High Speed Analysis of Fat-soluble Vitamins by Ultra High-performance Liquid Chromatography with Photodiode Array Detection

Introduction

Vitamins are essential nutrients to all living organisms and are organic compounds required in small amounts for healthy physiological activity. The class of fat-soluble vitamins is insoluble in water but readily dissolve in oil and thus when consumed in excess may have a detrimental impact on the human body, as they are difficult to excrete.

This application note describes the analysis of fat-soluble vitamins using UHPLC and PDA detection with a high speed data sampling rate of 100 spectra/sec.

Keywords:

UHPLC, Fat-soluble vitamin, Vitamin K3, Vitamin A, Retinol acetate, Vitamin K2, α-tocopherol, Vitamin D2, Vitamin D3, α-tocopherol, Tocopherol-α-acetate, Vitamin K1, C18 Column, PDF detector
Experimental

Equipment

- Pump: X-LC 3185PU x 2
- Degasser: X-LC 3080DG
- Mixer: X-LC 3180MX
- Column Oven: X-LC 3067CO
- Autosampler: X-LC 3159AS
- Detector: X-LC 3110MD

Conditions

- Column: ZORBAX SB-C18 (3.0 mmID x 50 mmL, 1.8 μm)
- Eluent: Acetonitrile/Methanol (70/30)
- Flow rate: 0.8 mL/min
- Column Temp.: 30 ºC
- Wavelength: 200-500 nm
- Injection Volume: 1 μL
- Standard Sample: 10 fat-soluble vitamin standards

Result

A contour plot and chromatogram of the fat-soluble vitamin standard mixture are shown in Figure 1. Excellent separation of the 10 vitamins was obtained in under 4 minutes.

Fig. 1. Chromatogram of Fat-soluble Vitamin Standard Mixture

- 1: Vitamin K3 (Menadione)
- 2: Vitamin A (Retinol)
- 3: Vitamin A acetate (Retinol acetate)
- 4: Vitamin K2 (Menaquinone)
- 5: Vitamin E (α-Tocopherol)
- 6: Vitamin D2 (Ergocalciferol)
- 7: Vitamin D3 (Cholecalciferol)
- 8: Vitamin E (α-Tocopherol)
- 9: Vitamin E acetate (α-Tocopherol acetate)
- 10: Vitamin K1 (Phytonadione) (0.1 mg/mL each)
The on-peak spectra of fat-soluble vitamin standard mixture are shown in Fig. 2.

Fig. 2. On-peak Spectra of Fat-soluble Vitamin Standard Mixture

1: Vitamin K3 (Menadione)  
2: Vitamin A (Retinol)  
3: Vitamin A acetate (Retinol acetate)  
4: Vitamin K2 (Menaquinone)  
5: Vitamin E (α-Tocopherol)  
6: Vitamin D2 (Ergocalciferol)  
7: Vitamin D3 (Cholecalciferol)  
8: Vitamin E (α-Tocopherol)  
9: Vitamin E acetate (α-Tocopherol acetate)  
10: Vitamin K1 (Phytonadione) (0.1 mg/mL each)