APPLICATION NOTE

Liquid Chromatography

Authors

Padmaja Prabhu
William Goodman
Wilhad Reuter

PerkinElmer, Inc.
710 Bridgeport Avenue
Shelton, CT 06484 USA

Fast Analysis of Fat-Soluble Vitamins Using Flexar FX-10 and Chromera CDS

Introduction

Vitamins are essential nutrients and used in numerous systems throughout the human body. Vitamins are divided into two groups: water-soluble (B-complex and C) and fat-soluble (A, D, E and K). Water-soluble vitamins have limited retention in the body and need regular replacement. Fat-soluble vitamins are stored in the liver and fatty tissues, allowing accumulation and consumption over time.

The determination of vitamin content in foods is important in quality, labeling and marketing. Europe and the United States require food products to be clearly labeled with nutritional information; the vitamin content is required on the label.

Supplements and functional foods are intended to provide health benefits to consumers–these products often contain vitamins. Vitamins, D and E are commonly marketed in supplements because of their role in calcium absorption of bones and antioxidant properties, respectively.

A reliable and fast determination of fat-soluble vitamins in food and supplement products is important in complying with regulations and controlling the quality and safety of products. This application note will present a fast and robust method for the determination of fat-soluble vitamins using UHPLC. An optimized separation of vitamins A acetate, K2, D2, D3, E succinate, E acetate and K1 using the PerkinElmer® Flexar™ UHPLC system is presented. Calibration of the UHPLC system and analysis of the vitamins in a commercial supplement will be presented.

PerkinElmer®
For the Better

Experimental

The PerkinElmer Flexar FX-10 UHPLC system was used throughout this application. A 1.5 µm particle, 50 mm length, 2.1 mm id, C18 column provided ample resolution and short run times. This column required an operating pressure of approximately 8000 psi resulting in a mobile phase flow rate of approximately 0.7 mL/min. A Flexar UV/Vis detector was operated at wavelengths specific to each vitamin, see Table 1. The instrument interaction, data analysis, and reporting was completed with the PerkinElmer Chromera® data system. The detailed operating parameters of the UHPLC system are presented in Table 1.

Table 1: Detailed analytical conditions for the Flexar LC.

Instrument	Flexar FX-10	
Autosampler	Flexar UHPLC	
Detector	Flexar UV-Vis	
Column	Grace Vision HL B C18, 100 mm x 1.5 µm, 2.1 mm i.d.	
Column temperature	40°C	
Detector wavelength	**Time (min)**	**Wavelength (nm)**
	0.00	325
	2.40	248
	3.60	265
	4.50	285
	6.20	248
	7.50	325
Injection volume	2 µL Fixed loop	
Flow rate	0.7 mL/min	
Mobile phase	A: Water, pH adjusted to 3.0 w/ o-phosphoric acid:Methanol (1:1)	
	B: Acetonitrile	

Gradient program

Type	**Time**	**Flow (min)**	**% A (mL/min)**	**% B**	**Curve**
Equil	3.0	0.8	20	80	0
Run	3.5	0.8	20	80	1
Run	1.0	0.8	0	100	1
Run	2.0	0.8	0	100	1
Run	1.0	0.8	20	80	1

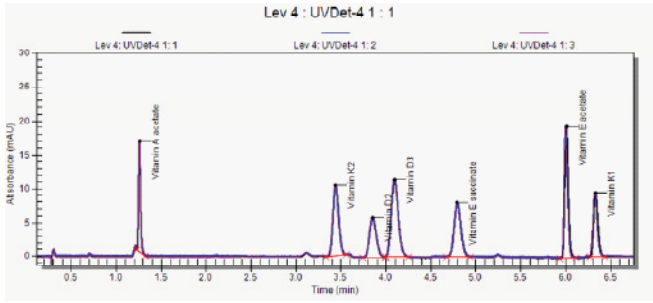

Figure 1: Overlaid chromatogram of three replicates analyses of calibration standard level 4.

Standard preparation (stock): The standards were procured from Chromadex. 10 mg of Vitamin A acetate, Vitamin K2, Vitamin D3, Vitamin E succinate and Vitamin E acetate were dissolved in water and acetonitrile (2:8) to give a solution of 1000 µg/mL (ppm). 5 mg of Vitamin D2, Vitamin K1 were dissolved in water and acetonitrile (2:8) to give a solution of 500 µg/mL each. These stock solutions were further diluted to create the calibration levels presented in Table 2.

Table 2: Detailed dilution scheme for preparation of calibration standards.

