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應用手冊

Chromatographic Separation of Fat-Soluble Vitamins, Including the Two Isomers D2 and D3

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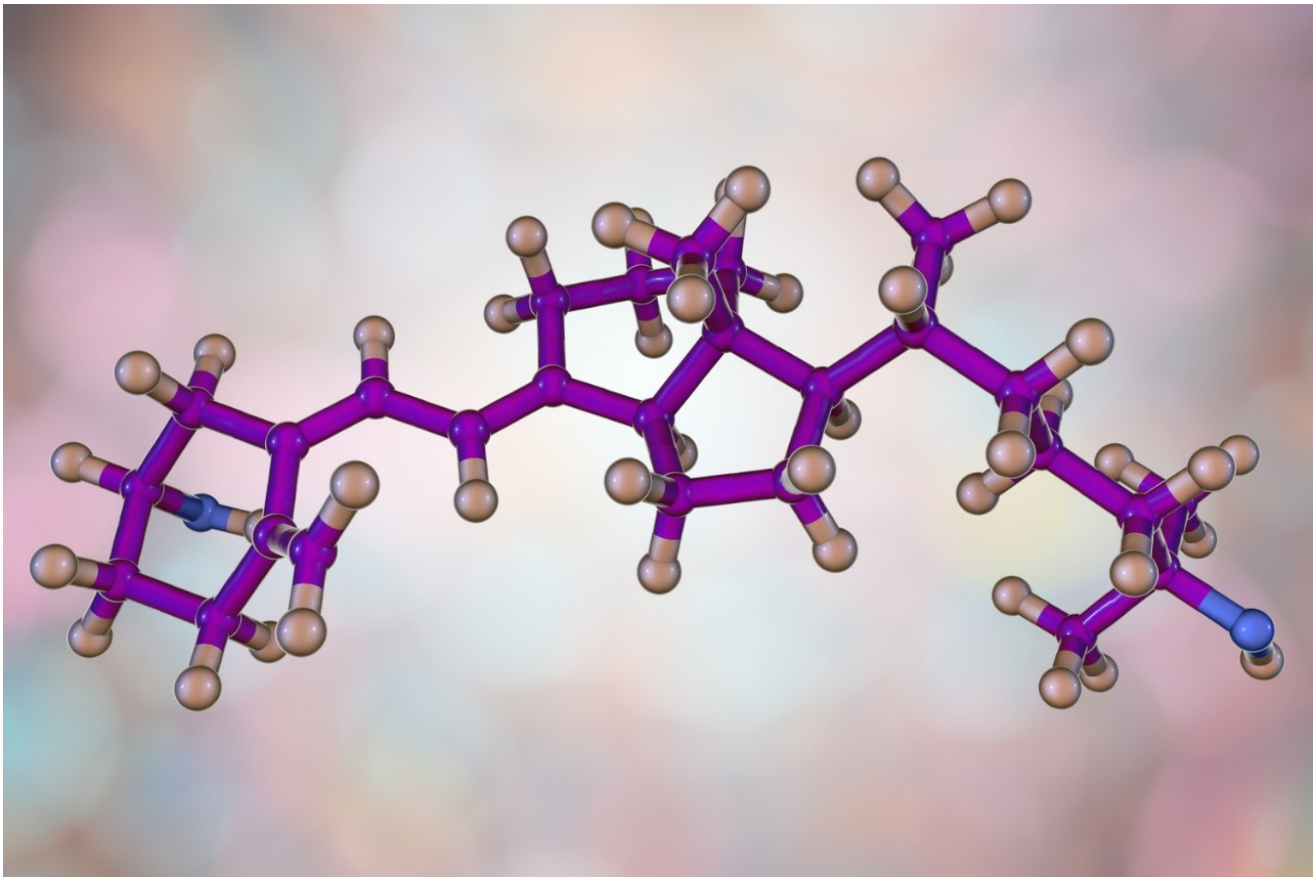

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief illustrates chromatographic separation of vitamin D2 and D3 using a Waters ACQUITY UPLC BEH C_{18} Column (150 mm).

Introduction

In a previous application note (720002021EN <https://www.waters.com/nextgen/us/en/library/application-notes/2009/analysis-of-fat-soluble-vitamins-using-uplc-with-pda-and-the-sq-detector.html> "Analysis of fat-soluble vitamins using UPLC-PDA-SQD"), a five minute method was described that showed the analysis of fat-soluble vitamins using UPLC-UV-MS.

