

Analysis of Fat-soluble Vitamins by Supercritical Fluid Chromatography System

Introduction

Since fat-soluble vitamins are easily dissolved in oils and fats, there is little loss of fat-soluble vitamins due to washing with water or heating, and cooking with oils and fats greatly increases the absorption rate at ingestion. However, excessive fat-soluble vitamins will be stored and accumulated in the liver, adipose tissue, etc., so it is necessary to be careful about hypervitaminosis. Vitamins A, D, E and K corresponding to fat-soluble vitamins are pointed out as possibility of disease or growth disorder due to overdose or deficiency. For example, with regard to vitamins A, D, and K, excessive intake may cause symptoms such as headache (vitamin A), nauseous (vitamin A), rough skin (vitamin A), kidney stone (vitamin D) and anemia (vitamin K).

Meanwhile, vitamin E acts as an antioxidant for fat-soluble substances in vivo, because it is very easily oxidized after absorption. Due to this antioxidant action, vitamin E is said to be a rejuvenating vitamin.

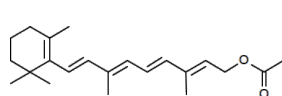
In this paper, the separation of 8 fat-soluble vitamins by using supercritical fluid chromatography (SFC) system was reported.

Keyword : SFC, fat-soluble vitamin, SFCpak Crest C18T-5, PDA detector

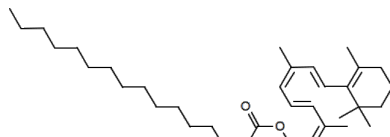
Experimental condition

Column:	SFCpak Crest C18T-5 (4.6 mmI.D. x 250 mmL , 5 μ m)
Eluent :	CO ₂ / Modifier
Modifier solvent:	Acetonitrile/Methanol(50/50)
Gradient (CO ₂ /Mod):	0 min(95/5) => 2 min(95/5) => 10 min(70/30) => 12 min(70/30) => 12.1 min(95/5) 1cycle; 17 min
Flow rate:	3.0 mL/min
Column temp.:	30 °C
Wavelength:	200 ~ 400 nm 220 nm: Vitamin E, Vitamin E acetate 248 nm: Vitamin K1, Vitamin K3 264 nm: Vitamin D2, Vitamin D3 320 nm: Vitamin A acetate, Vitamin A palmitate
Pressure:	15 MPa
Injection volume:	5 μ L
Standard sample:	8 fat-soluble vitamins 100 mg/L each in ethanol

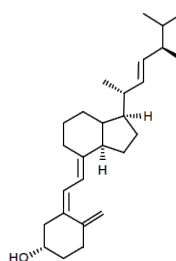
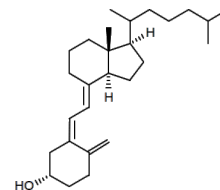
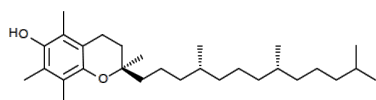
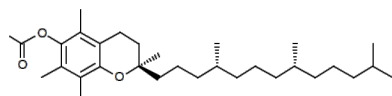
Structure



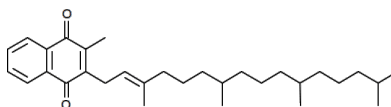
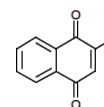
Vitamin A acetate



Vitamin A palmitate


 Vitamin D₂

 Vitamin D₃

 Vitamin E (α -tocopherol)


Vitamin E acetate


 Vitamin K₁

 Vitamin K₃

Results

Figure 1 shows the contour map of the standard solution. A good separation of 8 components within 8 minutes was achieved.

