For impurity analysis of oligonucleic acids using HPLC or LC-MS!

HFIP for Nucleic Acid Analysis

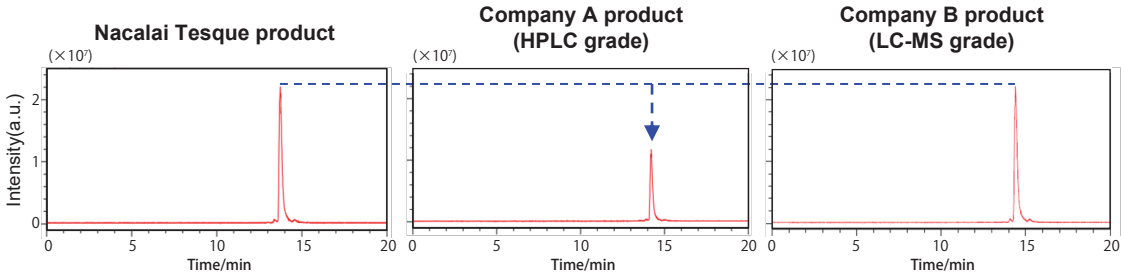
1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) is frequently used as a mobile phase additive for analysis of oligonucleotides below 100 nucleotides (nt) and their impurities, due to the high sensitivity and separation it enables. However, there are significant differences in LC-MS sensitivity and even detection of unidentified peaks depending on the manufacturer, product grade, and lot. This product is specifically tested using LC-MS for each batch, so researchers can be confident in their reagents.

Features

- **Suitable for analysis of oligonucleotides using HPLC and LC-MS**
- **Suitable for analysis of oligonucleotide impurities (short-mers, long-mers, phosphodiester [PO] impurities in phosphorothioated [PS] products)**
- **Each product lot tested using LC-MS**

Performance comparison

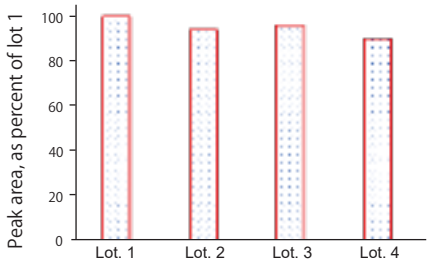
Using the oligonucleotide (Poly(dT)$_{19}$), LC-MS sensitivity was compared between this product and competitor HPLC and LC-MS grade products.



<LC conditions>
Column: COSMOCORE 2.6C$_{18}$ 2.1 mm I.D. × 100 mm
Mobile phase:
A: 100 mM HFIP-15 mM Triethylamine (TEA)
B: Solvent A / Methanol = 1 / 1 (v / v)
B conc. 20 → 50% (0 → 20 min)
Flow rate: 0.2 mL/min
Temperature: 40℃

<MS conditions>
Equipment: LCMS 2050 (Shimadzu)
Ionization: ESI/APCI (Negative), TIC
Mode: Scan
Mass range: 550-2000
Nebulizing gas flow: 2.0 L/min
Drying gas flow: 5.0 L/min
Heating gas flow: 7.0 L/min
DL temperature: 200 ºC
Desolvation temperature: 450 ºC
Interface voltage: -2.0 kV

Compared to the company A product (HPLC grade), our product showed about twice the sensitivity. Additionally, it had about the same sensitivity as the company B product (LC-MS grade).



Purity, non-volatile matter, water, absorbance, and metal content

Assay (GC)	> 99.8%	Ca	< 0.5 ppm	Mg	< 0.1 ppm
Non-volatile matter	< 0.01%	Cd	< 0.05 ppm	Mn	< 0.02 ppm
Water	< 0.05%	Co	< 0.02 ppm	Mo	< 0.1 ppm
Abs. (260 nm)	< 0.01	Cr	< 0.02 ppm	Na	< 1 ppm
Metal content		Cu	< 0.02 ppm	Ni	< 0.02 ppm
Al	< 0.5 ppm	Fe	< 0.1 ppm	Pb	< 0.1 ppm
Ba	< 0.1 ppm	K	< 0.5 ppm	Sr	< 0.1 ppm
Bi	< 0.1 ppm	Li	< 0.1 ppm	Zn	< 0.1 ppm

Comparing 4 different lots, the difference in sensitivity was found to be within 10%. Furthermore, there was no significant difference in purity and metal content.

