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Liquid Chromatograph Mass Spectrometer LCMS-9050

Application News

Reversed-Phase Ion-Pair LC/MS Analysis of siRNA under Denaturing and Non-Denaturing Conditions

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User Benefits

- The LCMS-9050 quadrupole time-of-flight mass spectrometer can be used to characterize siRNA.
- The elution of siRNA in double-stranded (non-denatured) or single-stranded (denatured) state can be confirmed by changing the column temperature.
- The LabSolutions Insight™ Biologics analysis software enables analysis of multiple oligonucleotide sequences at once.

■ Introduction

Among oligonucleotide therapeutics, antisense and siRNA therapeutics, which are composed of relatively short oligo nucleic acids, are attracting attention as new modalities for treating genetic and intractable diseases. Unlike single-stranded antisense, siRNA is double-stranded RNA and known to separate into single-stranded RNAs under heated or alkaline conditions. Quality control of siRNA drugs requires characterization of both single-stranded and double-stranded RNA.

This application describes an example of LC/MS analysis of siRNA under denaturing and non-denaturing conditions in reversed-phase ion pair chromatography. Multiple nucleic acid sequences (sense and antisense strands) were analyzed at the same time using LabSolutions Insight Biologics, a dedicated software for oligonucleotide characterization.

■ Samples

Double-stranded siRNA annealed with sense and antisense strands of the following sequences was 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-9050 quadrupole time-of-flight mass spectrometer systems. The analytical conditions are shown in Table 1.

Table 1 Analytical Conditions

UHPLC (Nexera XS inert)	
Column:	Shim-pack Scepter™ Claris C18-300*1 (100 mm x 2.1 mm I.D., 1.9 µm)
Mobile Phase A:	100 mM HFIP, 10 mM TEA - water
Mobile Phase B:	100 mM HFIP, 10 mM TEA - methanol
Gradient Program:	B Conc. 5 % (0 min) – 50 % (10 min) – 90 % (10.01-12 min) – 5 % (12.1-25 min)
Flowrate:	0.3 mL/min
Column Temp.:	25 or 60 °C
Injection Volume:	1 µL

*1: P/N 227-31209-02

MS (LCMS-9050)	
Ionization:	ESI negative
Mode:	MS *m/z* 550-2500
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
Interface Temp.:	350 °C
DL Temp.:	250 °C
Block Heater Temp.:	400 °C

■ Configuring the Data Analysis Parameters

LabSolutions Insight Biologics is data analysis software for characterizing oligonucleotides and oligonucleotide impurities. First, the user creates an oligonucleotide sequence in the parameter configuration window using the nucleobases, linkers, ribose and modifications provided by the software. Nucleobases, linkers, ribose, and base modifications can be added and removed in each tab 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).

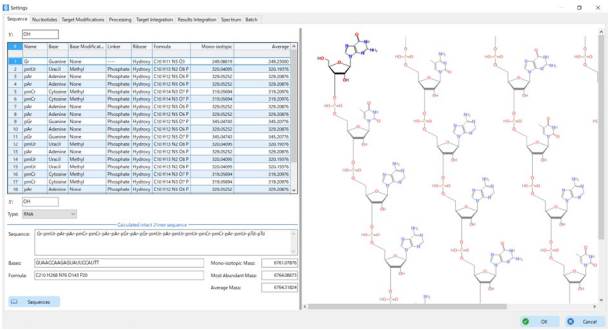

Fig. 1 Parameter Configuration Window

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.

