

アンチセンスオリゴ核酸と等モル不純物の分析

Analysis of antisense oligonucleotides and equimolar impurities such as PO or n – 1

近年、医薬品業界では低分子医薬や抗体医薬に続く新たなモダリティとして核酸医薬が注目されています。疾病の原因となるような遺伝子の作用をメッセンジャーRNAによる翻訳段階で抑制できるアンチセンス核酸は一本鎖で構成され、その多くは生体内におけるヌクレアーゼ耐性を向上させるためホスホロチオエート (PS) を含みます。今回は逆相イオン対 (IP-RP) HPLCにより、ヌシネルセン、ボラネソルセン及びアリカフォルセンと同じ配列及び修飾である合成PSオリゴ核酸 (NUS, VOL, ALI) と等モルの不純物 (PO又はn – 1、詳細はTableを参照) を分析しました。L-column3 C18 2 µm を用いて新規に開発されたトリブチルアミン及びヘプタン酸を用いるIPシステム [1]により、NUS, VOL及びALIは、等モルのPO又はn – 1不純物から良好に分離されました。

Key words : IP-RP HPLC, antisense oligonucleotide, tributylamine, heptanoic acid
Column : L-column3 C18 (USP category: L1)

[Analytical conditions]

Column : L-column3 C18 (2 µm, 12 nm); 2.1 mm I.D. × 50 mm L.; Cat. No. 813140
Eluent : A: 10 mmolL⁻¹ tributylamine, heptanoic acid (pH 7)
B: CH₃CN
A/B, 57/43-55/45 (0-20 min)
Flow rate : 0.2 mL/min
Temperature : 60°C
Detection : UV 265 nm
Injection volume : 0.2 µL
System : NEXERA XR (SHIMADZU CORPORATION)
Mixer volume : 180 µL
Sample : 10 µmolL⁻¹ NUS, NUS (3'-PO), NUS (3'-n – 1), VOL, VOL (3'-PO), VOL (3'-n – 1), ALI, ALI (3'-PO), and ALI (3'-n – 1) in H₂O

Table Sequences of PS oligonucleotides [1]

Sample	Sequences (5'-3')
NUS	MeU [^] MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] A [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^] G
NUS (3'-PO)	MeU [^] MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] A [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^] G
NUS (3'-n – 1)	MeU [^] MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] A [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^]
VOL	A [^] G [^] MeC [^] MeU [^] MeU [^] MeC [^] t [^] t [^] g [^] t [^] MeC [^] MeC [^] a [^] g [^] MeC [^] MeU [^] MeU [^] MeU [^] A [^] MeU [^]
VOL (3'-PO)	A [^] G [^] MeC [^] MeU [^] MeU [^] MeC [^] t [^] t [^] g [^] t [^] MeC [^] MeC [^] a [^] g [^] MeC [^] MeU [^] MeU [^] MeU [^] A [^] MeU [^]
VOL (3'-n – 1)	A [^] G [^] MeC [^] MeU [^] MeU [^] MeC [^] t [^] t [^] g [^] t [^] MeC [^] MeC [^] a [^] g [^] MeC [^] MeU [^] MeU [^] MeU [^] A [^]
ALI	g [^] c [^] c [^] c [^] a [^] a [^] g [^] c [^] t [^] g [^] g [^] c [^] a [^] t [^] c [^] c [^] g [^] t [^] c [^] a
ALI (3'-PO)	g [^] c [^] c [^] c [^] a [^] a [^] g [^] c [^] t [^] g [^] g [^] c [^] a [^] t [^] c [^] c [^] g [^] t [^] ca
ALI (3'-n – 1)	g [^] c [^] c [^] c [^] a [^] a [^] g [^] c [^] t [^] g [^] g [^] c [^] a [^] t [^] c [^] c [^] g [^] t [^] c

Upper- and lower-case letters indicate 2'-OH RNA and 2'-H DNA nucleotides, respectively.

"^": PS linkage, under lines: O-methoxyethyl nucleotides, MeC or Mec: 5-methyl cytosine,

MeU: 5-methyl uracil, PO: phosphodiester impurity, n – 1: n – 1 truncated sequence.

ALI has the same sequence as alicaforsen, whereas NUS and VOL have identical sequences or modifications as approved antisense oligonucleotides, such as nusinersen and volanesorsen, respectively.

