Oligonucleotide Separation on Zenix™ SEC and Proteomix® SAX

Oligonucleotide Separation on Zenix™ Size Exclusion Chromatography (SEC)

Highlighted FACTS:

► Zenix™ SEC is suitable for protein/peptide oligonucleotide conjugates separation.

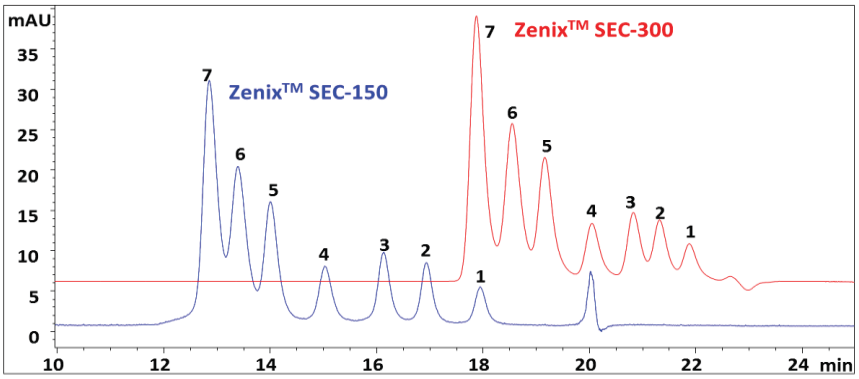
► Zenix™ SEC provides baseline separation for smaller oligonucleotides with a 5 base difference.

Technical Specifications:

Phase	Zenix and Zenix-C SEC
Material	Neutral, hydrophilic film bonded silica
Particle size (µm)	3
Pore sizes (Å)	80, 100, 150 and 300
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily)
Backpressure (psi)	~ 1,500 (at 0.35 mL/min on a 4.6 x 300 mm)
Maximum backpressure	~ 4,500
Maximum temperature	~ 80 °C
Mobile phase compatibility	Aqueous and organics

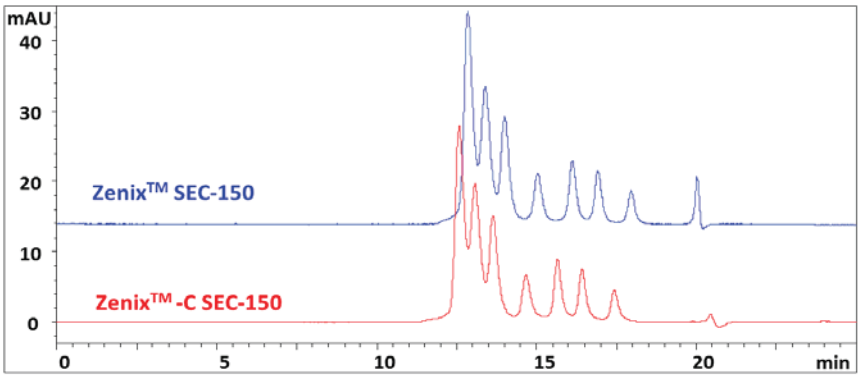
Zenix™ SEC-150 and Zenix™ SEC-300 Comparison

Column: Zenix™ SEC-150 and Zenix™ SEC-300 7.8 x 300 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: 150 mM sodium phosphate buffer pH 7.0, Injection Volume: 30 µL, Sample: 1) dA10, 2) dA15, 3) dA20, 5) dA40, 6) dA50, 7) dA60, 0.1 µM each in water 4) 5'-ATATCTACACGGCTACCCGTACCAATGCTGCTTCC-3' (35 nt)