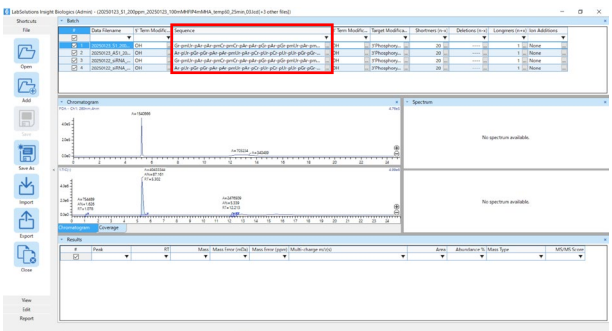

Fig. 2 Example of Multiple Sequence Settings

■ Separation of siRNA under Denaturing and Non-Denaturing Conditions

The UV chromatograms of sense, antisense, and double-stranded siRNA are shown in Fig. 3. The sense and antisense strands were eluted at different retention times at both 60 and 25 °C column oven temperatures. At a 60 °C column oven temperature, double-stranded siRNA was detected as denatured and dissociated into sense and antisense strands. On the other hand, at a 25 °C column temperature, double-stranded siRNA was eluted at a retention time of 7.5 minutes, which was slower than that of sense and antisense strands. In addition, the peak of the sense strand, which seemed to remain without forming a duplex during annealing, was also observed.

■ Results of Identification by LC/MS

Fig. 4 shows the component chromatograms obtained by LC/MS analysis of double-stranded siRNA. In Insight Biologics, the identified oligonucleotide sequence is displayed as a component chromatogram based on the MS1 spectrum and summed with different valences and isotopes. The sense strand and antisense strand sequences were identified at both column temperatures of 60 °C and 25 °C.

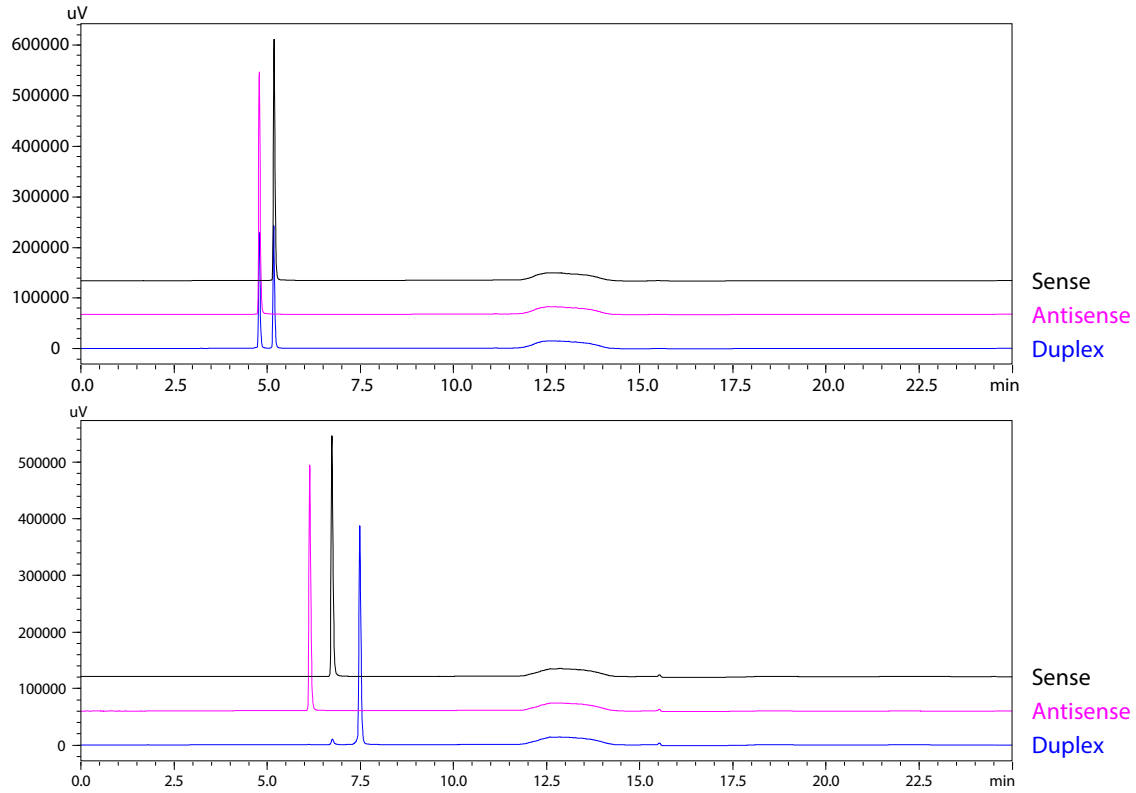

Fig. 3 UV Chromatograms (260 nm) of the Sense Strand, Antisense Strand and Duplex siRNA
Top: With 60 °C Column Oven Temp.; Bottom: With 25 °C Column Oven Temp.

