

## CHROMATOGRAPHIC SEPARATION OF FAT-SOLUBLE VITAMINS, INCLUDING THE TWO ISOMERS D2 AND D3

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### INTRODUCTION

In a previous application note (720002021EN “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.

This technical note illustrates chromatographic separation of vitamin D2 and D3 using a Waters® ACQUITY UPLC® BEH  $C_{18}$  column (150 mm).

### EXPERIMENTAL

#### LC conditions

LC system: Waters ACQUITY UPLC system  
Column: ACQUITY UPLC BEH  $C_{18}$  column  
2.1 x 150 mm, 1.7  $\mu$ m

Column temp.: 25 °C

Flow rate: 500  $\mu$ L/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

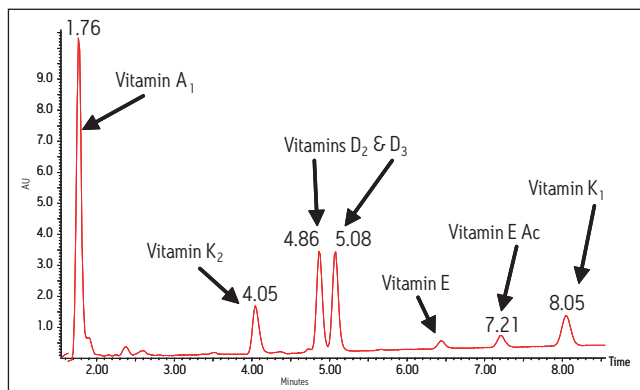


Figure 1. UV analysis of fat-soluble vitamins.

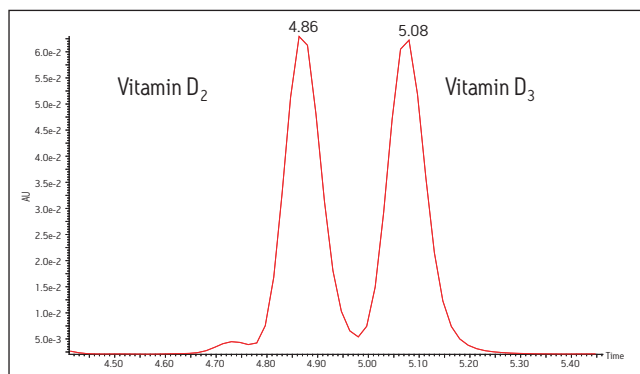


Figure 2. UV separation of vitamin D<sub>2</sub> and D<sub>3</sub>.

## CONCLUSION

A method has been developed which allows UV quantification of fat-soluble vitamins, in particular vitamins D<sub>2</sub> and D<sub>3</sub>.

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