

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

### Introduction

Vitamins are essential nutrient to living organism for living and growing and are also organic compounds indispensable for smooth physiological activity. Among those vitamins, the fat-soluble vitamins which are difficult to be dissolved in water but easily dissolved in oil may affect on the human body when those vitamins are consumed excessively, because they are not excreted in the urine.

This time, fat-soluble vitamins were analyzed by using PDA detector for Ultra High-Performance Liquid Chromatography, enabling 100 spectra/sec High-speed data sampling and the results are shown in this report.

**Keyword:** UHPLC, Fat-soluble vitamin, Vitamin K<sub>3</sub>, Vitamin A, Retinol acetate, Vitamin K<sub>2</sub>, δ-tocopherol, Vitamin D<sub>2</sub>, Vitamin D<sub>3</sub>, α-tocopherol, Tocopherol-α-acetate, Vitamin K<sub>1</sub>, 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 °C  
 Wavelength: 200 - 500 nm  
 Injection volume: 1 μL  
 Standard sample: 10 fat-soluble vitamin standards

### Result

The contour plot and chromatogram of fat-soluble vitamin standard mixture are shown in Figure 1. The good separation for 10 kinds of components were obtained only within 4 minutes.

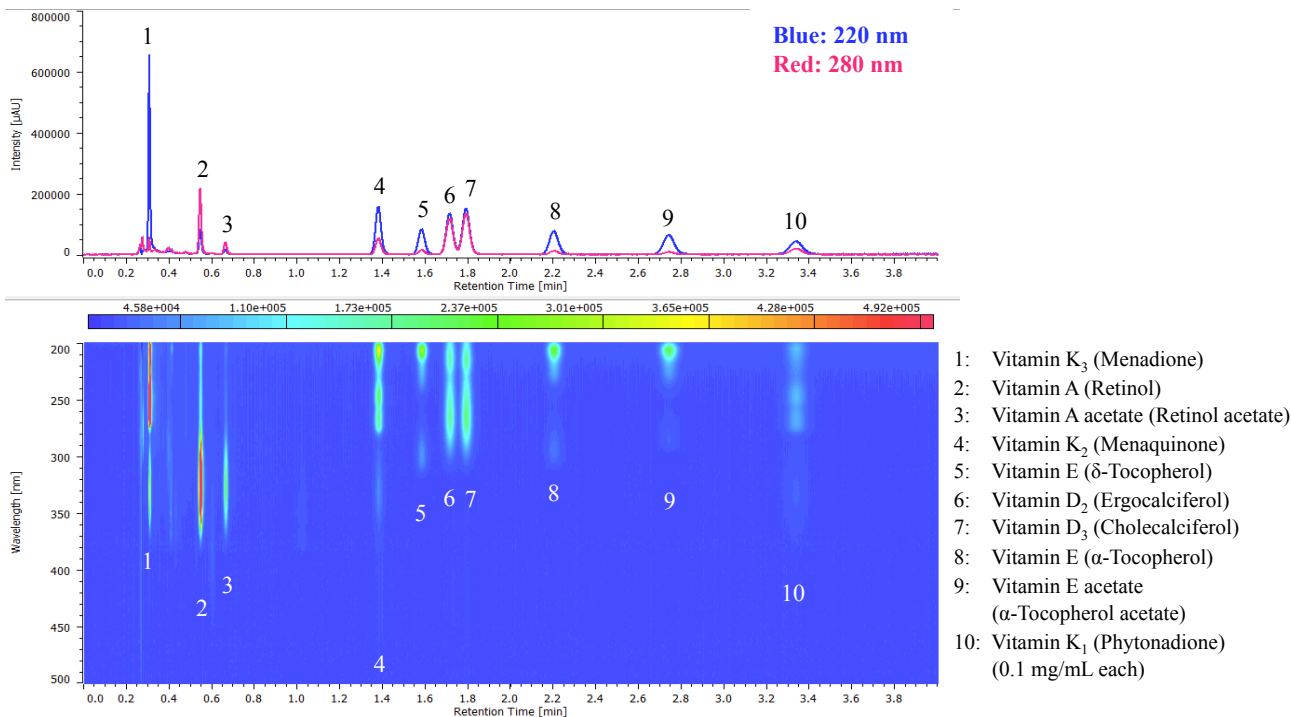


Fig. 1. Chromatogram of Fat-soluble Vitamin Standard Mixture

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

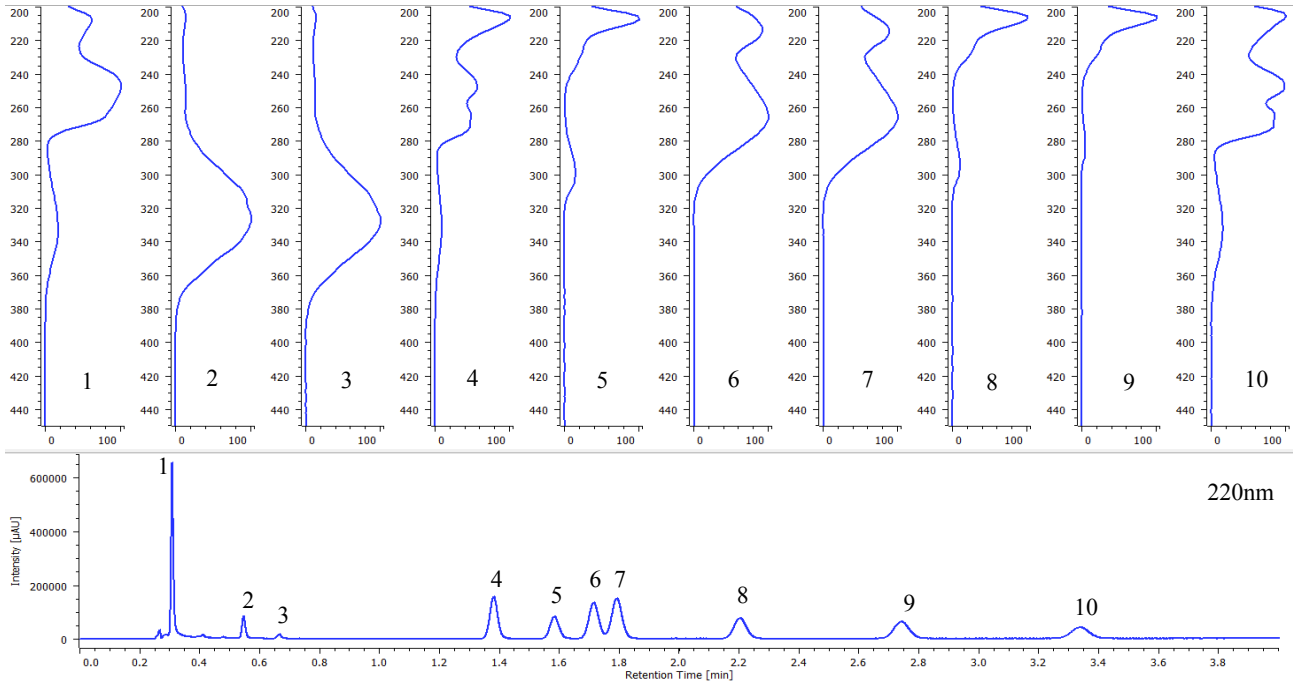


Fig. 2. On-peak Spectra of Fat-soluble Vitamin Standard Mixture

- |   |   |   |
|---|---|---|
| 1: Vitamin K <sub>3</sub> (Menadione)   | 5: Vitamin E (δ-Tocopherol)                 | 9: Vitamin E acetate<br>(α-Tocopherol acetate)                |
| 2: Vitamin A (Retinol)                  | 6: Vitamin D <sub>2</sub> (Ergocalciferol)  | 10: Vitamin K <sub>1</sub> (Phytonadione)<br>(0.1 mg/mL each) |
| 3: Vitamin A acetate (Retinol acetate)  | 7: Vitamin D <sub>3</sub> (Cholecalciferol) |   |
| 4: Vitamin K <sub>2</sub> (Menaquinone) | 8: Vitamin E (α-Tocopherol)                 |   |