

Chromaster

Simultaneous Analysis of Fat-soluble Vitamins

Vitamins are essential nutrients, and are classified as either fat-soluble vitamins or water-soluble vitamins. Major water-soluble vitamins include B vitamins and vitamin C. Major fat-soluble vitamins include vitamins A, D, E, and K. We have simultaneously analyzed 7 fat-soluble vitamin components by separation through a reverse phase column and diode array detection (DAD). DAD allows highly sensitive simultaneous analysis at optimal wavelengths. In addition, detected peaks can be identified from absorption spectra. Thus, food and other samples containing many contaminants can, in particular, be analyzed effectively. This analysis is suitable for qualitative analysis. Since some of the vitamins are unstable, it is recommended for quantitative analysis that each vitamin component in a sample is measured under the conditions specified in the analytical method of the corresponding component after pretreating the sample according to the method.

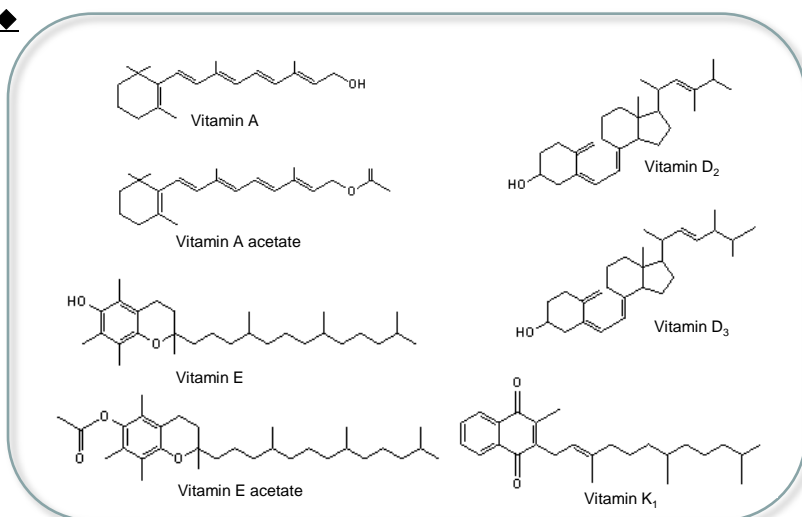
Simultaneous Analysis of Fat-soluble Vitamins

Standard Samples

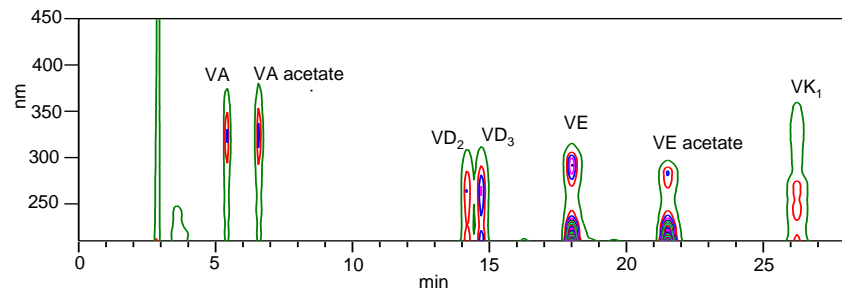
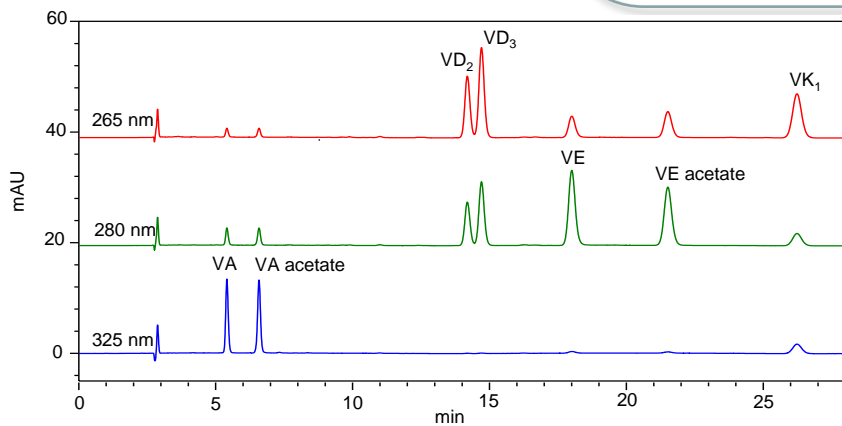
Concentrations and Structural Formulas

Component	Concentration
Vitamin A (retinol)	1 mg/L
Vitamin A acetate (retinol acetate)	10 mg/L
Vitamin D ₂ (ergocalciferol)	10 mg/L
Vitamin D ₃ (cholecalciferol)	10 mg/L
Vitamin E (dl- α -tocopherol)	100 mg/L
Vitamin E acetate (dl- α -tocopherol acetate)	100 mg/L
Vitamin K ₁ (phyloquinone)	10 mg/L

- The standard stock solution was prepared by dilution with ethanol.
- Standard solutions were prepared by diluting the standard stock solution with methanol.



