APPLICATION DATA

Nucleic acids
HPLC/IEX/SEC

Contents

Technical Data

- High resolution analysis of Oligonucleotides on reversed phase chromatography
- Separation of chemically modified oligonucleotides using YMC-Triart Bio C4
- Optimization of oligonucleotide setarations on ion-exchange chromatography

Analytical Data

YMC CO., LTD.
http://www.ymc.co.jp

YMC SEPARATION TECHNOLOGY

HPLC DATA SHEET

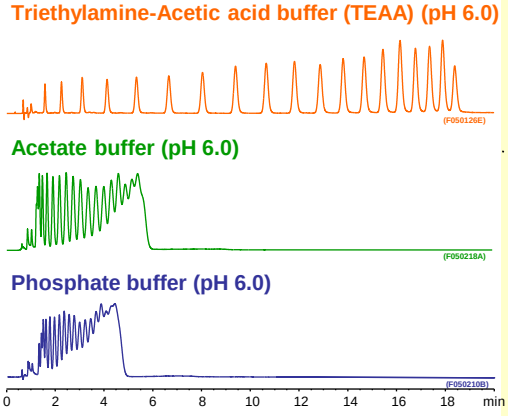
High resolution analysis of Oligonucleotides on reversed phase chromatography

F121018AE-2

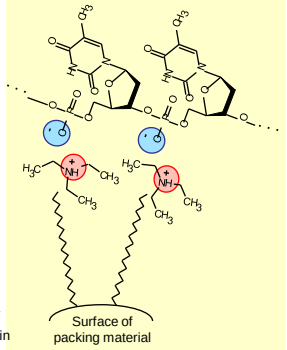
Oligonucleotides analysis on reversed-phase ion-pair chromatography (RP-IPC)

Comparison of retention and separation under various mobile phase conditions

Oligodeoxythymidylic acid, [d(T)$_{2\text{-}20}$]



Retention mechanism of oligonucleotide on RP-IPC (Image)



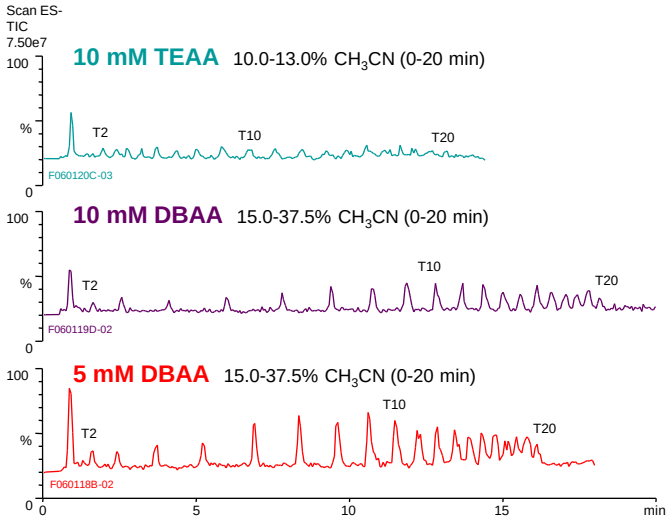
Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 4.6 mmI.D.	Flow rate	: 1.0 mL/min
Eluent	: A) 100 mM buffer B) 100 mM buffer/acetonitrile (80/20) 50-65%B (0-20 min)	Temperature	: 35ºC
		Detection	: UV at 269 nm
		Injection	: 5 µL (5 nmol/mL)

- Separations of oligodeoxythymidylic acid [d(pT)$_{2\text{-}20}$] under three different buffer systems are compared.
- d(pT)$_{2\text{-}20}$ is well retained under TEAA buffer system, and good separation even by one-nucleotide difference is achieved. On the other hand, retention and separation of d(pT)2-20 under acetate buffer and phosphate buffer are insufficient.
- Ion-pairing reagents that have both positive charge and hydrophobic moiety in molecule, such as triethylamine (TEA) or dibutylamine (DBA), form ion pair with negatively charged oligonucleotides. This interaction contributes to long retention and improvement on resolution.

Applicability to LC/MS analysis

Impact of concentration and types of ion-paring reagent on resolution and signal intensity

Oligodeoxythymidylic acid, [d(T)$_{2\text{-}20}$]

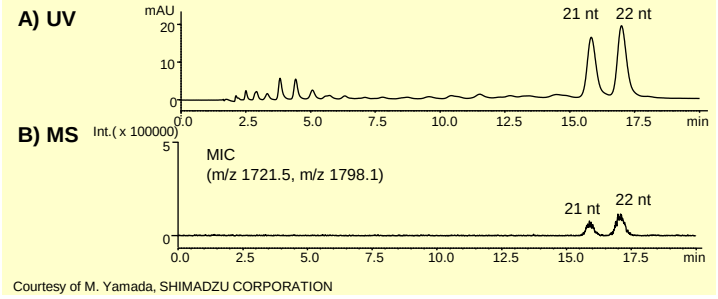


Column	: Hydrosphere C18 (3 µm, 12 nm), 50 X 2.0 mmI.D.
Eluent	: A) 10 mM triethylamine-acetic acid (pH 6.0) B) 10 mM triethylamine-acetic acid (pH 6.0)/acetonitrile (80/20) 50-65%B (0-20 min) A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) 10 mM di-n-butylamine-acetic acid (pH 6.0)/acetonitrile (50/50) 30-75%B (0-20 min) A) 5 mM di-n-butylamine-acetic acid (pH 6.0) B) 5 mM di-n-butylamine-acetic acid (pH 6.0)/acetonitrile (50/50) 30-75%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 35ºC
Detection	: ESI-negative mode
Injection	: 5 µL (5 nmol/mL)

- TEA and DBA are both volatile ion-pairing reagents, and applicable to LC/MS analysis. When comparing the separation characteristics of d(pT)$_{2\text{-}20}$ with those reagents under the same buffer concentration, signal intensity and retention with DBA is superior to that with TEA.
- At 5 mM dibutylamine-acetic acid buffer (DBAA) condition, higher signal intensity of oligonucleotides is achieved even though retention and resolution is slightly decreased.

LC/MS analysis of miRNA

5'-pUGG AGU GUG ACA AUG GUG UUG-3' (21 nt, MW 6890.1)
5'-pUGG AGU GUG ACA AUG GUG UUG U-3' (22 nt, MW 7196.3)



Courtesy of M. Yamada, SHIMADZU CORPORATION

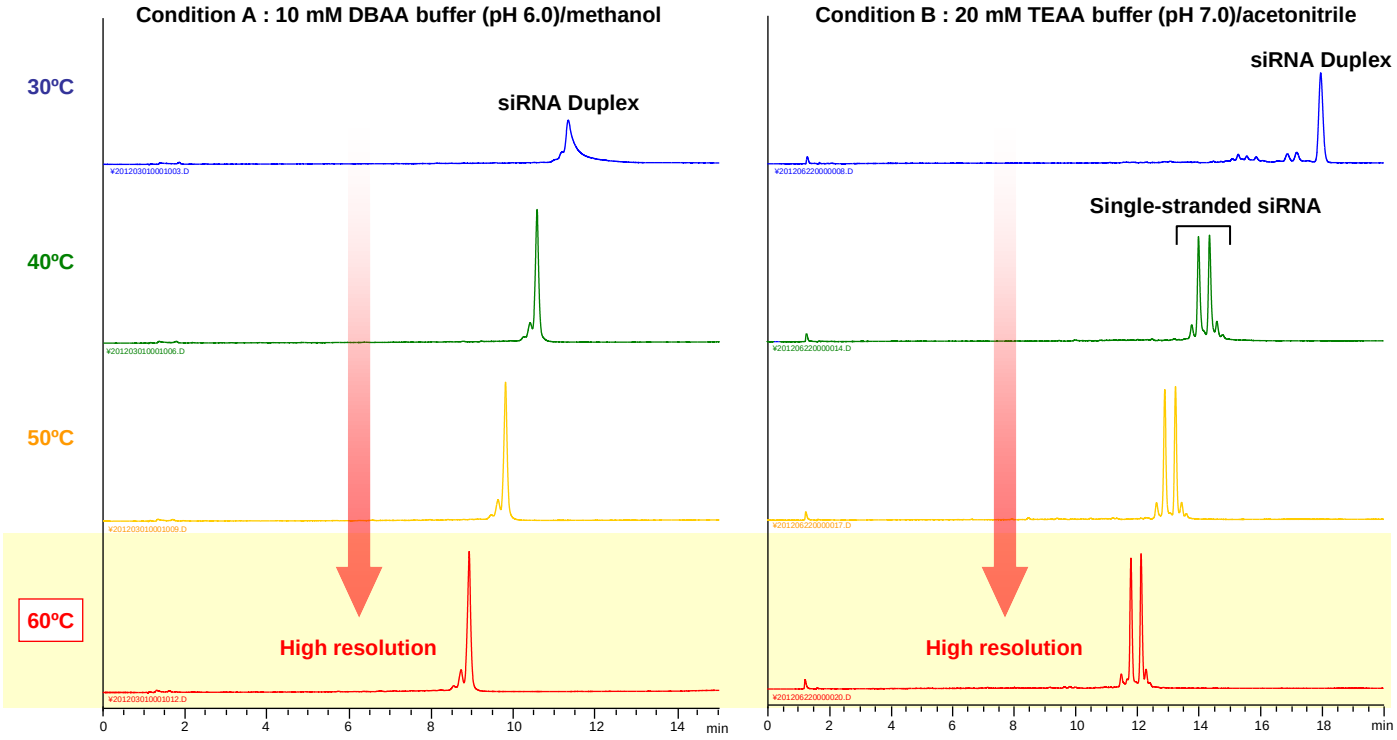
Column	: YMC-Triart C18 (3 µm, 12 nm), 150 X 2.0 mmI.D.
Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 7.5) B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50) 62-72%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 30ºC
Detection	: A) UV at 260 nm B) ESI-negative mode
Injection	: 4 µL (5 nmol/mL)
System	: LC) Shimadzu Prominence MS) Shimadzu LCMS2020

- Mixture of miRNA of 21 nt and 22 nt is separated by using 10mM DBAA/acetonitrile as a mobile phase and detected with MS.

High temperature analysis of oligonucleotides with YMC-Triart C18

Effect of mobile phase and column temperature on separation of siRNA duplex

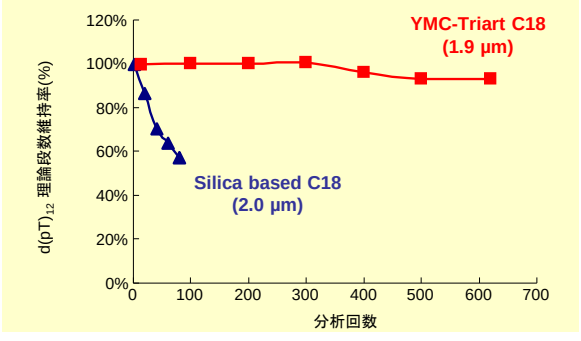
Crude synthetic siRNA duplex (19 bp) : 5'-CGU ACG CGG AAU ACU UCG AdTdT-3'
3'-dTdTGCA UGC GCC UUA UGA AGC U-5'



Column	: YMC-Triart C18 (1.9 µm, 12 nm) 100 X 2.0 mmI.D.	**Condition A** Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) methanol 35-60%B (0-15 min)
Flow rate	: 0.2 mL/min		
Detection	: UV at 269 nm	**Condition B** Eluent	: A) 20 mM triethylamine-acetic acid (pH 7.0) B) acetonitrile 5-12%B (0-20 min)
Injection	: 1 µL (5 nmol/mL)		
System	: Agilent 1290		

- Separation of siRNA duplex under different mobile phase conditions at various temperatures with YMC-Triart C18 is shown.
- Under both condition A and condition B, peak shape and resolution between immediate peaks is improved by increasing the column temperature.
- Due to the improvement of dispersion and distribution velocity when increasing column temperature, bio-macro molecules such as RNA and DNA generally exhibit sharper peak shape and improved resolution.
- Under condition B at 40 ℃ or higher temperature, two peaks of single-stranded RNA that is generated by denaturation of siRNA duplex are observed. This HPLC technique that is utilizing high temperature to generate single-stranded RNA is called "Denaturing HPLC", and widely used in the field of gene mutation analysis.
- As shown above, denaturation of duplex DNA or RNA is also influenced by ionic strength (type and concentration), pH and polarity as well as temperature. Those analysis conditions (temperature and mobile phase) are recommended to be optimized depending on characteristics of target analyte and purpose of analysis.

