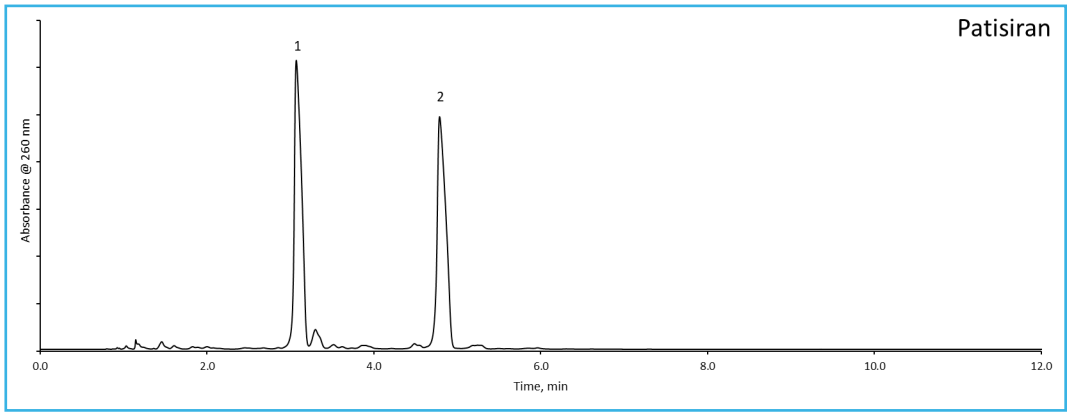
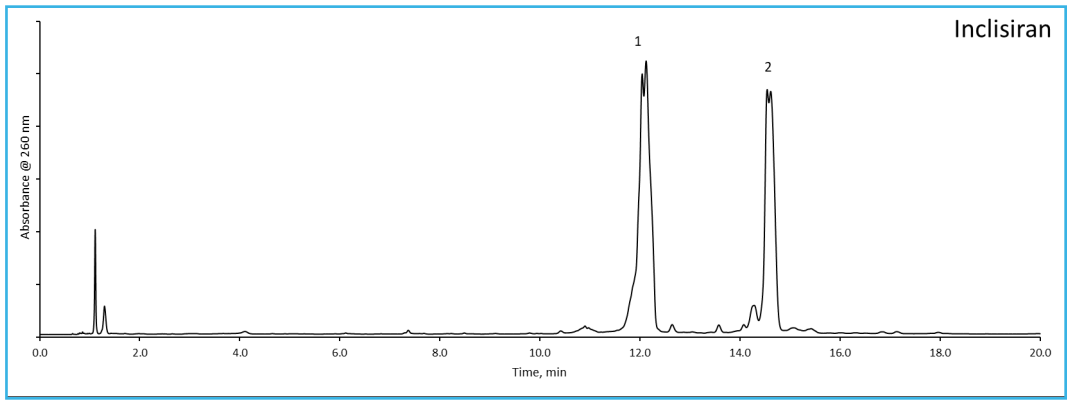
HALO®

BIOPHARMACEUTICALS

Evaluating Differences in Retention of siRNA Therapeutics on HALO 1000 Å OLIGO C18

420





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
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: 288 bar

Temperature: 70 °C
Injection Volume: 1.0 µL (1 mg/mL)
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 highlights the chromatographic differences between the siRNA therapeutics Patisiran and Inclisiran, emphasizing how chemical modifications influence retention. Inclisiran contains a higher density of 2'-O-methyl and 2'-fluoro substitutions compared to Patisiran, increasing modification density and strengthening interactions with the reversed-phase stationary phase. As a result, Inclisiran exhibits greater retention under identical chromatographic conditions.

In addition to these base-level modifications, Inclisiran carries a triantennary GalNAc conjugate used for hepatocyte targeting. Rather than increasing hydrophobicity, this large conjugate introduces significant steric bulk, which affects how the molecule interacts with the stationary phase and contributes to its increased overall retention. Patisiran, lacking this GalNAc moiety and containing fewer chemical modifications, elutes earlier with reduced retention.

The Inclisiran chromatogram also displays peak splitting, which is not attributable to a column performance issue. Instead, this splitting results from the separation of diastereomeric species generated by its phosphorothioate linkages.

These results clearly demonstrate how modification density, steric effects from conjugation, and backbone chemistry collectively drive the separation behavior of therapeutic siRNAs.