Optimisation of oligonucleotide separations using YMC´s non porous anion exchanger BioPro IEX QF

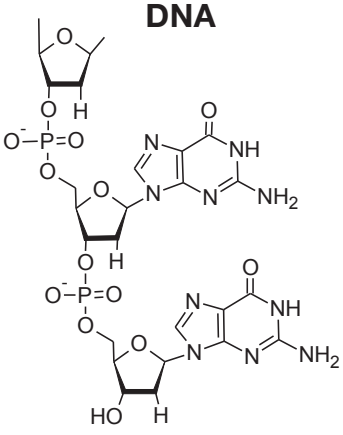
Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to play an important role as the next-generation pharmaceuticals together with antibody drugs.
These drugs demand chromatographic purification and analysis that can recognize slight structural differences following synthesis.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

Samples

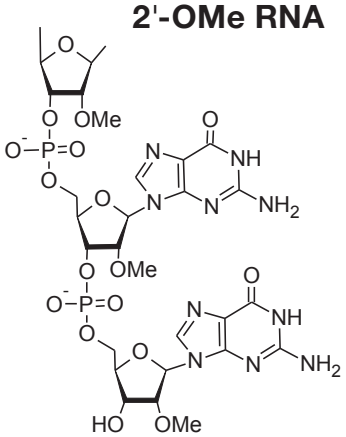
1	Single-strand DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20 mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21 mer)
3	Single-strand RNA	5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M) C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 20 mer)
4		5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)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

N(M)=2'-OMe RNA

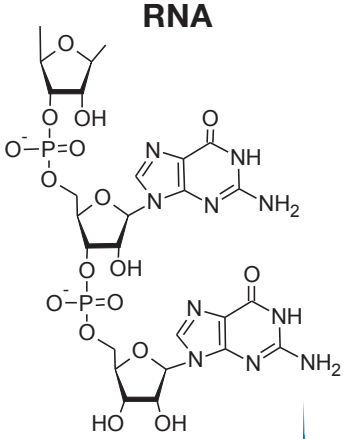
DNA



2'-OMe RNA



RNA



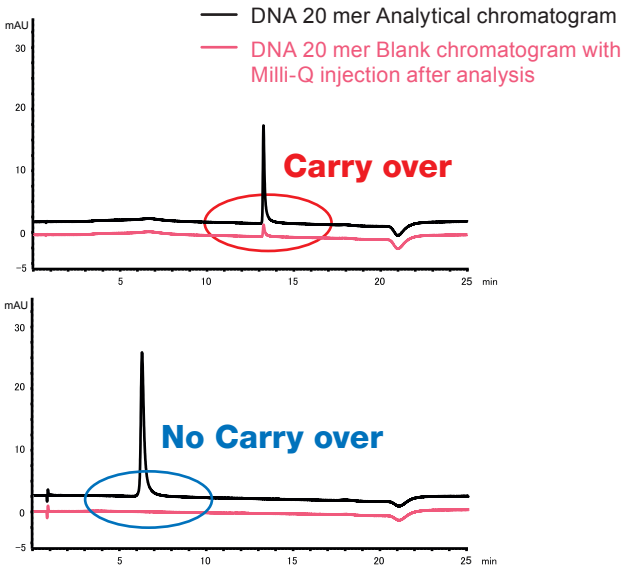
YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

APPLICATION NOTE

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

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

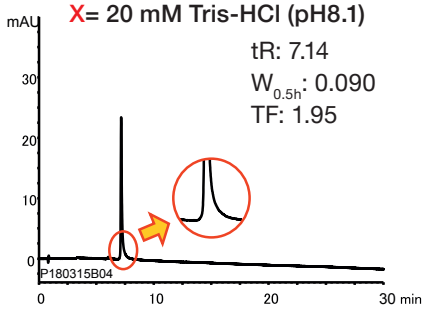


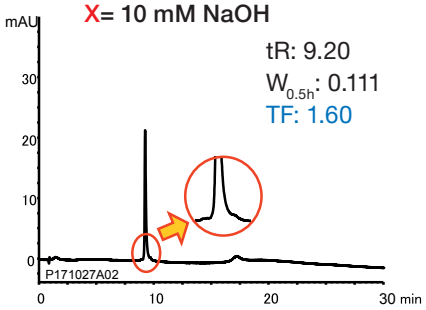
Column:	BioPro IEX QF 5 µm, 100 X 4.6 mm ID
Part No.:	QF00S05-1046WP
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 260 nm
Injection:	2 µL (10 nmol/mL)