Durability at pH 6.0 (DBAA buffer) and 65℃



Test condition	Column	: 1.9 µm or 2.0 µm, 12 nm, 50 X 2.0 mmI.D.
	Eluent	: A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) methanol 30-50%B (0-20 min)
	Flow rate	: 0.4 mL/min
	Detection	: UV at 269 nm
	Temperature	: 65ºC
	Sample	: Oligodeoxythymidylic acid, $[d(T)_{2\text{-}20}]$
	Injection	: 1 µL (5 nmol/mL)
	System	: Agilent 1290

- Combination of neutral buffer containing amino ion-paring reagent and high temperature is useful for high-throughput analysis of oligonucleotides or denaturing HPLC. However, conventional silica-based reverse-phase column can hardly used with such condition due to the poor durability.
- YMC-Triart C18 using inorganic/organic hybrid silica with thorough surface modification offers excellent durability at elevated temperature and pH. YMC-Triart C18 is ideal for oligonucleotides analysis.

Separation of chemically modified oligonucleotides using YMC-Triart Bio C4

U190829AE

Antisense DNA and siRNA have been widely used for gene silencing in basic research and in medicinal applications. Effective delivery of the oligonucleotides into cells is important for clinical applications. Previous methods took several hours or more to deliver oligonucleotides to cytoplasm. However, oligonucleotides modified with low molecular weight disulfide units at their terminuses reached the cytoplasm 10 minutes after administration to cell culture[1].
In RP-HPLC using C18 columns, such disulfide modified oligonucleotides show poor peak shape due to too strong interaction between highly hydrophobic their disulfide units and C18 phase. On the other hand, YMC-Triart Bio C4 show a good peak shape because of its short alkyl chains and low hydrophobic interaction.

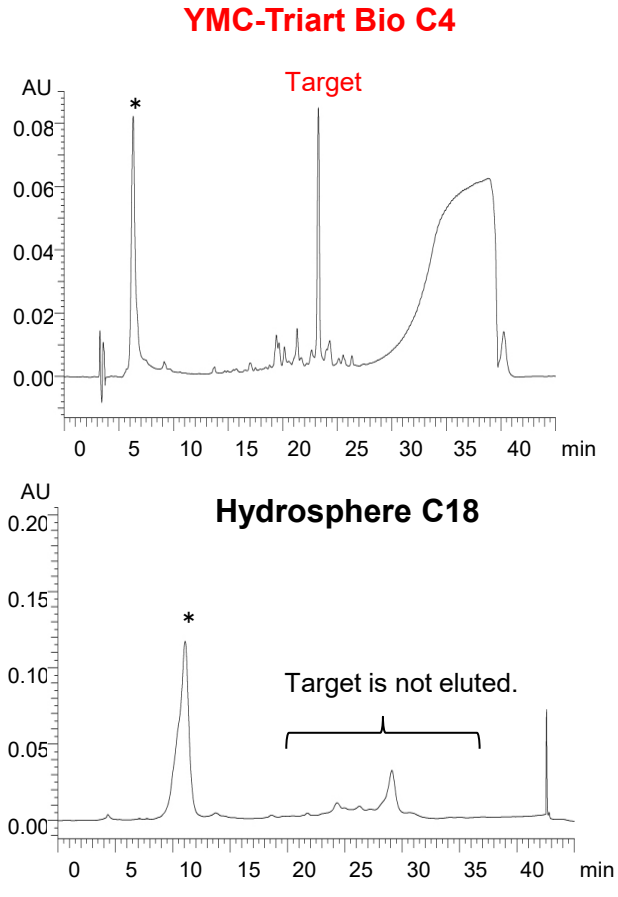
Separation of disulfide-modified oligonucleotides

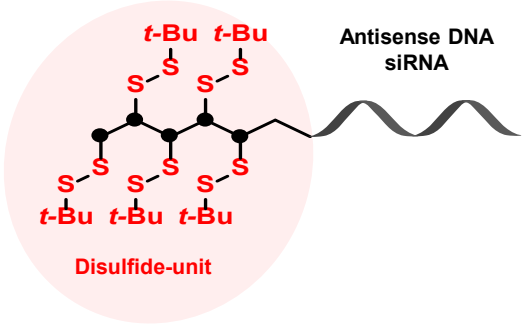
【Cell membrane permeable oligonucleotides】

Oligonucleotides are negatively charged polymers, resulting in low cell membrane permeable efficiency. Various strategies are being pursued including chemical modification of oligonucleotides itself. Recently, it is reported that the disulfide-modified oligonucleotides are efficiently internalized into cytoplasm through disulfide exchange reactions with thiol groups on cell surface. [1]

【Separation of highly hydrophobic oligonucleotides】

The chromatograms show the separation of phosphorothioate oligonucleotides modified with highly hydrophobic disulfide units. The target disulfide-modified oligonucleotides are not completely eluted from the C18 column due to the strong hydrophobic interaction. On the other hand, a good peak shape is obtained using YMC-Triart Bio C4, which has short alkyl chains, by the moderate interaction.





Disulfide-modified oligonucleotides

Column	: 5 µm, 250 X 4.6 mmI.D.
Eluent	: A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5) B) acetonitrile 5-95%B (0-30 min), 95%B (30-35 min), 95-5%B (35-35.1 min), 5%B (35.1-45 min)
Flow rate	: 1 mL/min
Temperature	: 50℃
Detection	: UV at 260 nm
Sample	: crude reaction mixture

*triethylammonium acetate

*phosphorothioate oligonucleotides without disulfide-unit

Reference 1)
Zhaome Shu et al. (2019) Disulfide-Unit Conjugation Enables Ultrafast Cytosolic Internalization of Antisense DNA and siRNA. *Angew. Chem,* 131, 6683-6687

Courtesy of Saki Kawaguchi, Chemistry Department, Nagoya University, Japan

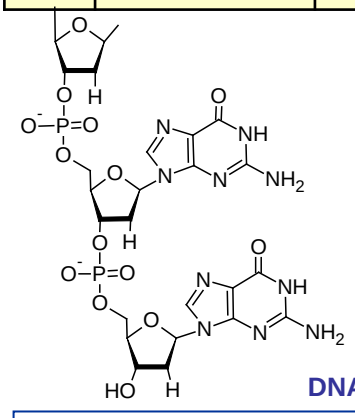
Optimization of oligonucleotide separations on ion-exchange chromatography

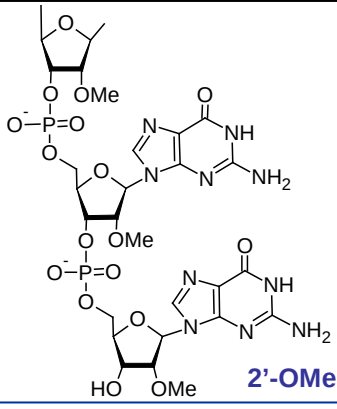
P180316AE

Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to play an important role as next-generation pharmaceuticals together with antibody drugs. These drugs demand chromatographic purification and analysis that can recognize slight structural differences following synthesis. In this report, we provide useful tips for optimization of ion-exchange chromatography methods for oligonucleotides.

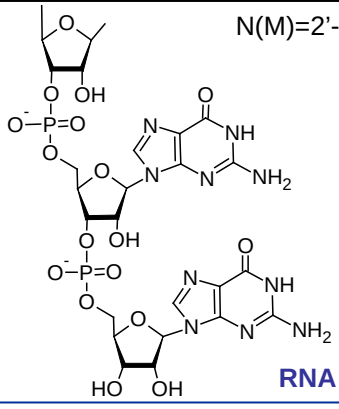
Samples

1	Single-strand DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20 mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21 mer)
3	Single-strand RNA	5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M) C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 20 mer)
4		5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M) A(M)C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 21 mer)
5		5'-UCAUCACACUGAAUACCAAU-3' (RNA 20 mer)
6		5'-GUCAUCACACUGAAUACCAAU-3' (RNA 21 mer)


DNA

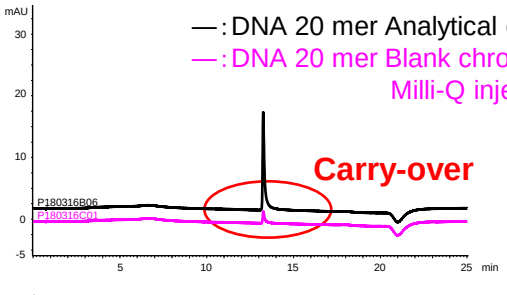

2'-OMe RNA

N(M)=2'-OMe RNA


RNA

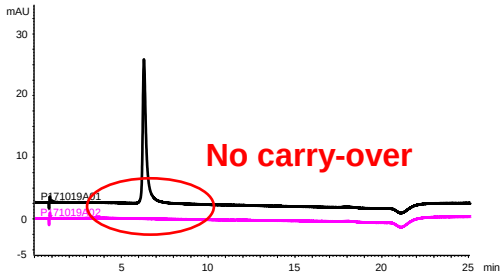
Reducing carry-over

A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl
5-70%B (0-15 min), 74%B (15-18 min), 5%B (18-33 min)
Initial : 50 mM NaCl



—: DNA 20 mer Analytical chromatogram
—: DNA 20 mer Blank chromatogram with Milli-Q injection after analysis

A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl
40-70%B (0-15 min), 74%B (15-18 min), 40%B (18-33 min)
Initial : 400 mM NaCl

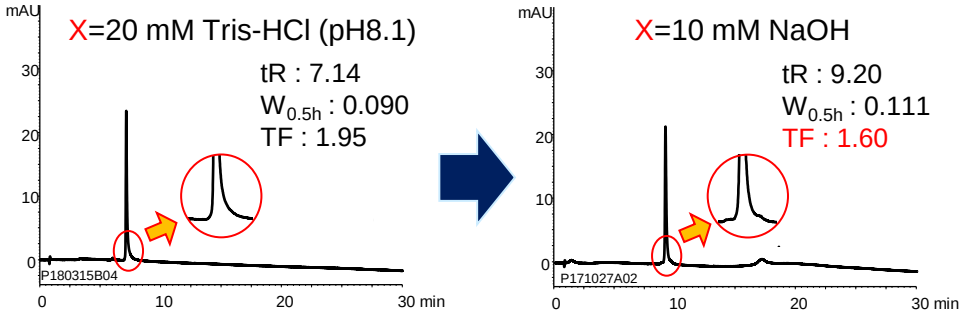


Column	: BioPro IEX QF 5 μm, 100 X 4.6 mm I.D.
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 2 μL (10 nmol/mL)

Carry-over is observed on gradient with low initial concentration of NaCl. But good separation with virtually no carry-over can be achieved by increasing the initial concentration (e.g. 300-400 mM NaCl).

Improving peak tailing

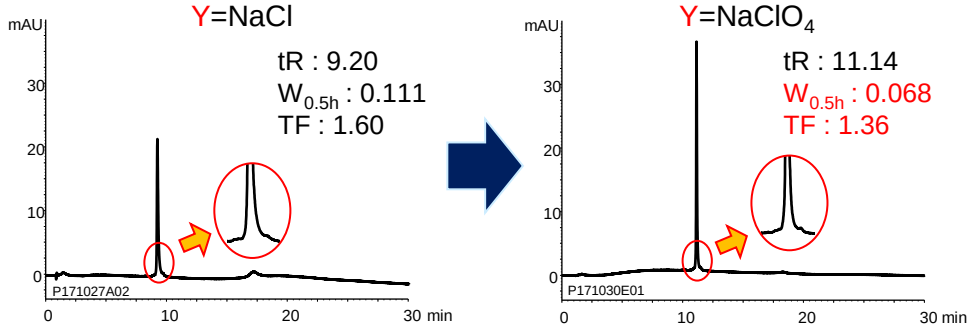
1) Influence of buffer type



Column	: BioPro IEX QF 5 μm, 100 X 4.6 mmI.D.
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 2 μL (10 nmol/mL)
Sample	: RNA 20 mer

Eluent	: A) **X** B) **X** containing 2.0 M NaCl 15-100%B (0-30 min)

2) Influence of counter ion type



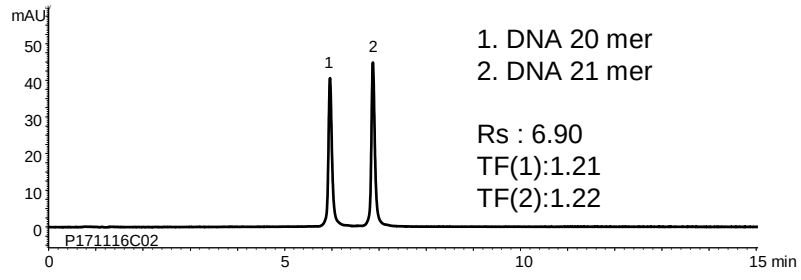
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 2.0 M **Y** 15-100%B (0-30 min) for NaCl 5-50%B (0-30 min) for $NaClO_4$

Gradient profile is adjusted because eluting strength of $NaClO_4$ is two to three times more than that of NaCl on ion exchange chromatography.

