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アプリケーションノート

Separation of Water-Soluble Vitamins on Legacy HPLC Columns Compared to CORTECS Premier HPLC Columns

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Abstract

Liquid chromatography is a powerful tool in the separation and analysis of complex mixtures. Applications can range from impurity assays for drug substances in pharmaceuticals to quantification of active ingredients in vitamin supplements. With advances in chromatography over the past few decades, analytical methods have become faster and more efficient. One of these advances is the development of stationary phases with solid-core particles.

In this work, it is demonstrated that the solid-core CORTECS™ Premier Column, in conjunction with MaxPeak™ High Performance Surfaces (HPS) technology, had better overall performance and selectivity compared to traditional fully porous silica or silica-hybrid particles. Specifically, the CORTECS Premier Column was able to successfully resolve the eight water-soluble vitamins and caffeine with much higher efficiency and lower peak tailing values compared to traditionally used columns.

Benefits

- Better overall efficiency and selectivity compared to fully porous particles
- Improved USP resolution, USP plate counts, and tailing factors
- Full separation of 7 water-soluble vitamins and caffeine using CORTECS Premier T3 Column

Introduction

In the modern analytical laboratory, scientists face many challenges in developing and running chromatographic methods. Not only can samples contain complex mixtures with a variety of excipients, but analyses are also often constrained by long run times and the copious use of toxic solvents. In the context of a pharmaceutical laboratory, this can mean high costs and long delays for producing quality medicines for patients.

Despite these challenges, the progression in particle technology has revolutionized liquid chromatography with new and exciting stationary phases. While traditional stationary phases contain fully-porous silica or silica-hybrid particles, the development of solid-core particles have been instrumental to increasing throughput. Solid-core particles have a nonporous, solid center, and a porous outer layer and offer many advantages, including higher efficiency, reduced solvent usage, and reduced instrument backpressure.[1]

The purpose of this work is to demonstrate the progression in particle technology by running an appropriate method for the analysis of vitamins using CORTECS solid-core technology, compared to two older legacy HPLC columns. The CORTECS Premier Column used employs MaxPeak High Performance Surfaces (HPS) technology, which mitigates the non-specific adsorption between analytes and the metal surfaces of the column. Overall, the CORTECS Premier Columns demonstrated higher efficiency along with improved peak shapes and resolution.

Experimental

Sample Preparation

Thiamine, nicotinic acid, pyridoxine, cyanocobalamin (B12), folic acid, and riboflavin standards were purchased from Sigma Aldrich. Caffeine was purchased from Alfa Aeser, and nicotinamide was purchased from Fluka.

A vitamin mix standard was prepared from individual stock solutions to a final concentration of 0.1 mg/mL in methanol. The individual stocks and the vitamin mix standard were stored at 4 °C, protected from light.

LC Conditions

LC system:	Arc HPLC System with 2998 Photodiode Array (PDA) Detector
Detection:	UV @ 254 nm
Columns:	CORTECS Premier T3, 4.6 x 150 mm, 5 µm (p/n :186010820) Symmetry™ C_{18}, 4.6 x 150 mm, 5 µm (p/n : WAT045905) XTerra™ MS C_{18}, 4.6 x 150 mm, 5 µm (p/n : 186000490)
Column temperature:	30 °C
Sample temperature:	5 °C
Injection volume:	10 µL
Flow rate:	0.50 mL/min
Mobile phase A:	25 mM Sodium Phosphate pH 3.0
Mobile phase B:	Methanol

Gradient Table

Time (min)	%A	%B	Curve
0.00	99	1	6
7.20	92	8	6
8.00	65	35	6
20.00	65	35	6
24.00	99	1	6
28.00	99	1	6

Data Management

Chromatography software:	Empower 3 Service Release 5

Results and Discussion

Eight water-soluble vitamins and caffeine were studied in the analysis. The structures of the analytes are shown in Figure 1. The chemical structures show some polar moieties, so analysis with a T3 or a C_{18} bonded column would be most appropriate for best retention. Specifically, the analytes can be stratified by the specific degree of hydrophobic interaction with the T3 or C_{18} columns. Theoretically, less polar analytes, such as caffeine, and larger structures with a higher degree of aromaticity should linger on the column and demonstrate longer retention times than thiamine or nicotinic acid, which are smaller and contain polar functional groups.

