High-Resolution Liquid Chromatography of Vitamin E Isomers[1]

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ABSTRACT

High-resolution liquid adsorption chromatography on a 2-m. Corasil II column was found to provide optimum separation of the eight naturally occurring vitamin E isomers. Columns were eluted by a single solvent mixture at a flow rate of 1 ml. per min. and separation was achieved in approximately 1.75 hr. The flow rate or the solvent polarity may be increased to speed elution when complete separation is not necessary. The procedure has been used for preparative purposes.

The natural occurrence of four tocopherols and four related tocotrienols is well established (1). Calculation of biological potency of vitamin E in foods from tocopherol content requires separation of the various tocopherols and tocotrienols prior to quantitation since they vary in their ability to produce the physiological response. Several separation procedures have been reported, and they have been reviewed by Bunnell (1). Thin-layer chromatography (TLC) is the most widely used method for separating vitamin E isomers. A TLC procedure has been accepted by the Association of Official Analytical Chemists (AOAC) as the official method for α-tocopherol and α-tocopherol acetate in foods and feeds (2). The primary disadvantage with the TLC procedure is its inability to separate γ-tocopherol and β-tocotrienol (3). Gas chromatography has also been employed as an analytical method after preliminary separation and clean-up of the samples by preparative TLC (4,5) and is also the basis of an official AOAC method (6). Since the tocopherols are antioxidants and are known to be light-sensitive, the TLC system has the disadvantage of leaving them vulnerable during the time they are on the plate. Gas chromatography requires derivatization prior to chromatography, making isolation of pure tocopherols difficult.

High-resolution liquid chromatography (HRLC) has been employed to separate fat-soluble vitamins (7), and a method for separating the four tocopherols has recently been described (8). In the present study, an HRLC system which separated all eight naturally occurring vitamin E isomers is described. This system has been used for tocopherol isolation using an analytical column, and we believe it should work with larger columns.

MATERIALS AND METHODS

Apparatus

All chromatography was performed on a Nester-Faust (Perkin-Elmer) Model 1200 liquid chromatograph equipped with dual high-pressure pumps. Two high-sensitivity ultraviolet (UV) detectors (Nester-Faust Model NFLC-250 and Instrument Specialty Co., Model UA-4) allowed recording at 254 and 280 nm. on separate recorders (Hewlett Packard Model 7123A). Solvents were degassed by shaking under vacuum just prior to placing in the constant displacement pumps. Sample application was made through a septum injector directly above the column using a 10 or 100 Hamilton Syringe (Hamilton Company, Whittier, Calif.).

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Column Preparation

The column was 2 mm. i.d., stainless steel (0.25 in., o.d.) by 2 m. long packed with Corasil II (37 to 50 μ, Waters Associates, Inc.) which had been activated at 116° C. for 24 hr. The columns were packed by hand using the dry support. Before use, the column was conditioned by eluting overnight at 0.5 ml. per min. Chromatographic conditions were: mobile phase, 0.5% tetrahydrofuran in hexane; flow rate, 1 ml. per min.; and inlet pressure, 300 to 500 p.s.i. After several months of use, the top 2 cm. of the column became contaminated; this resulted in excessive back pressure and required repacking of the column.

Tocopherol Isolation

Tocopherol-containing samples were extracted with acetone for 5 hr. using a Soxhlet extractor. The acetone solution of the oil was cooled in a dry ice-acetone bath and filtered through a cooled funnel. When large volumes of oil were handled, the freezing and filtering step was repeated several times. The samples were then flash-evaporated in a Renco rotary evaporator at 30° C. to remove solvent. The oil was taken up in cyclohexane and injected directly on the column for final purification. Sample identity was confirmed by TLC on silica gel plates (3).

Chemicals

α-, β-, γ-, and δ-Tocopherol were purchased from Supelco. α-Tocotrienol and β-tocotrienol were isolated from whole ground barley and wheat bran, respectively, as described under Tocopherol Isolation. γ-Tocotrienol and δ-tocotrienol were isolated from natural ammoniated latex, type S-4 (Firestone Synthetic Rubber and Latex Company) by the procedure of Chow et al. (3). All solvents were reagent grade quality and were used as received, with the exception of bulk hexane which was passed through a silica gel column before use and reagent-grade tetrahydrofuran which was distilled and passed through an alumina column.

