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The Comparison of SFC and HPLC Techniques Utilized for Separation of Tocopherols in Tenox GT-2

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Rapid SFC method for assay of α-, γ-, δ-tocopherols in the natural antioxidant Tenox GT-2 during 35 minutes run was described. The SFC and normal-phase HPLC methods for separation of individual tocopherols in this material are also compared.

Opisano szybką metodę oznaczania α-, γ-, δ-tokoferoli w naturalnym przeciwutleniaczu Tenox GT-2 przy użyciu SFC w ciągu 35 minut. Porównano również rozdział Tokoferoli w tym produkcie z wykorzystaniem SFC i HPLC.

Tocopherols known as natural antioxidants and products of vitamin-E activity are often used as addition to stabilize and to increase biological value of various foods, animal feeds, personal care products and drugs. Many commercial antioxidants containing tocopherols are concentrates obtained by distillation of vegetable oils condensates and few are synthesized as for example *d,l*-α-tocopherol.

Individual tocopherols display different biological and antioxidant properties. Therefore it is important to determine their concentrations in commercially available antioxidants. For example α-tocopherol is biologically the most active however its antioxidant properties are the weakest. Assay of tocopherols is usually carried out by utilizing TLC, GLC and reverse/ normal phase HPLC. Only normal-phase HPLC is capable of separating isocratically all four tocopherols [1], therefore that particular HPLC was chosen for the comparison with the new capillary SFC method. Supercritical fluid chromatography (SFC) is the chromatographic separation technique, which has been developed during last ten years. SFC combines the liquid-like solvating properties of supercritical fluids with the high efficiency of capillary columns to separate polar, non-polar thermally labile non-volatile compounds. SFC

is being used by increasing numbers of researches [2,3]. The technique was successfully applied to chromatograph such a difficult to analyze compounds like polysaccharides [4] and commercial dyes [5]. Gere [6] reported separation of vitamin A and D from α-tocopherol utilizing 50 μmSFC column and CO_2 methanol mobile phase. Separation of water soluble and lipid soluble vitamins on packed SFC column with the hexane/methanol mobile phase at 280°C was also reported [7], however stability of the vitamins at such high temperatures was not tested. HPLC separation of tocopherols in fats usually requires time consuming removal of interfering fatty substances. Unfortunately saponification causes hydrolysis of α-tocopheryl acetate, common vitamin E supplement, thus providing a measure of total tocopherol rather than α-tocopherol and its ester separately. On the other hand SFC technique is well suited for partition non-polar fatty matter from more polar unsaponifiable substances without sample cleanup. SFC is also more environmentally friendly than HPLC because it does not produce any solvent waste and usage of CO_2 is minimal. Attempt was made to develop such a method. In this study Tenox GT-2 food grade antioxidant which is sold as 70% concentrate of α-, γ-, δ-tocopherols in vegetable oil was analyzed by SFC using CO_2 as mobile phase. SFC data were compared with the results obtained by normal-phase HPLC.

EXPERIMENTAL

Materials and instrumentation

Tenox GT-2 (concentrate of α, γ-δ-tocopherols) was obtained from Eastman Chemical Company Kingsport, USA. HPLC grade *n*-hexane and 2-propanol were obtained from Fisher Scientific Itaska USA. Standards of α-, γ-and δ-tocopherols were purchased from Eastman Kodak Company, Rochester USA. Solid phase extraction (SPE) silica cartridges (SEP-PAK 500 mg) Part No. 51900 were purchased from Waters Corp. Milford USA. The HPLC chromatographic system consisted of Waters Model 600 gradient pump, Waters 996 Photodiode Array detector, Hitachi AS-2000 autosampler and Waters Millennium data acquisition system. LiChrosorb Si 60, 5 μm, 250×4.6 mm column (Phenomenex USA) was used for HPLC separation of Tocopherols.The SFC chromatographic system consisted of Model 600 SFC Chromatograph (Lee Scientific) equipped with FID and Dionex 4400 integrator. Ceramic pressure restrictor (Dionex Part No. 015354) was attached to the end of the column for flow restriction between the column and the flame detector. SP-Biphenyl-30, 100 μm ID, 0.25 μm film, 10 meters capillary column (Lee Scientific cat#15028) was used for SFC separations.

