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Determination of molecular weight of small interfering nucleotides siRNA by LCMS

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1. Overview

In this paper, a method for the determination of molecular weight of small interfering nucleotides siRNA by LCMS was established□

2. Introduction

In this paper, the molecular weight of small interfering nucleotides siRNA was determined by using Shimadzu biological inert ultra-high performance liquid chromatography Nexera XS inert in combination with LCMS-2050. The sense chain and antisense chain of siRNA were separated by adjusting the gradient of mobile phase. The siRNA was analyzed by DUIS (ESI+APCI) ion source. The interference mass spectrum peak was removed by optimizing the mobile phase and mass spectrum collection parameters. The molecular weight of siRNA was accurately determined by using the "mass spectrum multi-charge analysis" function of LabSolutions software.

3. Methods

Instrument: Nexera XS inert + LCMS-2050
Column: Shim-Pack Scepter C18-120[Metal free] (50 mm×2.1 mm I.D.，3μm);
Mobile phase: A-10mM DIPEA+25mM HFIP+10μM EDTA，B- Acetonitrile; Flow: 0.3 mL/min;
Eluent mode: Gradient;
MS scanning range: 50~2000; Qarray voltage: -50 V.

Miniaturization and high performance

Mass range: 2~2000 Da

Ionization method: DULS(ESI and APCI)

Time switching from positive to negtive：10 ms

Scanning speed：15000 u/s

Figure 1　LCMS-2050 Single quadrupole mass spectrometer

4. Result

4-1. Method development

The siRNA sample is a double-stranded RNA, consisting of a sense chain(ss) and an antisense chain(as), respectively. Under chromatographic conditions, it is separated into two peaks, with a TIC shown in Figure 2 and a mass spectrometry shown in Figure 3. From the mass spectrum, it can be seen that the mass spectrum peaks are very chaotic, which causes serious interference in determining the molecular weight of siRNA. Therefore, it is necessary to optimize the method and remove the interfering mass spectrum peaks.

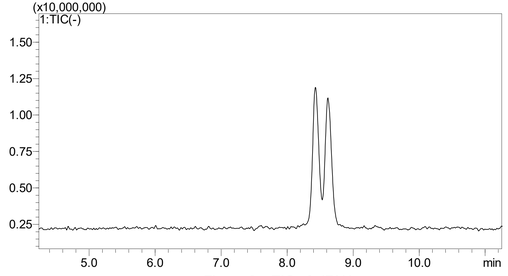

Figure 2　TIC of siRNA

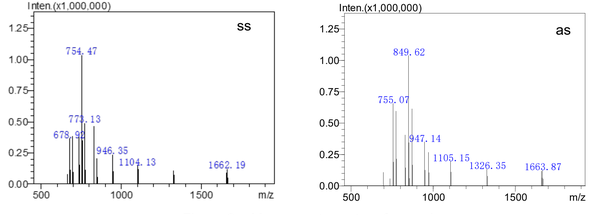

Figure 3　Mass spectrometry of ss and as

Taking the sense chain as an example, the optimization process of the method is described below. Deconvolution analysis was performed on the mass spectrum using the "Mass Spectrometry Multi Charge Analysis" function of Shimadzu LabSolutions™ software, and the analysis results are shown in Figure 4. In addition to the target molecular weight, there were also sodium addition peaks and hexafluoroisopropanol addition peaks in the deconvolution results

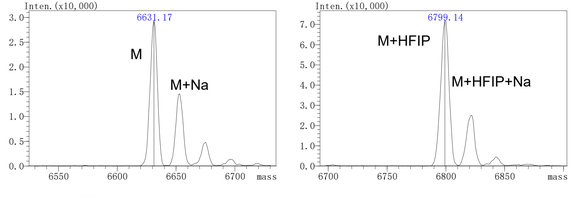

Figure 4　Molecular weight of sense chain obtained by deconvolution

In order to remove the additive ions in the mass spectrum, EDTA is added to the mobile phase for the complexation of sodium ions in the system. The multi charge deconvolution results are shown in Figure 5, showing that there is no obvious sodium addition peak in siRNA.

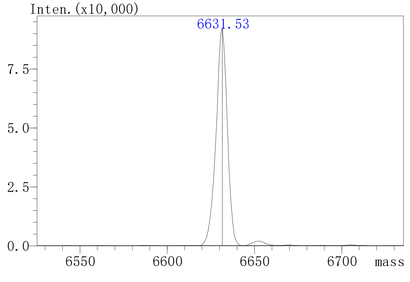

Figure 5　Deconvolution results of sense chain molecular weight after adding EDTA to the mobile phase

In order to remove the HFIP addition peak, the Qarray voltage was adjusted in this experiment. When the Qarray voltage is set to -50 V, the mass spectrum becomes very clean, removing the multi charge peaks of addition HFIP. The mass spectrum is shown in Figure 6,

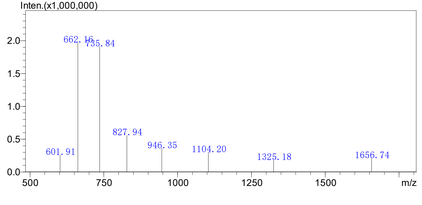

Figure 6　Mass spectrum when the Qarray voltage is set to -50 V

4-2. Mass spectrometry multi charge analysis results

Perform multi charge deconvolution analysis on the optimized mass spectrum, and the analysis results are shown in Figure 7. The results show that the siRNA sense chain mass spectrum contains eight ion peaks with different charges, and the number of charge is 4~11. The molecular weight calculated by software is 6631.65 Da. The mass spectrum of siRNA antisense chain contains seven ion peaks with different charges, and the number of charge is 4~10. The molecular weight calculated by software is 6637.53 Da.
The deviation between the molecular weight obtained by deconvolution and the theoretical value is 0.33 and 0.39 Da, respectively.

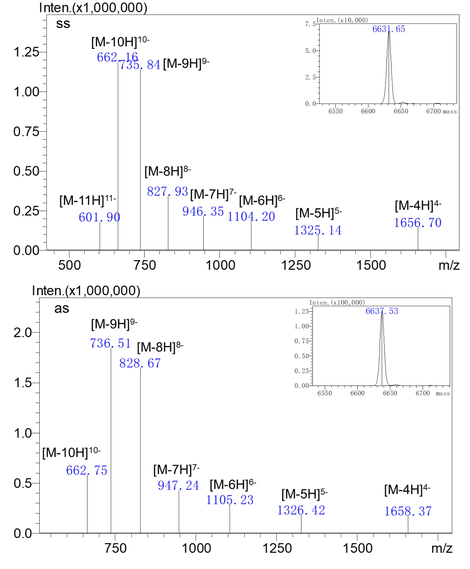

Figure 7 Multi charge and molecular weight deconvolution results of siRNA

5. Conclusions

This paper uses a biological inert ultra high performance liquid chromatography Nexera XS inert in combination with LCMS-2050 to determine the molecular weight of small interfering nucleotides siRNA. By optimizing the method, interference ion peaks in the mass spectrometry were removed, and the siRNA molecular weight was accurately measured using the mass spectrometry multi charge analysis function of LabSolutions software.