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Molecular Weight and Sequence Confirmation of Oligonucleotides by LCMS-9030 Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer

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1. Introduction

Oligonucleotides are short chain DNA or RNA molecule or their analogs, usually manufactured by chemical synthesis. Oligonucleotide therapeutics are a promising medicine to cure diseases by working at upper stream of action mechanism with fewer side effects. Molecular weight and molecular sequence are the critical quality attributes of the oligonucleotide therapeutics, which is usually analyzed by high-resolution mass spectrometry such as ESI-Q-TOF. However, spectral interpretation has become a bottleneck in mass spectrometry based sequencing experiments. In this study, a method are proposed for exact mass measurement and sequence confirmation of a 21 mer oligonucleotide by high-sensitivity and high-resolution LCMS-9030(ESI-Q-TOF) coupled with automatic spectral deconvolution and sequence confirmation software.

2. Experimental

2-1. System

MS and MS/MS spectrum of oligonucleotide were acquired by LCMS-9030 (ESI-Q-TOF, Shimadzu Corporation, Kyoto, Japan) via MS and MS/MS mode. The exact monoisotopic mass of oligonucleotide was confirmed by automatic deconvolution of MS spectrum using ReSpect algorithm in LabSolutions Insight Explore CSD software. The sequence of oligonucleotide was confirmed by Oligo module of the Protein Metrics software.

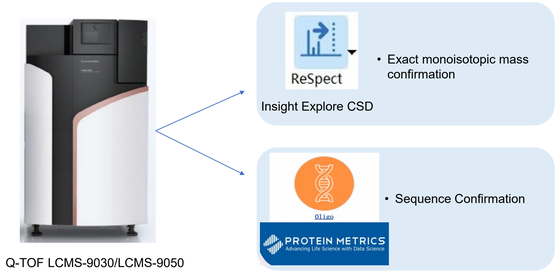

Figure1 LCMS-9030/LCMS-9050 (ESI-Q-TOF) coupled with molecular weight and sequence confirmation software

2-2. Sample preparation

A single strand and modified oligonucleotide (21 mer, Exact Mass: 6761.07876) was analyzed, the sequence was shown below.

5'-rGmUrArAmCmCrArArGrArGmUrAmUmUmCmCrAmUTT-3'

r: RNA; m: 2'-O-methyl modification.

Oligonucleotide was prepared at 100 pmol/μL in milliQ water.

2-3. Analytical condition

UHPLC（Nexera X3，Shimadzu）

Column:	XBridge Oligonucleotide C18 50 mm x 2.1 mm I.D., 2.5 μm
Mobile Phase A:	15 mM TEA, 400 mM HFIP prepared in H_2O
Mobile Phase B:	50% A in methanol
Flow Rate:	0.2 mL/min
Column Temperature:	60 ºC
Injection Volume:	1 μL
Gradient program:	

Time(min)	Module	Command	Value
10.00	Pumps	Pump B Conc.	40
11.00	Pumps	Pump B Conc.	40
12.00	Pumps	Pump B Conc.	100
13.00	Pumps	Pump B Conc.	100
13.10	Pumps	Pump B Conc.	20
15.00	Controller	Stop	

MS (LCMS-9030 ， ESI-Q-TOF ， Shimadzu)

Ionization:	ESI(-),MS&MS/MS		
Mass range:	MS1,500~2000 Da; MS2,100~2000 Da		
Nebulizing Gas Flow:	3.0 L/min	Interface Temperature:	350 ºC
Drying Gas Flow:	10.0 L/min	DL Temperature:	250 ºC
Heating Gas Flow:	10.0 L/min	HB Temperature:	400 ºC

3. Result

3-1. Molecular Weight confirmation

Oligonucleotide sample was subjected to an exact mass measurement using the LCMS-9030 in negative mode. The total ions chromatogram(TIC) and mass spectrum are shown in figure 2 and figure 3, respectively. Oligonucleotide target peak was observed clearly in the TIC. Multiple charged ions of oligonucleotide distributed from [M-4H] $^{4-}$ to [M-10H] $^{10-}$ were observed in the mass spectrum . The deconvoluted spectrum was obtained by "ReSpect" algorithm of LabSolutions Insight Explore CSD, as shown in figure 4. As shown in the figure, baseline separation was achieved for each isotope peak of the oligonucleotide. The exact monoisotopic mass was 6761.07876 Da, the accurate monoisotopic mass was 6761.07764 Da, the mass accuracy was about -0.16 ppm.

