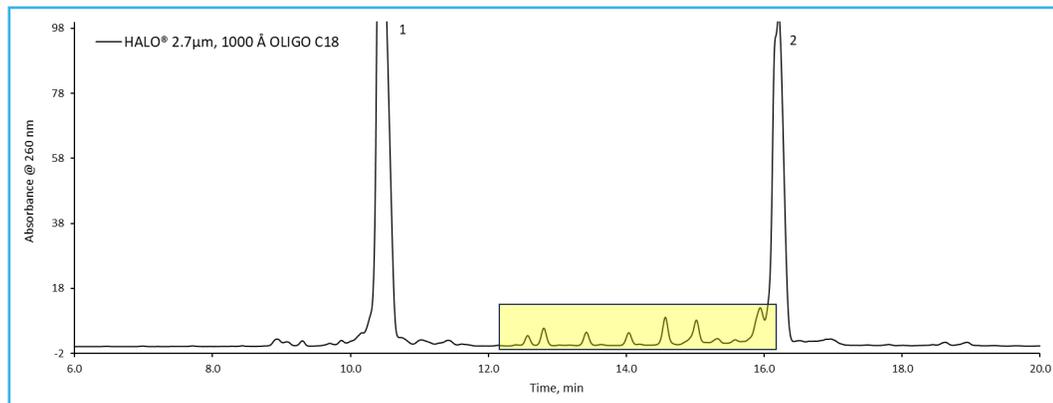




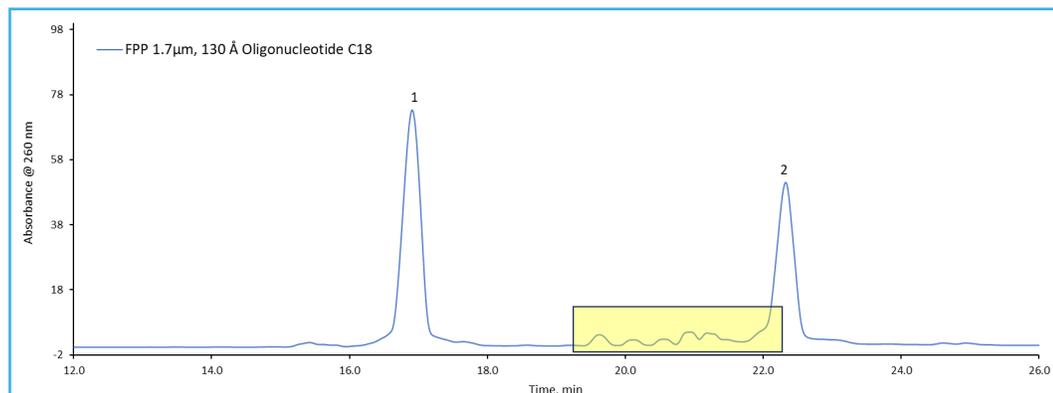
### Advantage of the HALO 1000 Å OLIGO C18 for siRNA Impurity Analysis

421



#### PEAK IDENTITIES

1. Anti-Sense Strand
2. Sense Strand



#### TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18  
 2.7 µm, 2.1 x 150 mm  
 Part Number: P2762-702  
 Column: FPP 1.7 µm, 130 Å Oligonucleotide C18  
 Mobile Phase A: 3 mM DiPEA/150 mM HFIP/5% MeOH  
 Mobile Phase B: 40/15/45 Water/IPA/MeOH

Gradient: Time % B  
 0.0 14  
 25 24  
 26 50  
 28 50  
 29 14  
 Flow Rate: 0.4 mL/min.  
 Pressure: HALO® - 288 bar  
 FPP - 543 bar

Temperature: 70 °C  
 Injection Volume: 1.0 µL (1 mg/mL of Lumasiran)  
 Sample Solvent: RNase Free Water  
 Wavelength: PDA, 260 nm  
 Flow Cell: 1 µL  
 Data Rate: 12.5 Hz  
 Response Time: 0.100 sec.  
 LC System: Shimadzu Nexera X2

This application note demonstrates the dramatic impact of pore size and particle morphology on the separation of Lumasiran siRNA and its related impurities. Using the HALO® 2.7 µm, 1000 Å OLIGO C18 column, in the highlighted regions, seven distinct impurity peaks are resolved between the antisense and sense strands, compared to only five impurity peaks observed on a fully porous 1.7 µm, 130 Å competitor oligonucleotide C18 column. The larger 1000 Å pore structure significantly improves mass transfer for higher molecular weight oligonucleotides, reducing diffusion limitations that commonly lead to peak broadening on smaller-pore, fully porous particles. In addition, the Fused-Core® particle design enhances efficiency by shortening diffusion paths while maintaining high efficiency. Together, these features result in sharper peaks, greater resolution, and far more detailed impurity profiling for Lumasiran. In contrast, the competitor's fully porous 130 Å column exhibits noticeable peak broadening and insufficient pore accessibility for large siRNA strands, masking finer impurity features and reducing confidence in impurity assessment while having a much higher backpressure. The "night-and-day" improvement achieved with the HALO 1000 Å column underscores the critical role of optimized pore architecture and mass-transfer performance in oligonucleotide separations.