Measurement examples for standard samples



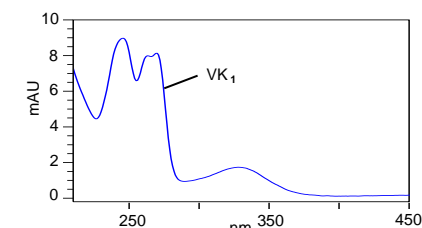
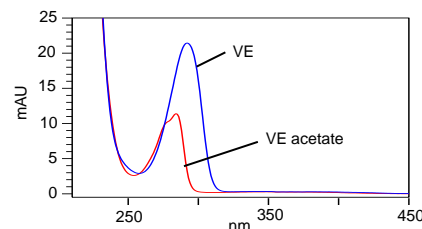
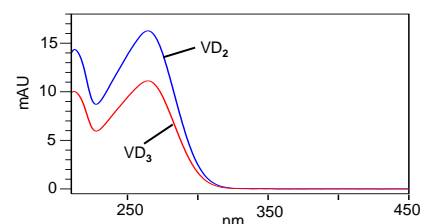
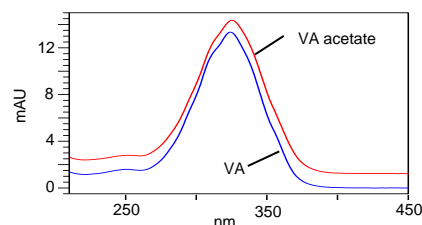
[Contour indication and extraction chromatogram]

[Analytical conditions]

Elute: CH₃CN / CH₃OH = 60 / 40 (v/v)
 Flow rate: 1.0 mL/min
 Injection rate: 10 μ L
 Column: HITACHI LaChrom C18 (5 μ m)
 (4.6 mm I.D. \times 250 mm)
 Column temperature: 40°C
 Detection: DAD 265, 280, and 325 nm

[Devices]

Chromaster
 5110 Pump
 5210 Automatic Sampler
 5310 Column Oven
 5430 Diode Array Detector
 Empower2 Data Processing System

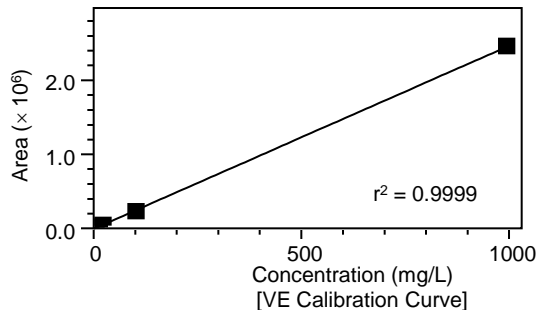
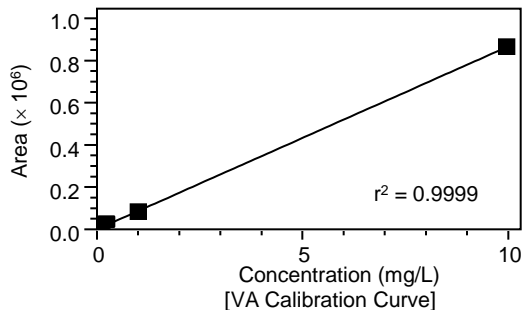


[Fat-Soluble Vitamin Spectra]

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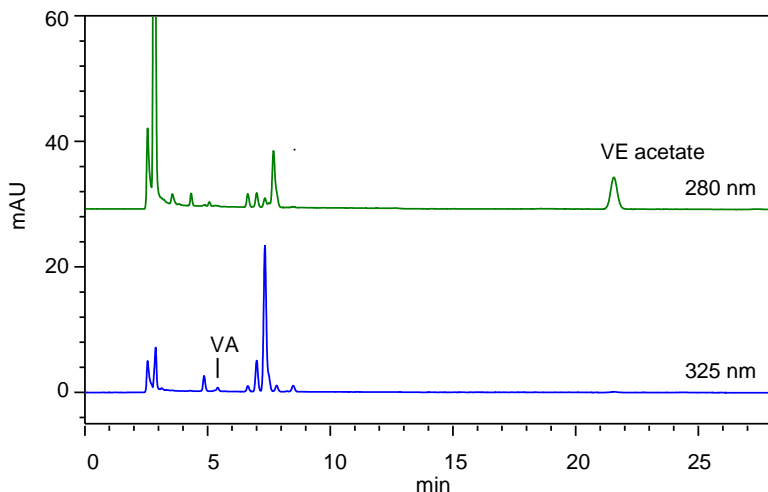
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Linearity



