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Application News

High Performance Liquid Chromatograph i-Series LC-2060

High Speed and Simultaneous Analysis for Fat-Soluble Vitamins

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User Benefits

- Enables simultaneous analysis of fat-soluble vitamins.
- The simplicity of using only methanol as the mobile phase reduces the effort of analytical preparation.
- The photodiode array (PDA) detector allows chromatograms of any wavelength to be obtained with a single analysis.

■ Introduction

Vitamins are nutrients that must be obtained from food because the body cannot synthesize them at all or in sufficient amounts. Vitamins are roughly classified as water-soluble or fat-soluble vitamins, which includes vitamins A, D, E, and K.

Fat-soluble vitamins are often analyzed using normal-phase chromatography, with different analytical conditions specified for each type of vitamin. In this article, vitamins A, D, and E were simultaneously analyzed using reversed-phase chromatography.

This article describes a simultaneous analysis of multiple fat-soluble vitamins.

■ Analysis of Standard Solution

Vitamins A, D, and E were dissolved in a small amount of tetrahydrofuran and then diluted with a mixture of methanol and tetrahydrofuran (97:3) to prepare the specified volume. Fig. 1 shows the chromatograms for the vitamins A, D, and E (50 IU, 100 IU, 0.1 mg/mL) obtained from the standard solution using the analytical conditions indicated in Table 1. A C18 column was used as the analytical column and methanol was used as the mobile phase. The PDA detector built into the integrated HPLC system was used. Using a PDA detector, chromatograms of any wavelength can be obtained after analysis, as long as it is within the specified wavelength range at the time of analysis. For this article, the maximum absorption wavelength of each compound was adopted as the detection wavelength. The system load pressure under these conditions was approximately 40 MPa.

Table 1 Analytical Conditions

System:	LC-2060C 3D
Column:	Shim-pack™ GIST-HP C18*1 (150 mm × 3.0 mm I.D., 2 µm)
Flowrate:	0.7 mL/min
Mobile Phase:	Methanol
Column Temp.:	40 °C
Injection Volume:	4 µL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA):	264 nm (Vitamin D), 280 nm (Vitamin E), 325 nm (Vitamin A)

*1 P/N: 227-30002-05 *2 P/N: 227-34001-01

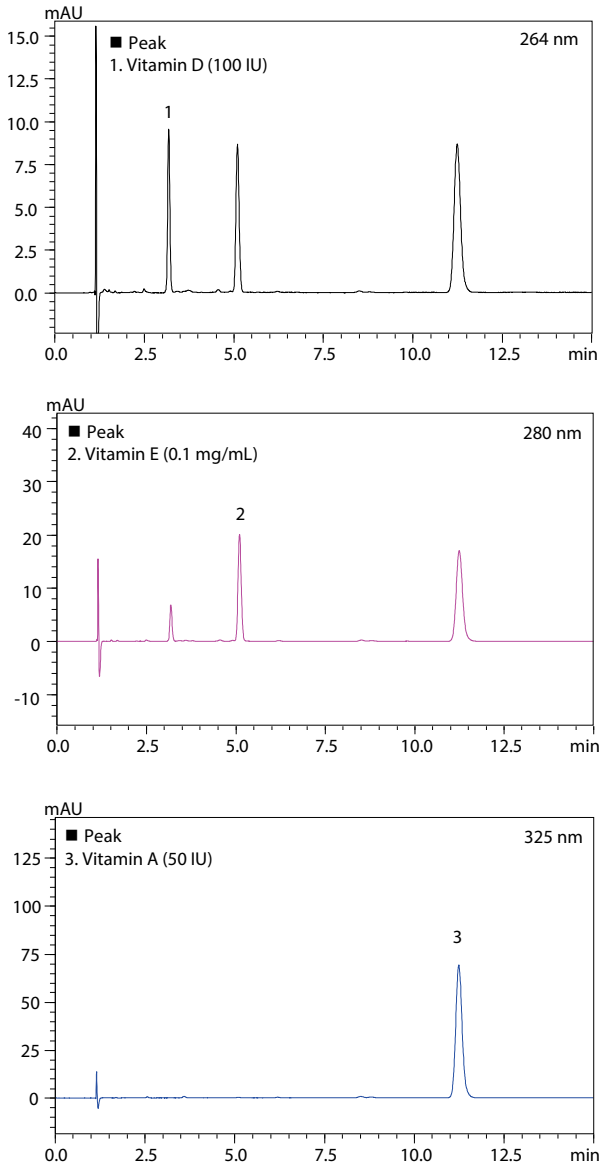

Fig. 1 Chromatograms of Standard Solution

■ Repeatability

Table 2 shows the repeatability (%RSD) of the retention time and the peak area in six repeated analyses of a standard solution of vitamins A, D and E (10 IU, 20 IU, 0.02 mg/mL). The repeatability of the retention time and the peak area was less than 0.7 % for all compounds.

Table 2 Repeatability (%RSD) in Six Repeated Analyses

Compound	Retention time	Peak area
Vitamin A	0.02	0.68
Vitamin D	0.04	0.49
Vitamin E	0.03	0.32

■ Calibration Curves

The calibration curves for the three target compounds were highly linear, with coefficients of determination (r^2) of 0.9999 or greater. Fig. 2 shows the calibration curves and Table 3 shows the concentration ranges of the calibration curves and coefficients of determination for the three target compounds.

