagent.

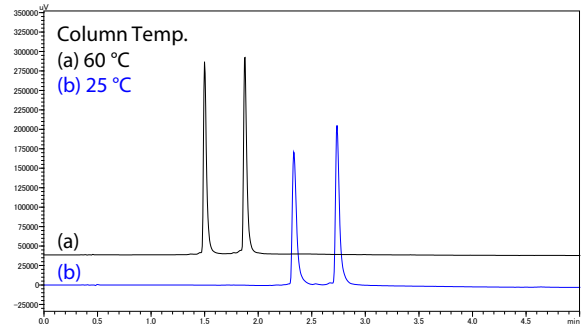

Fig. 3 UV Chromatogram (260 nm)

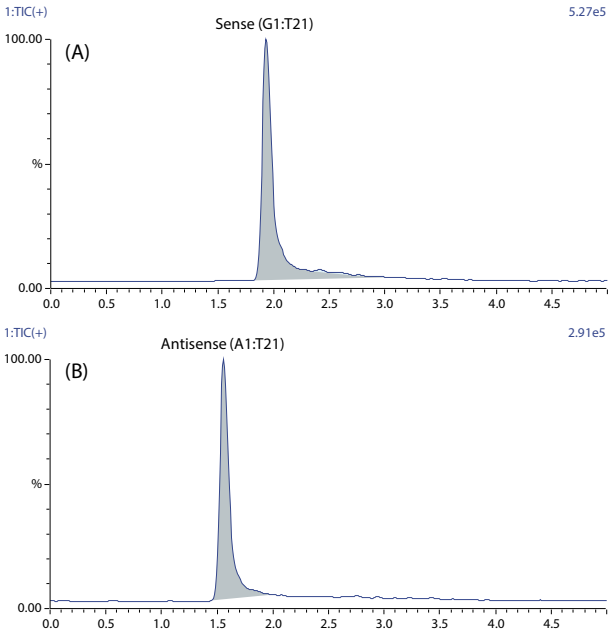

Fig. 4 Component Chromatograms with a Column Temperature of 60 °C
(A) Sense Strand; (B) Antisense Strand

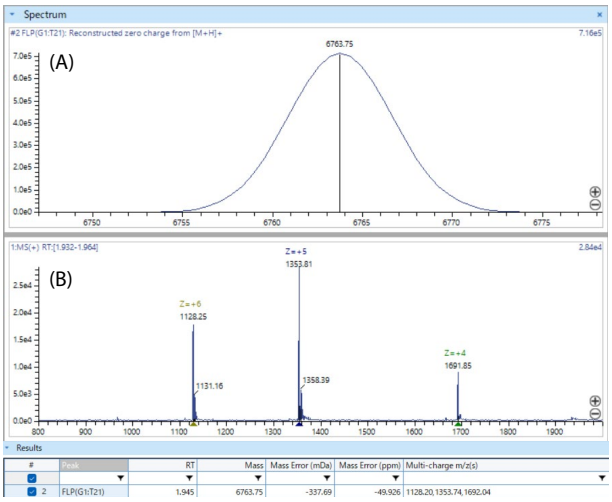

Fig. 5 Result of Identification for the Sense Strand with a Column Temperature of 60 °C
(A) Deconvoluted Spectrum; (B) Mass Spectrum

■ Conclusion

Ion-pair-free reversed-phase LC/MS analysis of siRNA was performed using an LCMS-2050 single quadrupole mass spectrometer to confirm molecular weight. The siRNA was eluted in a dissociated single-stranded state, with both sense and antisense strands detected within a mass error of less than 1 Da of the theoretical molecular weight values. The LabSolutions Insight Biologics software enables analysis of multiple oligonucleotide sequences at the same time.

■ Acknowledgments

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Related Applications

1. Ion-Pair Reversed-Phase LC/MS Analysis of GalNAc-siRNA Conjugates under Denaturing and Non-Denaturing Conditions
   Application News No. 01-01177-EN
2. Reversed-Phase Ion-Pair LC/MS Analysis of siRNA under Denaturing and Non-Denaturing Conditions
   Application News No. 01-00915-EN
3. Efficient Method Development for Separation of Capped mRNA Fragments
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