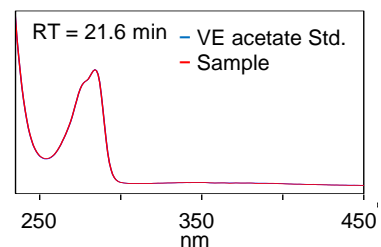
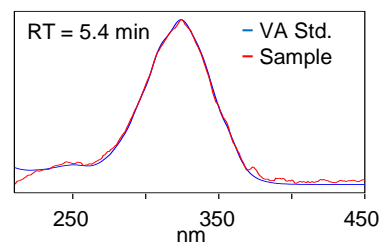
All the calibration curves (VA in the range of 0.01 to 10 mg/L; VA acetate, VD₂, VD₃ and VK₁, each in the range of 0.1 to 100 mg/L; and VE and VE acetate each in the range of 1 to 1000 mg/L) exhibited high linearity with $r^2 = 0.9999$ or more.

Analysis Example (1): Eye Drop Sample



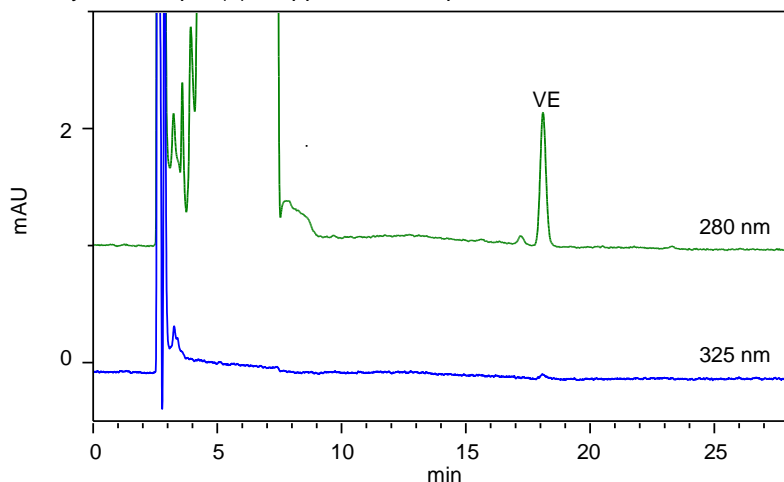
[Pretreatment of eye drops]

A sample was diluted 10 times with methanol and filtered through a 0.2 μ m filter.



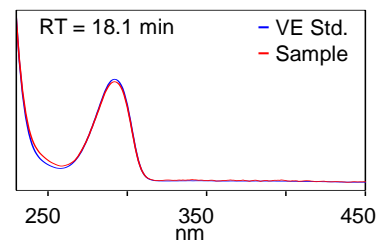
[Peaks and Spectra obtained for the standard sample]

Analysis Example (2): Supplement Sample



[Pretreatment of supplement sample]

A sample of 0.1 g was weighed out, filled up to 10 mL with methanol and filtered through a 0.2 μ m filter.



[Peaks and Spectra obtained for the standard sample]

Vitamin E

Natural: d- α -tocopherol

Extracted from vegetable oil and stabilized without treatment

Naturally derived: d- α -tocopherol acetate

Extracted from vegetable oil and acetic acid was attached for stabilization.

Synthesized: dl- α -tocopherol acetate

Chemically synthesized.

Bioactivity: synthesized VE < naturally derived VE < natural VE

Results: Vitamin A and vitamin E acetate were detected in the eye drop sample, and vitamin E was detected in the supplement sample. Each peak of the samples coincided well with that of the corresponding standard spectrum. Low-concentration samples such as VA in eye drops can be qualitatively analyzed satisfactorily. Vitamin Es used in pharmaceutical products include three types: natural; naturally derived; and synthesized. It will be found that any one of these types is used depending on the product.

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.

The system is for research use only, and is not intended for any animal or human therapeutic or diagnostic use.