High Sensitivity Detection of Synthetic Oligonucleotides in Biological Sample by HPLC-Chip/MS

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Introduction

Synthetic oligonucleotides have emerged as promising therapeutic agents for the treatment of a variety of diseases, including viral infections and cancer. Several classes of nucleic acids, such as antisense oligonucleotides, small interfering RNAs (siRNAs) and aptamers, are being investigated for therapeutic applications. While the different types of oligonucleotides work by distinct mechanisms of action, all are designed to modulate the expression of the targeted gene. As oligonucleotide drug discovery advances, efforts to develop more efficient, scalable and cost-effective synthesis and purification methods have intensified. LC/MS is an important characterization tool for oligonucleotide synthesis, enabling identification of process-related impurities and subsequent elucidation and optimization of process chemistries. Agilent 1200 Series LC platforms seamlessly couple with Agilent 6000 Series MS systems to deliver superior LC performance and mass accuracy and sensitivity for optimal LC/MS characterization of oligonucleotide. We demonstrate the characterization of synthesized three classes of oligonucleotide using Agilent 6530 Q-TOF platform.

Method

Agilent 1200 SL HPLC system and HPLC-Chip/MS Nano flow LC system were integrated with the Agilent 6530 Q-TOF . The POROSHELL 300Extend-C18 (1.0 x 75 mm, 5um) were used in Conventional LC system. The HPLC-Chip/MS integrates nano flow LC column, enrichment column and MS electrospray components into polyimide chip (Fig. 1). The nano flow LC column of HPLC-Chip was the ZORBAX 300SB-C18 (0.075 x 43 mm, 5um). The Agilent MassHunter Qualitative Analysis software was used for Q-TOF derived MS data analysis.

Table.2 HPLC and MS Analytical Condition (HPLC-Chip/MS system)

HPLC	: Agilent 1200
Column (Analysis)	: ZORBAX 300SB-C18 (0.075 x 43 mm, 3.5 um)
(Enrichment)	: ZORBAX 300SB-C18 (40 nL)
Mobile phase	: A: 5 mM Ammonium asetate buffer B: Acetonitrile 5 %B--(10min)--90 %B
Flow rate	: 0.6 uL/min
Mass spectrometer	: Agilent 6530 QTOF
Ionization	: ESI-Negative (HPLC-Chip)
Dry gas	: 5 L/min at 350℃
Fragmentor	: 250 v
Capillary Voltage	: 1850 v
Mass range	: *m/z* 200-3200
Acquisition mode	: High Resolution mode (4GHz) Extended Dynamic Range mode (2GHz)

Results and Discussion

1.Identification of Oligonucleotide by Agilent 1200SL HPLC system

Duplex and single strands in the synthesized oligonucleotide were chromatographically-separated with the LCMS system. From the result, it is possible to detect the single strand RNA as impurities. It is also identified the duplex RNA by the deconvolution using the measured duplex masses.

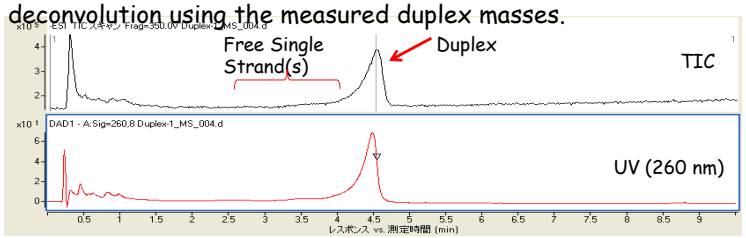
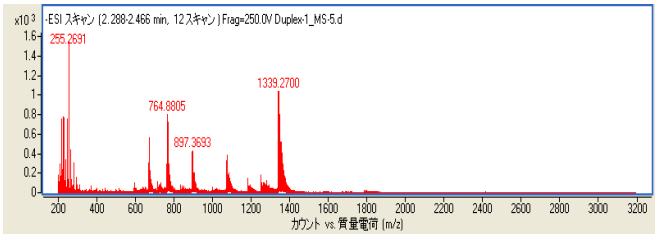


Fig. 2 Chromatography of Oligonucleotide

3.Identification and Sensitivity of Oligonucleotide by HPLC-Chip/MS system

It is necessary that high sensitivity may detect the oligonucleotide in biological sample (plasma, Urine and cell extract). We developed the Nano flow LC system to solve the problem. The Agilent HPLC-Chip/MS is an easy-to-use and high sensitivity nano flow LC/MS system, and the sensitivity was improveed 100 times compared with Agilent 1200 SL HPLC system (the pM level can be detected).