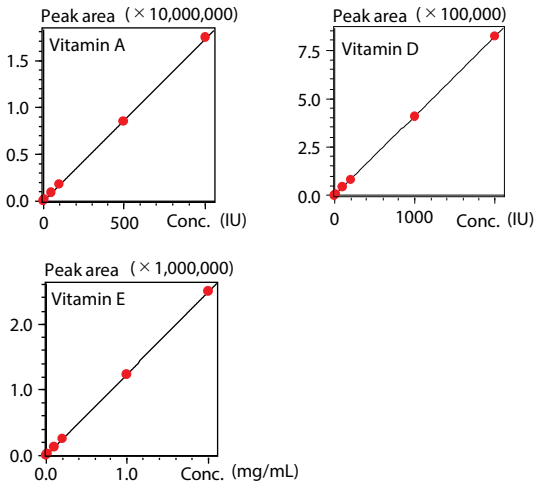

Fig. 2 Calibration Curves

Table 3 Concentration Ranges of Calibration Curves and Coefficients of Determination (r^2)

Compound	Conc. range	r^2
Vitamin A	1-1000 IU	0.9999
Vitamin D	2-2000 IU	0.9999
Vitamin E	0.002-2 mg/mL	0.9999

■ Analysis of Sample Containing Vitamins

0.25 g of the sample was dissolved in a small amount of tetrahydrofuran and then diluted with a 97:3 mixture of methanol/tetrahydrofuran (diluent) to make 10 mL using a volumetric flask. The resulting solution was diluted ten-fold with diluent before HPLC analysis.

The chromatograms of the sample are shown in Fig. 3. Target vitamins A, D, and E were separated and detected from the sample.

■ Conclusion

The combination of using an integrated HPLC system with simple analytical conditions allowed simultaneous analysis of fat-soluble vitamins (A, D, E). It is convenient to use the PDA detector built into the integrated HPLC because it can detect peaks at the optimum wavelength of each compound. Simultaneous analysis of multiple vitamins can improve the efficiency of the analysis process.

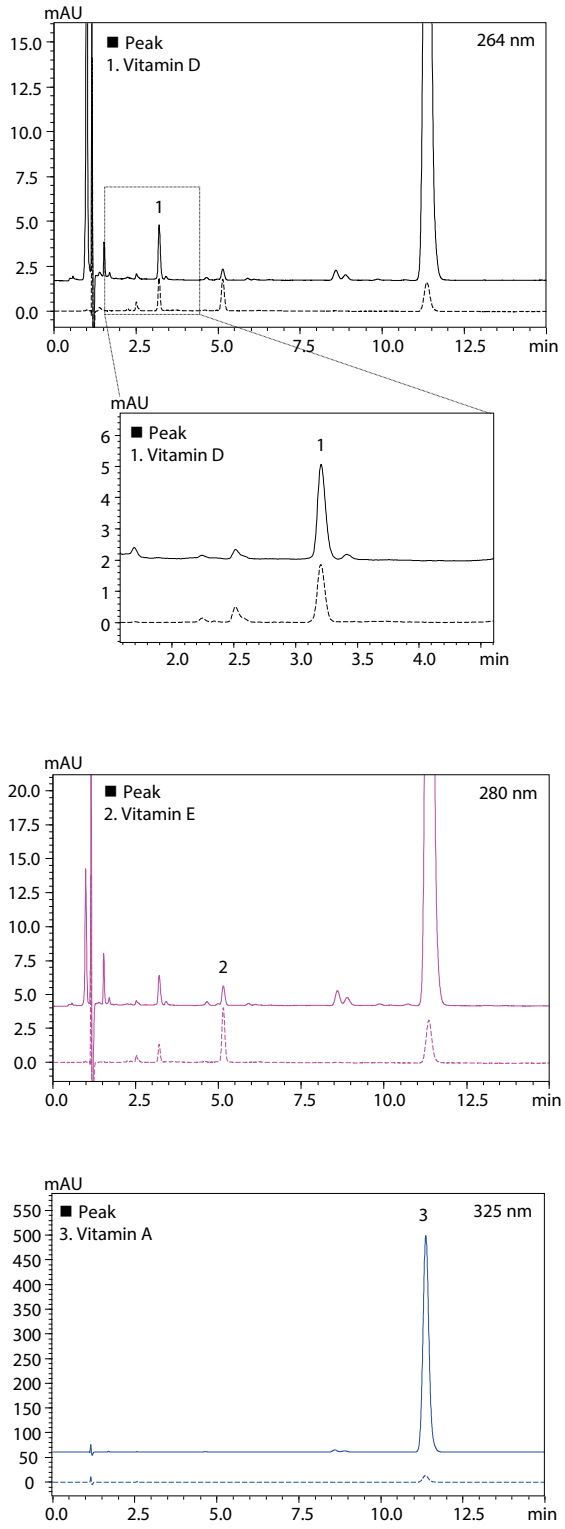

Fig. 3 Chromatograms of the Sample
(Solid Line: Sample, Broken Line: Standard Solution)

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