Cal level no.	**Concentration in µg/mL**						
	A	K2	D2	D3	E-s	E-a	K1
1.	0.5	2	2	2	20	20	2
2.	1.0	4	4	4	40	40	4
3.	1.5	6	6	6	60	60	6
4.	2.0	8	8	8	80	80	8
5.	2.5	10	10	10	100	100	10
6.	3.0	12	12	12	120	120	12

The UV detector was calibrated with a linear response across the range of 0.5-3.0 µg/mL for vitamin A; 2-12 µg/mL for vitamins K and D; and 20-120 µg/mL for vitamin E, the details are presented in Table 2.

Table 3: Calibration data for the determination of fat-soluble vitamins.

Vitamin	r^2
A	0.9997
K2	0.9989
D2	0.9999
D3	0.9999
E-s	0.9996
E-a	0.9997
K1	0.9994

Sample preparation: The developed method was used to determine the fat-soluble vitamin content of commercially available formulations. The samples were crushed, weighed and sonicated in 10 mL of (2:8) water : acetonitrile. All samples were filtered through 0.2 micron nylon filters. Furthermore, 1 mL of the sample solution was diluted to 5 mL. Finally, 1 mL of the latter solution is then further diluted to 10 mL and injected.

Discussion

Commercially available supplements were analyzed for fat-soluble vitamin content. Using the method developed here, the analytical run was 7.5 minutes with an elution order of Vitamin A acetate, Vitamin K2, Vitamin D2, Vitamin D3, Vitamin E succinate, Vitamin E acetate, and Vitamin K1. All the peaks were well resolved and the resolution between the closest eluting pair is 1.7. The conventional HPLC method, with a 50-minute gradient run, was reduced to a 6.5-minute run with the FX-10. This method has also decreased the solvent used per sample to 5.25 mL. When compared to conventional HPLC, this directly translates into solvent savings of approximately 45 mL per sample, or a reduction of 90% in solvent usage and waste generation.

Conclusions

An improved determination of vitamins using UHPLC is demonstrated in this application. Using a Flexar FX-10 UHPLC system in combination with a small particle (1.5 µm) LC column, five fat-soluble vitamins were rapidly determined in commercial vitamin supplements.

In comparison to conventional HPLC, this UHPLC method offers over 6-times faster chromatography while reducing solvent consumption by 90%. This not only improves productivity, but greatly reduces operating costs and the environmental footprint of the analysis.

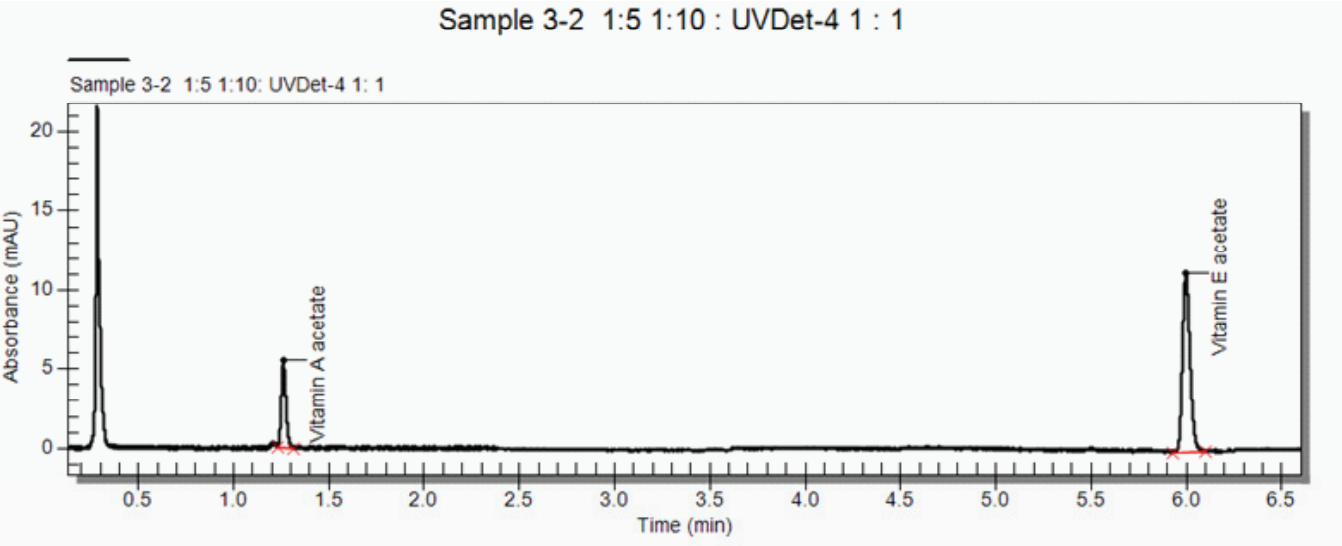

Figure 4: Example chromatogram of the analysis of fat-soluble vitamins in a commercial formulation.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009074_01 Printed in USA