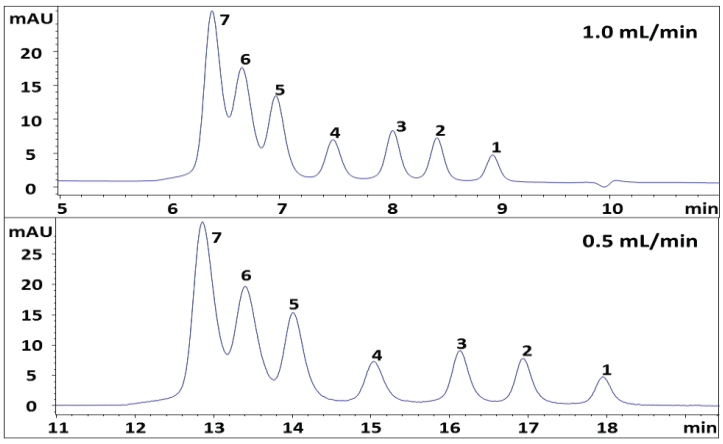
Zenix™ SEC-150 and Zenix™-C SEC-150 Comparison

Column: Zenix™ and Zenix™-C SEC-150 7.8 x 300 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: 150 mM sodium phosphate buffer pH 7.0, Injection Volume: 30 µL, Sample: 1) dA10, 2) dA15, 3) dA20, 5) dA40, 6) dA50, 7) dA60, 0.1 µM each in water 4) 5'-ATATCTACACGGCTACCCGTACCAATGCTGCTTCC-3' (35 nt)



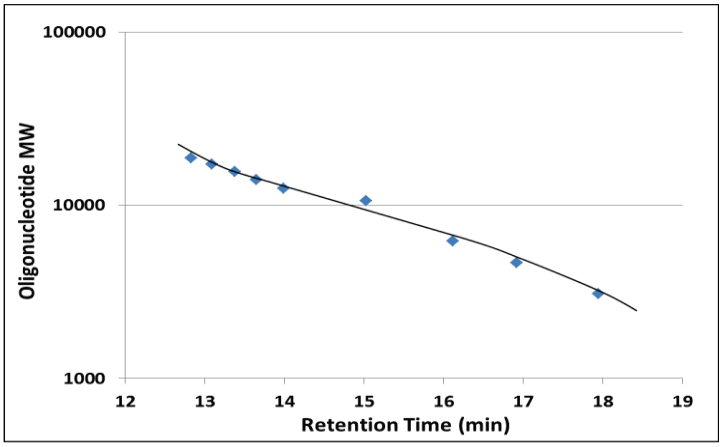
Separation on Zenix™ SEC-150 at varying Flow Rates

Column: Zenix™ and Zenix™-C SEC-150 7.8 x 300 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: 150 mM sodium phosphate buffer pH 7.0, Injection Volume: 30 µL



Oligonucleotide Calibration Curve on Zenix™ SEC-150

Column: Zenix™ and Zenix™-C SEC-150 7.8 x 300 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: 150 mM sodium phosphate buffer pH 7.0

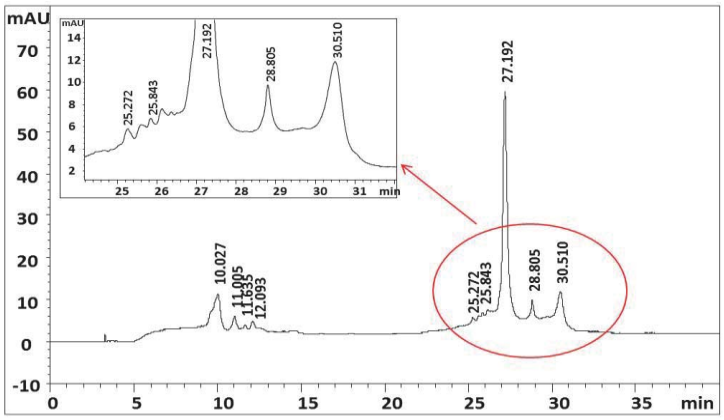


Oligonucleotide Separation on Zenix™ SEC and Proteomix® SAX

Oligonucleotide Separation on Proteomix® Strong Anion Exchange (SAX)

