Comparison of a chromatographic performance for bioinert HPLC column hardware to standard HPLC hardware

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Abstract

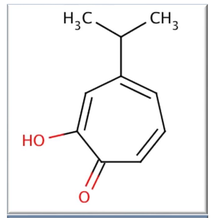
Many large biomolecules, such as nucleotides, proteins/peptides, and metal-coordinating compounds, are prone to surface interactions. These unwanted interactions can have a significantly negative impact on the performance of chromatographic methodology. Over the years, several approaches have been proposed to mitigate the issues caused by these unwanted interactions. A very effective technique is to utilize a surface coating on the column body and frit that makes them bioinert. The work presented here will compare chromatographic performance for HPLC hardware with a bioinert surface coating and typical stainless steel HPLC column hardware. This coating shows excellent peak shapes for highly adsorptive substances. Additionally, it is especially ideal for use in highly sensitive LC/MS analyses since it prevents carry over and ensures complete recovery without column preconditioning.

Benefits to using a surface-coated column versus the most commonly-used stainless steel column:

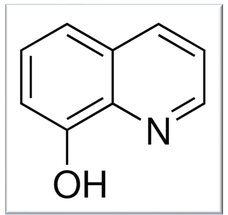
- Surface-coated columns outperform most stainless steel columns
- The bioinert hardware provides a superior alternative for compounds that contain phosphate groups such as oligonucleotides
- Enhanced peak shape and response
- Improved performance of coordination compounds

Introduction

Large molecules, like proteins and oligonucleotides, often require an inert flow path for good peak shape and reproducible HPLC analysis. In this work, we present a quick comparison of the YMC-Accura Triart C18 bioinert column versus the standard stainless steel YMC-Triart C18 column.



Hinokitiol



8-Quinolinol

Two stock solutions of 1mg/mL containing hinokitiol and 8-quinolinol were prepared in 50:50 water:acetonitrile diluent. A sample mix of both compounds with a final concentration of 0.1mg/mL was used for analysis.

Experimental Parameters

For this experiment, a mixture of hinokitiol and 8-quinolinol were prepared in water/ acetonitrile diluent. Both compounds are chelating agents, which can bind to metal sites.

System	Agilent 1200 Series HPLC
DAD detector	310nm
Columns	YMC-Accura Triart C18 100 x 4.6mm ID, 3µm, 120Å YMC-Triart C18 100 x 4.6mm ID, 3µm, 120Å
Mobile Phase	60:40 water:acetonitrile
Column Temp	30°C
Flow	1.0mL/min
Injection Volume	10µl

Results

Figure 1. YMC-Triart C18 stainless steel column

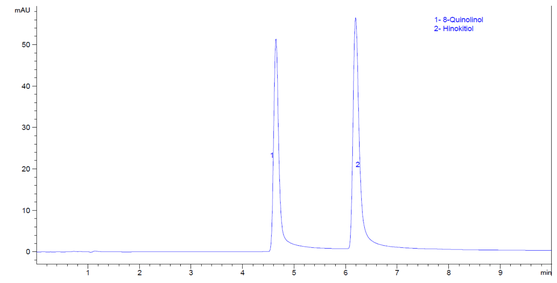


Figure 2. YMC-Accura Triart C18 bioinert column

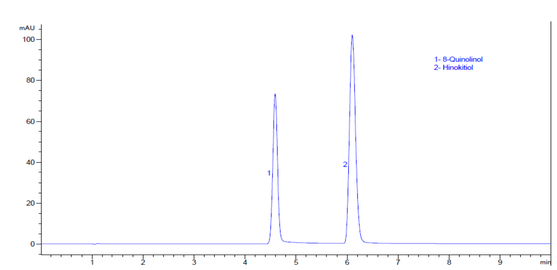


Table 1. Stainless steel to Accura (bioinert) comparison

	YMC-Triart C18 Stainless Steel Column			
	Retention Time (min)	Theoretical Plates	USP Symmetry	Resolution
8-Quinolinol	4.65	11,448	0.57	--
Hinokitiol	6.19	13,523	0.54	7.98

	YMC-Accura Triart C18 Bioinert Column			
	Retention Time (min)	Theoretical Plates	USP Symmetry	Resolution
8-Quinolinol	4.59	11,726	0.98	--
Hinokitiol	6.10	12,269	0.86	7.75

Discussion

Comparing the chromatograms in Figure 1 for stainless steel and Figure 2 for the Accura column, it is evident that the peak shape of both compounds is much improved on the Accura column versus the stainless steel column. Both fronting and tailing are significantly higher in the stainless steel column. In addition, the peak intensity and areas for both compounds are improved with the Accura column compared to the stainless steel.

When evaluating chromatographic performance, no significant difference was found in terms of theoretical plates and resolution between the two columns; however, the symmetry, which is the peak shape characteristic, was significant. As Table 1 shows, we obtained symmetry values on the stainless steel column of 0.57 and 0.54 for 8-Quinolinol and Hinokitiol respectively. When the Accura column was used, these values improved to 0.98 and 0.86 for the same compounds.

Conclusion

The results clearly show that the YMC-Accura Triart C18 columns outperformed the standard YMC-Triart C18 stainless steel columns in the analysis of chelating agents, such as 8-quinolinol and hinkitiol. In another preliminary experiment (data not shown), it was observed that the YMC-Accura Triart C18 columns also outperformed the stainless steel column for compounds that contain AMP, ADP and ATP. Another compelling reason to consider using the YMC-Accura Triart C18 columns, aside from the improved chromatographic performance, is the time saved by the method developer. This reduction in time spent can subsequently reduce costs to the funding laboratory.