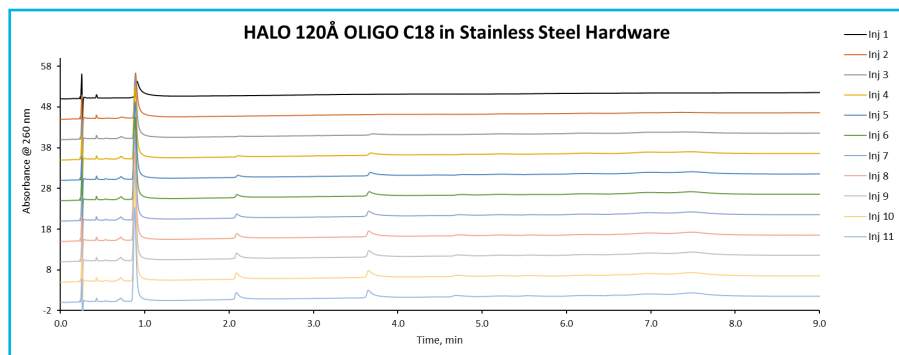




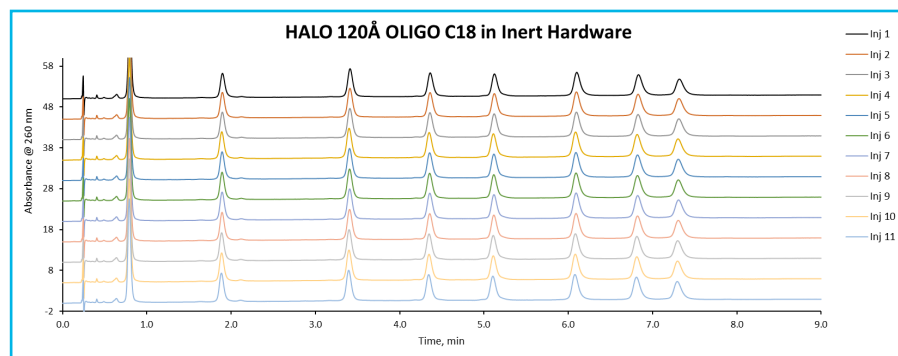
Effects of Inert Hardware on Low Ion-Pair Oligonucleotide Separations

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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 120 Å OLIGO C18, 2.7 µm, 2.1x50 mm
(Stainless Steel)
 HALO 120 Å OLIGO C18, 2.7 µm, 2.1x50 mm
(INERT)

Mobile Phase A: 25mM HAA @ pH 7.0

Mobile Phase B: 50/50 25mM HAA/Acetonitrile

Gradient:	Time	%B
	0.0	8
	12.0	22
	12.5	8
	16.0	8

Flow Rate: 0.4 mL/min.

Back Pressure: 130 bar

Temperature: 60 °C

Injection: 1 µL of 1:10 Dilution of Ladder (10µg/mL)

Sample Solvent: RNAse free water

Wavelength: PDA, 260 nm

Flow Cell: 1 µL

Data Rate: 40 Hz

Response Time: 0.05 sec.

LC System: Shimadzu Nexera X2

This application highlights the critical role of column hardware material in the separation and recovery of oligonucleotides. An inert column was compared directly to a stainless steel column using identical chromatographic conditions. The inert hardware (tube, inlet and outlet frits) delivered high recovery on the very first injection, indicating minimal analyte interaction with the hardware. In contrast, the stainless steel column exhibited poor recovery on the initial injection and showed only gradual improvement with repeated use. Even after 11 injections, recovery from the stainless steel column remained significantly lower and never matched the performance achieved with the inert hardware. These results demonstrate that oligonucleotides can interact strongly with stainless steel surfaces, leading to adsorption, delayed equilibration, and reduced quantitative performance. Inert hardware effectively eliminates these nonspecific interactions, providing immediate, consistent recovery and improved method robustness. For applications requiring accurate quantitation, high sensitivity, and minimal carryover, particularly during method development, inert column hardware offers a clear advantage over traditional stainless steel column hardware components.