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

Improving peak shape

❶ Influence of buffer type

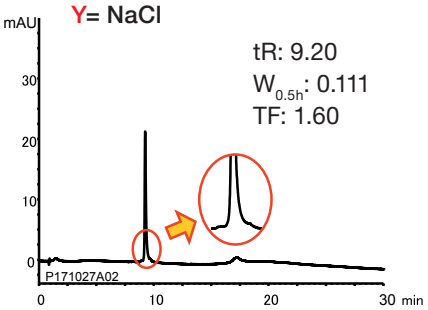


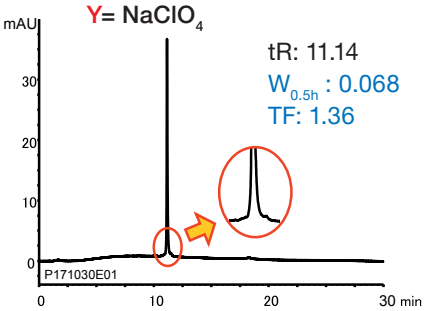


Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
Flow rate:	1.0 mL/min
Temperatur:	25 °C
Detection:	UV at 260 nm
Injection:	2 µL (10 nmol/mL)
Sample:	RNA 20 mer

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

❷ Influence of counter ion type





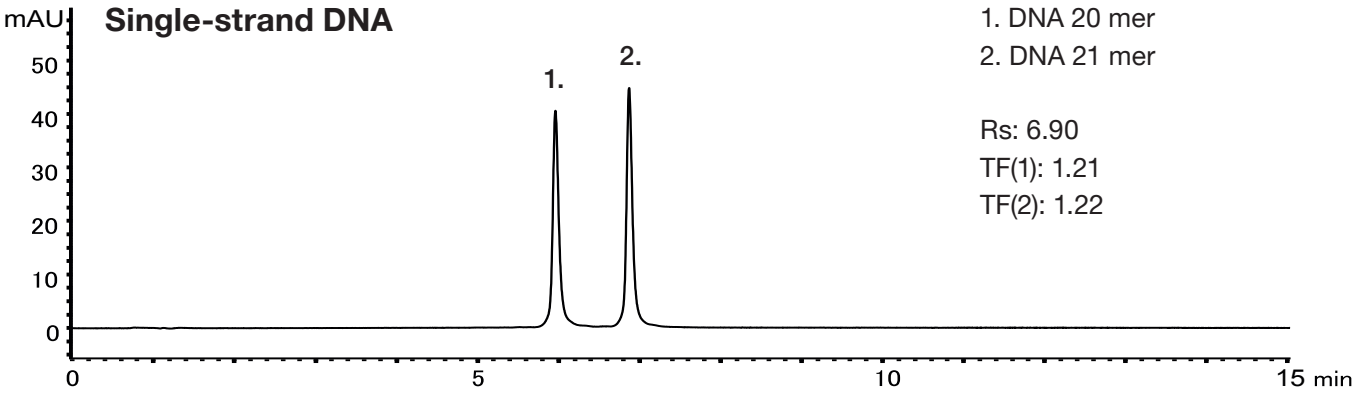
Eluent: A) 10 mM NaOH
B) 10 mM NaOH containing 2.0 M **Y**
15-100%B (0-30 min) for NaCl
5-50%B (0-30 min) for $NaClO_4$

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, the tailing factor of the oligonucleotide peak was improved. In addition, changing the counter ion from NaCl to $NaClO_4$ is effective.
It is important to optimise buffer and counter ion for excellent peak shape of oligonucleotides.