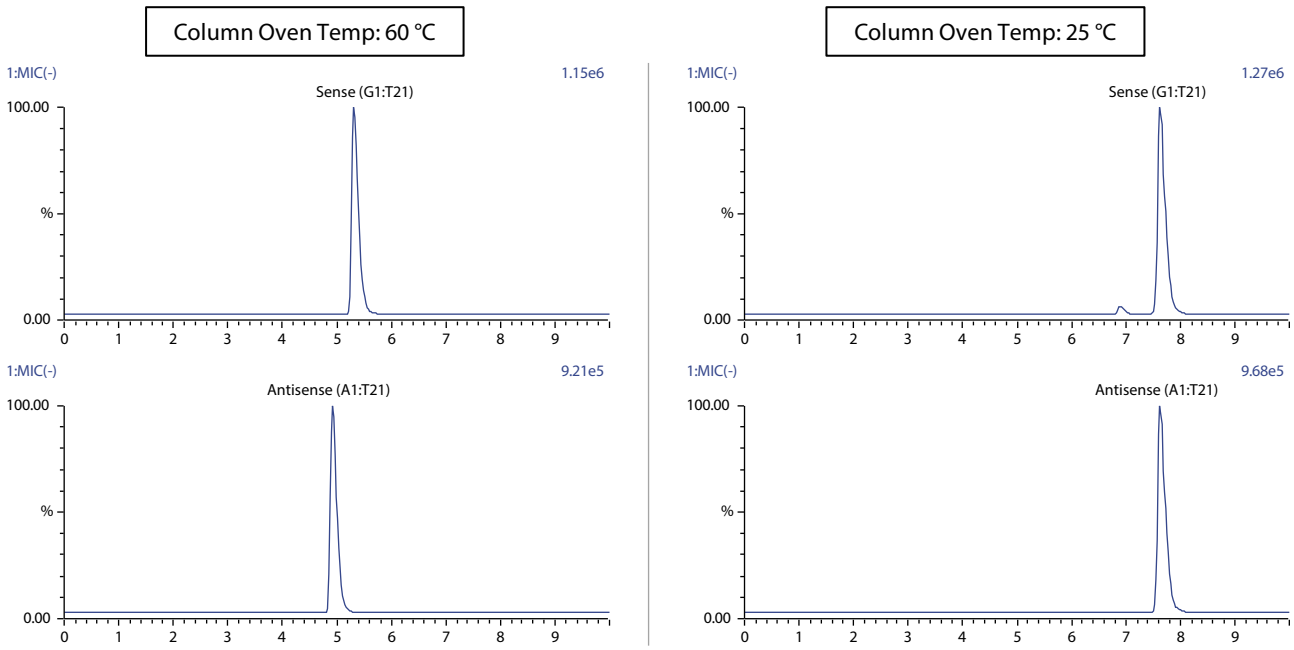

Fig. 4 Component Chromatograms of Duplex siRNA (Results of Identification by LC/MS)
Left: With 60 °C Column Oven Temp.; Right: With 25 °C Column Oven Temp.

■ Conclusion

LC/MS analysis of siRNA under denaturing and non-denaturing elution conditions was performed using an LCMS-9050 quadrupole time-of-flight mass spectrometer. Under denaturing conditions at a column temperature of 60 °C, siRNA was eluted in a dissociated single-stranded state, but at a column temperature of 25 °C, siRNA was eluted in a double-stranded state. By using the oligonucleotide analysis software LabSolutions Insight Biologics, multiple nucleic acid sequences can be analyzed at the same time.

■ Acknowledgments

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Related Applications

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