By changing the buffer from 20 mM Tris-HCl (pH 8.1) to 10 mM NaOH, tailing factor of the oligonucleotide peak was improved. In addition, changing counter ion from NaCl to $NaClO_4$ is effective.
→ **It is important to optimize buffer and counter ion for excellent peak shape of oligonucleotides.**

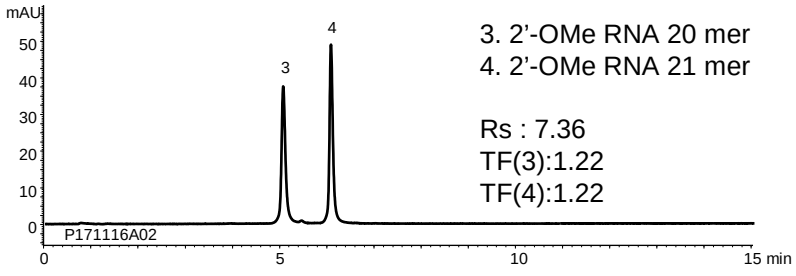
Analysis examples with the optimized conditions

Single-strand DNA



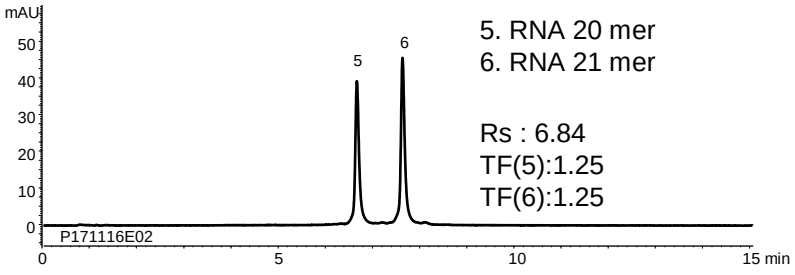
Column	: BioPro IEX QF 5 μm, 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$ 25-55%B (0-15 min), 100%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 4 μL (5 nmol/mL each)

Single-strand 2'-OMe RNA



Good separation without carry-over and peak tailing of oligonucleotides was achieved by optimization of buffer/counter ion in the mobile phase and gradient profile, and by using BioPro IEX QF, non porous anion exchange column.

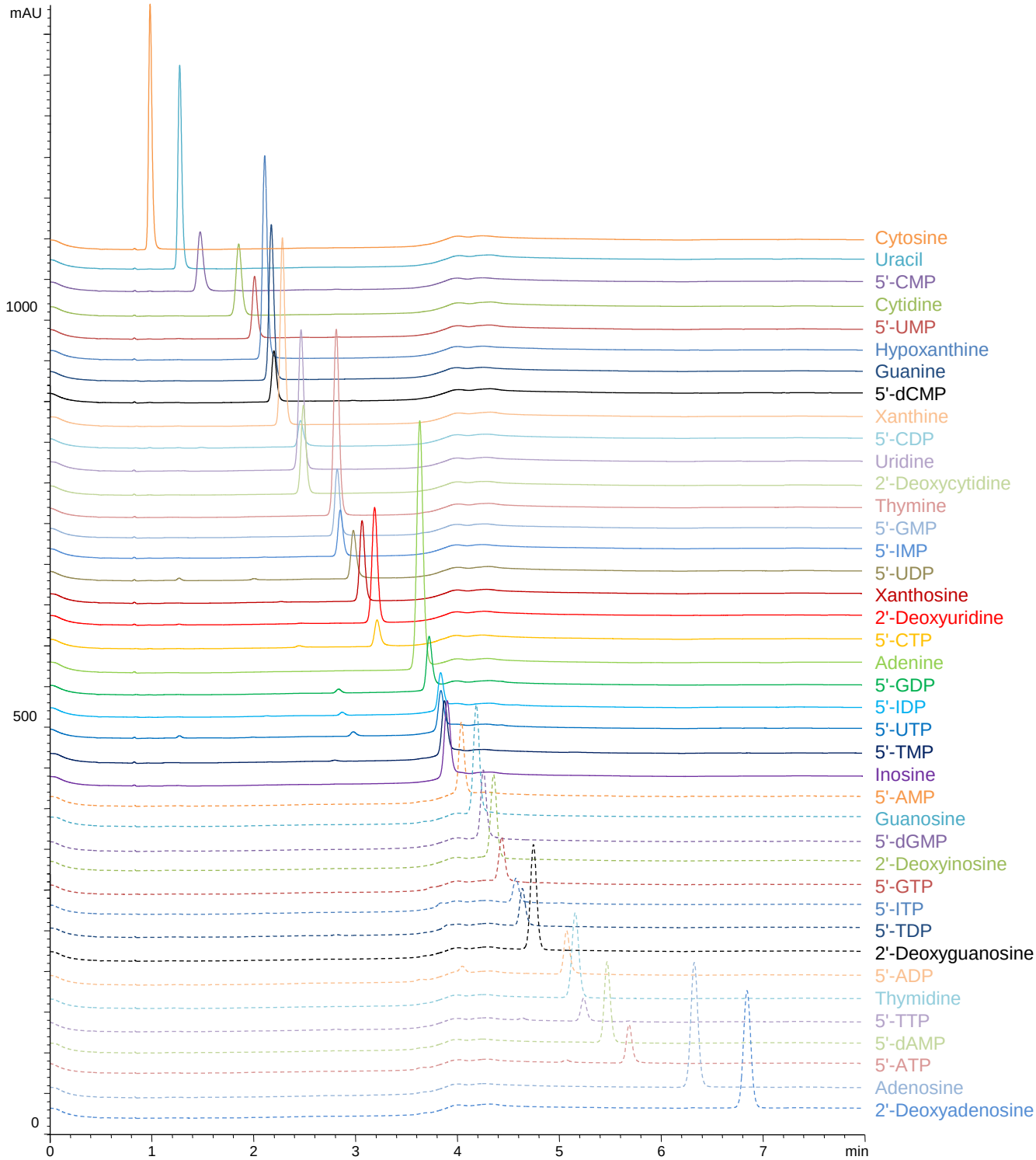
Single-strand RNA



Analytical Data

核酸塩基・ヌクレオシド・ヌクレオチド
Nucleic acid bases, nucleosides and nucleotides

C140730A

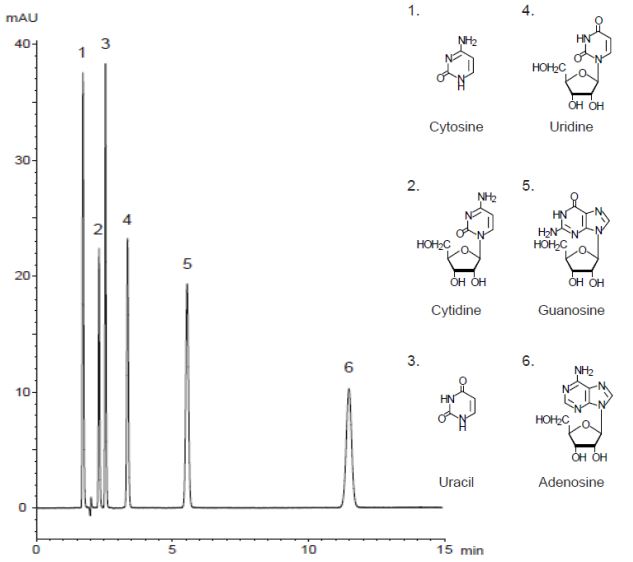


Column	: YMC-Triart C18 (3 µm, 12 nm) 50 X 3.0 mmI.D.
Eluent	: A) 50 mM TEAA* (pH 7.0) B) 50 mM TEAA* (pH 7.0)/acetonitrile (80/20) 0-40%B (0-8 min)
Flow rate	: 0.425 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 2 µL (50 µg/mL)

*TEAA: triethylamine-acetic acid

核酸塩基・ヌクレオシド
Nucleic acid bases and nucleosides

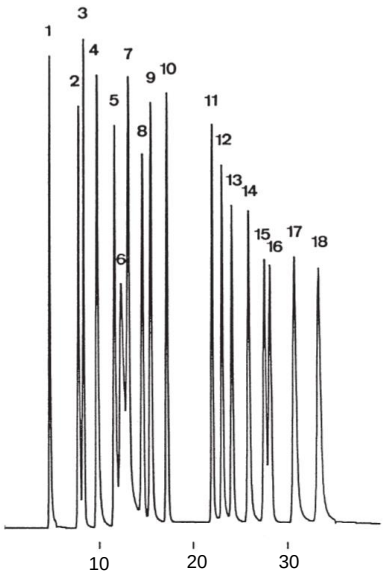
R090205G



Column	: Hydrosphere C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM CH_3COONH_4-CH_3COOH (pH 4.1)/methanol (90/10)
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 254 nm
Injection	: 5 µL
Sample	: Cytosine (0.01 mg/mL), Cytidine (0.01 mg/mL), Uracil (0.005 mg/mL), Uridine (0.01 mg/mL), Guanosine (0.01 mg/mL), Adenosine (0.01 mg/mL)

核酸塩基・ヌクレオシド
Nucleic acid bases and nucleosides

T910404A

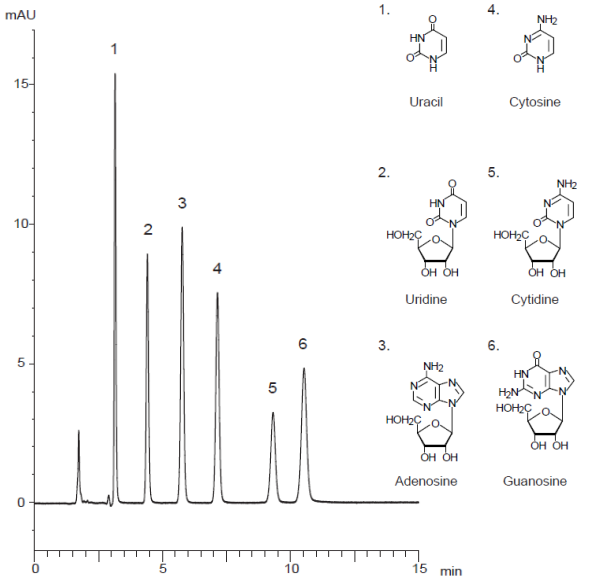


1. Cytosine
2. Cytidine
3. Uracil
4. Deoxycytidine
5. Guanine
6. Adenine
7. Hypoxanthine
8. Uridine
9. Xanthine
10. Thymine
11. Inosine
12. Guanosine
13. Deoxyinosine
14. Deoxyguanosine
15. Xanthosine
16. Thymidine
17. Adenosine
18. Deoxyadenosine

Column	: YMC-Pack ODS-AQ (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: A) 20 mM CH_3COOH-CH_3COONH_4 (pH 3.5) B) 20 mM CH_3COOH-CH_3COONH_4 (pH 3.5)/methanol (90/10) 30%B (0-5 min), 30-100%B (5-13 min), 100%B (13-40 min)
Flow rate	: 0.7 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 12 µL (0.001-0.05 mg/mL)

核酸塩基・ヌクレオシド
Nucleic acid bases and nucleosides

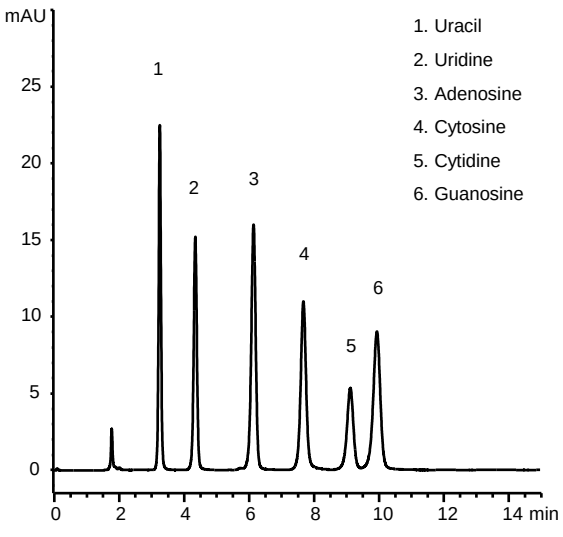
R090116P



Column	: YMC-Pack Diol-NP (5 µm, 12 nm) 150 X 2.0 mmI.D.
Eluent	: water/acetonitrile (10/90) containing 10 mM CH_3COONH_4
Flow rate	: 0.2 mL/min
Temperature	: 30℃
Detection	: UV at 254 nm
Injection	: 1 µL
Sample	: Uracil (0.005 mg/mL), Uridine (0.01 mg/mL), Adenosine (0.01 mg/mL), Cytosine (0.01 mg/mL), Cytidine (0.01 mg/mL), Guanosine (0.01 mg/mL)