Deconvoluted Sense strand Deconvoluted Antisense strand

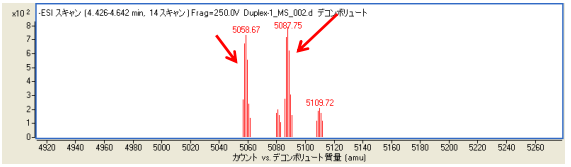
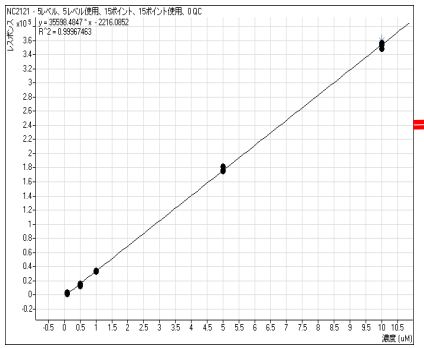


Fig. 5 Mass Spectrum of Oligonucleotide and Deconvolution result



Duplex concentration : 0.1 uM – 10 uM

LOD : 0.00009 uM

Linearity :R^2=0.9989

Fig. 6 Linearity of Oligonucleotide

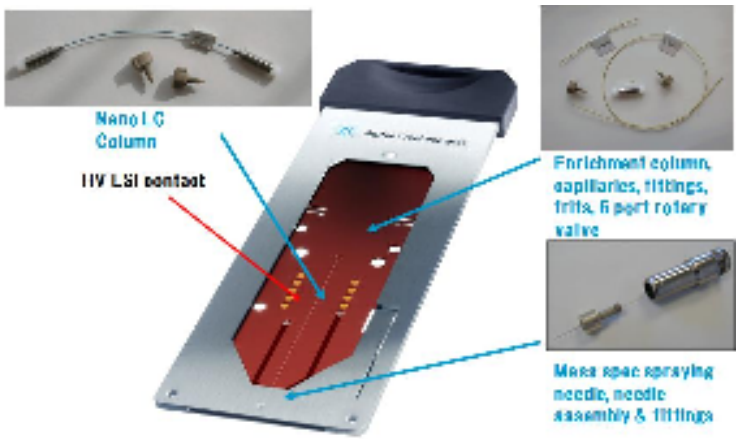


Fig. 1 HPLC-Chip

Table.1 HPLC and MS Analytical Condition (Agilent 1200 SL HPLC system)

HPLC	: Agilent 1200 SL
Column	: POROSHELL 300Extend-C18 (1.0 x 75 mm, 5 um)
Oven temp	: 40 ℃
Mobile phase	: A:100 mM Triethylammonium asetate buffer B: Acetonitrile 5 %B--(20min)--30 %B
DAD	: 260 nm
Flow rate	: 0.2 mL/min
Mass spectrometer	: Agilent 6530 QTOF
Ionization	: Dual ESI-Negative
Nebulizer gas	: 35 psi
Dry gas	: 9 L/min at 350℃
Fragmentor	: 250 v
Capillary Voltage	: 3500 v
Mass range	: *m/z* 400-3200
Acquisition mode	: High Resolution mode (4GHz) Extended Dynamic Range mode (2GHz)

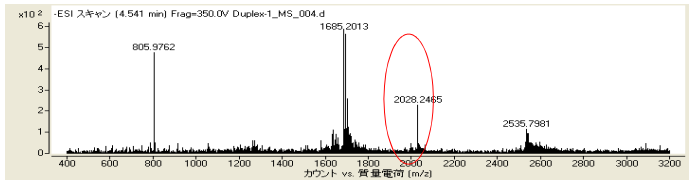
Experimental Sequence

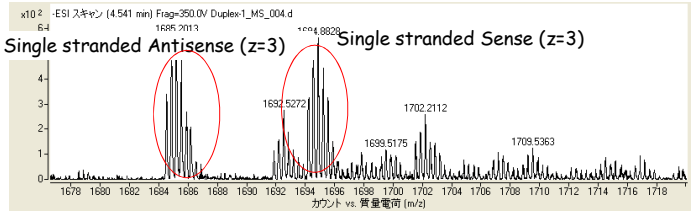
Sense Strand

5'-ccc-acg-aaa-agu-uug-a-3'

