



Oligonucleotides Analysis using SAX and RP

Sepax Technologies





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Sample Information: Oligonucleotide Standards

- Molecular Weight Range: 4,000 ~ 13,000 Da;
- 6 standard solutions ranging from 15 ~ 40 mer, with a gap of 5 bases each;
- Concentration: 10 nmol/mL; dissolved in water

Objective: *Baseline separation for each standard's chromatographic peak*

Experiments: Reversed Phase & Ion Exchange

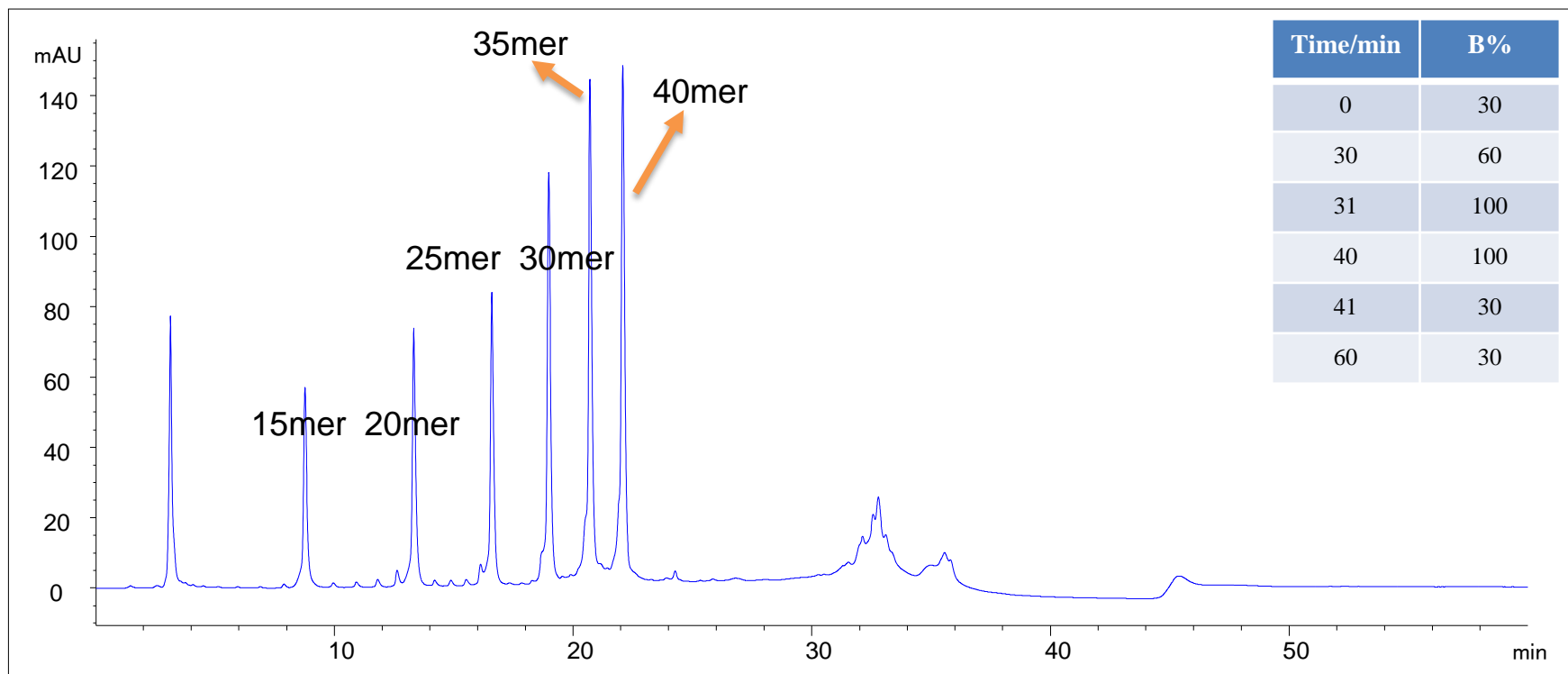
Reversed Phase	Ion Exchange
Column	
Bio-C18	Proteomix SAX
Mobile Phase	
A: 100mM TEAA, pH 7.0	A: 20mM Tris, pH 8.5
B: 95% Acetonitrile + 5% A, pH 8.3	B: 20mM Tris + 1.5M NaCl, pH 8.5
Required Injections	
Blank, Two injections for each sample, Blank	