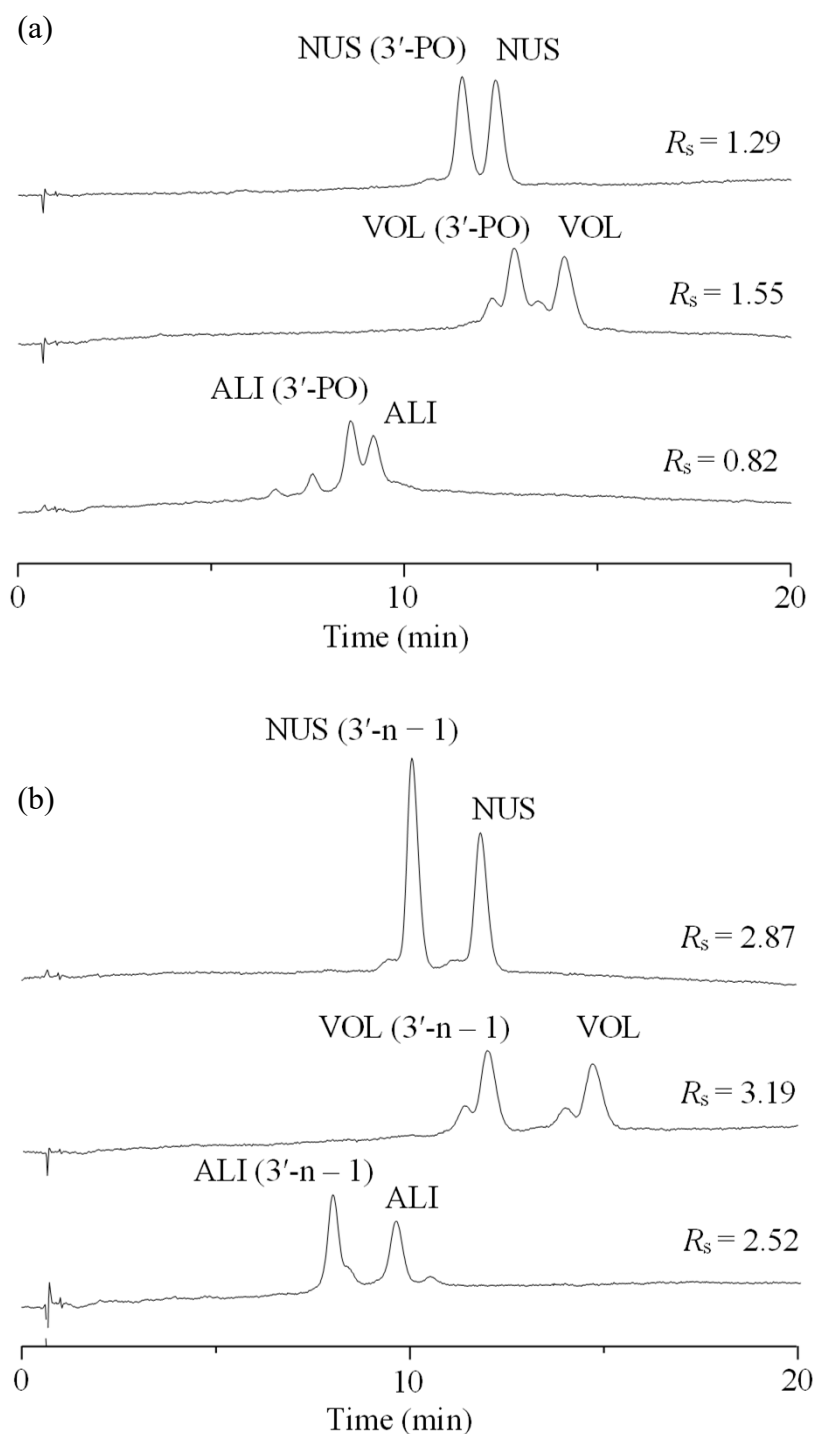


Figure Separations of PS oligonucleotides from equimolar (a) phosphodiester impurity (PO) and (b) $n - 1$ truncated sequence ($n - 1$) using IP system with tributylamine and heptanoic acid, optimized for selectivity [1].

Figure はNUS、VOL及びALIの等モルのPO又は $n - 1$ 不純物からの良好な分離を実証しています。トリブチルアミンとヘプタン酸を用いるIPシステムは選択性が最適化されているため、PSオリゴ核酸と不純物はシャープなピーク形状で効果的に分離されます [1]。

[1] Y. Obata, H. Sakamaki, J. Chromatogr. A 1750 (2025) 465915. <https://doi.org/10.1016/j.chroma.2025.465915>.

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