核酸塩基・ヌクレオシド
Nucleic acid bases and nucleosides

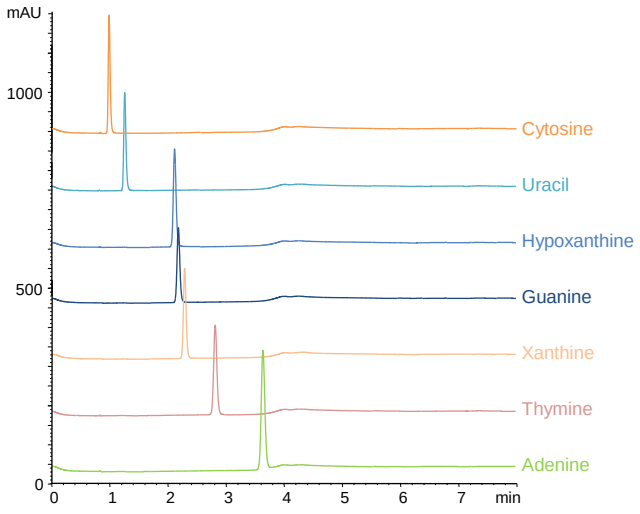
V120501A



Column	: YMC-Triart Diol-HILIC (5 µm, 12 nm) 150 X 3.0 mmI.D.
Eluent	: 100 mM CH_3COONH_4/acetonitrile (10/90)
Flow rate	: 0.425 mL/min
Temperature	: 30℃
Detection	: UV at 254 nm
Injection	: 2 µL (5-10 µg/mL)

核酸塩基
Nucleic acid bases

C140730B

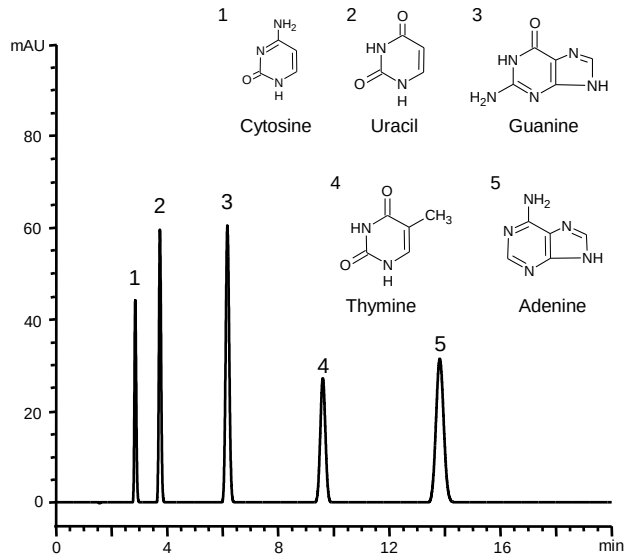


Column	: YMC-Triart C18 (3 μm, 12 nm) 50 X 3.0 mmI.D.
Eluent	: A) 50 mM TEAA* (pH 7.0) B) 50 mM TEAA* (pH 7.0)/acetonitrile (80/20) 0-40%B (0-8 min)
Flow rate	: 0.425 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 2 μL (50 μg/mL)

*TEAA: triethylamine-acetic acid

核酸塩基
Nucleic acid bases

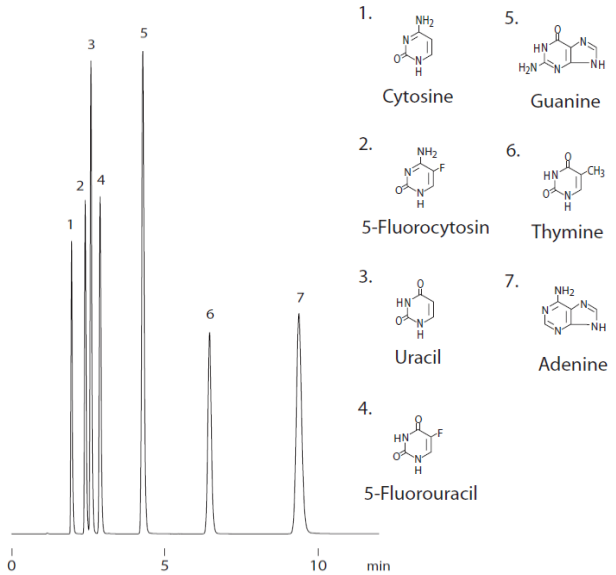
B111219E



Column	: YMC-Triart C18 (5 μm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 254 nm
Injection	: 8 μL (0.01-0.02 mg/mL)

核酸塩基
Nucleic acid bases

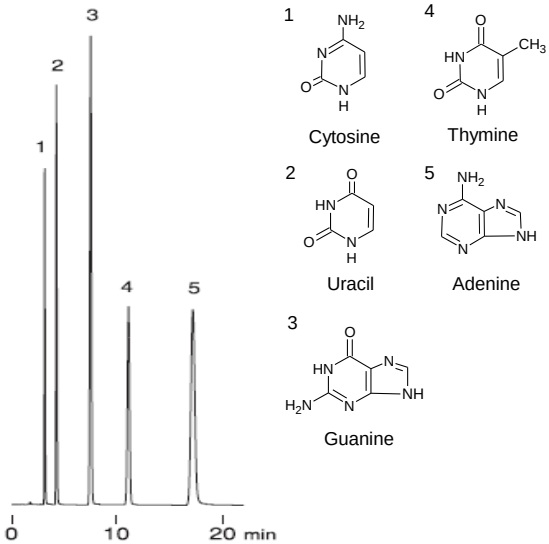
J010209E



Column	: Hydrosphere C18 (3 μm, 12 nm) 100 X 4.6 mmI.D.
Eluent	: 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 254 nm
Injection	: 10 μL (0.01-0.02 mg/mL)

核酸塩基
Nucleic acid bases

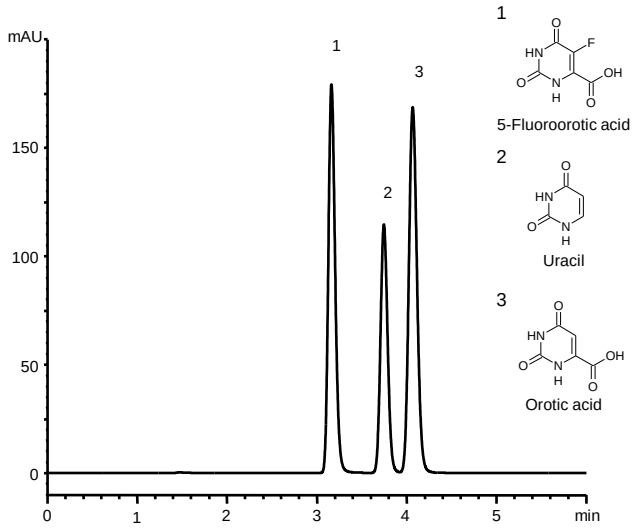
S991029A



Column	: Hydrosphere C18 (5 μm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 254 nm
Injection	: 8 μL (0.01-0.02 mg/mL)

オロチン酸
Orotic acid

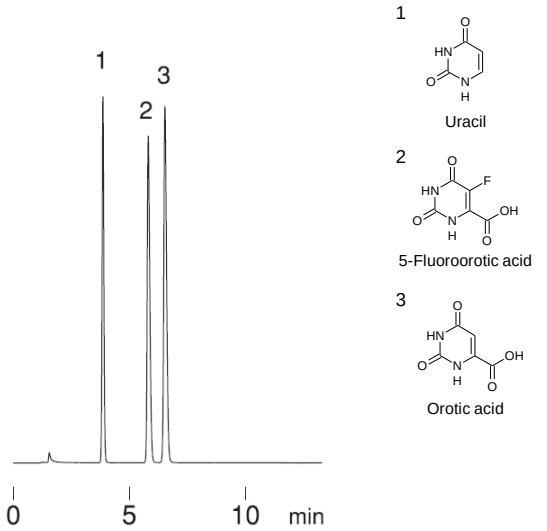
B120210B



Column	: YMC-Triart C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM phosphoric acid
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 254 nm
Injection	: 10 µL (0.015-0.1 mg/mL)

オロチン酸
Orotic acid

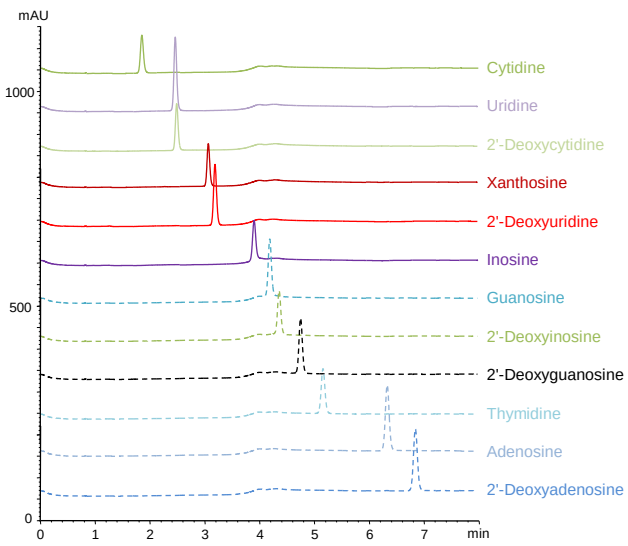
A000711A



Column	: Hydrosphere C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM H_3PO_4
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 254 nm
Injection	: 10 µL (0.015-0.1 mg/mL)

ヌクレオシド
Nucleosides

C140730C

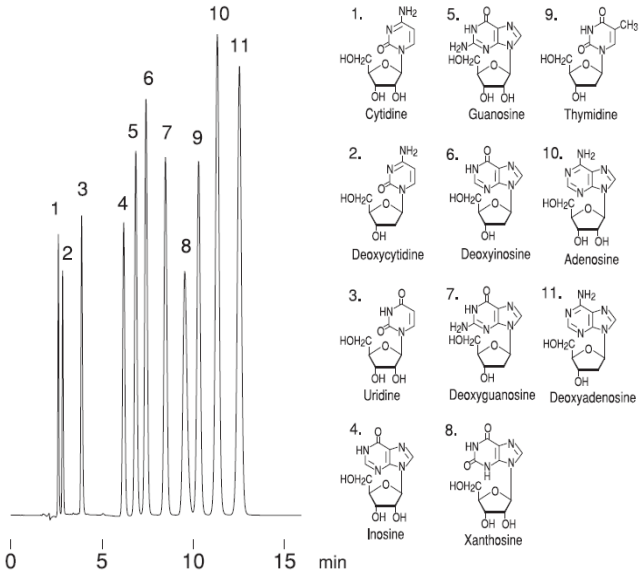


Column	: YMC-Triart C18 (3 µm, 12 nm) 50 X 3.0 mmI.D.
Eluent	: A) 50 mM TEAA* (pH 7.0) B) 50 mM TEAA* (pH 7.0)/acetonitrile (80/20) 0-40%B (0-8 min)
Flow rate	: 0.425 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 2 µL (50 µg/mL)

*TEAA: triethylamine-acetic acid

ヌクレオシド
Nucleosides

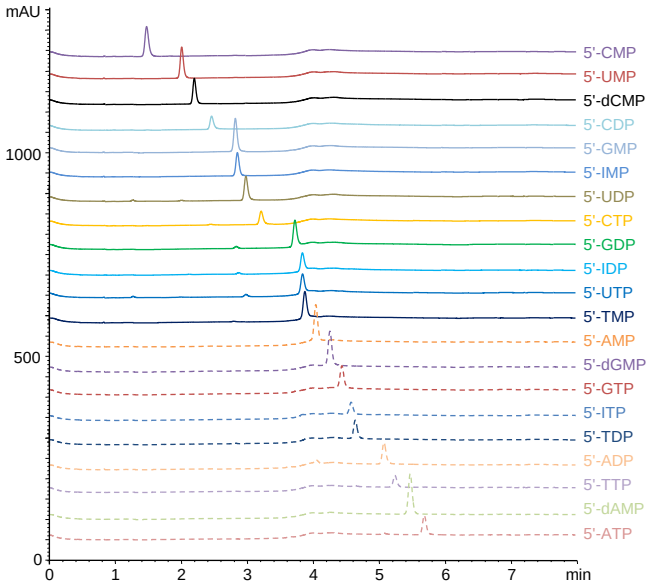
A000802B



Column	: Hydrosphere C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM $NH_4H_2PO_4$-H_3PO_4 (pH 3.5)/methanol (92/8)
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 15 µL (5-15 µg/mL)

ヌクレオチド
Nucleotides

C140730D

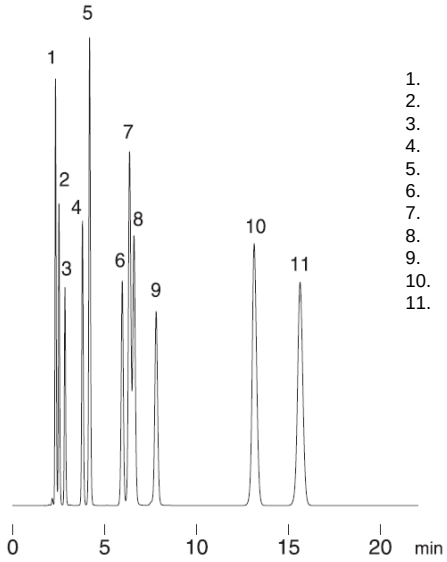


Column	: YMC-Triart C18 (3 µm, 12 nm) 50 X 3.0 mmI.D.
Eluent	: A) 50 mM TEAA* (pH 7.0) B) 50 mM TEAA* (pH 7.0)/acetonitrile (80/20) 0-40%B (0-8 min)
Flow rate	: 0.425 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 2 µL (50 µg/mL)

