HPLC DATA SHEET

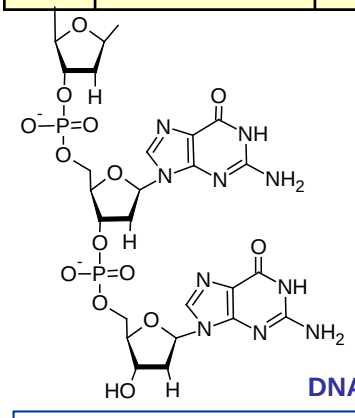
Optimization of oligonucleotide separations on ion-exchange chromatography

P180316AE

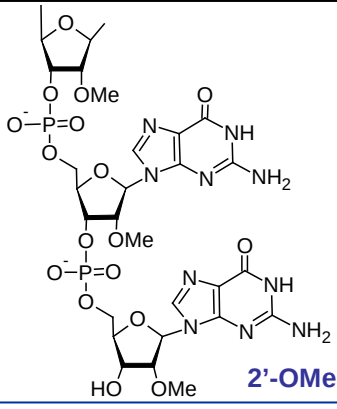
Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to play an important role as next-generation pharmaceuticals together with antibody drugs. These drugs demand chromatographic purification and analysis that can recognize slight structural differences following synthesis. In 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)

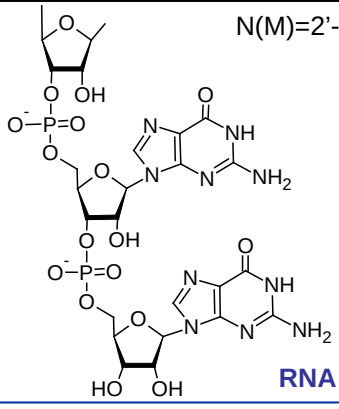


DNA



2'-OMe RNA

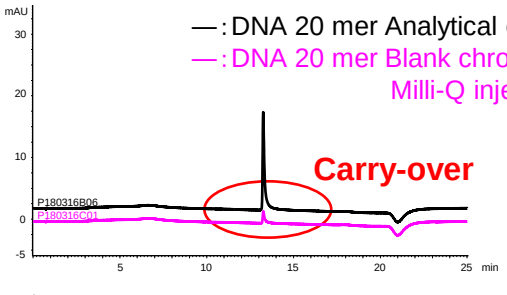
N(M)=2'-OMe RNA



RNA

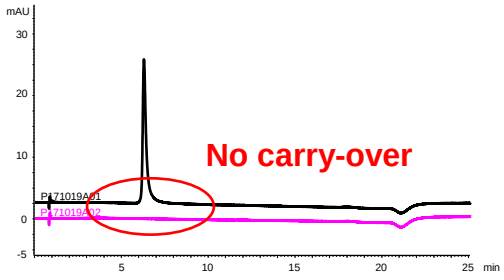
Reducing carry-over

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

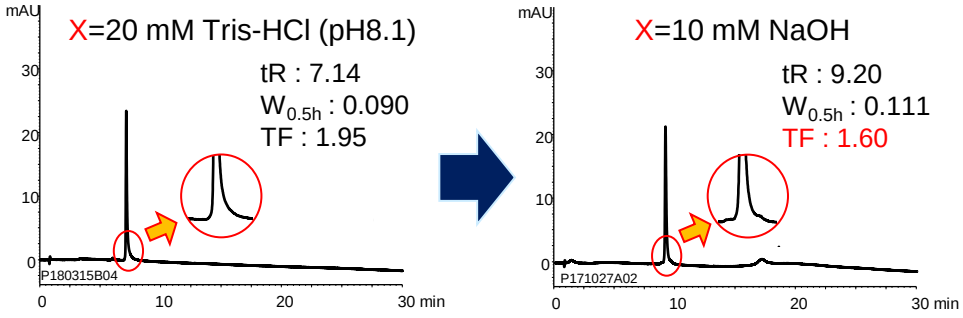


Column	: BioPro IEX QF 5 μm, 100 X 4.6 mm I.D.
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 2 μL (10 nmol/mL)

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

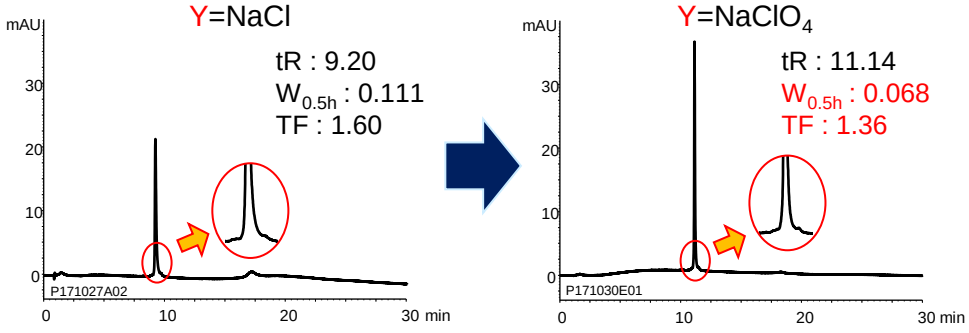
1) Influence of buffer type



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



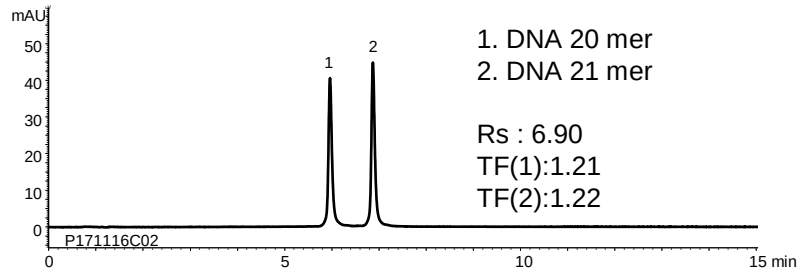
Eluent	: A) 10 mM NaOH B) 10 mM NaOH containing 2.0 M **Y** 15-100%B (0-30 min) for NaCl 5-50%B (0-30 min) for $NaClO_4$

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.**

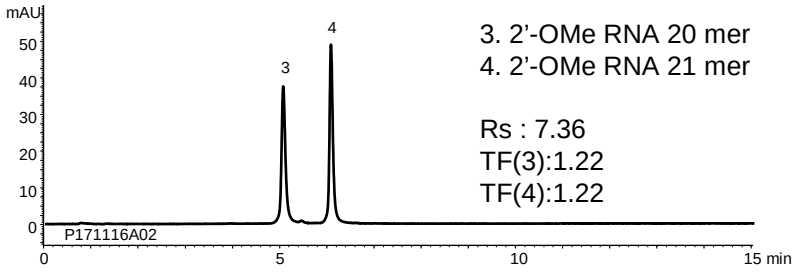
Analysis examples with the optimized conditions

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