Sample and standards preparation

Vegetable oil was removed from Tenox GT-2 by utilizing SPE silica cartridge. 20 mg of Tenox GT-2 dissolved in 1 ml of *n*-hexane was deposited on the silica SEP-PAK cartridge previously activated with 3 ml *n*-hexane. Oil was washed off from the cartridge by 10 ml of *n*-hexane and oil free fraction was selectively eluted with *n*-hexane/2-propanol [85+15 (V/V)] solvent. The solvent was evaporized under nitrogen and a residue was dissolved in 50 ml of *n*-hexane prior to analyses. Total recovery of tocopherols from the cartridge was 98.7%. For independent SFC analysis sample of Tenox GT-2 was just diluted in *n*-hexane without oil removal. Calibration curve of α-tocopherol in *n*-hexane (0.5, 2.5, 5, 10 μg per injection) was constructed on both instruments for assay of individual tocopherols.

SFC analysis

SFC-grade carbon dioxide (Scott Specialty Gasses Plumsteadville USA) was used as the mobile phase. Samples and standards were chromatographed by pressure programming the mobile phase from 12 MPa to 20 mpa at 0.4 MPa min^{-1} and from 20 MPa to 35MPa 1 MPa min^{-1}. FID temperature was 350°C and oven temperature 175°C, respectively.

HPLC analysis

The mobile phase *n*-hexane/2-propanol 97.5+2.5 (V+V) was used with isocratic flow of 1 ml min^{-1}. PDA UV scans (230 nm–360 nm) were collected every second during data acquisition. UV spectra of αβ-, γ-, δ-tocopherol standards were acquired and stored in the memory with the retention time information. The spectra were utilized during identification of individual tocopherols in the Tenox GT-2 antioxidant.

RESULTS AND DISCUSSION

SFC separation of tocopherols from Tenox GT-2 sample without oil removed is shown in Fig. 1. Individual tocopherols were identified by addition of tocopherol standards to sample and monitoring increase of peak areas and heights. Chromatopgraphic data showed that removal of fatty material from the sample by SPE extraction was not necessary for the separation of tocopherols by SFC. Sample with and without oil had the same separation of tocopherols and almost baseline resolution without oil interference.

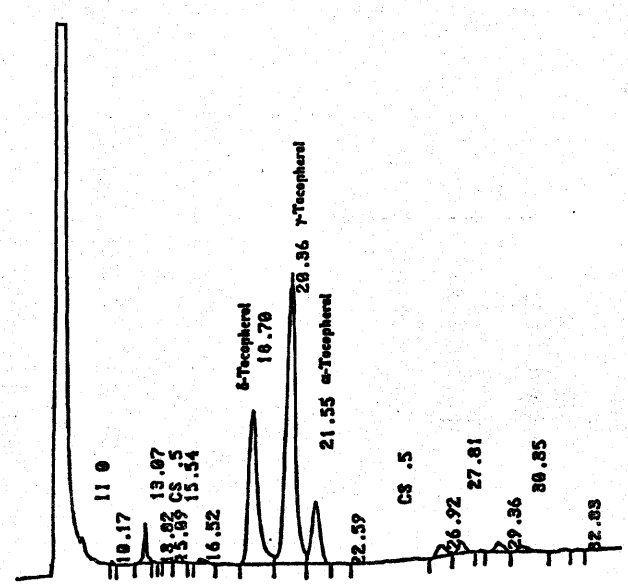

Figure 1. Supercritical fluid chromatography analysis of Tenox GT-2 antioxidant with the SP-Biphenyl-30-100 μm column and flame-ionization detector

Fatty material was eluted from the SFC column at the end of run at 335–35 MPa. Multiple injections displayed good reproducibility of results with the RSD < 3%. Sensitivity of the SFC method using above mentioned conditions was calculated as 0.05 μg/injection. HPLC separation of tocopherols from Tenox GT-2 sample is shown in Figs. 2 and 3. Identification of α-, γ-, δ-tocopherols was accomplished by standard addition and confirmed by library search of previously stored UV spectra of standards.