*TEAA: triethylamine-acetic acid

ヌクレオチド
Nucleotides

A000922A

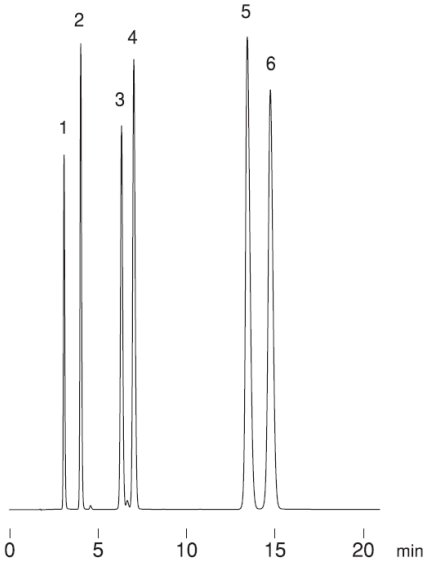


1. 5'-UTP
2. 5'-UDP
3. 5'-CMP
4. 5'-UMP
5. 5'dCMP
6. 5'-GMP
7. 5'-ATP
8. 5'-IMP
9. 5'-ADP
10. 5'-TMP
11. 5'-AMP

Column	: Hydrosphere C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 100 mM KH_2PO_4- K_2HPO_4 (pH 5.5)
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 10 µL (0.05-0.15 mg/mL)

ヌクレオチド
Nucleotides

A000912B

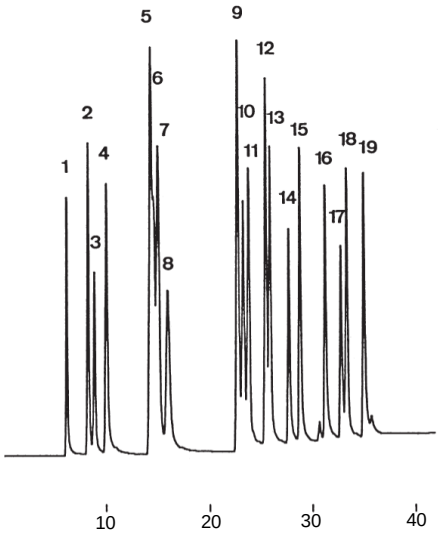


1. 5'-CMP
2. 5'-UMP
3. 5'-GMP
4. 5'-IMP
5. 5'-TMP
6. 5'-AMP

Column	: Hydrosphere C18 (5 µm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 100 mM KH_2PO_4
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 10 µL (0.1-0.3 mg/mL)

ヌクレオチド
Nucleotides

T910424A



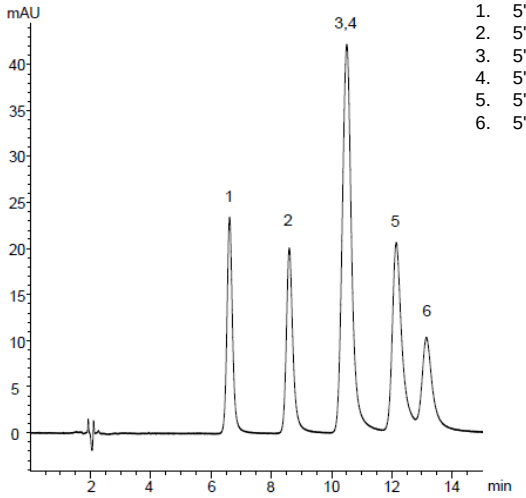
1. 5'-CMP
2. 5'-UMP
3. 5'-dCMP
4. 5'-CDP
5. 5'-GMP
6. 5'-UDP
7. 5'-IMP
8. 5'-CTP
9. 5'-UTP
10. 5'-GDP
11. 5'-TMP
12. 5'-AMP
13. 5'-dGMP
14. 5'-GTP
15. 5'-TDP
16. 5'-ADP
17. 5'-TTP
18. 5'-dAMP
19. 5'-ATP

Column	: YMC-Pack ODS-AM (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: A) 0.2 M TEAA* (pH 6.6) B) 0.2 M TEAA* (pH 6.6)/acetonitrile (95/5) 4%B (0-10 min), 4-100%B (10-35 min), 100%B (35-50 min)
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 14 µL (0.029-0.16 mg/mL)

*TEAA: triethylamine-acetic acid

ヌクレオチド
Nucleotides

R100209O

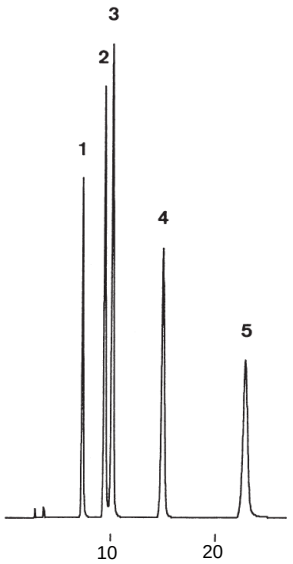


1. 5'-TMP
2. 5'-UMP
3. 5'-IMP
4. 5'-AMP
5. 5'-GMP
6. 5'-CMP

Column	: YMC-Pack Diol-NP (5 µm, 12 nm) 150 X 2.0 mmI.D.
Eluent	: 100 mM CH_3COONH_4/acetonitrile (25/75)
Flow rate	: 0.2 mL/min
Temperature	: 40℃
Detection	: UV at 254 nm
Injection	: 1 µL (0.1 mg/mL)

ヌクレオチド
Nucleotides

T920525C

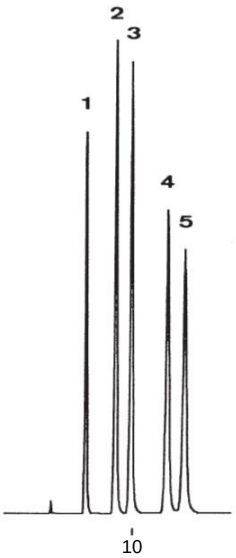


1. 5'-CMP
2. 5'-AMP
3. 5'-UMP
4. 5'-IMP
5. 5'-GMP

Column	: YMC-Pack Polyamine Ⅱ (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: 50 mM KH_2PO_4-H_3PO_4 (pH 3.5)
Flow rate	: 1.0 mL/min
Temperature	: 40℃
Detection	: UV at 260 nm
Injection	: 10 µL (0.05-0.1 mg/mL)

ヌクレオチド
Nucleotides

T920525D

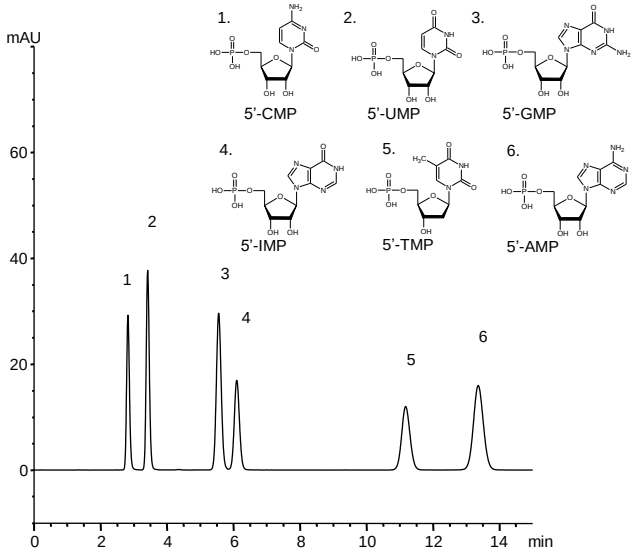


1. 5'-CMP
2. 5'-AMP
3. 5'-UMP
4. 5'-IMP
5. 5'-GMP

Column	: YMC-Pack NH_2 (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: 50 mM KH_2PO_4-H_3PO_4 (pH 3.5)
Flow rate	: 1.0 mL/min
Temperature	: 40℃
Detection	: UV at 260 nm
Injection	: 10 µL (0.05-0.1 mg/mL)

ヌクレオチド
Nucleotides

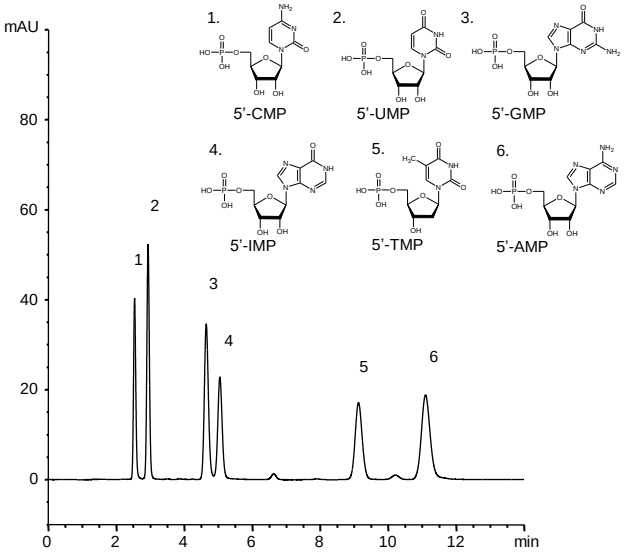
C130530G



Column	: YMC-Triart C18 (5 µm, 12 nm) 150 X 3.0 mmI.D.
Eluent	: 10 mM KH_2PO_4 (pH 4.6)
Flow rate	: 0.425 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 2 µL (50 µg/mL)

ヌクレオチド
Nucleotides

C130729D

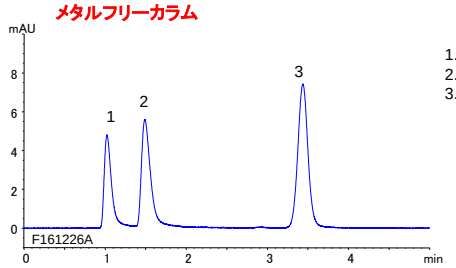


Column	: YMC-Triart C18 (5 µm, 12 nm) 150 X 3.0 mmI.D.
Eluent	: 20 mM CH_3COOH-CH_3COONH_4 (pH 4.7)
Flow rate	: 0.425 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 2 µL (50 µg/mL)

ヌクレオチド
Nucleotides

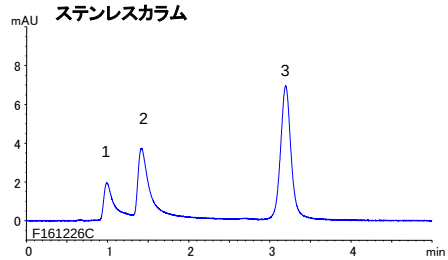
F161226

メタルフリーカラム



1. 5'-ATP
2. 5'-ADP
3. 5'-AMP

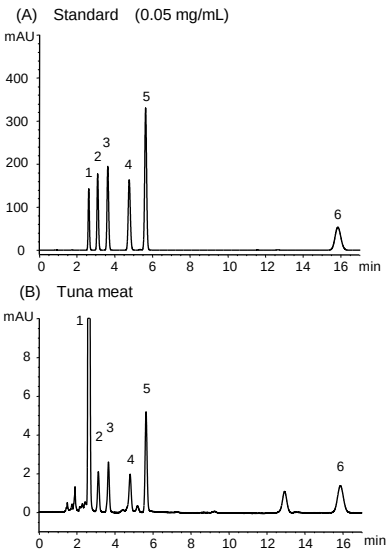
ステンレスカラム



Column	: YMC-Triart C18 (3 µm,12 nm) 50 X 2.1 mmI.D.
Eluent	: 5 mM $HCOONH_4$
Flow rate	: 0.21 mL/min
Temperature	: 25℃
Detection	: UV at 265 nm
Injection	: 1 µL (10 µg/mL)

マグロ肉中のATPとその関連物質
ATP and its related compounds in Tuna meat

C130912L



1. 5'-IMP
2. 5'-ATP
3. 5'-ADP
4. 5'-AMP
5. Hypoxanthine
6. Inosine

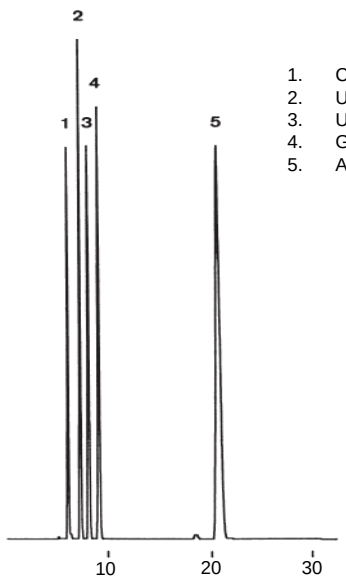
Sample preparation procedure

1 g of Tuna meat
↓←1 M $HClO_4$ 5 mL
Homogenized
↓
Centrifuged (3000 rpm, 5 min)
↓
Supernatant
↓
Adjusted pH to 7.0 with 1 M $NaHCO_3$
↓
Diluted to 100 mL with eluent
↓
Filtered

