Oligonucleotides

Terminal base dependence of oligonucleotide separations

Application Note

BioPharma

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Introduction
The ability of a column to separate a 29mer from a 30mer of the same sequence (with one additional 3' base) depends on the identity of the 3' terminal base of the 30 mer. In general, the separation follows the rule A>T>C>G. This effect is shown on Agilent Polaris C18-A. Further, temperature has a significant impact on oligonucleotide separation. As the temperature is raised to 60 °C, the oligonucleotide selectivity is improved thereby resulting in a good separation.
Conditions

Technique: Agilent Polaris C18-A, 4.6 x 50 mm, 5 µm
Eluent:
Solvent A: 100 mM TEAA, 0.1 mM EDTA,
Solvent B: 25% CH3CN, 100 mM TEAA, 0.1mM EDTA,
Gradient:
30 - 35%B in 10 min
(0:00 - 25% B, 0:30 - 30% B, 10:30 - 35% B, 11:00 - 35% B, 11:01 - 25% B; 13:00 - 25% B)
Flow: 1.0 mL/min,
Temperature: 60 °C
Detection: 260 nm
Samples: 50 pmol/μL, injected 50 pmol each

Peak identification

29mer: 5’ - CGT GGC CCT ATG CTT GTA AAC CTT GG
30mer:
A: 5’ - CGT GGC CCT ATG CTT GTA AAC CTT GGA
C: 5’ - CGT GGC CCT ATG CTT GTA AAC CTT GGC
G: 5’ - CGT GGC CCT ATG CTT GTA AAC CTT GGG
T: 5’ - CGT GGC CCT ATG CTT GTA AAC CTT GGT