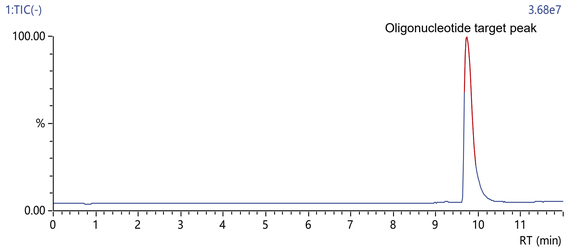

Figure 2 TIC of oligonucleotide sample

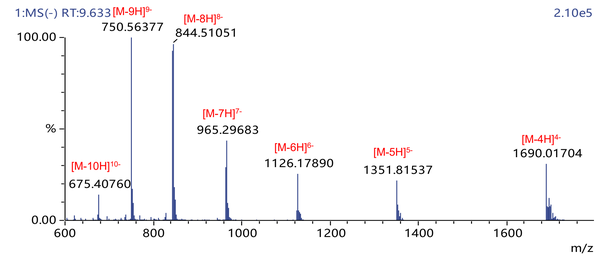

Figure 3 Mass spectrum of oligonucleotide sample

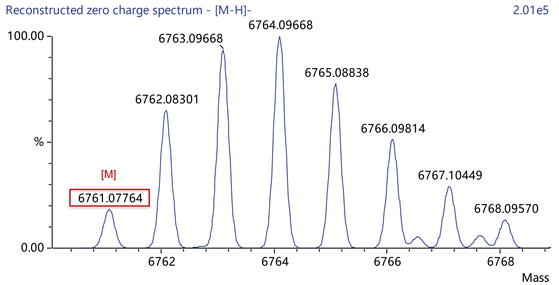

Figure 4 Deconvoluted spectrum of oligonucleotide sample

3-2. Sequence confirmation

The LCMS-9030 raw data containing secondary mass spectrum in the collision energy range of 10-80V together with oligonucleotide sequence were imported to the oligo module in Protein Metrics software. Next, sequence confirmation was conducted automatically via some simple parameter settings. The sequence confirmation results are shown in figure 5. A rich array of oligonucleotide fragment ions were observed and the fragment ion types were marked in the secondary mass spectrum. Oligonucleotide sequence was shown in the top right-hand corner. A set of red and blue L-shaped line symbols represent that the measured fragment ion at this position is consistent with the theoretical fragment ion. As shown in the figure, good matching between oligonucleotide sequence and the designed sequence was achieved.

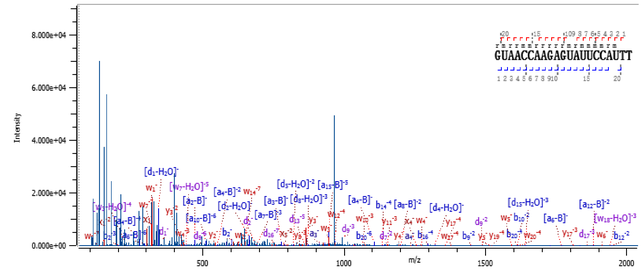

Figure 5 Sequence confirmation results of oligonucleotide

4. Conclusions

- A automatic and fast method was proposed for exact mass determination and sequence confirmation of modified oligonucleotide using LCMS-9030 Q-TOF coupled with LabSolutions Insight Explore CSD and Oligo module of Protein Metrics.
- LCMS-9030 has the advantages of high resolution, high mass accuracy and high sensitivity, can measure the accurate mass of oligonucleotides and their fragment ions. Baseline separation was achieved among oligonucleotide isotope peaks through automatic deconvolution processing of Insight Explore CSD. Consequently, high mass accuracy was achieved in this study just about -0.16 ppm.
- Oligo module of Protein Metrics can batch confirm oligonucleotide sequences automatically and quickly, with intuitive and accurate results.