Column	: YMC-Triart C18 (5 µm, 12 nm) 150 X 3.0 mmI.D.
Eluent	: 50 mM KH_2PO_4-K_2HPO_4 (pH 6.8)
Flow rate	: 0.425 mL/min
Temperature	: 40℃
Detection	: UV at 260 nm
Injection	: 6 µL

糖ヌクレオチド
Sugar nucleotides

T911206B



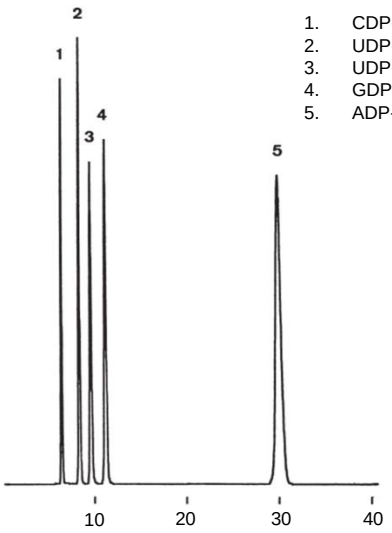
1. CDP-D-glucose
2. UDP-D-glucose
3. UDP-*N*-acetyl-D-glucosamine
4. GDP-D-mannose
5. ADP-D-glucose

Column	: YMC-Pack ODS-A (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: 20 mM TEAA* (pH 5.7)/acetonitrile (99/1)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 260 nm
Injection	: 5 µL (0.27-0.71 mg/mL)

*TEAA: triethylamine-acetic acid

糖ヌクレオチド
Sugar nucleotides

T911206E



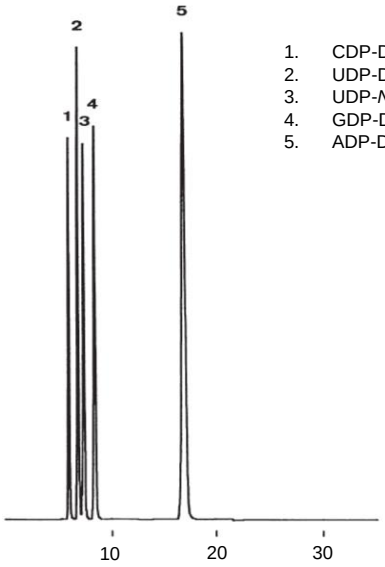
1. CDP-D-glucose
2. UDP-D-glucose
3. UDP-*N*-acetyl-D-glucosamine
4. GDP-D-mannose
5. ADP-D-glucose

Column	: YMC-Pack ODS-A (5 µm, 30 nm) 250 X 4.6 mmI.D.
Eluent	: 100 mM TEAA* (pH 6.0)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 260 nm
Injection	: 5 µL (0.27-0.71 mg/mL)

*TEAA: triethylamine-acetic acid

糖ヌクレオチド
Sugar nucleotides

T911206D



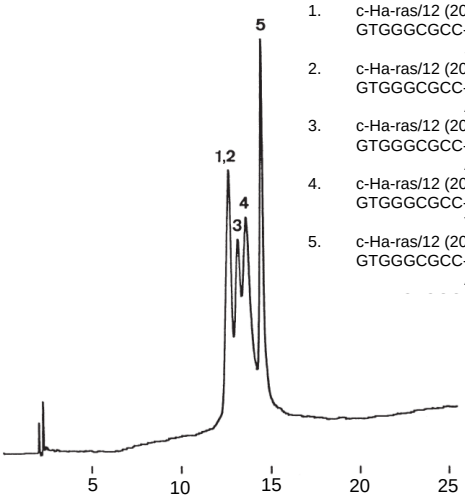
1. CDP-D-glucose
2. UDP-D-glucose
3. UDP-*N*-acetyl-D-glucosamine
4. GDP-D-mannose
5. ADP-D-glucose

Column	: YMC-Pack Ph (5 µm, 12 nm) 250 X 4.6 mmI.D.
Eluent	: 100 mM TEAA* (pH 6.0)
Flow rate	: 1.0 mL/min
Temperature	: 37℃
Detection	: UV at 260 nm
Injection	: 5 µL (0.27-0.71 mg/mL)

*TEAA: triethylamine-acetic acid

オリゴヌクレオチド
Oligonucleotides

G910620C



1. c-Ha-ras/12 (20 mer) GTGGGCGCC-AGC-GGTGTGGG Ser
2. c-Ha-ras/12 (20 mer) GTGGGCGCC-GCC-GGTGTGGG Ala
3. c-Ha-ras/12 (20 mer) GTGGGCGCC-AGC-GGTGTGGG Asp
4. c-Ha-ras/12 (20 mer) GTGGGCGCC-AGC-GGTGTGGG Val
5. c-Ha-ras/12 (20 mer) GTGGGCGCC-AGC-GGTGTGGG Arg

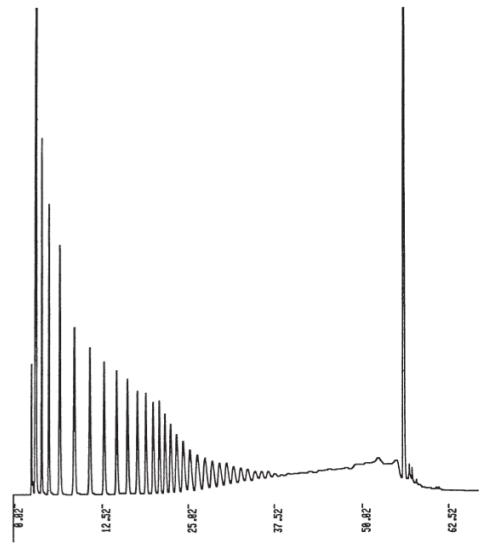
Column	: YMC-Pack ODS-A (5 µm, 30 nm) 150 X 4.6 mmI.D.
Eluent	: A) 100 mM TEAA* (pH 6.5)/acetonitrile (94/6) B) 100 mM TEAA* (pH 6.5)/acetonitrile (85/15) 0-100%B (0-25 min)
Flow rate	: 1.0 mL/min
Temperature	: 30℃
Detection	: UV at 260 nm
Injection	: 15 µL (2.0 pmol/µL)
Sample	: TAKARA ras Gene Probe set

*TEAA: triethylamine-acetic acid

※TAKARA ras Gene Probe set, manufactured by TAKARA SHUZO CO., LTD.

オリゴヌクレオチド, poly(A)
Oligonucleotides, Poly(A)

Y920608A



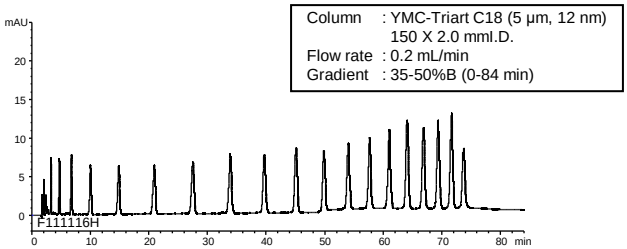
Column	: YMC-Pack ODS-AM (5 µm, 12 nm) 150 X 6.0 mmI.D.
Eluent	: A) 0.1 M phosphate buffer (pH 7) B) 0.1 M phosphate buffer (pH 7)/acetonitrile (75/25) 20-27%B (0-15 min), 27-35%B (15-90 min)
Flow rate	: 1.0 mL/min
Temperature	: ambient
Detection	: UV at 260 nm
Injection	: 10 µL
Sample	: Poly(A)

※Courtesy of Dr. Y. Baba, Kobe Women's College of Pharmacy

オリゴヌクレオチド, $d(T)_{2-20}$
Oligonucleotides, $d(T)_{2-20}$

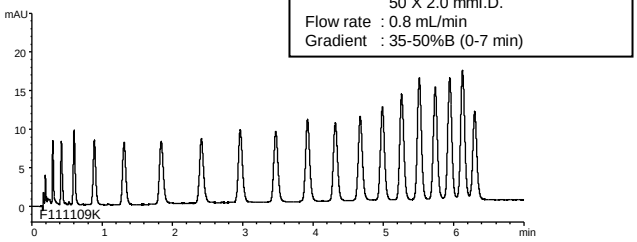
F111118A

Conventional LC method



Column	: YMC-Triart C18 (5 µm, 12 nm) 150 X 2.0 mmI.D.
Flow rate	: 0.2 mL/min
Gradient	: 35-50%B (0-84 min)

UHPLC method



Column	: YMC-Triart C18 (1.9 µm, 12 nm) 50 X 2.0 mmI.D.
Flow rate	: 0.8 mL/min
Gradient	: 35-50%B (0-7 min)

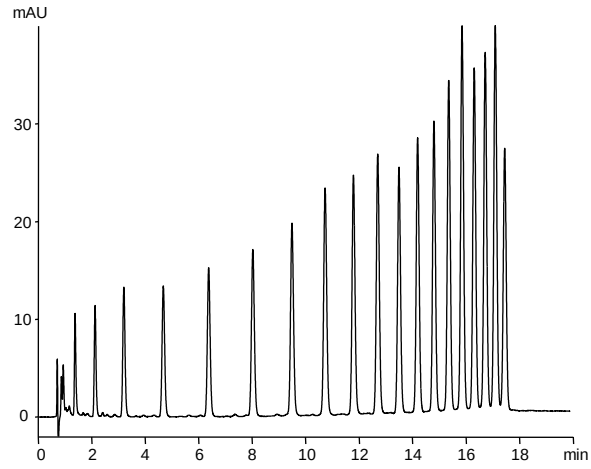
Eluent	: A) 10 mM DBAA* (pH 6.0) B) methanol
Temperature	: 35℃
Detection	: UV at 269 nm
Injection	: 1 µL (5 nmol/mL)
Sample	: Oligodeoxythymidylic acid [$d(T)_{2-20}$]

*di-*n*-butylamine-acetic acid

オリゴヌクレオチド, $d(T)_{2-20}$
Oligonucleotides, $d(T)_{2-20}$

F050423A

Oligodeoxythymidylic acid
[$d(T)_{2-20}$]



Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 4.6 mmI.D.
Eluent	: A) 10 mM DBAA* (pH 6.0) B) 10 mM DBAA* (pH 6.0)/methanol (20/80) 42-70%B (0-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 35℃
Detection	: UV at 269 nm
Injection	: 5 µL (5 nmol/mL)

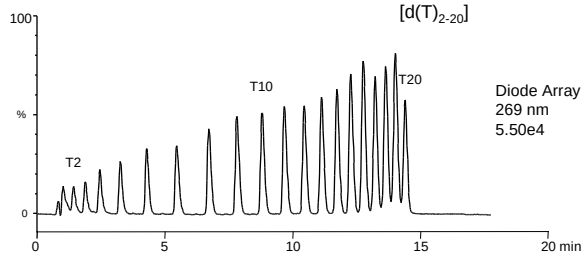
*di-*n*-butylamine-acetic acid

オリゴヌクレオチド $d(T)_{2-20}$のLC/MS分析
LC/MS analysis of oligonucleotides $d(T)_{2-20}$

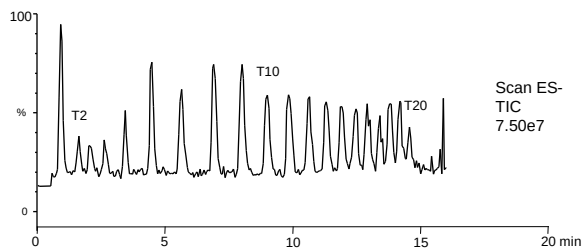
F060118A

A) UV

Oligodeoxythymidylic acid
[$d(T)_{2-20}$]



B) MS



Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 2.0 mmI.D.
Eluent	: A) 5 mM DBAA* (pH 6.0) B) 5 mM DBAA* (pH 6.0)/methanol (20/80) 42-70%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 35℃
Detection	: A) UV at 269 nm B) ESI negative-mode
Injection	: 5 µL (5 nmol/mL)

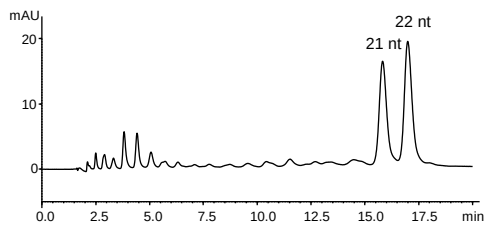
*di-*n*-butylamine-acetic acid

オリゴヌクレオチド（miRNA）のLC/MS分析
LC/MS analysis of oligonucleotides (miRNA)