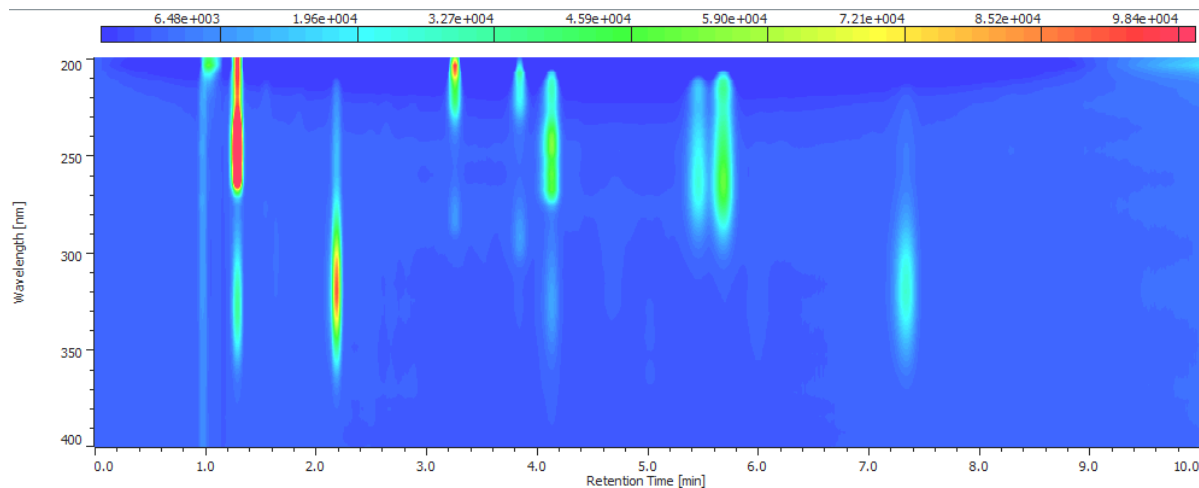


Fig. 1 Contour map of standard solution

1: Vitamin K₃, 2: Vitamin A acetate, 3: Vitamin E acetate, 4: Vitamin E,
5: Vitamin K₁, 6: Vitamin D₂, 7: Vitamin D₃, 8: Vitamin A palmitate

Figure 2 show the chromatograms of each wavelength.

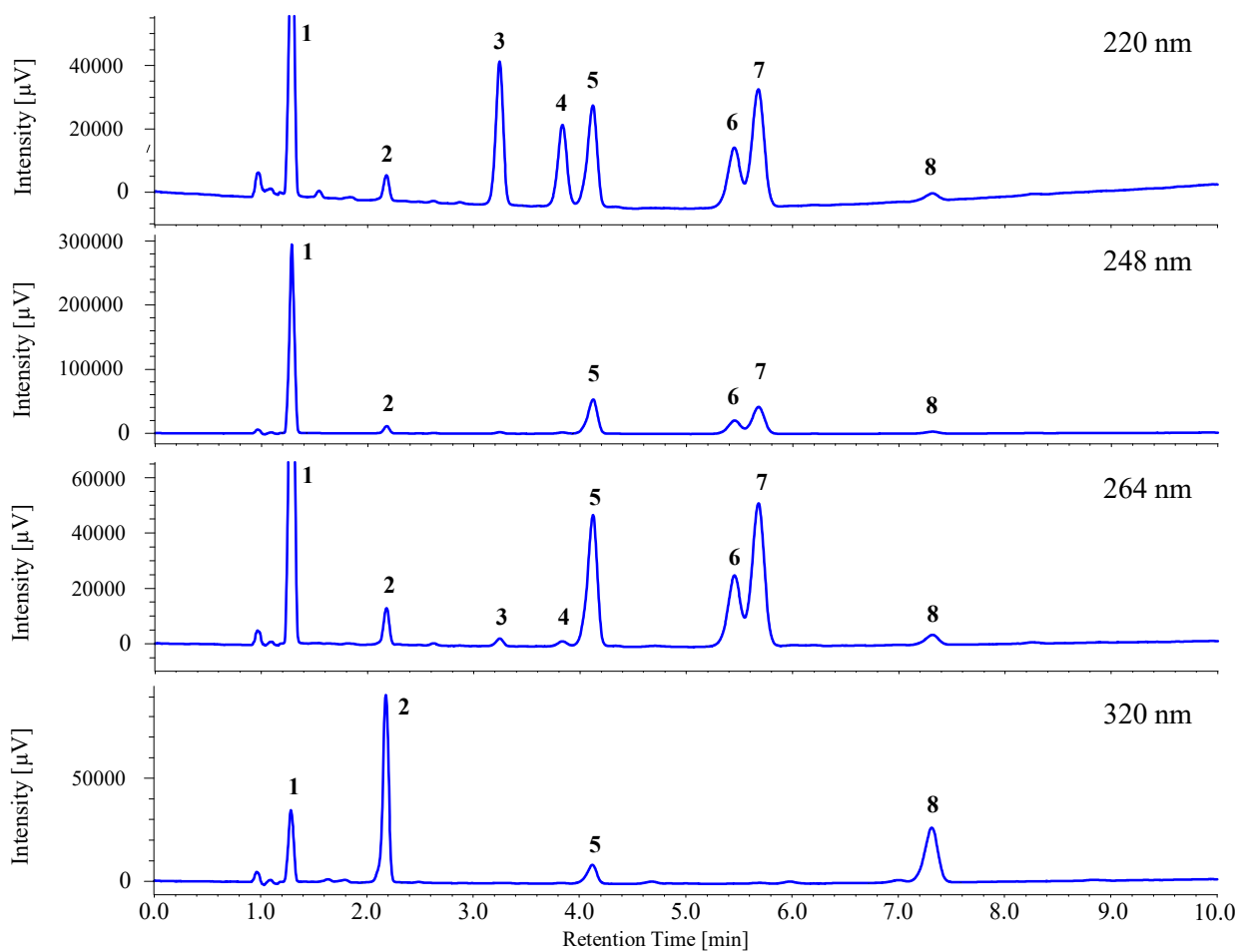


Fig. 2 Chromatograms of standard solution

1: Vitamin K₃, 2: Vitamin A acetate, 3: Vitamin E acetate, 4: Vitamin E,
5: Vitamin K₁, 6: Vitamin D₂, 7: Vitamin D₃, 8: Vitamin A palmitate

On-peak spectrum of each component is shown in Figure 3.

