Protocol for Separating Phosphorothioate Diastereoisomers via HPLC

Method 1: Reverse phase, using Waters C18 µ-Bondapak, 3.9 mm * 300 mm column
Ref. Sam Butcher communication; Slim and Gait, Nucleic Acids Res 19 pp1183-1188, 1991

Buffer A: 100 mM ammonium acetate, pH 6.8
Buffer B: 20% A, 80% acetonitrile

HPLC settings:

1) Use 100 µL injection loop, ~50 µL injection volume
2) Run about ¼ of the deprotected RNA prep from a 0.4 µm synthesis (~150 µg)
3) Flow rate of 1.5 mL/min (pressure is around 1.000)
4) 0-8 min, 0% B
   8-11 min, 0-5% B
   11-41 min, 5-7% B (optimized for 19mer RNA)
   41-49 min, 20% B
5) Chart rate of 30 cm/hr, 20 mV on the medium sensitivity channel
6) Run at room temperature

Method 2: Anion exchange, using Dionex DNAPac PA-100, 9 mm * 250 mm column
Ref. Dionex user's manual

Buffer A: 100 mM ammonium acetate, pH 8; 370 mM KCl; and 2% acetonitrile
Buffer B: 100 mM ammonium acetate, pH 8; 700 mM KCl; and 2% acetonitrile

HPLC settings:

1) Use 100 µL injection loop, ~50 µL injection volume
2) Run about ¼ of the deprotected RNA prep from a 0.4 µm synthesis (~150 µg)
3) Flow rate of 1.5 mL/min (pressure is around 0.500)
4) Gradient of 0 → 56% B over 30 min (optimized for 19mer RNA)
5) Chart rate of 30 cm/hr, 20 mV on the medium sensitivity channel
6) Heat column to 65 ˚C, then equilibrate in buffer A

Materials:
Ammonium acetate: Fisher, HPLC grade, cat. # A639
KCl: Sigma, 99% pure, cat. # P-9541
Acetonitrile: Fisher, Optima grade, cat # A996

Notes:

1) Scale down to less than 100 µg if resolution is poor
2) Make a more shallow gradient around the %B that you know the oligo will elute
3) Always filter samples through a 0.22 µm filter before injection
4) Use Waters "SepPak" Cartridge for desalting, but beware of high acetonitrile content in your eluate after reverse phase HPLC
5) If Method 1 doesn't give good separation, try Method 2

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