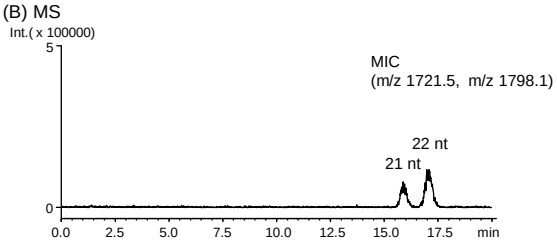
F120202A

5'-pUGG AGU GUG ACA AUG GUG UUG-3' (21 nt, MW 6890.1)
5'-pUGG AGU GUG ACA AUG GUG UUG U-3' (22 nt, MW 7196.3)

(A) UV



(B) MS



Courtesy of M.Yamada, SHIMADZU CORPORATION

Column	: YMC-Triart C18 (3 µm, 12 nm), 150 X 2.0 mmI.D.
Eluent	: A) 10 mM DBAA* (pH 7.5) B) 10 mM DBAA* (pH 7.5)/acetonitrile (50/50) 62-72%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 30℃
Detection	: (A) UV at 260 nm (B) ESI-negative mode
Injection	: 4 µL (5 nmol/mL)
Instrument	: LC) Shimadzu Prominence, MS) Shimadzu LCMS2020

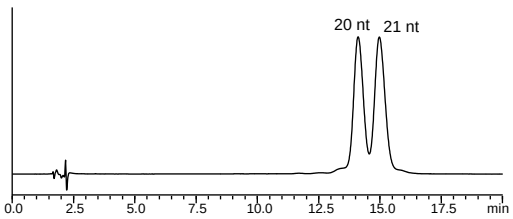
*di-*n*-butylamine-acetic acid

オリゴヌクレオチド（siRNA）のLC/MS分析
LC/MS analysis of oligonucleotides (siRNA)

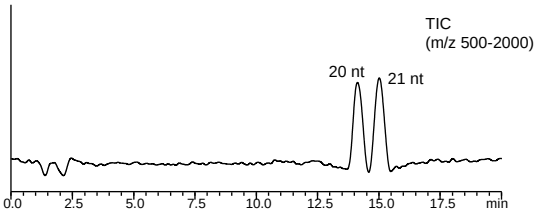
F120227A

5’-GU ACG CGG AAU ACU UCG AdTdT-3’ (20 nt)
5’- CGU ACG CGG AAU ACU UCG AdTdT-3’ (21 nt)

(A) UV



(B) MS



Courtesy of M.Yamada, SHIMADZU CORPORATION

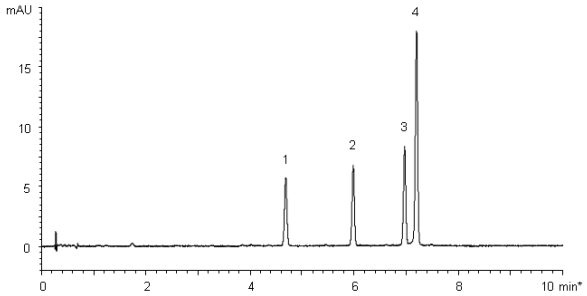
Column	: YMC-Triart C18 (3 µm, 12 nm) 150 X 2.0 mmI.D.
Eluent	: A) 5 mM DBAA* (pH 7.5) B) 5 mM DBAA* (pH 7.5)/acetonitrile (50/50) 55-65%B (0-20 min)
Flow rate	: 0.2 mL/min
Detection	: (A) UV at 260 nm (B) ESI-negative mode
Instrument	: LC) Shimadzu Prominence MS) Shimadzu LCMS-IT-TOF

*di-*n*-butylamine-acetic acid

オリゴヌクレオチド（14～21mer）
oligonucleotides (14-21mer)

F180403J

1. 5’-CAC UGA AUA CCA AU-3’ (14 mer)
2. 5’-UCA CAC UGA AUA CCA AU-3’ (17 mer)
3. 5’-UCA UCA CAC UGA AUA CCA AU-3’ (20 mer)
4. 5’-GUC AUC ACA CUG AAU ACC AAU-3’ (21 mer)



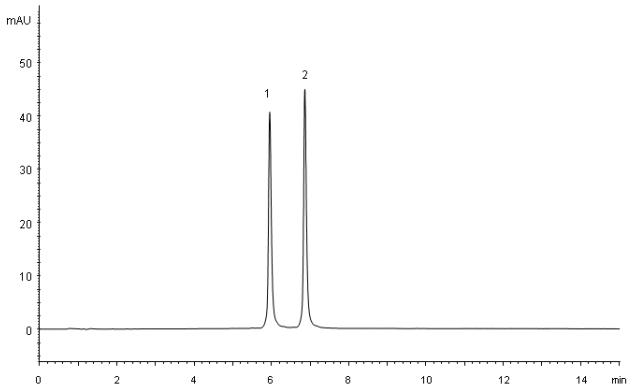
Column	: YMC-Triart C18 (1.9 µm, 12 nm) 50 X 2.1 mmI.D.
Eluent	: A) 200 mM HFIP*-8 mM triethylamine B) methanol 10-20%B (0-10 min)
Flow rate	: 0.42 mL/min
Temperature	: 65℃
Detection	: UV at 260 nm
Injection	: 1 µL (2-4 nmol/mL)

* 1,1,1,3,3,3-hexafluoro-2-propanol

合成オリゴヌクレオチド（一本鎖DNA）
Synthetic oligonucleotides (Single-strand DNA)

P171116C

1. 5’-TCATCACACTGAATACCAAT-3’ (20 mer)
2. 5’-GTCATCACACTGAATACCAAT-3’ (21 mer)

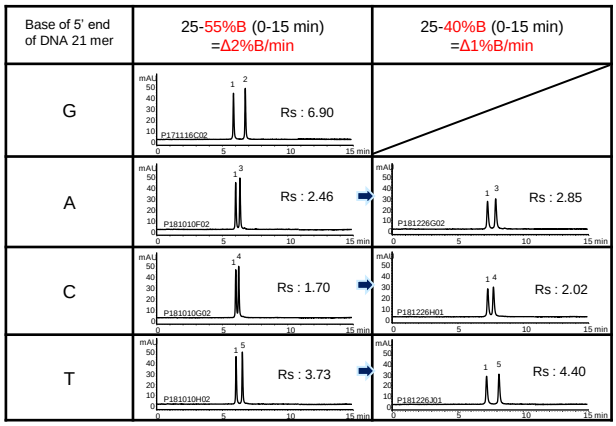


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$ 25-55%B (0-15 min), 100%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 4 µL (5 nmol/mL)

合成オリゴヌクレオチド（一本鎖DNA）
Synthetic oligonucleotides (Single-strand DNA)

U190801A

1. 5’-TCATCACACTGAATACCAAT-3’ (DNA 20 mer)
2. 5’-GTCATCACACTGAATACCAAT-3’ (DNA 21 mer)
3. 5’-ATCATCACACTGAATACCAAT-3’ (DNA 21 mer)
4. 5’-CTCATCACACTGAATACCAAT-3’ (DNA 21 mer)
5. 5’-TTCATCACACTGAATACCAAT-3’ (DNA 21 mer)

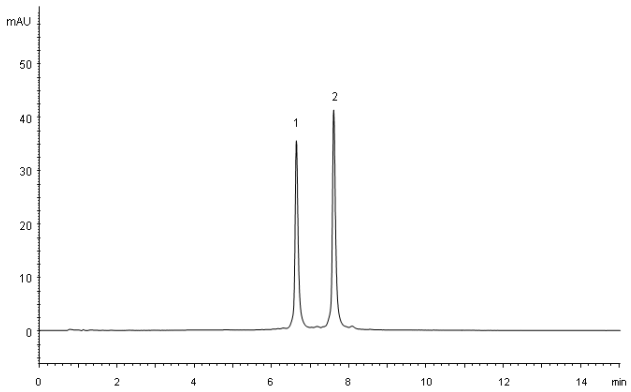


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 4 µL (each 5 nmol/mL)

合成オリゴヌクレオチド（一本鎖RNA）
Synthetic oligonucleotides (Single-strand RNA)

P171116E

1. 5'-UCAUCACACUGAAUACCAAU-3' (20 mer)
2. 5'-GUCAUCACACUGAAUACCAAU-3' (21 mer)

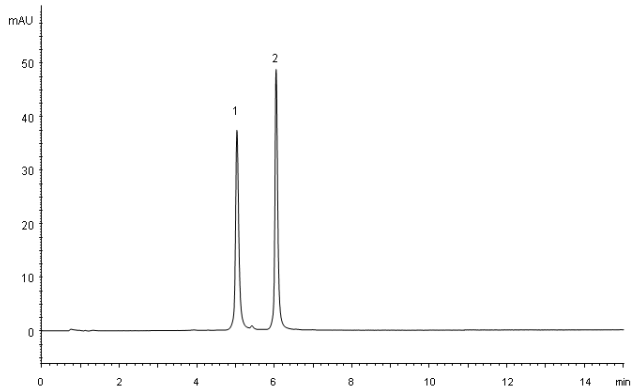


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$ 25-55%B (0-15 min), 100%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 4 µL (5 nmol/mL)

合成オリゴヌクレオチド（一本鎖RNA）
Synthetic oligonucleotides (Single-strand RNA)

P171116A

1. 5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (20 mer)
2. 5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (21 mer)
N(M)=2'OMe RNA

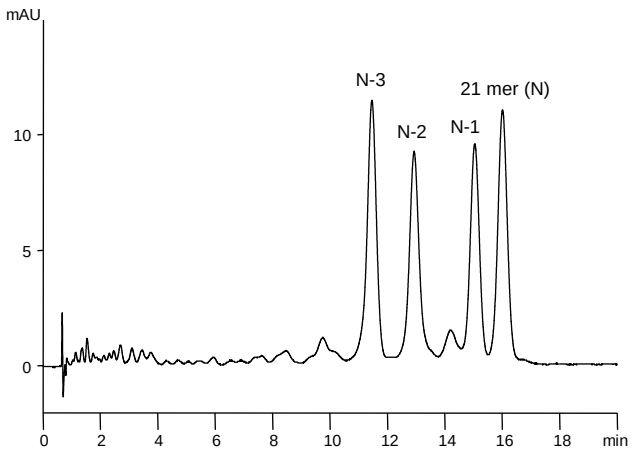


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$ 25-55%B (0-15 min), 100%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 4 µL (5 nmol/mL)

合成オリゴヌクレオチド
Synthetic oligonucleotides

F050502A

5'-CCCGTGTTCCTTGCCACAGAC-3' (21 mer)



Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 4.6 mmI.D.
Eluent	: A) 10 mM DBAA* (pH 6.0) B) 10 mM DBAA* (pH 6.0)/methanol (20/80) 55-60%B (0-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 35℃
Detection	: UV at 269 nm
Injection	: 3 µL (10 nmol/mL)
Sample	: primer of DNA sequencing

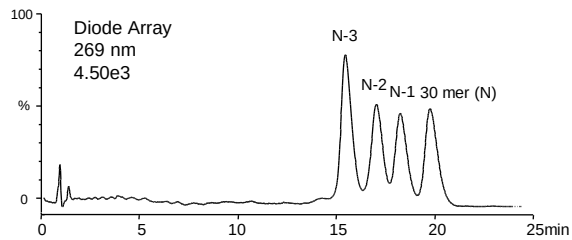
*di-*n*-butylamine-acetic acid

合成オリゴヌクレオチドのLC/MS分析
LC/MS analysis of synthetic oligonucleotides

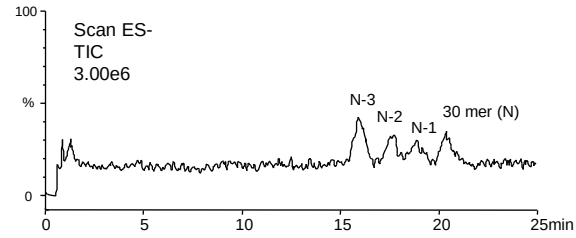
F060213C

5'-CCGCTCGAGCTAAAAAAAGCCTGTGTTACC-3' (30 mer)

A) UV



B) MS



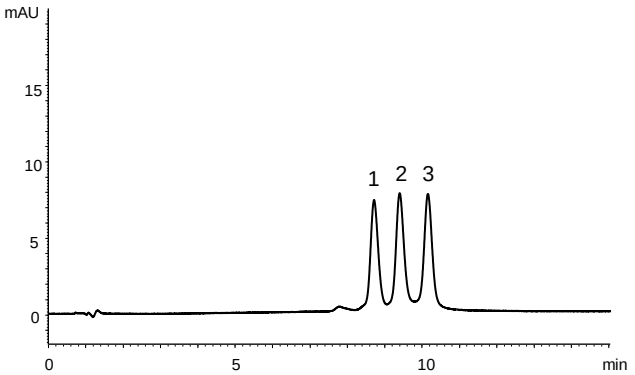
Column	: Hydrosphere C18 (3 µm, 12 nm) 50 X 2.0 mmI.D.
Eluent	: A) 10 mM DBAA* (pH 6.0) B) 10 mM DBAA* (pH 6.0)/acetonitrile (50/50) 58-62%B (0-20 min), 62%B (20-25 min)
Flow rate	: 0.2 mL/min
Temperature	: 35℃
Detection	: A) UV at 269 nm, B) ESI negative-mode
Injection	: 1 µL (10 nmol/mL)
Sample	: primer of DNA sequencing

