Water-Soluble Vitamin Analysis on ZirChrom®-SAX

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Technical Bulletin #305

Traditionally the analysis of water-soluble vitamins by reversed-phase HPLC has been complicated by the lack of retention for these compounds on conventional silica C18 columns. Other analytical approaches, such as ion-pair chromatography, have also failed to yield successful and reproducible results. Here we demonstrate efficient baseline resolution of six water-soluble vitamins in six minutes using a ZirChrom®-SAX column. This method can be combined with ZirChrom's ProTain® In-Line Protein Removal System for the analysis of these compounds in biological samples.

Introduction

In this application note we focus on the HPLC analysis of Vitamin C and five B-complex vitamins; Vitamin B_1 (thiamine), Vitamin B_2 (riboflavin), Vitamin B_3 (nicotinic acid form), Vitamin B_3 (nicotinamide form), and Vitamin B_6 (pyridoxine). All of these vitamins are water-soluble.

Chromatographers oftentimes struggle in their attempts to analyze water-soluble vitamins by HPLC. Many water-soluble vitamins are very polar. Thiamin (Vitamin B_1), pyridoxine (Vitamin B_6) and ascorbic acid (Vitamin C), for example, show almost no retention on conventional C18 columns. Reversed-phase analytical methods employing ion-pair reagents have been offered as a potential solution to this problem, but these methods tend to suffer from column-to-column reproducibility problems due to the somewhat unpredictable way ion-pairing reagents interact with the silica surface and the bonded phase.

In this technical bulletin we present a unique method for the analysis of water-soluble vitamins using a ZirChrom®-SAX HPLC column. ZirChrom®-SAX, an anion exchange material, is polyethleneimine-coated zirconia containing a substantial amount of hydrophobic cross-linker which imparts both ion-exchange and reversed-phase characteristics. The mixed mode retention characteristics of the ZirChrom®-SAX column create the unique selectivity ideal for this application (Figure 1).

For the analysis of water-soluble vitamins in serum, or other samples containing biological matrices, we recommend the addition of the ProTain® In-Line Protein Removal System; consisting of one guard holder (part# 850-00-2) and a set of three ProTain® inserts (part# PT01-0246). Please see technical bulletins #275 and #291 for further information on the use of ZirChrom's ProTain® In-Line Protein Removal System.

Experimental

Six water-soluble vitamin standards were prepared in an aqueous solution and injected on a ZirChrom®-SAX column. The separation conditions are as follows.

Column:	ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546)
Mobile Phase:	50 mM Ammonium dihydrogenphosphate, pH 4.5
Flow rate:	1.0 ml/min.
Temperature:	30 °C
Injection Vol.:	5.0 µl
Detection:	UV at 254 nm

The separation is shown in Figure 1. Under these conditions the separation of Vitamin C and the four B-complex vitamins is achieved, with good peak shape and baseline resolution, in 6 minutes.

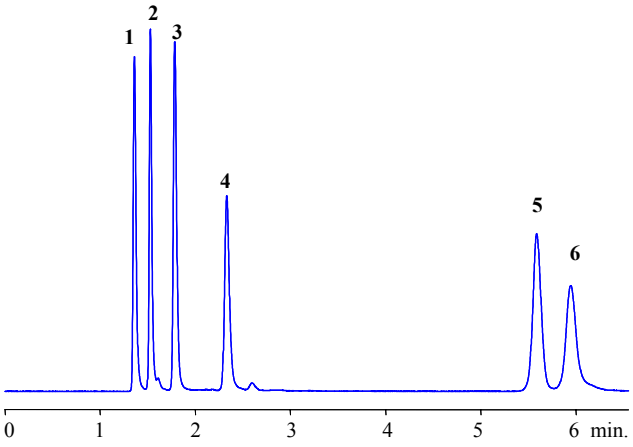

Figure 1. Analysis of Water-Soluble Vitamins.
1=Thiamine (Vit. B_1), 2=Pyridoxine (Vit. B_6),
3= Nicotinamide (form of Vit. B_3), 4=Riboflavin (Vit. B_2),
5=Nicotinic acid (form of Vit. B_3), 6=Ascorbic acid (Vit. C)

ZirChrom columns combine the high efficiency usually associated with silica columns with complete chemical and thermal stability.

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Fast Resolution of Vitamin D_2 from Vitamin D_3 on ZirChrom®-CARB

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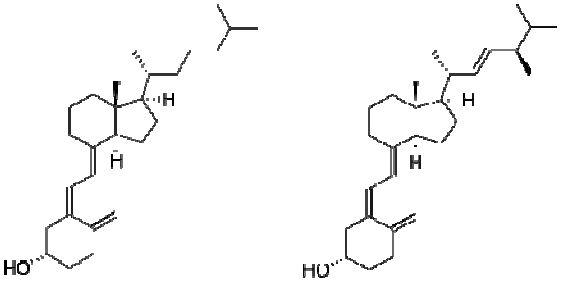
Technical Bulletin # 338

In this application we examine the superior selectivity of the ZirChrom®-CARB phase for two closely related compounds; vitamin D_2 and D_3.



Cholecalciferol Ergocalciferol

Figure 1: Structures of cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2).

Introduction

Vitamin D refers to a group of fat-soluble secosteroids which are critical for enhancing intestinal absorption of important nutients. The most biologically relevant members of this group for humans are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3).

These closely related compounds are difficult to separate quickly using traditional reversed phase columns. Additionally, these highly reactive compounds are difficult to quantify precisely using LC/MS as the ionization process causing instability, lowing the robustness of the method (1).

Here we present an isocratic method that provides baseline resolution of a mix of vitamin D_2 and D_3 standards in two minutes using a ZirChrom®-CARB phase with UV detection at 275 nm.

Experimental

A mixture of two standards, cholecalciferol and ergocalciferol, was separated at 70 ºC temperature using a ZirChrom®-CARB column. The separation conditions were as follows:

Column:	ZirChrom®-CARB, 50 mm x 4.6 mm i.d. (Part Number: ZR01-5046)
Mobile Phase:	A: 50/50 ACN/IPA B: THF
Temperature:	70 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	74 bar
Detection:	UV at 275 nm

ZirChrom®-CARB separation of cholecalciferol and egocalciferol allows for baseline resolution of the compounds in two minutes using isocratic conditions and UV detection.

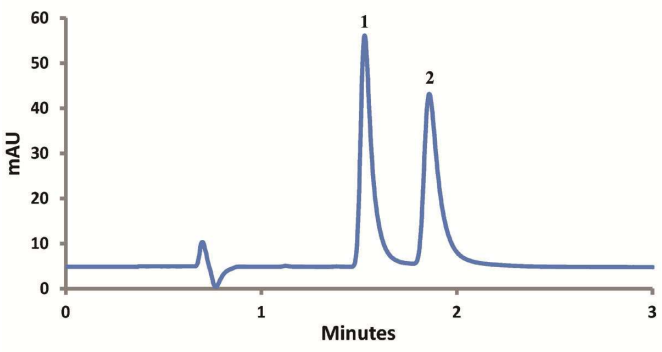


Figure 2: 1 = Cholecalciferol (vitamin D_3), 2 = Egocalciferol (vitamin D_2)

This method can be tailored to your specific application needs. ZirChrom technical support can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

References

(1) Brydwell, C.W. et al, Am J Clin Nutr, Vol. 88 no.2, 5545-5575 (2008)

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