Rapid Analysis of Mivelsiran, a lipid conjugated siRNA, using the HALO 1000 Å OLIGO C18 column

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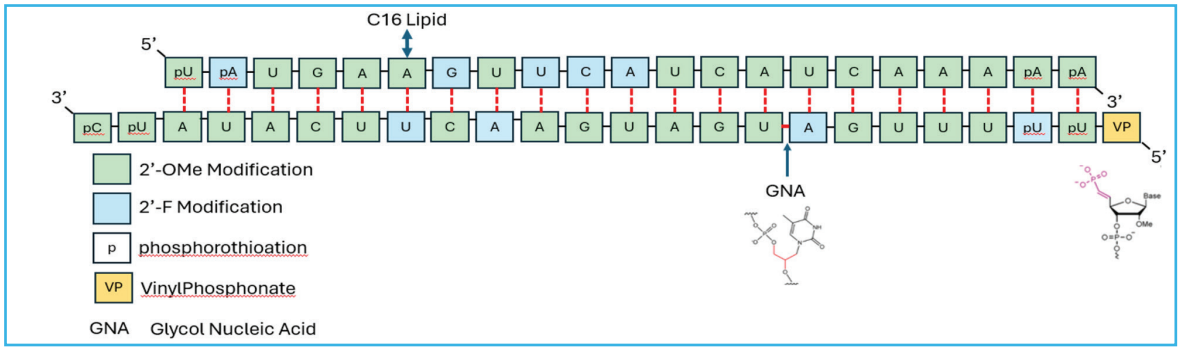

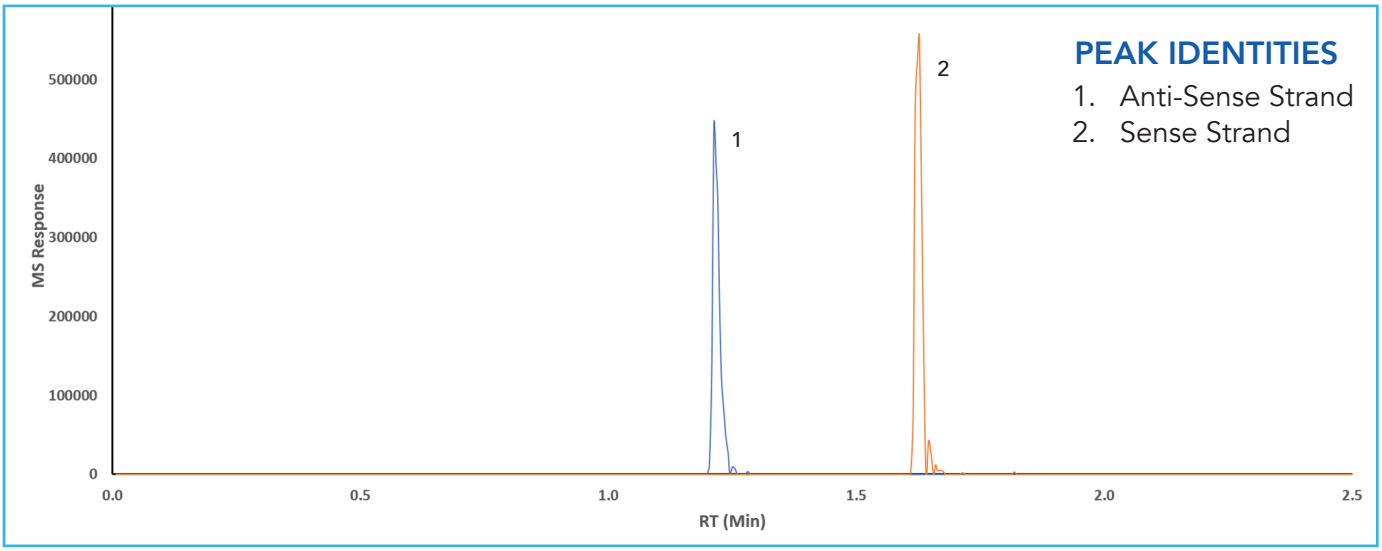

TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18 , 2.7 µm, 2.1x50mm
Part Number: P2762-402
Mobile Phase A: 15mM Hexylamine, 50mM HFIP
Mobile Phase B: 90% ACN/10%H_2O
Gradient:

Time	% B
0.0	15
0.5	15
1.5	40
1.8	50
2.0	100
2.5	100
3.0	15

Flow Rate: 0.85 mL/min.
Pressure: 230 bar
Temperature: 70 °C
Injection Volume: 10 µL (50 ng/mL)
Sample Solvent: 1x Tris-EDTA (10mM Tris pH 8.0, 1mM EDTA) (1mg/ml)

LC System: Shimadzu Nexera X2

MS Method Conditions:

Mass Spectrometer: Thermo Q-Exactive HF
Ion Mode: Negative polarity
Source: HESI II probe
Sheath Gas Flow Rate: 35
Aux Gas Flow Rate: 20
Sweep Gas Flow Rate: 1
Spray Voltage: 3.5kV
Capillary Temp: 400 °C
S-lens RF Level: 60
Aux Gas Heater Temp: 325 °C
MS1 Resolution: 120,000
AGC Target: 3e6
Maximum IT: 200ms
Scan Range: 800-2000 *m/z*

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The number of oligonucleotide therapeutics in development is rising rapidly as many of the pharmacological challenges are being better understood and solved using novel chemistries. Many of the chemical modifications used in the earliest FDA approved therapeutics included phosphorothiolation, 2'-OMe and 2'-F for nuclease resistance and GalNac conjugated linkers for targeted delivery, particularly for hepatocytes.

More recently, additional chemical modifications are being incorporated for additional nuclease resistance, minimizing off-target effects and expanding tissue and cell specific targeting modalities. Mivelsiran (ALN-APP) represents a next generation siRNA therapeutic that takes advantage of lessons learned from the previous generation siRNA therapeutics. Mivelsiran, currently in clinical trials, targets Amyloid Precursor Protein (APP) mRNA which has been implicated in the progression of Alzheimer's disease. Mivelsiran contains many of the previously mentioned chemical modifications, however, in order to target APP in the central nervous system, Mivelsiran contains a 2'-conjugated C16 lipid on the sense strand allowing passage across the Blood-brain barrier. Additionally, the antisense strand contains a 5'-E-Vinylphosphonate for additional nuclease resistance as well as a glycol nucleic acid 8 bases downstream which has been shown to reduce off-target interactions of the "Seed" region of the anti-sense strand.

The addition of a C16 lipid to a single strand of an oligo duplex presents a challenge from an analytical perspective given the significant difference in hydrophobicity between the strands. The ability to analyze both strands in a single chromatographic run is desired for both impurity analysis and rapid screening for pharmacokinetic purposes.

Here we demonstrate a method using the HALO 1000 Å OLIGO C18 column that is able to efficiently separate both strands in less than 3 minutes using a 2.1 x 50 mm column. Extracted ion chromatograms were generated from the (-6) charge state of both strands. This demonstrates the capabilities of the HALO 1000 Å OLIGO C18 column and a basis for building methods to analyze these increasingly complicated therapeutics.