*di-*n*-butylamine-acetic acid

ホスホロチオエート型オリゴヌクレオチド
Phosphorothioate oligonucleotides

P180606C

1. 5'-T^T^T^T^T^T^T^T^T^T^T^T^TTT-3' (DNA 15 mer 12PS, 2PO)
2. 5'-T^T^T^T^T^T^T^T^T^T^T^T^TT-3' (DNA 15 mer 13PS, 1PO)
3. 5'-T^T^T^T^T^T^T^T^T^T^T^T^T-3' (DNA 15 mer All PS)
   ^=Phosphorothioated

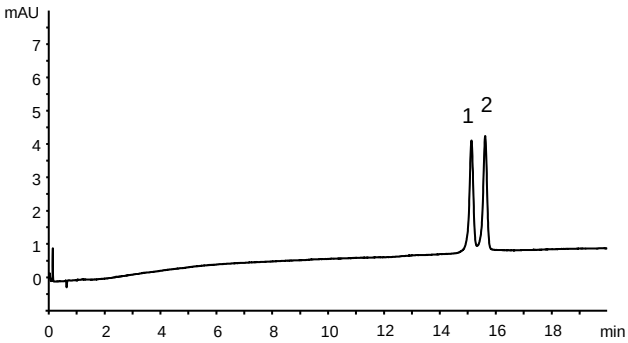


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 1.0 M $NaClO_4$ 40-70%B (0-15 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 6 µL (each 3.3 nmol/mL)

ホスホロチオエート型オリゴヌクレオチド
Phosphorothioate oligonucleotides

AB190618B

1. 5'-U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3' (RNA 20 mer)
2. 5'-G^U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3' (RNA 21 mer)
   ^=Phosphorothioated



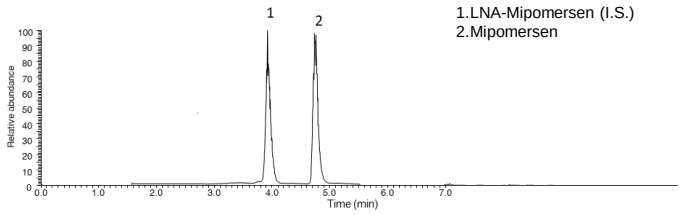
Column	: YMC-Triart C8 [Metal free column] (1.9 µm, 12 nm) 100 X 2.1 mmI.D.
Eluent	: A) 15 mM triethylamine-400 mM HFIP* B) methanol 10-20%B (0-20 min)
Flow rate	: 0.42 mL/min
Temperature	: 70℃
Detection	: UV at 260 nm
Injection	: 1 µL (each 1.25 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

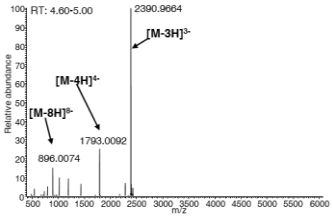
アンチセンスオリゴヌクレオチド（ミポメルセン）のLC-HRMS分析
LC-HRMS analysis of antisense oligonucleotides (mipomersen)

R201223A

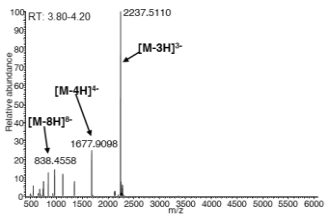
Total ion chromatogram



Mass spectrum of mipomersen



Mass spectrum of LNA-mipomersen



Courtesy of Y. Sun, National Institute of Health Sciences

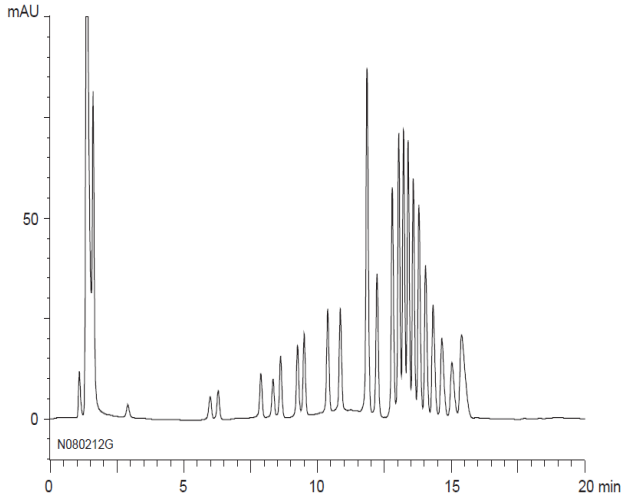
Column	: YMC-Triart C8 [Metal free column] (1.9 µm, 12 nm)*1 100 X 2.1 mmI.D.
Eluent	: A) water/triethylamine/HFIP*2 (100/0.4/2; triethylamine 28.0 mM, HFIP 135.8 mM) B) methanol/triethylamine/HFIP (100/0.4/2)
Gradient	: [Sample separation step] 10-40%B (0-5.0 min) [Column wash steps] 40-70%B (5.0-5.1 min), 70%B (5.1-7.0 min), 70-10%B (7.0-7.1 min), 10%B (7.1-8.0 min), 10-90%B (8.0-8.1 min), 90%B (8.1-9.0 min), 90-10%B (9.0-9.1 min), 10%B (9.1-10.0 min), 10-90%B (10.0-10.1 min), 90%B (10.1-11.0 min), 90-10%B (11.0-11.1 min)
Flow rate	: 0.3 mL/min
Temperature	: 50℃
Injection	: 10 µL (1000 ng/mL)
System	: LC) Vanquish Binary Pump H system (Thermo Fisher Scientific) HRMS) Orbitrap HRMS Q Exactive Plus (Thermo Fisher Scientific)

*1 Prewashed the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour
*2 1,1,1,3,3,3-hexafluoro-2-propanol

Reference :
Y. Sun et al, Development of a bioanalytical method for an antisense therapeutic using high-resolution mass spectrometry, Bioanalysis, 2020 NOV 26, doi: 10.4155/bio-2020-0225.

DNAフラグメント
DNA fragments

N080212G

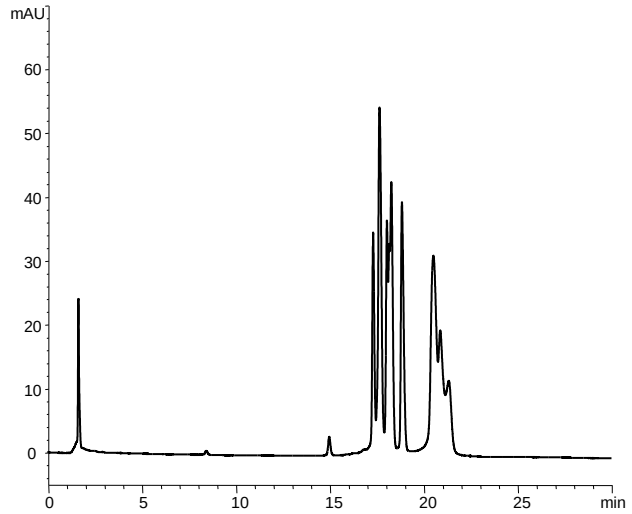


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mmI.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 40-100%B (0-30 min)
Flow rate	: 0.5 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 20 µL (0.25 mg/mL)
Sample	: 1 kb DNA ladder (75-12,216 bp)

ラムダDNA制限酵素*Hind* IIIおよび*EcoR* I分解物
Lambda DNA *Hind* III/*EcoR* I restriction fragments

P081023B

Lambda DNA *Hind* III/*EcoR* I digest (130-23,130 bp)

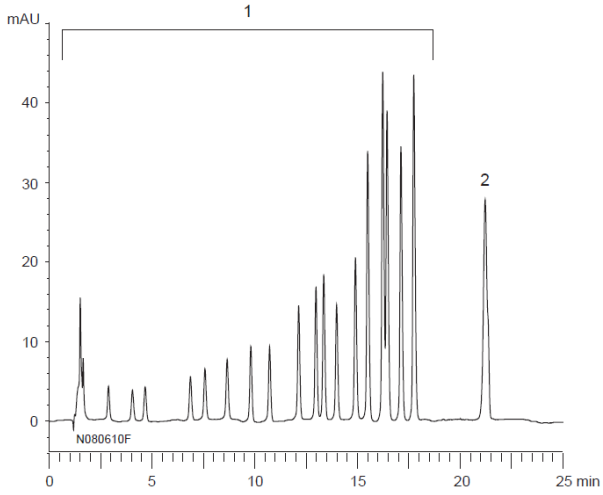


Column	: BioPro IEX QF (5 µm), 100 X 4.6 mmI.D.
Eluent	: A) 20 mM Glycine-NaOH (pH10.6) B) 20 mM Glycine-NaOH (pH10.6) containing 1.0 M NaCl 70-90%B (0-30 min), 100%B (30-35 min), 0%B (35-65 min), 70%B (65-95 min)
Flow rate	: 0.5 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 20 µL (0.1 mg/mL)
Sample	: Lambda DNA digested by *Hind* III and *EcoR* I (130-23,130 bp)

プラスミドpBR322の制限酵素*Hae* III分解物
Plasmid pBR322 and pBR322 *Hae* III restriction fragments

N080610F

1. Plasmid pBR322 *Hae* III digest (8-587 bp)
2. Plasmid pBR322 (4,361 bp)

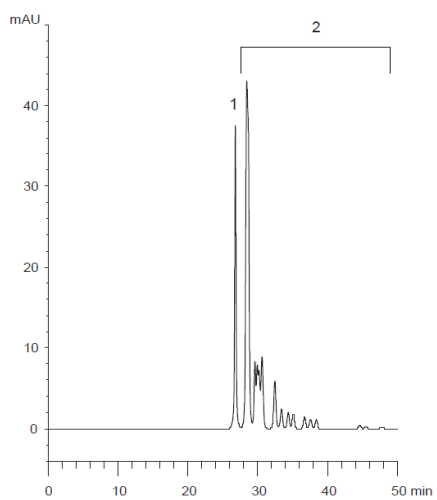


Column	: BioPro IEX QF (5 µm) 100 X 4.6 mm I.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 70-85%B (0-20 min), 85%B (20-25 min)
Flow rate	: 0.5 mL/min
Temperature	: 35℃
Detection	: UV at 260 nm
Injection	: 10 µL
Sample	: Plasmid pBR322 *Hae* III digest (0.13 mg/mL) Plasmid pBR322 (0.03 mg/mL)

プラスミドpBR322の制限酵素*Hae* III分解物
Plasmid pBR322 and pBR322 *Hae* III restriction fragments

P080617A

1. Plasmid pBR322 (4,361 bp)
2. Plasmid pBR322 *Hae* III digest (8-587 bp)



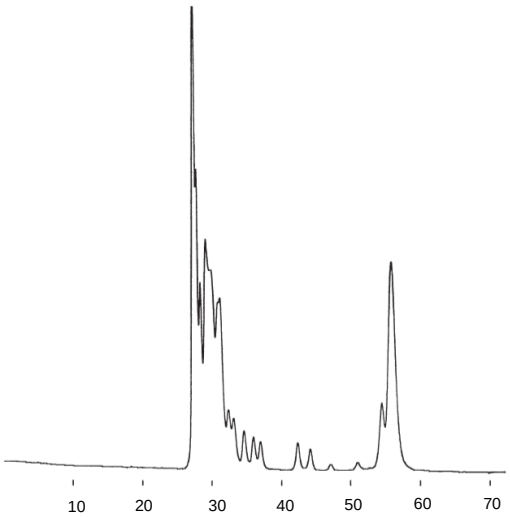
Column	: YMC-Pack Diol-300 + Diol-200 (5 µm) 500 X 8.0 mmI.D. X 2
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (25℃)
Detection	: UV at 260 nm
Injection	: 10 µL
Sample	: Plasmid pBR322 (0.03 mg/mL) Plasmid pBR322 *Hae* III digest (0.13 mg/mL)

プラスミドpBR322の制限酵素*Msp* I分解物

Plasmid pBR322 and pBR322 *Msp* I restriction fragments

G920109B

1. Plasmid pBR322 cleaved with restriction endonuclease *Msp* I



Column	: YMC-Pack Diol-300 + Diol-200 (5 µm) 500 X 8.0 mmI.D. X 2
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (26℃)
Detection	: UV at 260 nm
Injection	: 3 µL (0.49 mg/mL)
Sample	: Plasmid pBR322 cleaved with restriction endonuclease *Msp* I

YMC CO., LTD.
YMC Karasuma-Gojo Bldg., 284 Daigo-cho
Karasuma Nishiiru Gojo-dori, Shimogyo-ku
Kyoto, 600-8106, Japan
TEL:+81-75-342-4515 FAX:+81-75-342-4550
www.ymc.co.jp sales@ymc.co.jp

Distributor