RESULTS AND DISCUSSION

Conventional liquid chromatography lacks the selectivity required to achieve

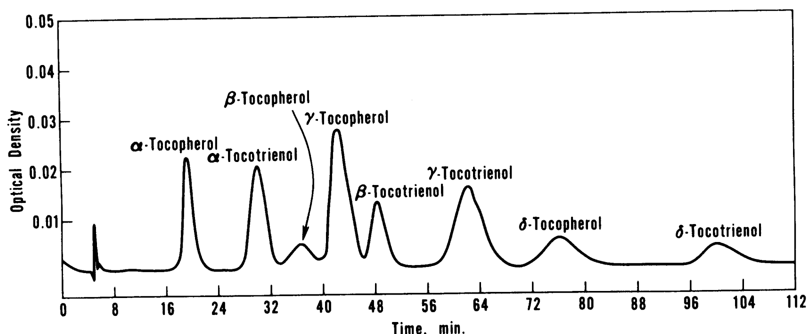

Fig. 1. Separation of tocopherol standards. Conditions: column Corasil II, 2 m. × 2 mm., stainless steel; mobile phase: 0.5% tetrahydrofuran in hexane; flow rate: 1 ml. per min.; column inlet pressure: 500 p.s.i.; wavelength: 254 nm.

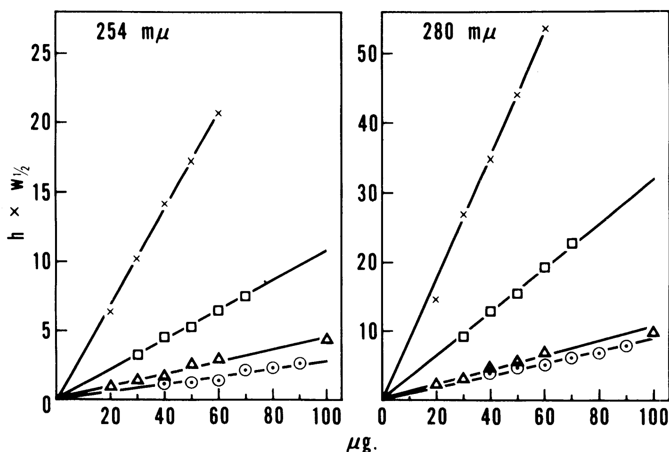

Fig. 2. Ultraviolet detector response for various tocopherols at 254 and 280 nm. Triangles = α-tocopherol; crosses = β-tocopherol; squares = γ -tocopherol; circles = δ-tocopherol.

complete resolution of vitamin E isomers. High-pressure liquid-liquid chromatography on Permaphase ODS with methanol-water elution described by Williams et al. (7), and evaluated in this study, also failed to resolve all the tocopherols and tocotrienols. Preliminary investigations with adsorption HRLC on a 0.5-m. column packed with high-quality silica gel and elution with the TLC solvent system did not give the desired separation. We found it necessary to use silica gel-coated glass beads (Corasil II), increase the column length to 2 m., and

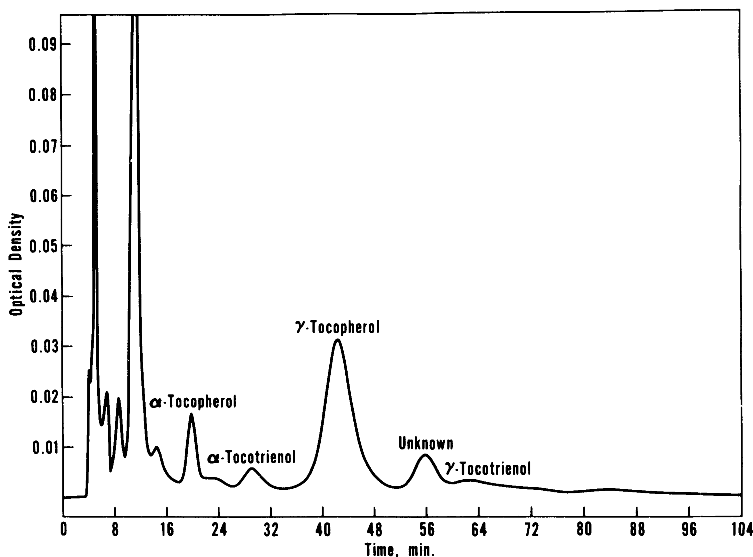

Fig. 3. Separation of corn oil tocopherols. Conditions same as Fig. 1 above.

