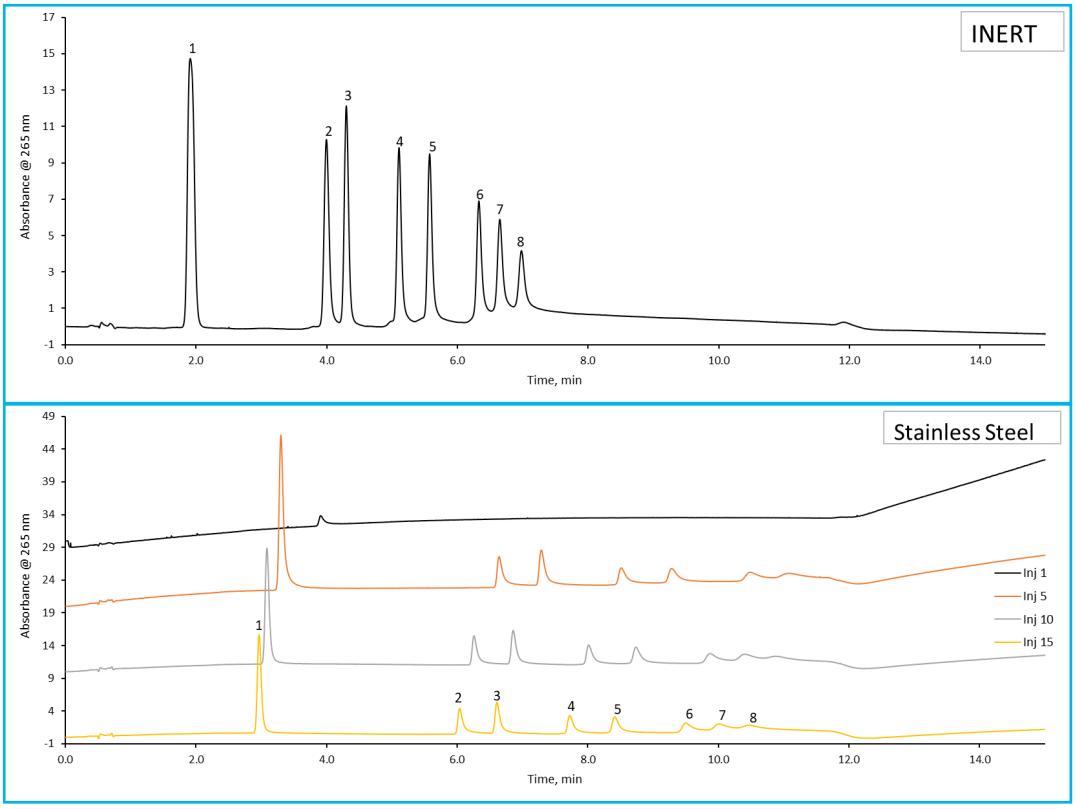
HALO®

BIOPHARMACEUTICALS

Advantages of Inert under HILIC Conditions

401



PEAK IDENTITIES

1. 10 mer
2. 15 mer
3. 20 mer
4. 25 mer
5. 30 mer
6. 40 mer
7. 50 mer
8. 60 mer

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 100 mm - INERT
Part Number: P2812-605
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-605
Mobile Phase A: 30/70 Water/ACN, 50mM Ammonium Acetate
Mobile Phase B: 90/10 Water/ACN, 50mM Ammonium Acetate

Gradient:

Time	%B
0.0	20
10.0	50
11.0	50
11.1	20
15.0	20

Flow Rate: 0.4 mL/min
Back Pressure: 141 bar
Temperature: 60 °C

Injection: 1.0 µL, (10µg/mL)
Sample Solvent: RNAse Free Water
Wavelength: PDA, 265 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.1 sec.
LC System: Shimadzu Nexera X2

A separation of a single-stranded oligonucleotide ladder under HILIC conditions was performed using two HALO® Penta-HILIC columns. The first chromatogram shows results from a standard stainless steel column. Notably, multiple injections were required to obtain sufficient recovery for detecting the later-eluting peaks. Even after 15 injections, quantifying the final oligonucleotide remained difficult. This example illustrates the importance of inert hardware for HILIC separations of phosphorylated compounds. In addition to poor recovery, increased retention was observed on the stainless steel column compared to the inert one, likely due to interactions between the oligonucleotides and the metal surface. In contrast, the second chromatogram—using an inert Penta-HILIC column—demonstrates that a single injection yields improved peak shapes and significantly higher recovery of the later-eluting oligonucleotides. Clearly, using inert column hardware from the outset saves both time and sample compared to repeated injections on stainless steel.

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