



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.



JASCO UHPLC System
at www.jascoinc.com

Keywords:

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

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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 °C
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.

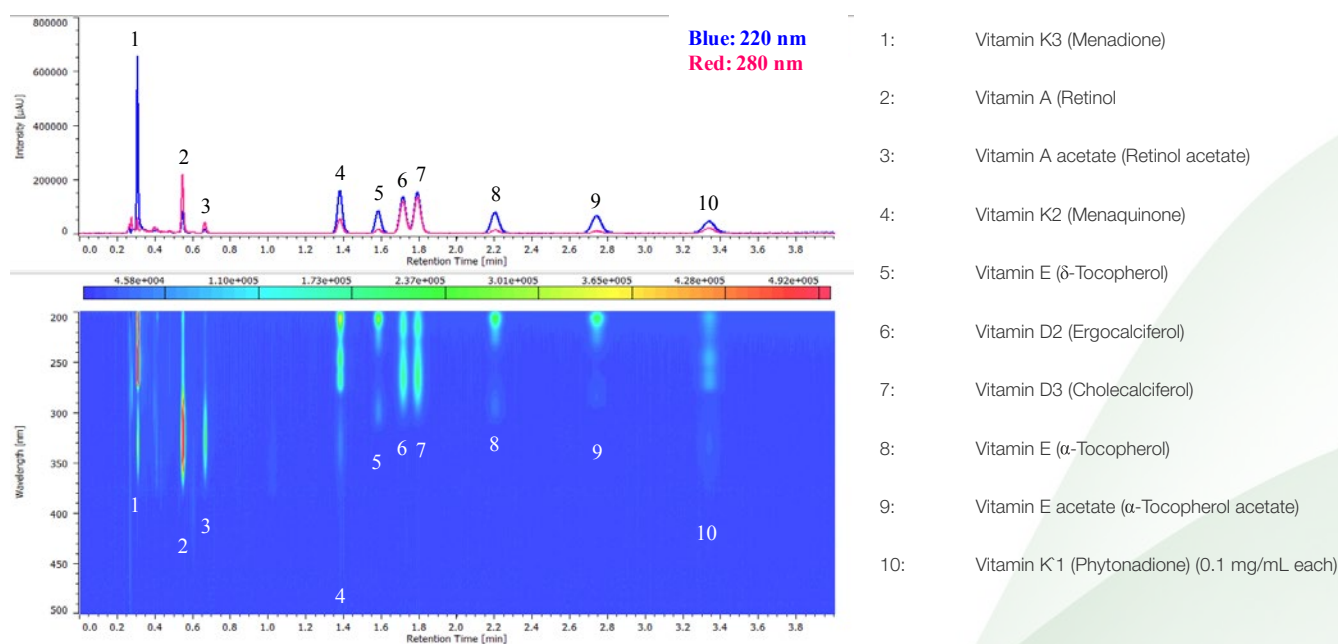


Fig. 1. Chromatogram of Fat-soluble Vitamin Standard Mixture

The on-peak spectra of fat-soluble vitamin standard mixture are shown in Fig. 2.

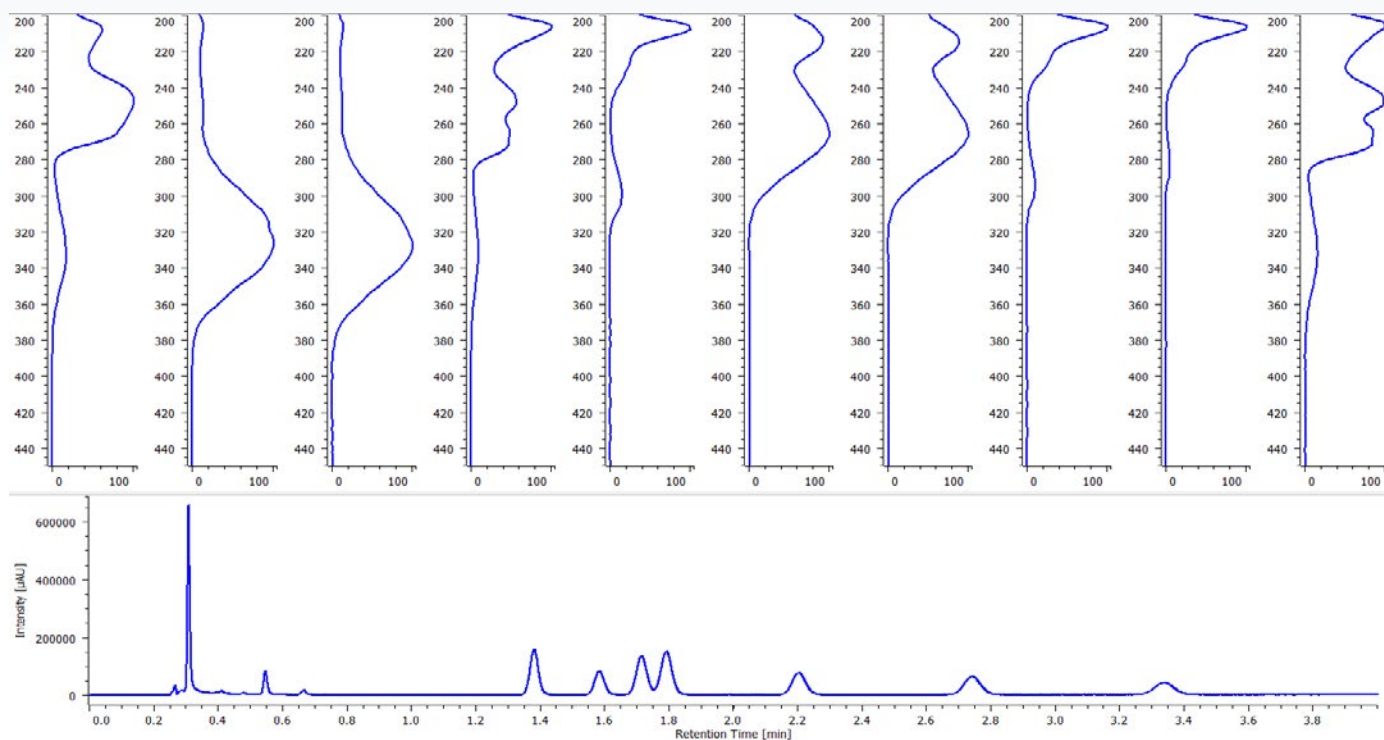


Fig. 2. On-peak Spectra of Fat-soluble Vitamin Standard Mixture

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