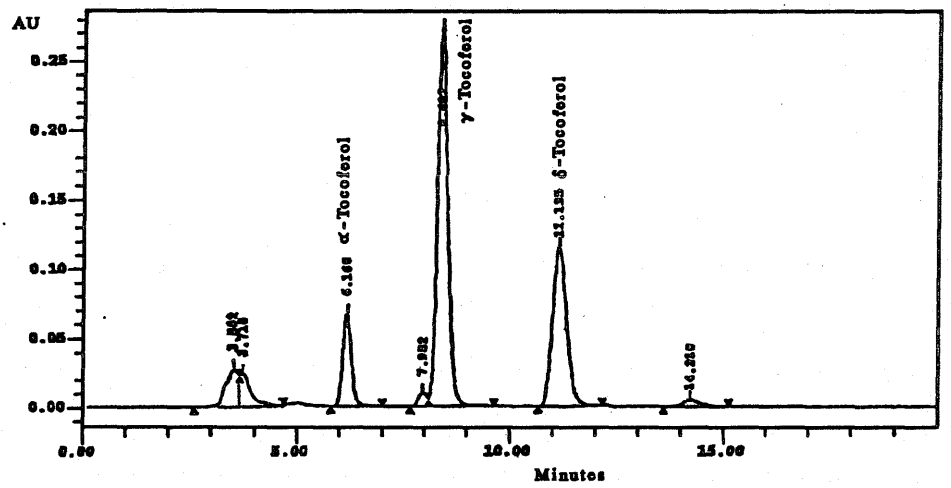

Figure 2. HPLC analysis of Tenox GT-2 antioxidant with LiChrosorb Si 60-5 μm column; UV detection at 295 nm

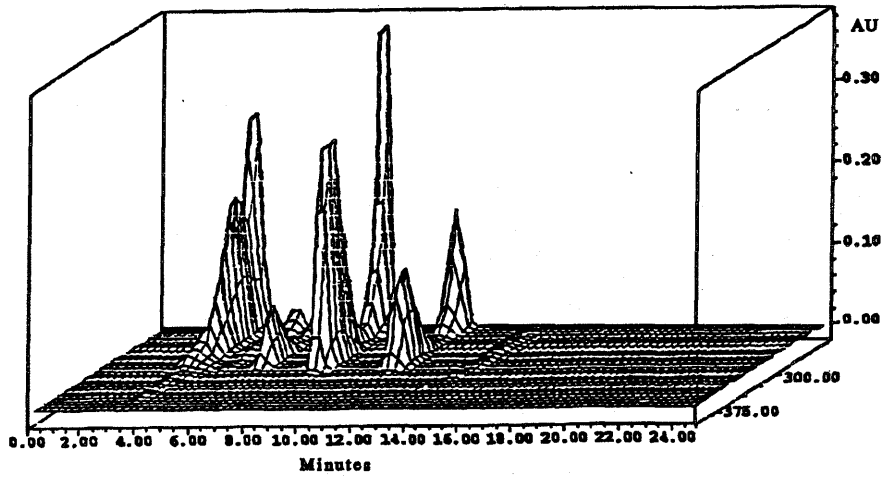

Figure 3. HPLC analysis of Tenox GT-2 antioxidant with LiChrosorb Si 60-5 μm column; photodiode array detector, scans from 230 to 360 nm

Sensitivity of HPLC method was calculated as 0.04 μg/injection. Concentrations of individual tocopherols in the Tenox GT-2 antioxidant were calculated utilizing α-tocopherol calibration curve and are presented in Table 1. The results showed slightly higher concentration of δ-tocopherol by SFC in comparison with the HPLC method. UV spectral data indicated that the small peak eluted before γ-tocopherol (Rt 7.952) was β-tocopherol. During SFC analysis, β-tocopherol was not entirely separated from tocopherol under current analytical conditions and was contributing to elevated result of δ-tocopherol concentration in the Tenox GT-2. In the conclusion SFC technique enables rapid (under 35 minutes) determination of α-, γ- and δ-tocopherols in the oil matrixes without sample preparation. On the other hand normal-phase HPLC methods require sample cleanup procedures since saponifiable matters often interfere with the UV detection. Both methods displayed comparable sensitivities (0.04 and 0.05 μg/injection) for HPLC and SFC respectively, however HPLC chromatogram displayed better resolution. Using the modified carbon dioxide as mobile phase, analyses time by SFC could be reduced. Smaller diameter SFC columns would improve resolution due to a higher theoretical plates number and therefore would enhance separation of β- from δ-tocopherol.

Table 1. Composition of tocopherols in Tenox GT-2 antioxidant by HPLC and SFC

Tenox GT-2	Concentration of tocopherols by HPLC	Concentration of tocopherols by SFC
α-Tocopherol	7.88	7.97
γ-Tocopherol	39.67	39.82
δ-Tocopherol	21.77	22.72
Total Tocopherols	69.32	70.51

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