Analysis of Various Oligonucleotides by LC/UV/MS (VN-50 2D)

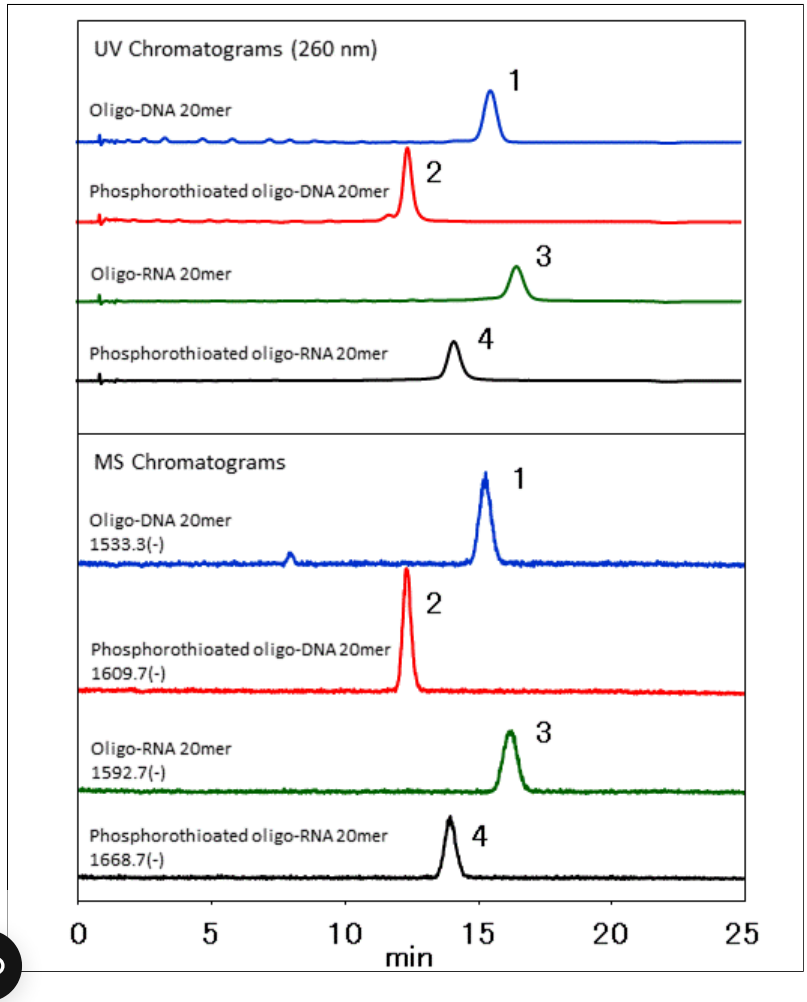
Nucleic acids are composed of pentacarbonate, phosphoric acid, and base. While pentacarbonate in RNA is ribose, in DNA, it is deoxyribose (hydroxyl group (OH) at the 2-position of ribose is replaced by hydrogen (H)). Phosphorothioated oligonucleic acids are often used in nucleic acid drugs, because they are more stable than regular oligonucleic acids.

In this application, synthetic oligo-DNA, synthetic phosphorothioated oligo-DNA, synthetic oligo-RNA, and synthetic phosphorothioated oligo-RNA were analyzed by LC/UV/MS using HILICpak VN-50 2D, a polymer-based HILIC column.

Under HILIC mode, the higher the hydrophilicity, the stronger the retention becomes. Thus, since synthetic oligo-RNA has higher hydrophilicity than synthetic oligo-DNA, its retention becomes longer (compare peaks 1 and 3). Meanwhile, in a phosphorothioated oligonucleotide's phosphate linkage, an oxygen atom is replaced by a sulfur atom. This makes it more hydrophobic, and thus its retention becomes weaker than non-phosphorothioated types (compare peaks 1 and 2 and peaks 3 and 4).

The application developed here does not require a use of ion-pairing reagent nor highly concentrated salt in the eluent. Therefore, it is suitable for LC/MS analysis of oligonucleotides.

[Analysis of 2'-OMe and 2'-MOE Modified Phosphorothioated Oligo-RNA by LC/UV/MS (VN-50 2D)]



Sample : 0.1 mg/mL each (in H_2O), 1 μL

1. Synthesized oligo-DNA 20mer(crude),
ATACCGATTAAGCGAAGTTT

2. Synthesized phosphorothioated oligo-DNA 20mer(crude),
A*T*A*C*C*G*A*T*T*A*A*G*C*G*A*A*G*T*T*T

3. Synthesized oligo-RNA 20mer(cartridge purified),
AUACCGAUUAAGCGAAGUUU

4. Synthesized phosphorothioated oligo-RNA 20mer(cartridge purified),
A*U*A*C*C*G*A*U*U*A*A*G*C*G*A*A*G*U*U*U

* means phosphorothioated position

Column	: Shodex HILICpak VN-50 2D (2.0 mm I.D. x 150 mm)
Eluent	: (A) 50 mM $HCOONH_4$ aq. (pH9.8) / (B) CH_3CN
	Linear gradient ;
	(B %) 64 to 56 % (0 to 10 min), 56 % (10 to 20 min),
	56 to 64 % (20 to 20.01 min), 64 % (20.01 to 25 min)
Flow rate	: 0.3 mL/min
Detector	: UV (260 nm) (small cell volume), ESI-MS (SIM Negative)
Column temp.	: 40 °C

Sample Name Index

Oligodeoxyribonucleotide, Oligo-DNA

Oligoribonucleotide, Oligo-RNA

Phosphorothioated oligodeoxyribonucleotide, Phosphorothioated oligo-DNA

Phosphorothioated oligoribonucleotide, Phosphorothioated oligo-RNA

Product Name Index

VN-50 2D (HILICpak)

Applications

High Sensitive Analysis of Synthetic Oligo-DNAs by LC/MS (Comparison between VN-50 1D and VN-50 2D)

LC/UV/MS Analysis of Oligo-DNA (VN-50 2D)

Analysis of Phosphorothioated Oligo-DNA (VN-50 2D)

Analysis of Oligo-DNAs (VN-50 2D)

Analysis of 2'-OMe and 2'-MOE Modified Phosphorothioated Oligo-RNA by LC/UV/MS (VN-50 2D)

Analysis of 10 - 50mer Oligo-DNAs (VN-50 2D)

Analysis of Oligonucleotides and Their Impurities (1) Truncated Oligonucleotides (VN-50 2D)

Analysis of Oligonucleotides and Their Impurities (2) Base Alteration (VN-50 2D)

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