Analysis of fat-soluble vitamins is important for many application areas such as ADME (adsorption, distribution, metabolism, excretion) studies within the food and beverage industry as well as the clinical environment.

Vitamin D deficiency often occurs when there is inadequate intake from food-derived sources, or from sunlight, or from a combination of the two. When there is a deficiency of Vitamin D, bone softening results, manifesting as rickets in children and osteomalacia (and possibly osteoporosis) in adults.

The two most common isomers of vitamin D are D2 and D3. They come from different sources (vitamin D2 is derived from fungal and plant sources, and is not produced by the human body, vitamin D3 is derived from animal sources). Though D2 and D3 are well documented, it is a challenge to separate them chromatographically.

MS provides the better selectivity needed to quantify vitamin D2 and vitamin D3 because they have different *m/z* values (397 and 385 respectively), even when LC resolution is not optimal. If the two isomers are poorly resolved chromatographically, quantification of the two isomers using UV is difficult as they both have a UV maxima at 260 nm.

Experimental

LC Conditions

LC system:	Waters ACQUITY UPLC system
Column:	ACQUITY UPLC BEH C_{18} column 2.1 x 150 mr µm
Column temp.:	25 °C
Flow rate:	500 µL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient

Time (min)	%A	%B	Curve
Initial	10	90	Initial
0.10	10	90	9
3.00	0	100	6
7.00	0	100	6
7.50	10	90	6
10.00	10	90	6

Detection Conditions

PDA system:	Waters 2996 Photodiode Array (PDA)

	detector
Wavelength range:	230 to 450 nm
Resolution:	1.2 nm
Sampling rate:	20 spectra/second

