Advantages of Inert Hardware Under HILIC Conditions for Oligonucleotide Separations

Hydrophilic interaction liquid chromatography (HILIC) is widely used for the separation of highly polar analytes, including oligonucleotides. While HILIC provides strong retention and resolution for these molecules, interactions between analytes and column hardware can adversely impact recovery and reproducibility.

In particular, stainless steel column components can adsorb oligonucleotides, resulting in reduced peak areas for initial injections and necessitating multiple "conditioning" runs before achieving consistent recovery.

Recent evaluations comparing HALO® columns with inert hardware against standard stainless steel hardware under HILIC conditions demonstrate a clear advantage for inert column designs, especially in minimizing analyte loss due to adsorption.

Keywords: HILIC, oligonucleotides, inert hardware, stainless steel, recovery, conditioning, HALO®

EXPERIMENTAL

Test Conditions:

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

Gradient:

Time (min)	%B
0.0	50
10.0	20
11.0	20
11.1	50
15.0	50

Flow Rate: 0.4 mL/min
Back Pressure: 141 bar
Temperature: 60 °C
Injection: 1.0 µL (10 µg/mL of ssDNA 10/60 Ladder)
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

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

DISCUSSION AND RESULTS

When comparing the performance of inert and stainless steel hardware under HILIC conditions for oligonucleotide separations, a striking difference in recovery behavior emerges. The inert hardware achieved full recovery of all oligonucleotide peaks from the very first injection, as shown in Figure 1, which displays a clean separation of each oligonucleotide with no loss of peak area.

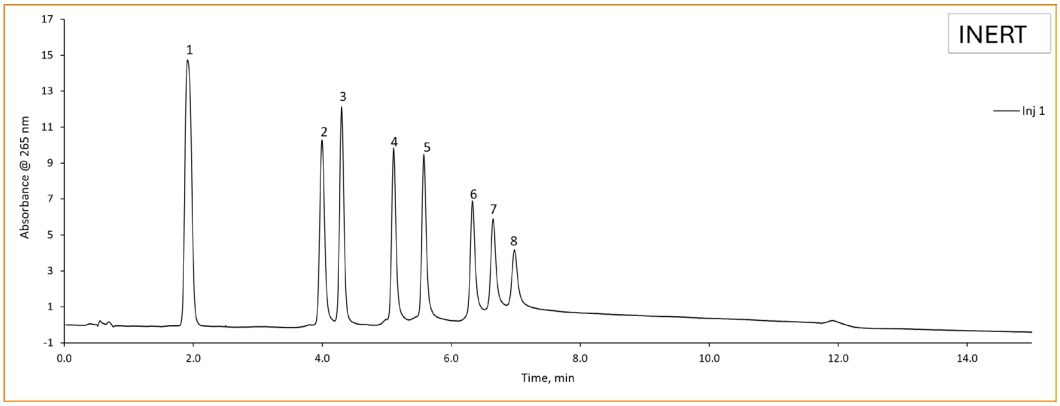


Figure 1. A separation of oligonucleotides on a Penta-HILIC column that is loaded with inert hardware. Each oligonucleotide is well resolved and shows full recovery on the first injection.

In contrast, the stainless steel hardware exhibited significantly reduced recovery in the initial runs. The first injection produced only a fraction of the expected peak area, and recovery gradually improved over the course of six or more injections. This progression is illustrated in Figure 2, where the chromatogram series shows how additional injections slowly improve peak area, yet the peak shapes and intensities never fully match those of the inert hardware.

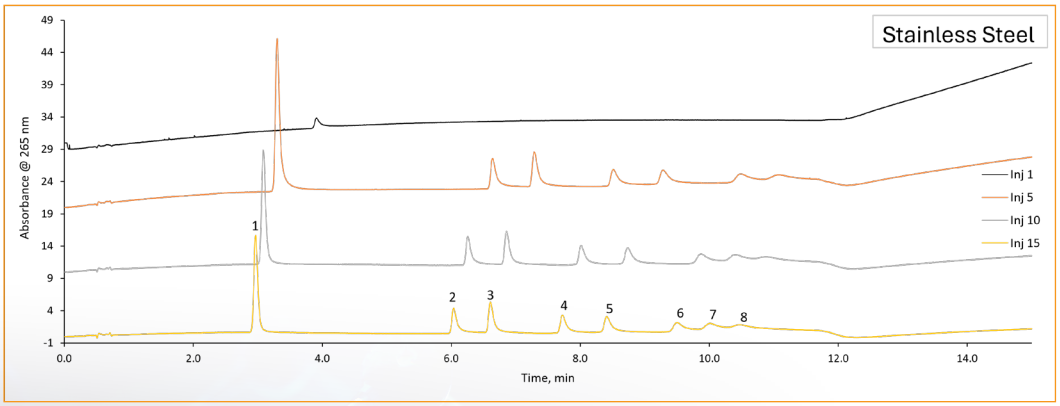
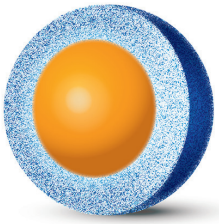


