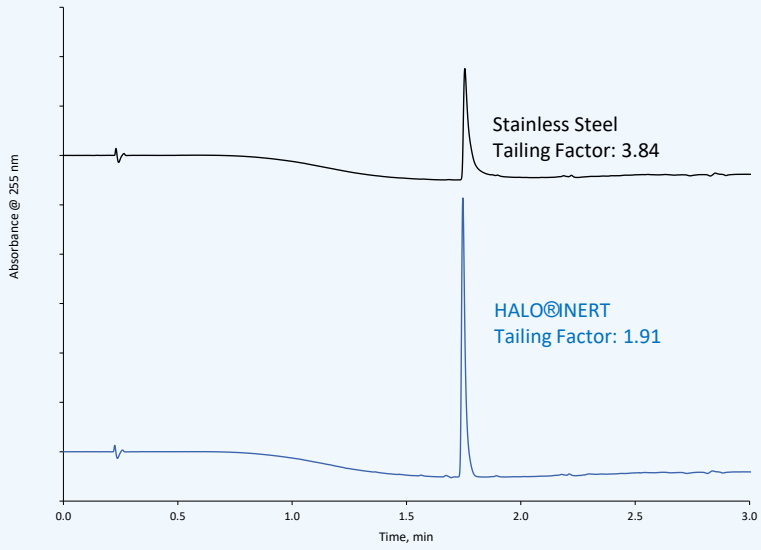
Benefits of HALO® INERT Column Hardware

Analytes that can chelate to metal such as phosphorylated and carboxylated compounds, and some biomolecules, e.g., oligonucleotides, can exhibit non-specific adsorption to stainless steel column hardware (column body and frits). In the most extreme cases, these interactions can cause no signal to be observed to less dramatic effects such as reduced peak areas. In all cases, tailed peak shapes are a symptom of the non-specific adsorption. Multiple injections of either the target analyte or a peptide/protein may need to be made to obtain reproducible results, which is commonly known as column conditioning. For more information, see reference 1. To combat non-specific adsorption, the HALO® INERT Column Hardware has recently been introduced. This hardware consists of the stainless steel column body and frits that have been coated to create an inert surface, thus eliminating the need for column conditioning.

To demonstrate the effectiveness of the HALO® INERT Column Hardware, the same manufactured lot of HALO 90 Å C18, 2.7 µm material was loaded into stainless steel hardware and HALO® INERT hardware. Injections of hydrocortisone 21-phosphate sodium salt were made on each column. Figure 1 shows a comparison of the results obtained using the stainless steel column (black trace) to the HALO® INERT column (blue trace). The tailing factor is ~ 50% smaller with the HALO® INERT column compared to the stainless steel column.

Figure 1. Comparison of stainless steel column results to HALO® INERT results for hydrocortisone 21-phosphate sodium salt. HALO® INERT shows both improved tailing and area.



TEST CONDITIONS

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm, INERT
Part Number: P2812-402
Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-402
Mobile Phase:
A: 10 mM ammonium formate, pH 3.2
B: Acetonitrile
Gradient: 5-55% B in 3 min
Flow Rate: 0.5 mL/min
Back Pressure: 206 bar
Temperature: 30 °C
Injection: 1.0 µL of 25 ng/µL hydrocortisone sodium phosphate
Sample Solvent: 90/10 water/methanol
Wavelength: PDA, 255 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

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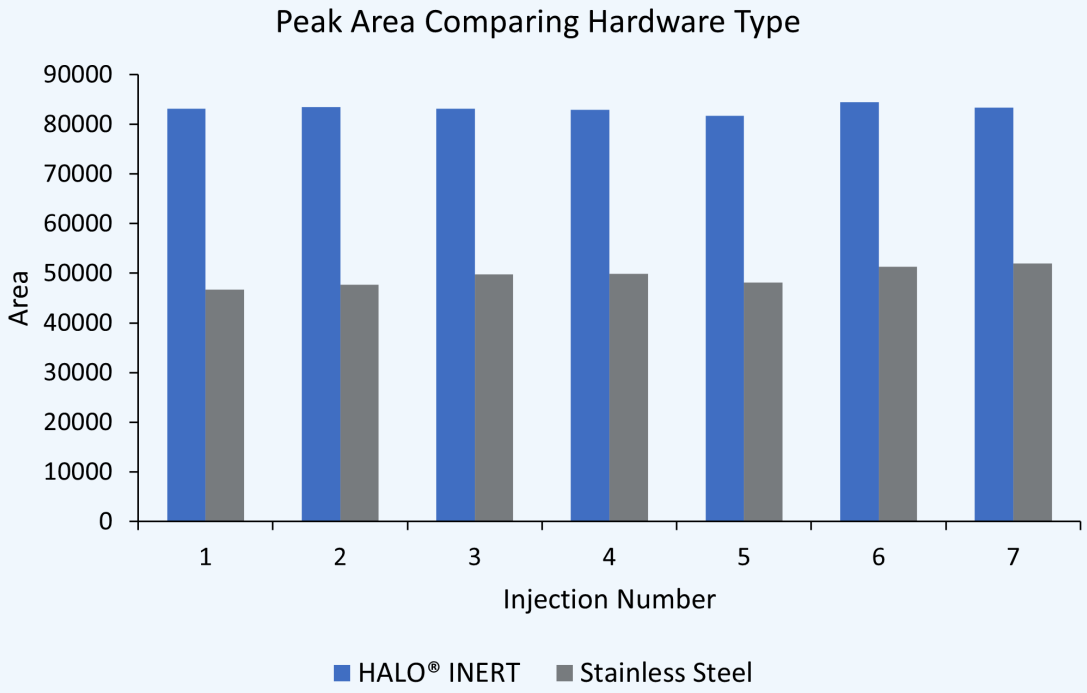
Figure 2. Consistently higher areas observed with HALO® INERT hardware compared to stainless steel hardware for hydrocortisone 21-phosphate sodium salt for multiple injections.

