



ZirChrom®

Fast Resolution of Vitamin D₂ from Vitamin D₃ on ZirChrom®-CARB

Merlin Bicking, Ph.D. and Kelly S. Johnson
ACCTA and ZirChrom Separations, Inc.

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In this application we examine the superior selectivity of the ZirChrom®-CARB phase for two closely related compounds; vitamin D₂ and D₃.

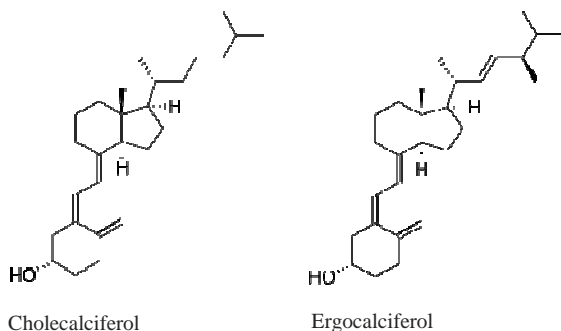


Figure 1: Structures of cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂).

Introduction

Vitamin D refers to a group of fat-soluble secosteroids which are critical for enhancing intestinal absorption of important nutrients. The most biologically relevant members of this group for humans are ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃).

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, lowering the robustness of the method (1).

Here we present an isocratic method that provides baseline resolution of a mix of vitamin D₂ and D₃ 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 °C 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 °C
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 ergocalciferol allows for baseline resolution of the compounds in two minutes using isocratic conditions and UV detection.

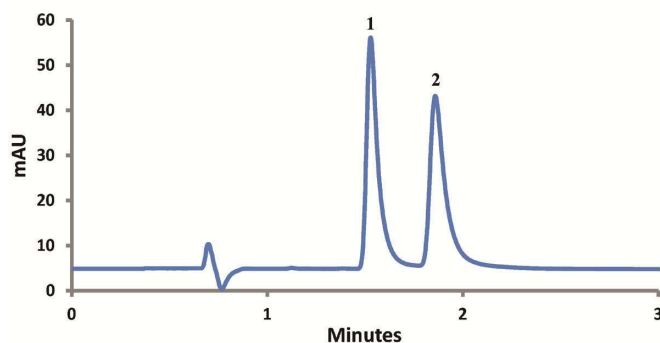


Figure 2: 1 = Cholecalciferol (vitamin D₃), 2 = Ergocalciferol (vitamin D₂)

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)

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

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