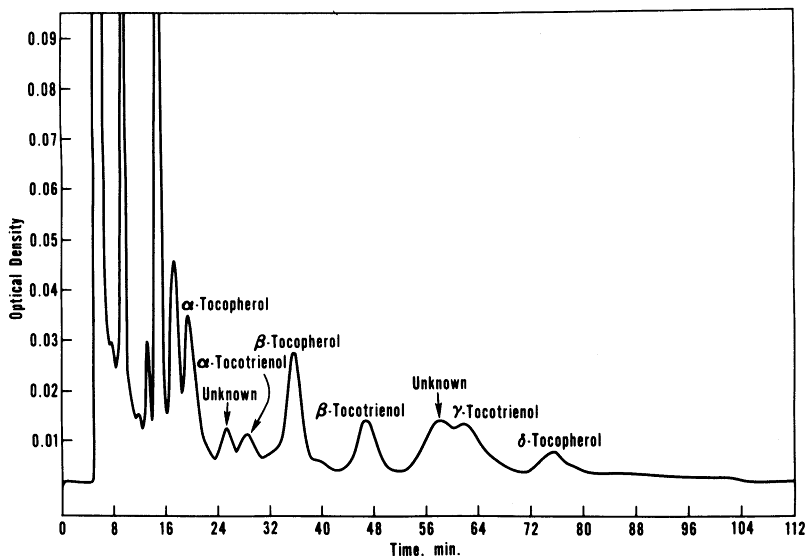

Fig. 4. Separation of wheat bran tocopherols. Conditions same as Fig. 1 above.

elute with a low-polarity solvent to obtain complete resolution. The separation we obtained is shown in Fig. 1.

Glass beads coated with silica gel are an excellent column packing material for tocopherol separation. Column packing procedures were found to be reproducible, and a 1-m. column prepared as described gave 370 theoretical plates. The hand-packed columns described in this study generally developed 300 to 500 p.s.i. Shorter columns packed with smaller-particle-size supports, but having equivalent theoretical plates, could also produce the separation described here.

The separation shown in Fig. 1 can be modified if the separation desired need not be as complete or if only a few specific isomers are of interest. Increasing the flow rate to 1.5 or 2.0 ml. per min. will speed the separation but result in loss of resolution between γ-tocopherol and β-tocotrienol and cause some overlap of α-tocotrienol and β-tocopherol. Increasing the tetrahydrofuran concentration to 1.0% will improve the chromatography for δ-tocopherol and δ-tocotrienol but will destroy resolution of the earlier eluting isomers.

Tocopherols were detected by UV absorption using two detectors set at 254 and 280 nm., respectively. The detector response for different quantities of tocopherols was determined and the results are plotted in Fig. 2. Since most tocopherols have their UV maximum in the 290 nm. region, the detector response is much better at the 280 nm. wavelength than at 254 nm. This difference can be used as an aid in peak identification. A fluorometer would also be a valuable detector since extraneous UV peaks would not be detected (8).

As a test of this separation procedure, acetone extracts of wheat bran and corn were treated to remove saponifiables and cyclohexane solutions of the isolates chromatographed. Figure 3 is a chromatogram of corn-oil tocopherols. γ-Tocopherol is the main constituent with lesser amounts of α-tocopherol, α-

tocotrienol, and γ-tocotrienol. Figure 4 shows a chromatogram of wheat bran oil. Wheat bran has a total of six tocopherols including a substantial amount of the β-isomers. These results are in agreement with TLC procedures. In both samples a UV absorbing peak, eluting just before γ-tocotrienol, was detected but has not been identified.

The results of this study indicate that vitamin E can be separated by HRLC into the various isomers without elaborate clean-up and derivatization procedures. The separation itself provides a criterion of identification by a characteristic retention time and this could be further enhanced by using a fluorometric detector in conjunction with the UV detector. As an analytical technique HRLC appears to be applicable to a variety of grain oils. The analytical column employed in this study was used to prepare tocotrienol standards by combining peaks from several runs, each producing 25 to 100 γ. Appropriate scaling-up of column dimensions and sample size should allow isolation and purification of larger quantities of tocopherols.

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