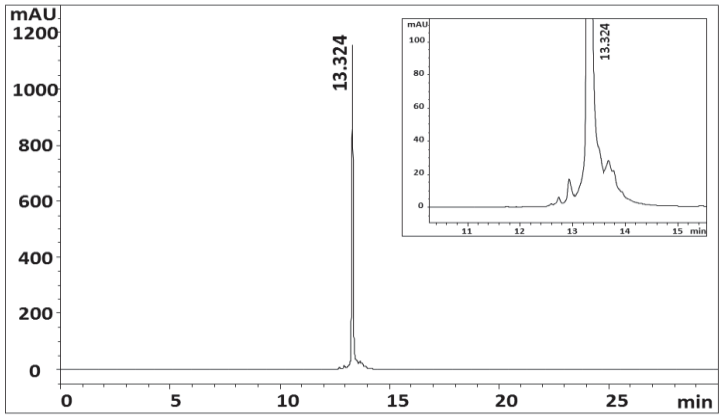
Analysis of ssDNA on Proteomix® NP5 SAX

Column: Proteomix® NP5 SAX 4.6 x 250 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: A: 20 mM Tris pH 8.0 and B: A + 1 M NaCl, Gradient: 0-100% B in 30 min, Injection Volume: 10 µL, Sample: ssDNA, diluted 100 times with water



Analysis of siRNA on Proteomix® NP5 SAX

Column: Proteomix® NP5 SAX 4.6 x 250 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: A: 20 mM Tris pH 8.0 and B: A + 0.5 M $NaClO_4$, Gradient: 25-75% B in 30 min, Injection Volume: 2 µL, Sample: siRNA



Highlighted FACTS:

► Proteomix® SAX separates varieties of oligonucleotides such as single stranded DNA fragments and small interfering RNAs.

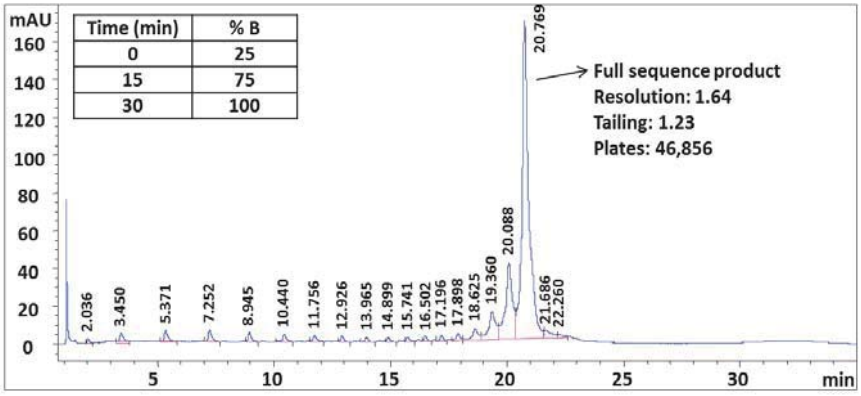
► High separation resolution on Proteomix® SAX is achieved between full length oligonucleotides and their degradation products.

Technical Specifications:

Phase	Proteomix® SAX NP5
Material	PS/DVB resin chemically bonded with $N(CH_3)_3$ functional groups
Particle size (µm)	5
Pore sizes (Å)	Non-porous
pH stability	2 – 12
Maximum backpressure	~ 6,000
Maximum temperature	~ 80 °C
Mobile phase compatibility	aqueous solutions and mixtures of water with acetonitrile, acetone or methanol

Analysis of Oligonucleotides on Proteomix® NP5 SAX

Column: Proteomix® NP5 SAX 4.6 x 250 mm, Flow rate: 1.0 mL/min, Detection: UV 260 nm, Mobile phase: A: 20 mM Tris pH 8.0 and B: A + 0.5 M NaCl, Injection Volume: 50 µL, Sample: 60 µg/mL Oligonucleotides in water (6,000 Da, at least one base difference)



Effect of Different Salts on Proteomix® NP5 SAX

Column: Proteomix® NP5 SAX 4.6 x 250 mm, Flow rate: 0.5 mL/min, Detection: UV 260 nm, Mobile phase: A: 20 mM Tris pH 8.0 and B: A + 0.5 M (salt), Gradient: 0-100% B in 30 min, Injection Volume: 5 µL, Sample: Mixture of poly dA10-18, dA20, 10 µM each