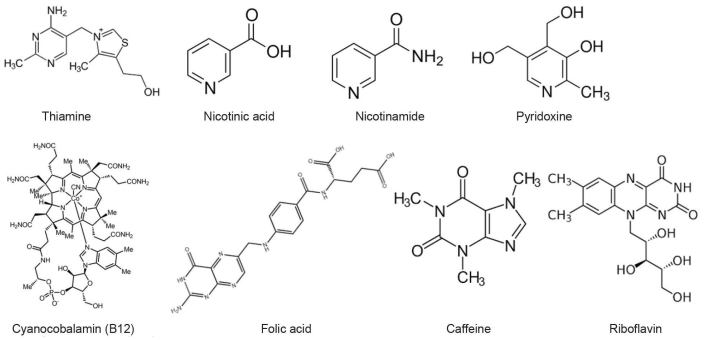

Figure 1. Chemical structures of water-soluble vitamins and caffeine.

For the vitamins analysis, a solid-core CORTECS Premier T3 Column was chosen and compared to two traditional fully-porous columns, a XTerra MS C_{18} Column and a Symmetry C_{18} Column. These columns contain a hybrid, fully porous particle stationary phase, and are legacy L1-classified columns for the analysis of water-soluble vitamins in industry.[2] All columns used were 4.6 mm x 150 mm with 5 µm particle sizes, a particle size that is ideal for scaling methods between the chromatographic modalities (HPLC, UHPLC).[1]

The results of the chromatographic analyses are shown in Figure 2. As expected, the three columns show little

difference in retentivity, as the retention times of the analytes are very similar. However, it should be noted that the XTerra MS C_{18} Column failed to resolve nicotinamide and pyridoxine (peaks 3 and 4). The analytical chemist would require more time for method optimization to resolve the two peaks. The CORTECS Premier T3 Column and the Symmetry C_{18} Column successfully resolved all 8 vitamins, although the Symmetry Column to a lesser extent due to lower USP resolution of pyridoxine. Overall, the CORTECS Premier T3 Column had superior USP resolution values compared to the other two columns, as summarized in Table 1.

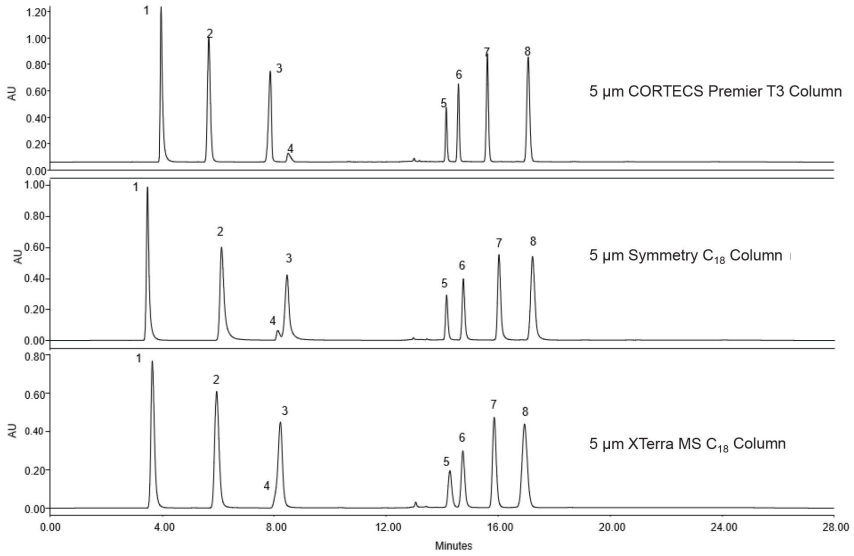

Figure 2. Chromatographic analysis on three different columns. 1) Thiamine 2) Nicotinic Acid 3) Nicotinamide 4) Pyridoxine 5) Cyanocobalamin (B12) 6) Folic Acid 7) Caffeine 8) Riboflavin.

