

ヌシネルセンのスパイク不純物の分析

Analysis of spiked impurities of nusinersen such as PO and 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アート（PS）を含みます。今回は逆相イオン対（IP-RP）HPLCにより、ヌシネルセンと同じ配列及び修飾である合成PSオリゴ核酸（NUS）とスパイク不純物（PO及び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] は、NUSにスパイクされたPO及びn – 1不純物を良好に分離することができるため優れた定量性を示します。

Key words : IP-RP HPLC, Phosphorothioate oligonucleotide, tributylamine, heptanoic acid, Metal-free column
Nusinersen
Column : L-column3 C18 (USP category: L1)

[Analytical conditions]

Column : L-column3 C18 (2 µm, 12 nm); 2.0 mm I.D. × 150 mm L. Metal-free; Cat. No. 863020
Eluent : A: 10 mmolL⁻¹ tributylamine, heptanoic acid (pH 7)
B: eluent A/CH₃CN (50/50)
A/B, 12/88-6/94 (0-30 min)
Flow rate : 0.2 mL/min
Temperature : 60°C
Detection : UV 265 nm
Injection volume : 1 µL
System : NEXERA XR (SHIMADZU CORPORATION)
Mixer volume : 180 µL
Sample : (a) 100 µmolL⁻¹ NUS, 1 µmolL⁻¹ NUS (5'-n – 1)/NUS (m-n – 1)/NUS (3'-n – 1) in H₂O
100 µmolL⁻¹ NUS, 1 µmolL⁻¹ NUS (5'-PO)/NUS (m-PO)/NUS (3'-PO) in H₂O
(b) 100 µmolL⁻¹ NUS, 0.2-5 µmolL⁻¹ NUS (5'-PO)/NUS (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 (m-PO)	MeU [^] MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] A [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^] G
NUS (5'-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 [^] G
NUS (m-n – 1)	MeU [^] MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^] G
NUS (5'-n – 1)	MeC [^] A [^] MeC [^] MeU [^] MeU [^] MeU [^] MeC [^] A [^] MeU [^] A [^] A [^] MeU [^] G [^] MeC [^] MeU [^] G [^] G

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

"^": PS linkage, under lines: O-methoxyethyl nucleotides, MeC: 5-methyl cytosine, MeU: 5-methyl uracil,
PO: phosphodiester impurity, n – 1: n – 1 truncated sequence. NUS has identical sequences or modifications as approved antisense oligonucleotides, such as nusinersen.

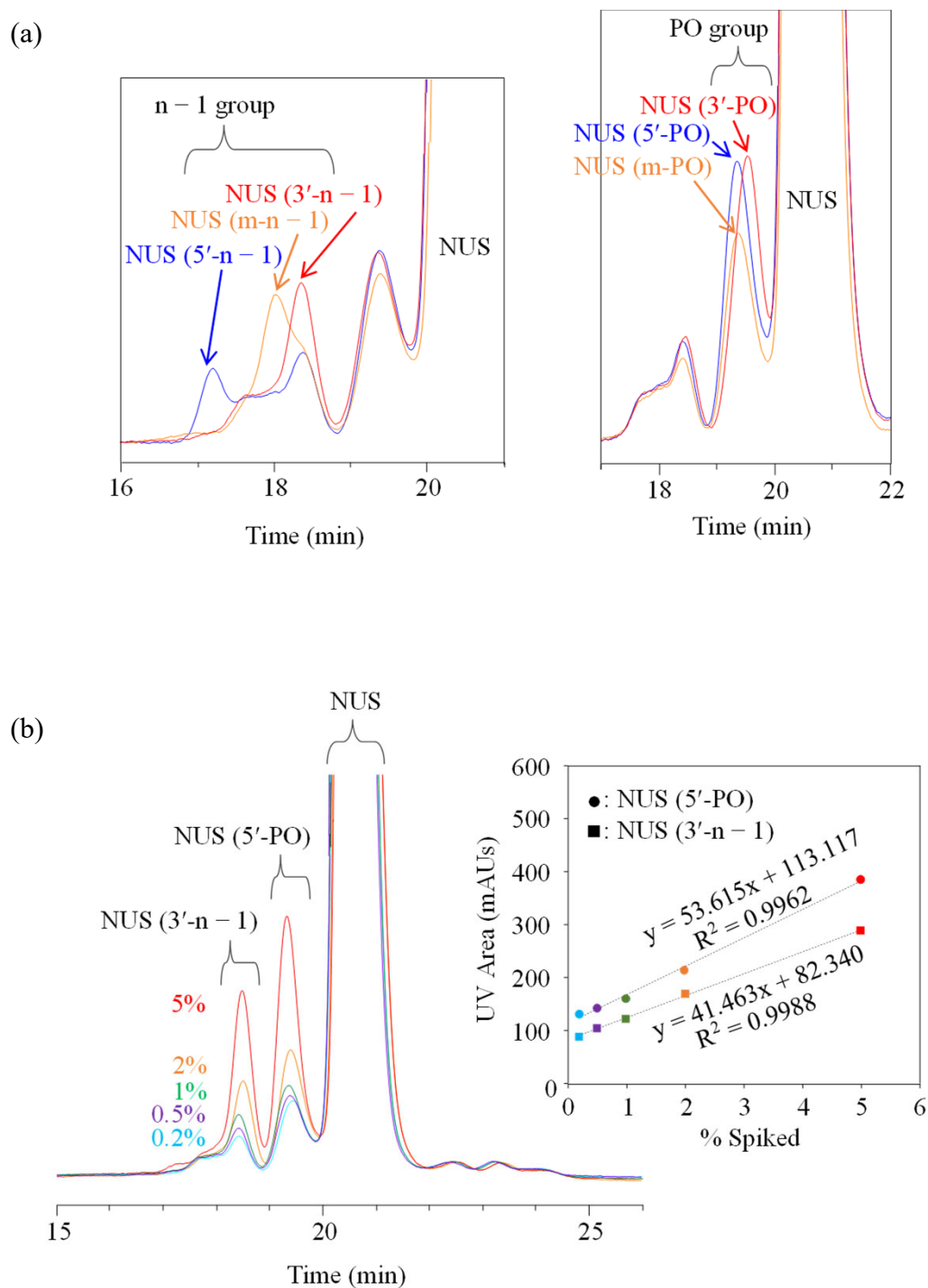


Figure Analysis of spiked impurities, such as PO and $n - 1$, using the IP system with tributylamine and heptanoic acid for optimized selectivity. (a) Effect of different positions of PO or $n - 1$ on the separation for 1% spiked impurity and (b) quantification of spiked impurities (0.2%–5%) [1].

PO及び $n - 1$ 不純物をスパイクさせた $100 \mu\text{molL}^{-1}$ NUS溶液を分析しました。不純物の非特異性吸着損失を防ぐため、メタルフリーカラムを使用しています。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、1%スパイクされたすべての異なる位置のPO又は $n - 1$ はNUSから効果的に分離できます (Figure (a))。さらに、0.2%–5%のスパイクされたNUS (5'-PO)及びNUS (3'- $n - 1$) に対して、このIPシステムは優れた分離に基づく優れた定量的性能を発揮します (Figure (b)) [1]。

[1] Y. Obata, H. Sakamaki, J. Chromatogr. A 1750 (2025) 465915. <https://doi.org/10.1016/j.chroma.2025.465915>. 2025.05 Oba