

Analysis of Fat Soluble Vitamins Using a Thermo Scientific Accucore XL C18 4 μm HPLC Column

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Key Words

Accucore XL C18, fused core, superficially porous, fat soluble vitamins, large solid core particle

Abstract

This application note demonstrates the use of the Accucore™ XL C18 4 μm HPLC column for the analysis of fat soluble vitamins.

Introduction

Based on Core Enhanced Technology™ using 4 μm solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5 μm or even 3 μm fully porous particles. Very high separation efficiencies using standard HPLC instruments and conditions provide increased peak resolution and lower limits of detection. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility.

Vitamins are biologically active compounds that act as controlling agents for an organism's normal health and growth. The level of vitamins in food may be as low as a few micrograms per 100 g. Vitamins are often accompanied by an excess of compounds with similar chemical properties. Thus, not only quantification but also identification is mandatory for the detection of vitamins in food. Vitamins generally are labile compounds that should not be exposed to high temperatures, light, or oxygen. HPLC separates and detects these compounds at room temperature and blocks oxygen and light.



Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade methanol	M/4056/17
Thermo Scientific Finnpiptette F2 pipettor kit	PMP-020-220F
Thermo Scientific Finntip pipette tips, 200 μL	PMP-107-600F
Thermo Scientific Finntip pipette tips, 1000 μL	PMP-103-206K
Thermo Scientific National Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Preparation

A mixed working standard of vitamin K1 (500 µg/mL), vitamin K2 (500 µg/mL), vitamin E (500 µg/mL), vitamin E acetate (500 µg/mL), vitamin D2 (1500 µg/mL) and vitamin D3 (25 µg/mL) was prepared in 80:20 v/v acetonitrile:methanol

Separation Conditions

Part Number

Instrumentation:	Thermo Scientific Dionex UltiMate 3000 RSLC HPLC system	
Columns:	Thermo Scientific Accucore XL C18 4 µm, 150 x 4.6 mm Fully porous C18 5 µm, 150 x 4.6 mm	74104-154630
Mobile phase A:	80:20 (v/v) acetonitrile:methanol	
Column temperature:	30 °C	
Injection volume:	5 µL	
Flow rate:	1 mL/min	
UV detection:	280 nm	

Results

The analysis of six fat soluble vitamins on an Accucore XL C18 4 µm column gave resolution of greater than 2.5 for all compounds (Figure 1). Resolution of the critical pair (vitamin D2 and D3) increased by 30% to 2.5 for the Accucore XL C18 4 µm compared to 1.92 for the 5 µm fully porous C18 column. Table 1 shows that use of the Accucore XL provided an average increase in efficiency of 82% for all six compounds when compared to the fully porous column. This was achieved with a minimal backpressure increase, from 47 bar with the 5 µm fully porous column to 62 bar for the 4 µm Accucore XL column.

