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## Note

# Separation of vitamin E isomers by high-performance liquid chromatography

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Previous analyses for vitamin E in biological materials have predominately relied upon spectrophotometric[1,2], indirectly measuring total tocopherols, thin-layer chromatographic–gas–liquid chromatographic[3,4] or thin-layer chromatographic–spectrophotometric[5] combination techniques that are too time-consuming for routine work. Several researchers have reported high-performance liquid chromatographic analyses of mixtures of fat-soluble vitamins[6,7] but the isolation of the different tocopherols has received little attention. Since it may be important to differentiate between the various tocopherols, particularly in foods, in order to assess vitamin E activity accurately, we propose this simple rapid liquid chromatographic technique for analyses of vitamin E — $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-tocopherols.

## EXPERIMENTAL

### *Materials*

Hexanes (HPLC grade) and 2-propanol (spectro-grade) were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.), $\alpha$-, $\gamma$-, and $\delta$-tocopherols, from Hoffmann-La Roche (Nutley, N.J., U.S.A.), and a $\beta$- and $\gamma$-tocopherol mixture, from Eisai (Tokyo, Japan).

### *Apparatus*

Spectra-Physics (Santa Clara, Calif., U.S.A.) Models 3500 and 3100 liquid chromatographs equipped with a Model 230 mixed wavelength UV spectrophotometer were employed. A Chromatronix HP sample valve was purchased with the above instrument; the 20-$\mu$l loop was used for initial separations and under calibration conditions, a large loop (30 $\mu$l) was prepared by the investigators. A stainless-steel column (25 cm × 2.0 mm I.D.) was purchased from Spectra-Physics packed with 5-$\mu$m Spherisorb. The mobile phase was hexane–isopropanol (99.75:0.25) at a flow-rate of 0.8 ml/min. The absorbance at 280 nm was monitored at a chart speed of 1 in./min on a 10-mV linear recorder (Linear Instruments, Irvine, Calif., U.S.A.). Peak heights were measured.

## RESULTS AND DISCUSSION

Six standard solutions of each of the four tocopherols were prepared in hexane and analyzed by high-performance liquid chromatography. Solutions were blown down under nitrogen gas immediately following injections and kept in amber-colored sample bottles; when not in use, solutions were stored under nitrogen at 5°. These calibration solutions produced linear calibration curves between 0.03 and 15 $\mu$g (Fig. 1).

Good separation of $\alpha$-, $\beta$-, $\gamma$- and $\delta$-tocopherols was obtained (Fig. 2). The $\beta$- and $\gamma$-tocopherol peaks exhibited a resolution of 1.5 with the column yielding 1987

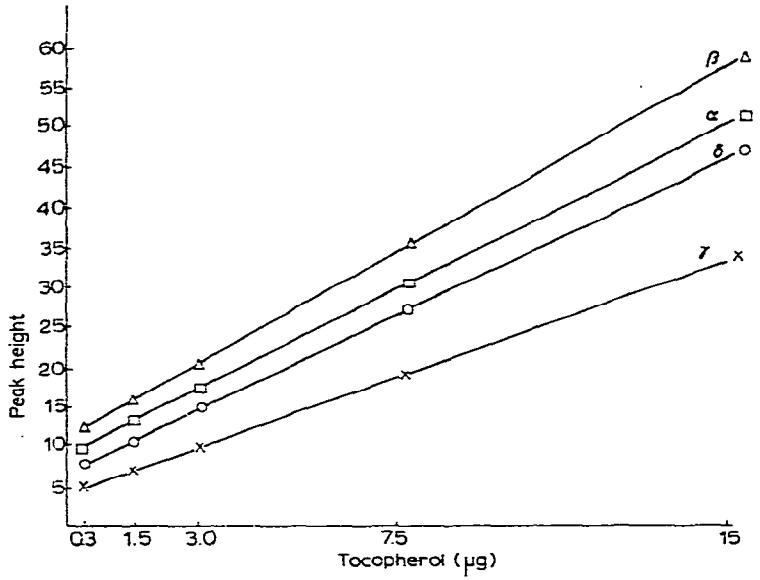


Fig. 1. Ultraviolet detector response for various tocopherols at 280 nm. □ = $\alpha$-; △ = $\beta$-; × = $\gamma$-; and ○ = $\delta$- tocopherols.

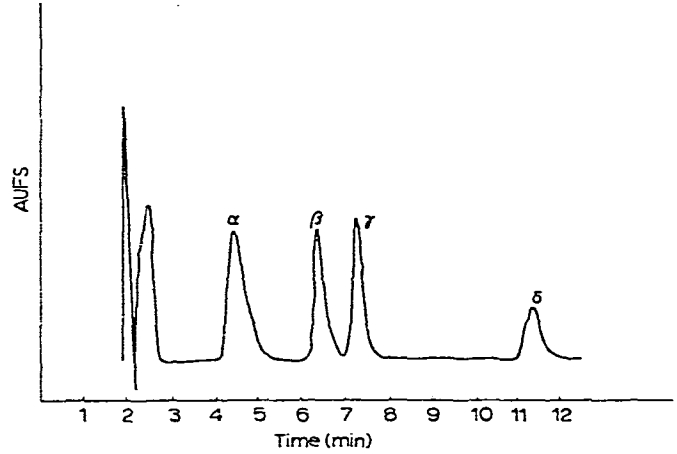


Fig. 2. High-performance liquid chromatogram of tocopherol standards at 280 nm. Experimental conditions are given in the text.

effective plates for the $\beta$-tocopherol peak. Retention ratios relative to $\alpha$-tocopherol were as follows: $\beta$-, 1.44; $\gamma$-, 1.59; and $\delta$-, 246. The retention time for $\alpha$-tocopherol was 4.5 min.

The lower limit of detection was found to be approximately 1 $\mu$g tocopherol/ml. It has been proposed that smaller quantities of tocopherols would be detect3d through use of a spectrofluorometer[8,9]; however with the universal equipment used in this study, one can expect to detect and quantitate the vitamin E compounds in plasma, platelet and food samples, injecting between 10 and 30 $\mu$l. With such unsophisticated inexpensive instrumentation, an 8-min baseline separation of the positional isomers ($\beta$- and $\gamma$-tocopherols) was easily achieved.

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