USP resolution	CORTECS Premier T3	Symmetry C_{18}	XTerra MS C_{18}
Thiamine			
Nicotinic acid	10.2	12.4	8.8
Nicotinamide	11.4	9.0	7.6
Pyridoxine	2.8	1.5	
Vit B12	30.7	27.4	15.1
Folic acid	3.8	3.6	1.9
Caffeine	7.9	6.8	4.6
Riboflavin	8.7	5.0	3.6

Table 1. Comparison of USP Resolution values for the CORTECS Premier T3, Symmetry C_{18}, and XTerra MS C_{18} columns.

Another important factor of an analysis is the USP tailing. The tailing factor should be close to a value of 1.0, which mimics a perfect Gaussian distribution curve and shows sufficient interaction of the analyte with the column, with very little to no non-specific interactions to cause peak tailing. The absence of peak fronting also indicates an appropriate mass load of the analyte onto the columns stationary phase, leading to the efficient chromatographic separation of the analyte. Tailing factors are summarized in the table in Table 2.

USP tailing	CORTECS Premier T3	Symmetry C_{18}	XTerra MS C_{18}
Thiamine	1.8	1.5	1.4
Nicotinic acid	1.2	1.7	1.2
Nicotinamide	0.8	1.2	0.9
Pyridoxine	1.9	1.4	
Vit B12	1.1	1.2	1.1
Folic acid	1.0	1.2	1.2
Caffeine	1.1	1.3	1.2
Riboflavin	1.1	1.2	1.1

Table 2. Comparison of USP Tailing factors for the CORTECS Premier T3, Symmetry C_{18}, and XTerra MS C_{18} Columns.

The CORTECS Premier T3 Column has improved tailing factors and better overall peak shape for most of the analytes. Although the tailing factor for pyridoxine (4) was 1.9 on the CORTECS Column, it was not resolved on the Xterra Column, and it is tailing into the next peak (nicotinamide, 3) on the Symmetry Column. All peaks on

the CORTECS are also visually much sharper and taller than the other columns, along with superior resolution.

The improved peak shapes, along with much higher USP plate counts (Table 3), attest to the efficiency of the solid-core particle. The USP plate count is a function of the retention time of the peak divided by the peak width at half-height squared, and can only be calculated under isothermal, isocratic or isodense conditions.[4] Ideally, the sharper the peak and the longer the analyte retains on the column, the higher the efficiency and plate count value. The CORTECS Column solid-core particle can separate the eight analytes with much higher efficiency at double the flow rate, without increasing the backpressures or compromising resolution or peak shape.

USP plate count	CORTECS Premier T3	Symmetry C_{18}	XTerra MS C_{18}
Thiamine	12,770	7,170	4,099
Nicotinic acid	13,944	8,855	6,673
Nicotinamide	26,779	30,449	11,601
Pyridoxine	17,733	17,289	Not calculated
Vit B12	254,182	129,304	57,292
Folic acid	236,174	115,445	60,951
Caffeine	193,148	101,681	66,805
Riboflavin	125,577	62,499	36,993

Table 3. Comparison of USP Plate Counts for the CORTECS Premier T3, Symmetry C_{18}, and XTerra MS C_{18} columns.

Conclusion

CORTECS Premier Columns, when combined with MaxPeak High Performance Surfaces (HPS) Technology, are a powerful tool in the analytical chemist's toolbox. It was successfully demonstrated that a reversed-phase LC method for water-soluble vitamins can be run with higher efficiency using CORTECS solid-core particle technology. Specifically, the CORTECS Premier T3 Column was able to successfully resolve the eight vitamins and caffeine with much higher plate counts and lower peak tailing values compared to traditionally used columns.

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