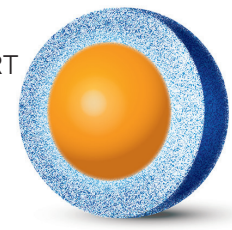


Additionally, the HALO® INERT column showed > 40% increase in peak area as shown in the graph in Figure 2 (above).

The benefits of the HALO® INERT column hardware were also observed when comparisons using a 15 mer of Oligo dT were run using HALO® OLIGO C18 in both inert and stainless steel hardware. Sample loads of 0.25 to 2 ng were injected on each column. At all of the concentrations, the area was greater and the tailing was lower with the HALO® INERT column hardware. See Table 1 for the comparative results.

ng injected on column	% Difference (Area)	% Difference (Tailing Factor)
0.25	57%	-29%
0.5	46%	-26%
1	26%	-39%
2	20%	-26%
5	8%	-23%

Table 1. Comparison of results of Oligo dT 15 mer injected at different amounts on HALO® INERT hardware compared to stainless steel hardware.



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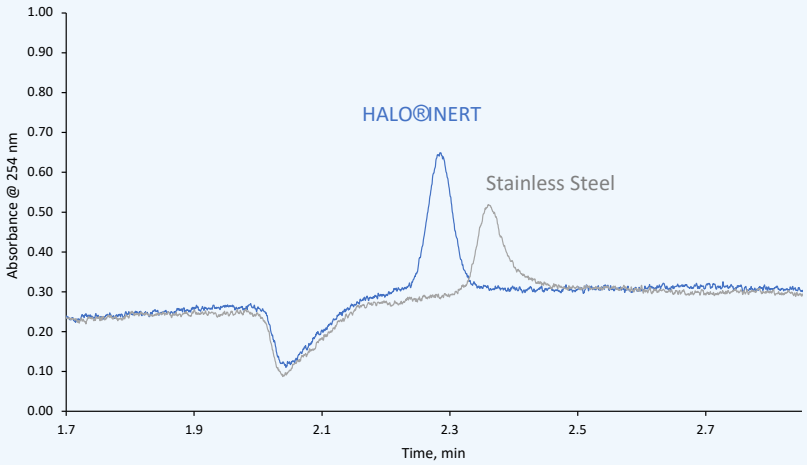
As the sample load increases, the difference in area decreases. A possible explanation for this may be that as more sample is injected on the stainless steel column, a conditioning effect is observed in which the active metal sites are occupied and cannot bind additional sample. Another observation from this comparison is that the retention time of the Oligo dT 15 mer is reduced with the HALO® INERT hardware compared to the stainless steel hardware. See example chromatograms in Figure 3.

Figure 3. Chromatograms showing the 0.5 ng sample load level of Oligo dT 15 mer on the HALO® OLIGO C18 column in inert hardware (blue) compared to the HALO® OLIGO C18 in stainless steel column hardware (gray). The area is 46% greater and tailing factor is 26% lower with the inert hardware. Retention time is decreased since non-specific metal interactions are reduced when using the inert hardware.



TEST CONDITIONS

Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Part Number: P2A62-402
Mobile Phase:
A: 100mM TEAA, Adjusted to pH = 8.4
B: ACN
Gradient:

Time	%B
0.0	8
3.0	10
3.5	20
4.0	20
4.1	8
8.0	8

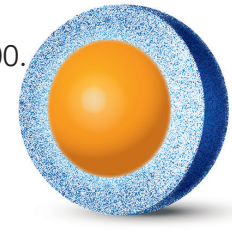
Flow Rate: 0.5 mL/min
Back Pressure: 146 bar
Temperature: 60 °C
Injection: 1.0 µL
Sample Solvent: 10mM Tris HCl/1mM EDTA pH=8.0
Wavelength: PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

CONCLUSIONS

In summary, the HALO® INERT column hardware shows advantages in terms of improved peak shape and recovery of analytes, such as phosphorylated analytes and oligonucleotides, that are likely to exhibit non-specific adsorption to metal sites on stainless steel. By using the HALO® INERT columns, reproducibility is improved and there is no need for column conditioning. HALO® INERT improves the reliability of the data generated and reduces the need for additional sample injections, thus increasing the efficiency of the experiments.

REFERENCE

1. Powell, M. J. and Timperman, A. T. (2004) Proteome Analysis. In Aguilar, M-I. (editor) HPLC of Peptides and Proteins Methods and Protocols. Humana Press, 387-400.



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