' Antisense Strand

5'-uca-aac-uuu-ucg-ugg-g-3'





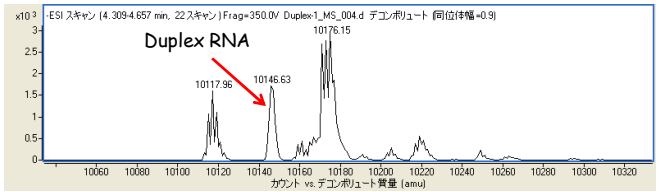


Fig. 3 Mass Spectrum of Oligonucleotide and Deconvolution result

2.Sensitivity of Oligonucleotide

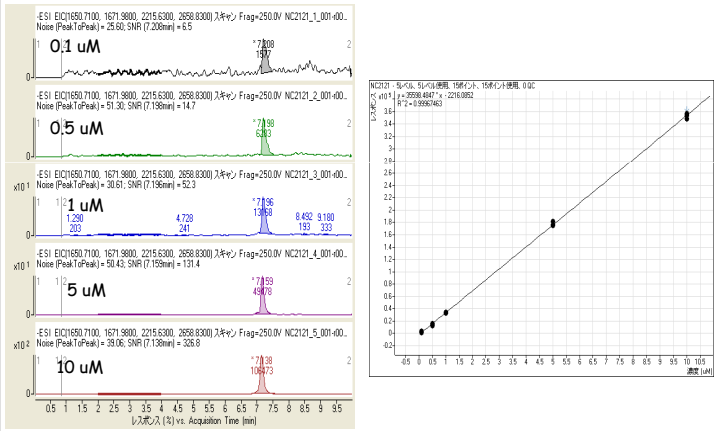


Fig. 4 Linearity of Oligonucleotide

Duplex concentration : 0.1 uM – 10 uM

LOD : 0.009 uM

Linearity :R^2=0.9996

4.Preparation of Oligonucleotide in Biological Sample

The preparation for synthesized oligonucleotide in biological sample is important, we propose a new preparation method by using oligonucleotide spiked sample to the plasma.

Workflow

The oligonucleotide spiked Plasma

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The high abundant protein is removed to the MARS column

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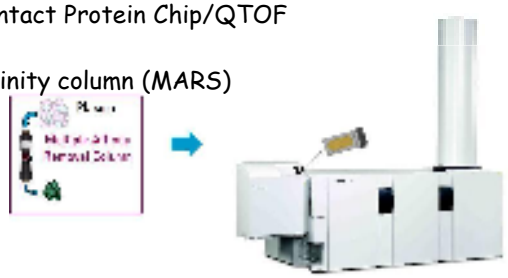
Analysis of Intact Protein Chip/QTOF

Affinity column (MARS)



Intact Protein Chip/QTOF System

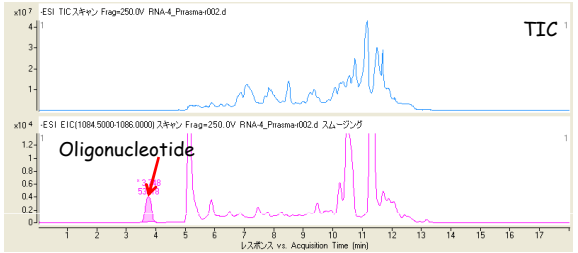


Fig. 7 Detection of oligonucleotide in plasma

Summary

1. Oligonucleotide (Duplex RNA) could be identified with the LCMS system.
2. The high sensitivity analysis of oligonucleotide was possible by the use of HPLC-Chip/MS.
3. The oligonucleotide in the plasma could be detected by a new preparation.