Oligonucleotides Analysis using Proteomix SAX

Column: Proteomix SAX-NP5, 5 μ m, 4.6 \times 250 mm (PN: 403NP5-4625), Mobile Phase: A: 20mM Tris, pH8.5, B: 20mM Tris +1.5M NaCl, pH8.5, Flow Rate: 0.5 mL/min; Detector: UV 260nm; Column Temperature: 40°C; Sample: Oligonucleotide Standards; Injection Volume: 10 μ L



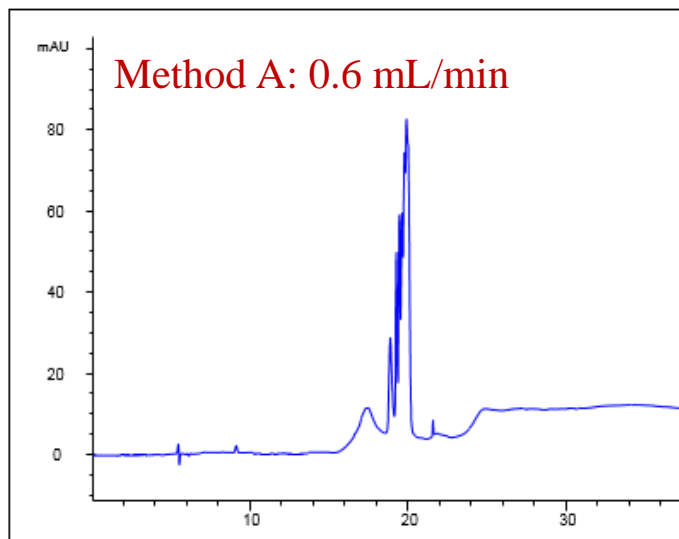
PEAK	1 st	2 nd	3 rd	4 th	5 th	6 th
RT (min)	8.767	13.319	16.588	18.973	20.705	22.080
Tailing	0.99	0.96	0.93	0.84	0.91	0.84
Resolution		18.12	12.74	9.34	6.85	5.43



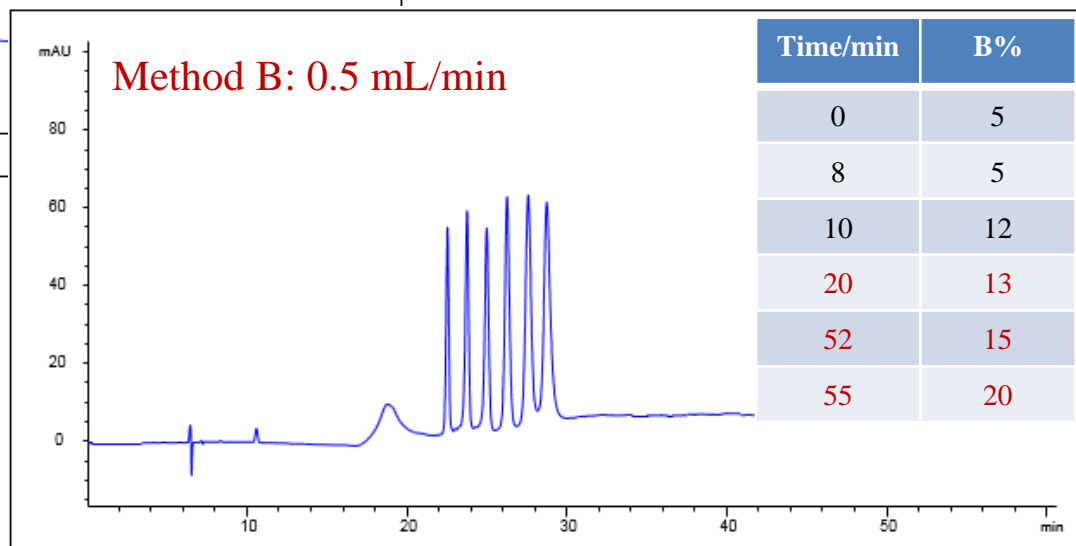


Oligonucleotides Analysis using Bio-C18

Column: Bio-C18, 5 μm , 300 \AA , 4.6 x 250 mm (PN: 106185-4625); Mobile Phase: A:100 mM TEAA, pH 7.0; B:95%ACN + 5%A, pH 8.3; Flow Rate: **Method A: 0.6 mL/min**; **Method B: 0.5 mL/min**; Detector: UV 260nm; Column Temperature: 50°C; Sample: Oligonucleotide Standards; Injection volume: 5 μL