Results and Discussion

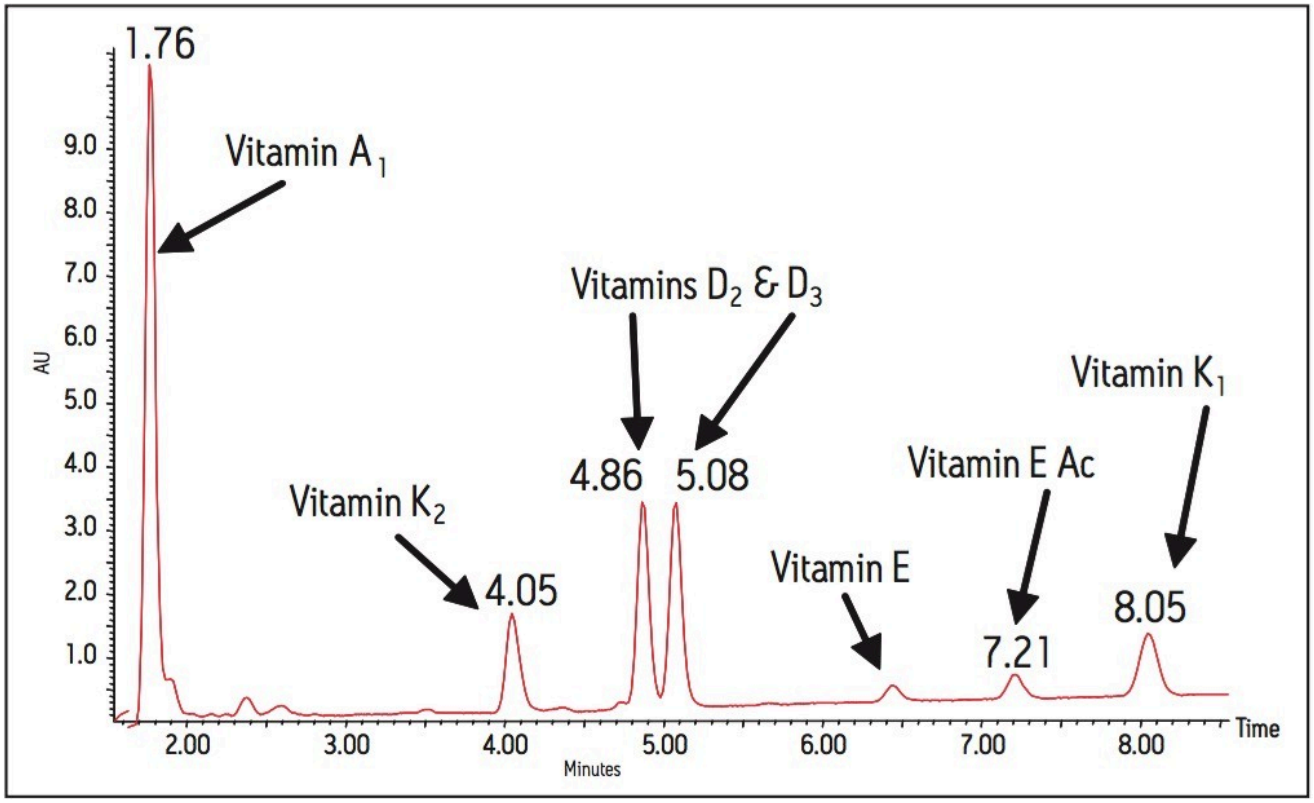

Figure 1. UV analysis of fat-soluable vitamins.

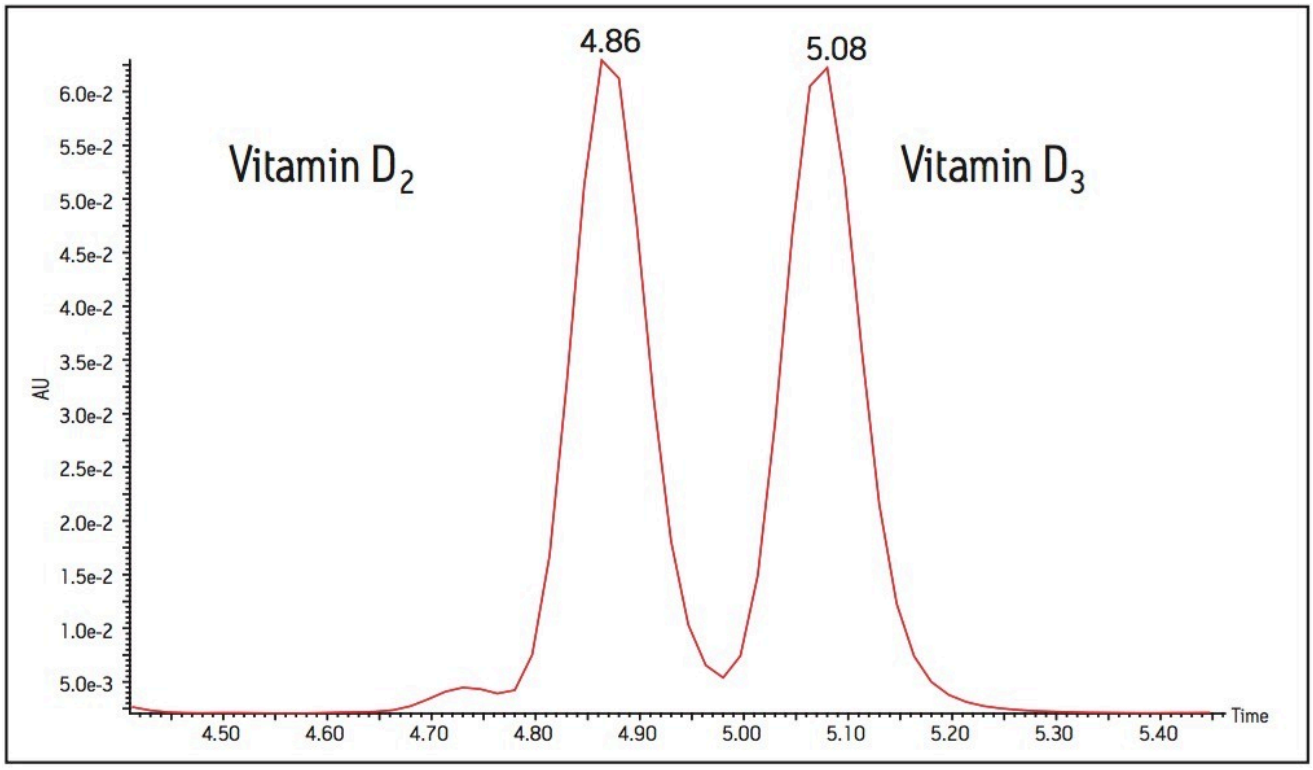

Figure 2. UV separation of vitamin D2 and D3.

Conclusion

A method has been developed which allows UV quantification of fat-soluble vitamins, in particular vitamins D2 and D3.

Featured Products

- ACQUITY UPLC System <https://www.waters.com/514207>
- 2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

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