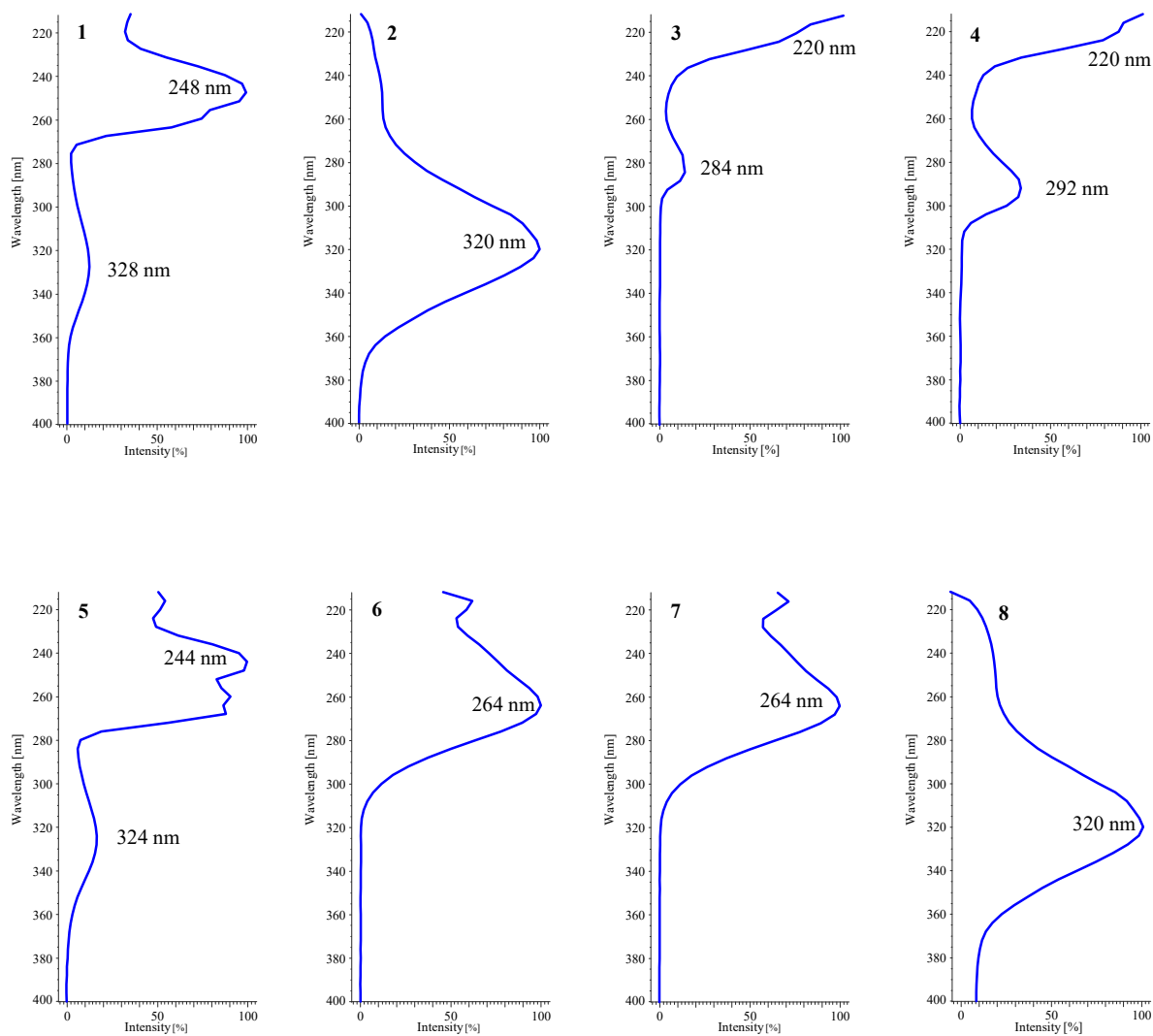


Fig. 3 On-peak spectra of each component
 1: Vitamin K₃, 2: Vitamin A acetate, 3: Vitamin E acetate, 4: Vitamin E,
 5: Vitamin K₁, 6: Vitamin D₂, 7: Vitamin D₃, 8: Vitamin A palmitate