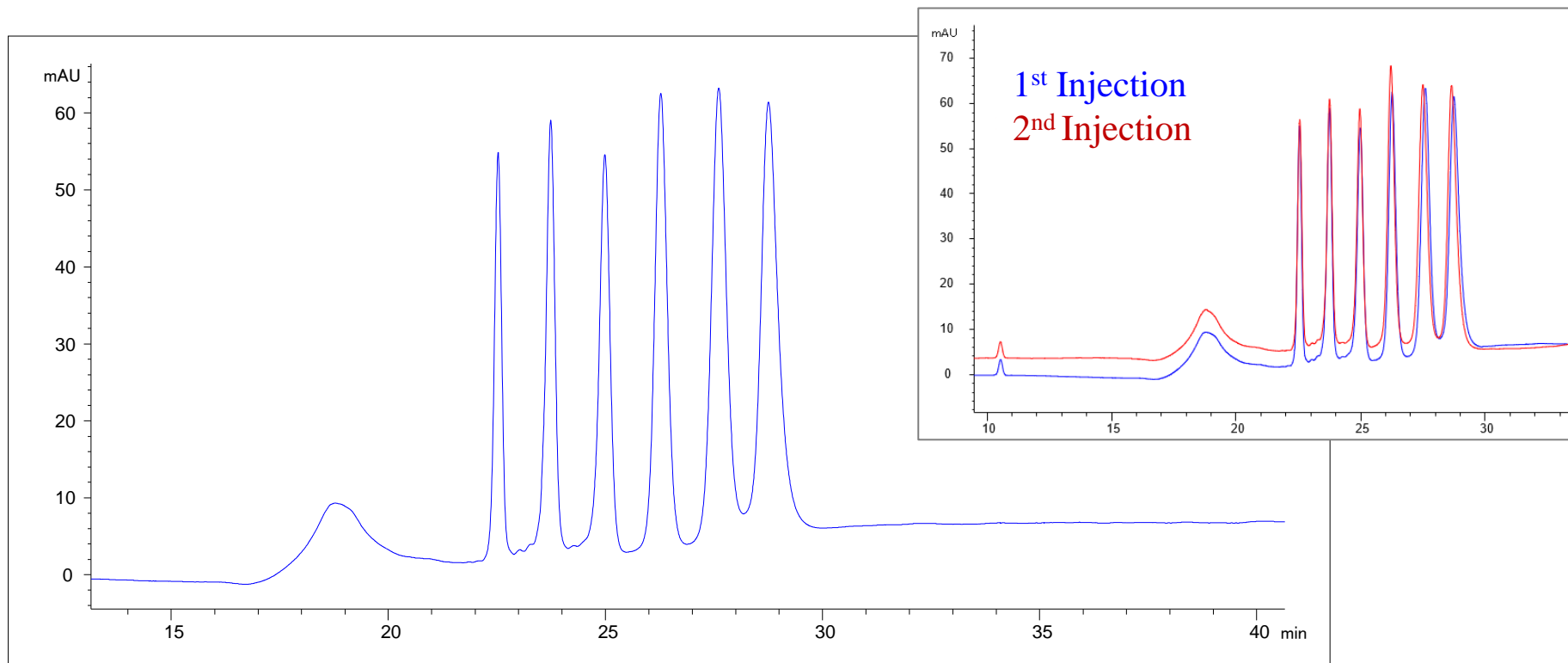
Time/min	B%
0	5
8	5
10	12
12	14
60	18





Oligonucleotides Analysis using Bio-C18

Column: Bio-C18, 5 μm , 300 \AA , 4.6 x 250 mm (PN: 106185-4625); Mobile Phase: A:100 mM TEAA, pH 7.0; B:95% ACN + 5%A, pH 8.3; Flow Rate: Method B: 0.5 mL/min; Detector: UV 260nm; Column Temperature: 50°C; Sample: Oligonucleotide Standards; Injection Volume: 5 μL



PEAK	1 st	2 nd	3 rd	4 th	5 th	6 th
RT (min)	22.53	23.74	24.989	26.279	27.614	28.758
Tailing	0.97	0.89	0.9	1.06	1.24	1.13
Resolution		3.48	3	2.61	2.28	1.7





siRNA Analysis using GP-C18 (RP)-UPLC

Column: GP-C18, 1.8 μ m, 120 A, 2.1 x 50 mm (PN: 101181-2105); Mobile Phase: **A: 7mM TEA+100mM HFIP+10mL ACN, B: 70%A+30%ACN**; Flow Rate: 0.2 mL/min; Detector: UV 260nm; Column Temperature: Room temperature; Sample: 21 nt siRNA (1.2 mg/mL); Injection volume: 2 μ L; Column Pressure: 247 bar