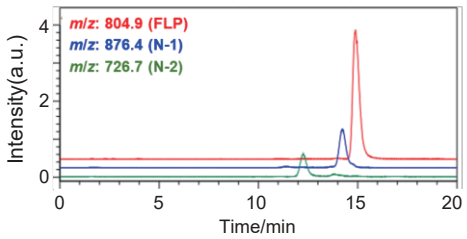
Analysis by LC-MS

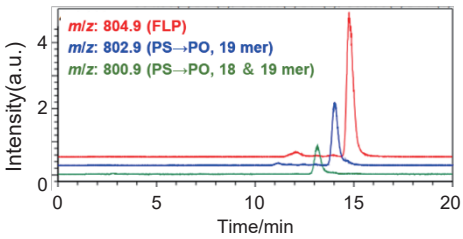
■ Phosphorothioated oligonucleotides

Sequence

FLP:	5'-A^T^C^G^G^G^A^G^G^G^C^T^T^A^C^C^A^T^C-3'
N-1:	5'-A^T^C^G^G^G^A^G^G^G^C^T^T^A^C^C^A^T-3'
N-2:	5'-A^T^C^G^G^G^A^G^G^G^C^T^T^A^C^C^A-3'
PS→PO, 19 mer:	5'-A^T^C^G^G^G^A^G^G^G^C^T^T^A^C^C^A^T-C-3'
PS→PO, 18＆19 mer:	5'-A^T^C^G^G^G^A^G^G^G^C^T^T^A^C^C^A-T-C-3'

* Phosphodiester bonds (PO) converted to phosphorothioate (PS)　^ : PS bond　- : PO bond

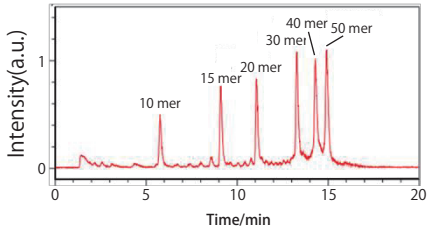




<Conditions>
Column: COSMOCORE 2.6C$_{18}$ 2.1 mm I.D. × 100 mm
Mobile phase: A: 100 mM HFIP-15 mM Triethylamine (TEA)
B: Solvent A / Acetonitrile / Methanol = 2 / 1 / 1 (v / v / v)
B conc. 14 → 20%（0 → 20 min）
Flow rate: 0.2 mL/min　Sample conc.: 100 μM (FLP) and 25 μM (others)
Temperature: 65℃　Inj. Vol.: μL
Ionization: ESI/APCI (Negative), SIM

By optimizing the analysis conditions, we were able to separate the target compound from impurities that are produced during synthesis.

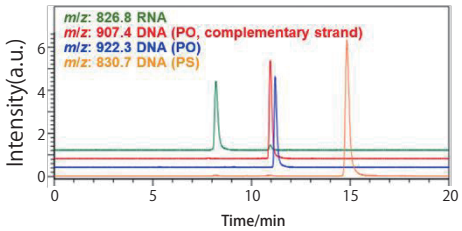
■ DNA ladder



<Conditions>
Column: COSMOCORE 2.6C$_{18}$　2.1 mm I.D. × 100 mm
Mobile phase: A: 100 mM HFIP-15 mM Triethylamine (TEA)
B: Solvent A / Acetonitrile / Methanol = 2 / 1 / 1 (v / v / v)
B conc. 7.5 → 27.5%（0 → 20 min）
Flow rate: 0.2 mL/min　Sample conc.: 10 μM
Temperature: 65℃　Inj. Vol.: 3 μL
Ionization: ESI/APCI (Negative), TIC

Because hydrophobicity increases with oligonucleotide length, longer strands are retained for longer.

■ ssDNA (PO and PS forms), ssRNA



<Conditions>
Column: COSMOCORE 2.6C$_{18}$　2.1 mm I.D. × 100 mm
Mobile phase: A: 100 mM HFIP-15 mM Triethylamine (TEA)
B: Solvent A / Methanol = 1 / 1 (v / v)
B conc. 20 → 60%（0 → 20 min）
Flow rate: 0.2 mL/min　Sample conc.: 25 μM
Temperature: 40℃　Inj. Vol.: 3 μL
Ionization: ESI/APCI (Negative), SIM

Hydrophobicity increases in the order of RNA < DNA (PO) < DNA (PS), and the strands elute in the same order. Hydrophobicity also changes depending on the DNA sequence, so strands with similar length may have different retention times.

Reference

Separation and purification of short-, medium-, and long-stranded RNAs by RP-HPLC using different mobile phases and C$_{18}$ columns with various pore sizes
Ozaki M, et al. Anal. Methods. 2024;16:1948-1956.
https://doi.org/10.1039/D4AY00114A　※ Featured on Front Cover

Separation of long-stranded RNAs by RP-HPLC using an octadecyl-based column with super-wide pores
Kuwayama T, et al. Anal. Sci. 2023;39:417-425.
https://doi.org/10.1007/s44211-022-00253-w　※ Selected as Hot Articles 2023

Ozaki M, et al. Medical Science Digest December Special Issue. 2023, 49, p.40-43.

Cover art on the front cover of Analytical Methods and the award certificate for being selected as one of the Hot Articles 2023.

Ordering Information

Product Name	Grade	Storage	Catalog No.	PKG Size
1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) for nucleic acid analysis	SP	Room temp.	22941-42	25 mL

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The quality for certainty.

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