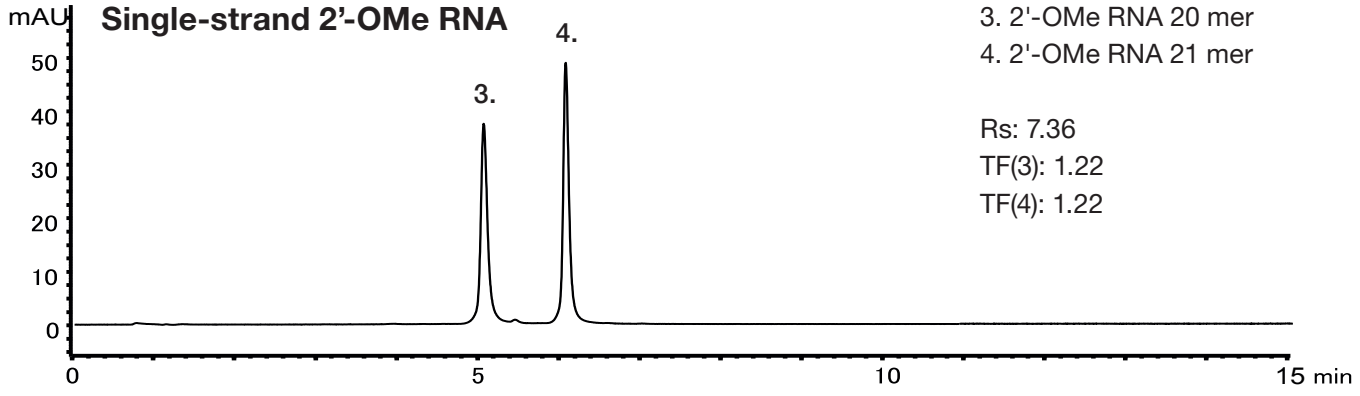
YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

Analysis examples with the optimised conditions



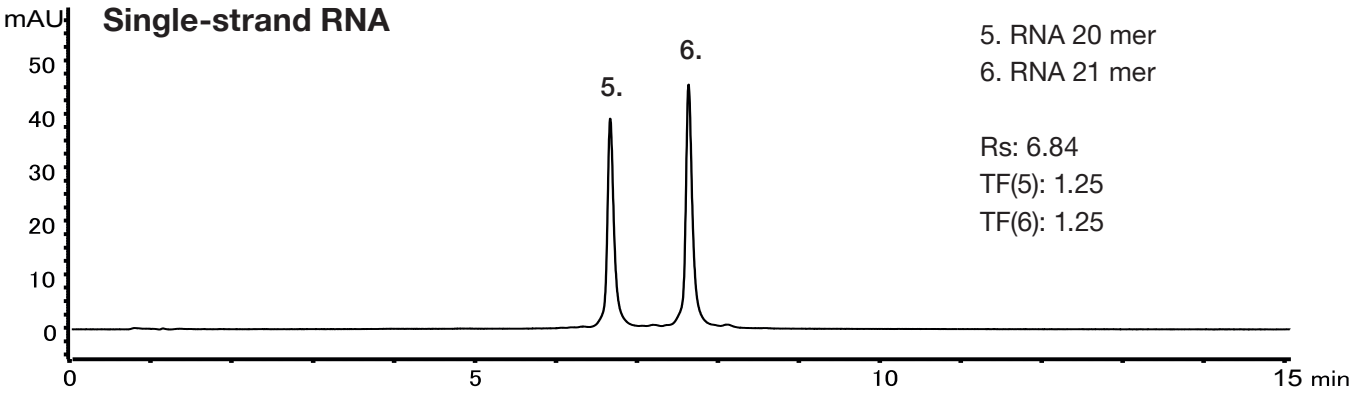
1. DNA 20 mer
2. DNA 21 mer

Rs: 6.90
TF(1): 1.21
TF(2): 1.22



3. 2'-OMe RNA 20 mer
4. 2'-OMe RNA 21 mer

Rs: 7.36
TF(3): 1.22
TF(4): 1.22



5. RNA 20 mer
6. RNA 21 mer

Rs: 6.84
TF(5): 1.25
TF(6): 1.25

Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
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 °C
Detection:	UV at 260 nm
Injection:	4 µL (5 nmol/mL each)

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