Analysis of Phosphorothioated DNA Oligonucleotides

Oligonucleotide research has exploded in recent years, requiring new and sophisticated techniques not only in their application, but also analysis. Phosphorothioate modification of oligonucleotides (S-oligos) is a technique used to improve stability and slow degradation by nucleases. In the synthesis of S-oligos it is imperative that modifications are as directed, whether it be a partially or fully modified S-oligo. Cosmocore C18 columns are shown here in a method to elucidate minute differences in chemical modification with excellent resolution.

Acquisition Method

Column:	Cosmocore C18
Column Size:	2.1 x 100 mm
Mobile Phase:	A: 100mM TEAA water; B: Acetonitrile
Gradient:	10 → 25 %B (0 → 10 min)
Flow Rate:	0.4 mL/min
Temperature:	50 Celsius
Detection:	UV 260
Sample:	Phosphorothioate modified DNA oligos
Inj. Vol.:	1 µL

Sample Sequences

A: 20-mer All PS
T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T

B: 20-mer 1PO 5'
TT*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T*T

C: 20-mer 2PO 5'+ middle
TT*T*T*T*T*T*T*T*T* T*T**T**T*T*T*T*T*T*T*T*T

T* = phosphorothioate linkage

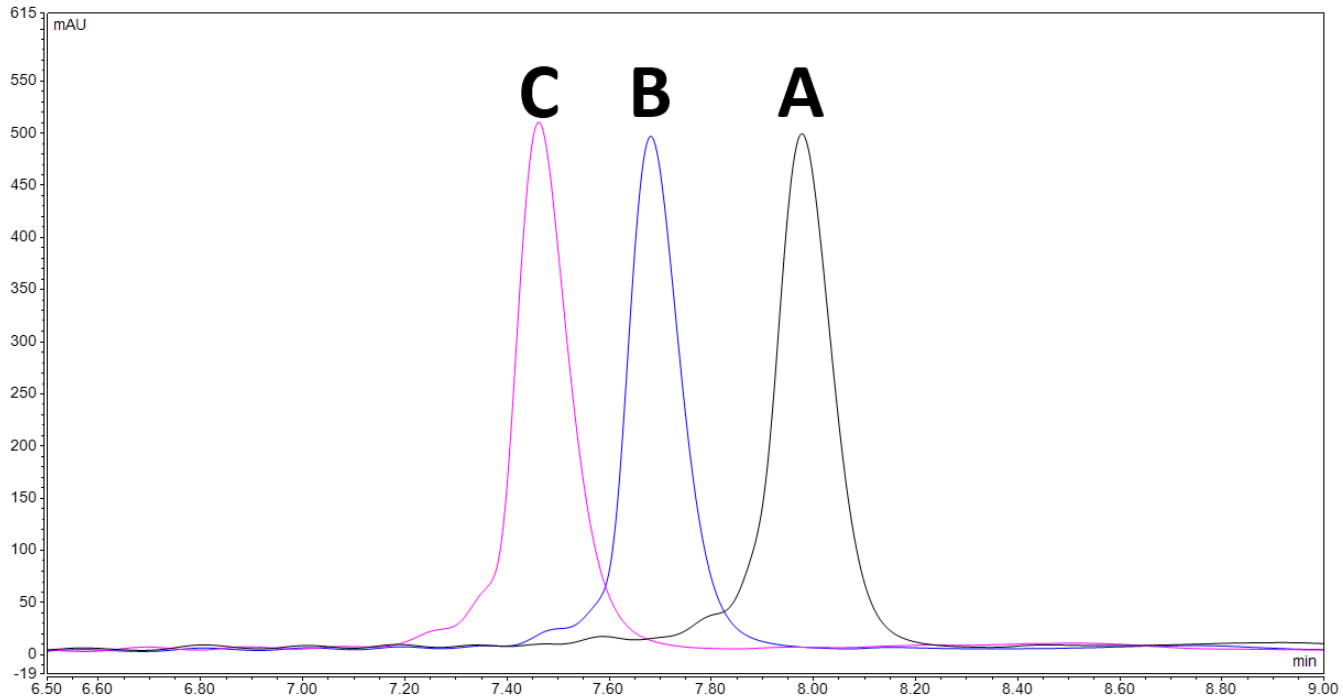

Figure 1: Overlaid chromatograms of fully and partially phosphorothioated DNA oligos. Excellent resolution is observed between oligos of the same length and differing number of modifications.