Figure 2. A separation of oligonucleotides on a Penta-HILIC column using the standard stainless steel hardware. The chromatogram shows the progression of multiple injections on the column starting from injection 1 and going to injection 15. The recovery of the oligonucleotides is low and even after continuous injections the peak shapes don't match the inert column.

This discrepancy is most likely due to surface interactions between the oligonucleotides and the metal components in stainless steel hardware including the tube and frits. Oligonucleotides contain phosphate backbones and, in some cases, exposed nucleobase functionalities that can interact strongly with exposed metal oxide layers on stainless steel. Under the high organic solvent content typical of HILIC mobile phases, solvation layers are reduced, making these interactions more prominent.



Over multiple injections, these active metal sites gradually become passivated by adsorbed material, reducing the extent of interaction and leading to the observed increase in recovery — a phenomenon commonly referred to as "column conditioning." However, this process is inefficient, consumes valuable sample, and still does not fully eliminate the interaction.

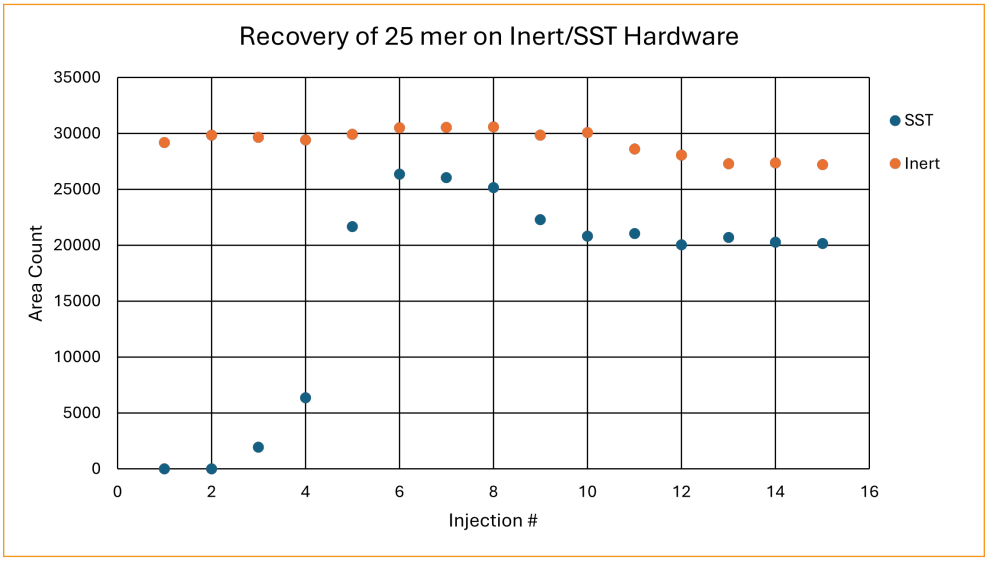


Figure 3. A plot comparing the recovery of the 25 mer oligonucleotide over 15 injections on both the inert hardware (Orange) and the stainless steel hardware (Blue)

The impact of these differences on quantitative performance is highlighted in Figure 3, where the peak area of the 25 mer oligonucleotide is plotted over 15 sequential injections. The inert hardware (orange) delivers consistently high recovery from the first injection, while the stainless steel hardware (blue) shows a gradual climb toward full recovery, never quite matching the inert column's performance.

Inert hardware avoids these issues by using surface treatments or alternative materials that effectively mask or eliminate reactive metal sites. By preventing interactions with the analyte, inert hardware maintains consistent and high recovery from the very first injection, preserving both sensitivity and reproducibility in HILIC oligonucleotide separations.

CONCLUSION

For oligonucleotide separations under HILIC conditions, inert hardware delivers immediate and complete analyte recovery, eliminating the need for time- and sample-consuming conditioning injections. Stainless steel hardware, in contrast, exhibits significant under-recovery in early injectionsand requires at least six runs to approach full recovery—yet still underperforms compared to inert designs. Adopting inert column hardware for HILIC applications enhances reproducibility, maximizes sample utilization, and reduces startup time, making it the superior choice for oligonucleotide analysis.

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