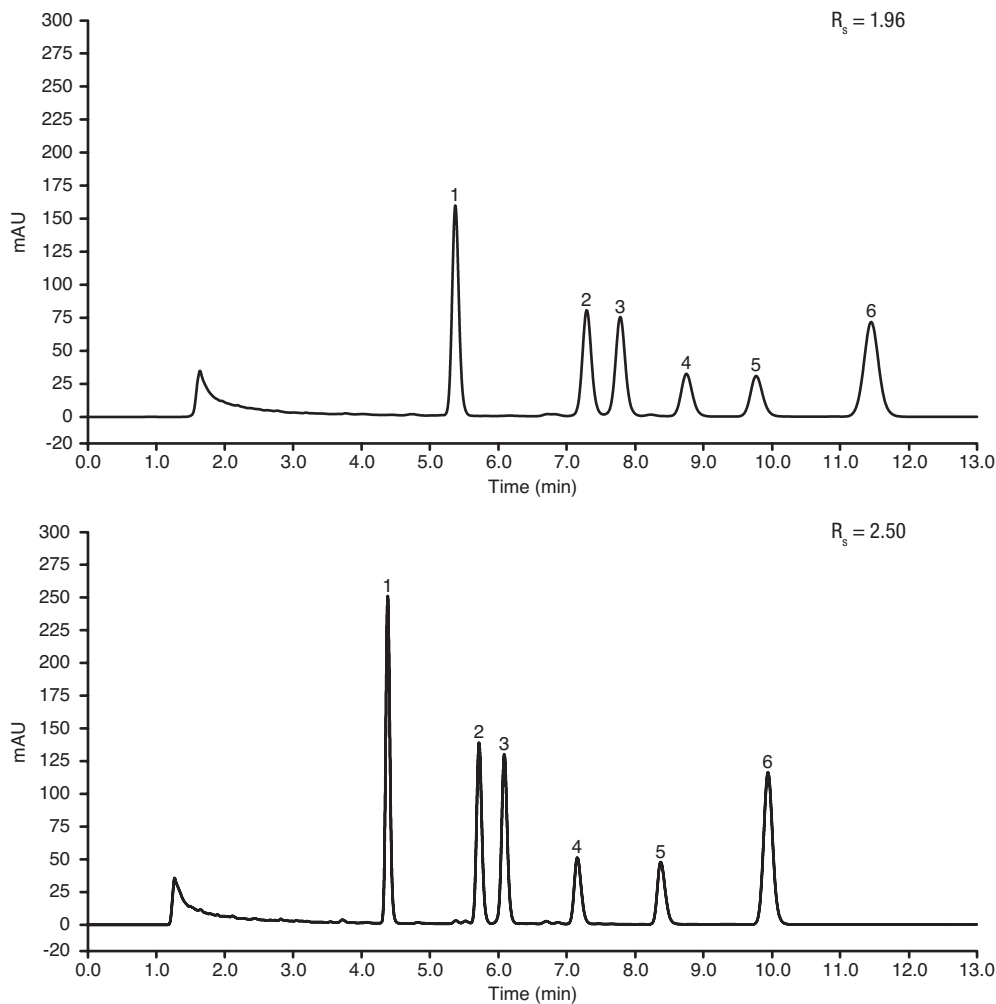
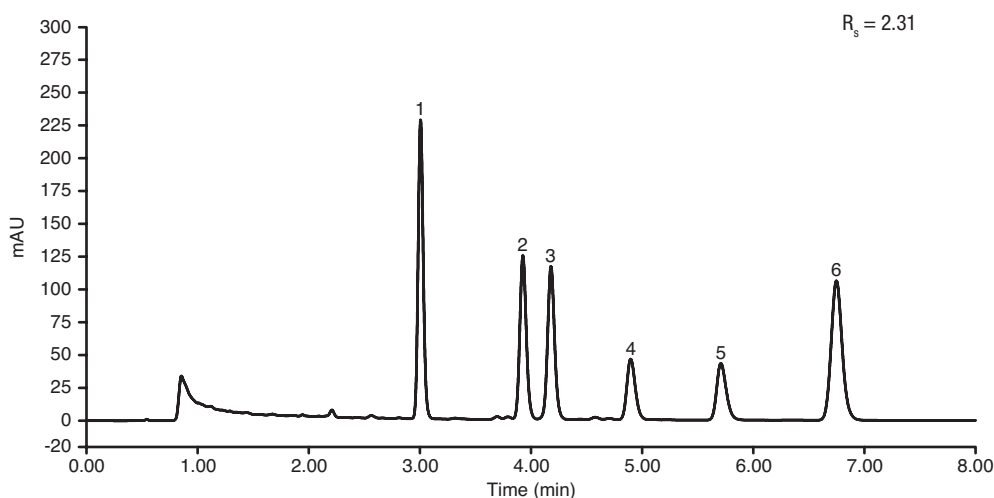


Figure 1: Chromatograms for 5 µm fully porous C18 (top) and 4 µm Accucore XL C18 (bottom)

		Efficiency HETP (USP)	
		Accucore XL	Fully Porous
1	Vitamin K2	23826	13599
2	Vitamin D2	25566	13963
3	Vitamin D3	25710	13985
4	Vitamin E	22788	13288
5	Vitamin E acetate	24568	13880
6	Vitamin K1	26179	12776

Table 1: Efficiency for six fat soluble vitamins

Increasing the flow rate to 1.5 mL/min gave a 3 minute reduction in run time with only minor impact on efficiency and resolution (Figure 2). The critical pair of vitamin D2 and D3 was still well resolved with a value of 2.3 and the back pressure increased to only 97 bar.

Figure 2: Chromatogram for Accucore XL C18 4 μ m with a flow rate of 1.5 mL/min

Conclusion

The use of an Accucore XL C18 4 μ m column gave significant performance improvement over a conventional 5 μ m fully porous column under the same chromatographic conditions with no changes in system configuration. Resolution improved by 30% and efficiency improved by an average of 82%. Run time can be reduced while maintaining superior resolution and efficiency by increasing flow rate.

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