Determination of Free Tocopherols in Deodorizer Distillate by Capillary Gas Chromatography

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The determination of free tocopherols in deodorizer distillate by capillary gas chromatography is described. Samples are silylated and chromatographed on a 30 M, DB-5 capillary column using temperature programming. No interferences were found with any of the tocopherols or the internal standard. A complete analysis requires less than one hr. No saponification is necessary. This method is offered as a replacement for AOCS Method Ce 3-74.

Deodorizer distillate, obtained mainly from the processing of crude soybean oil, is the raw material for the manufacture of natural vitamin E products. Deodorizer distillate is a complex material consisting of many components, including sterols, steryl esters, sterol hydrocarbons, tocopheryl esters, free fatty acids, mono-, di- and triglycerides, waxy hydrocarbons, etc., with only 2–15% being tocopherols. Distillates may also contain large amounts of water and foreign particles. There are two main reasons for a quantitative measurement of tocopherols in deodorizer distillate: (i) The price paid for deodorizer distillate is dependent on its tocopherol content, and (ii) tocopherol content in deodorizer distillate is important in monitoring yield and manufacturing costs.

The current method (1,2) for tocopherols in deodorizer distillate, utilizing packed column chromatography, is labor-intensive, often affected by interferences to both the tocopherols and the internal standard peaks. Saponification is necessary to reduce these interferences (unknown species) under any of the tocopherol peaks, while interferences with the internal standard, usually cholesterol or brassicasterol, are not affected by saponification, thus requiring correction. Total assay time per sample is ca. four hr.

The objective of this work has been to develop a method for the determination of tocopherols in deodorizer distillate utilizing capillary gas chromatography and to eliminate interferences and reduce labor time.

EXPERIMENTAL

Chromatographic equipment. The analyses were performed on a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector. The capillary column, DB-5, 30M, 0.25 mm i.d., 0.25 μm film thickness, was purchased from J. & W. Scientific Co., Folsom, California. Separations were performed using temperature programming.

Chemicals. Chrom AR grade pyridine, Mallinckrodt, was purchased from American Scientific Products, McGraw Park, Illinois. Sylon BFT was purchased from Supelco Inc., Bellefonte, Pennsylvania. Sylon BFT is a mixture of 99 parts bis(trimethylsilyl)trifluoroacetamide to one part trimethylchlorosilane. α-Tocopherol, USP Reference standard, was purchased from USP, Rockville, Maryland. Chloroform, 1-heptadecanol and stearoyl chloride were purchased from Eastman Kodak Co., Rochester, New York.

Preparation of internal standard. Weigh 50 g of melted 1-heptadecanol into a one-l beaker. Allow it to solidify. Add 30 g of stearoyl chloride. Heat the mixture on a steam bath for two hr. Note: As the reactants melt, hydrogen chloride is vigorously liberated. After bubbling subsides, recrystallize the product using 3A alcohol until analysis, using capillary gas chromatography, indicates >99% purity, by area percent. The final product should be oven dried at 150°C until a constant weight is obtained.

Accurately weigh an appropriate amount of heptadecyl stearate and dissolve it in chloroform to give a final concentration of ca. 4.0 mg/ml.

Preparation of standards. Accurately weigh 20–30 mg of pure α-tocopherol into a 50-ml Erlenmeyer flask. Add one ml of pyridine and two ml of Sylon BFT. Heat contents to 50°C for ca. 10 min. Cool the mixture slightly, then add 4.00 ml of internal standard solution and 15 ml of chloroform. Inject one-μl aliquots into the gas chromatograph. Calculate response factors as follows: $Rf = A(IS) \times Wt(S)/A(S) \times Wt(IS)$ where: Rf is the response factor, A(IS) is the area internal standards, Wt(S) the weight, g, tocopherol standard, A(S) the area tocopherol standard and Wt(IS) the weight internal standard, g. Response factors for δ-, β- and γ-tocopherols are taken to be equivalent to that of α-tocopherol.

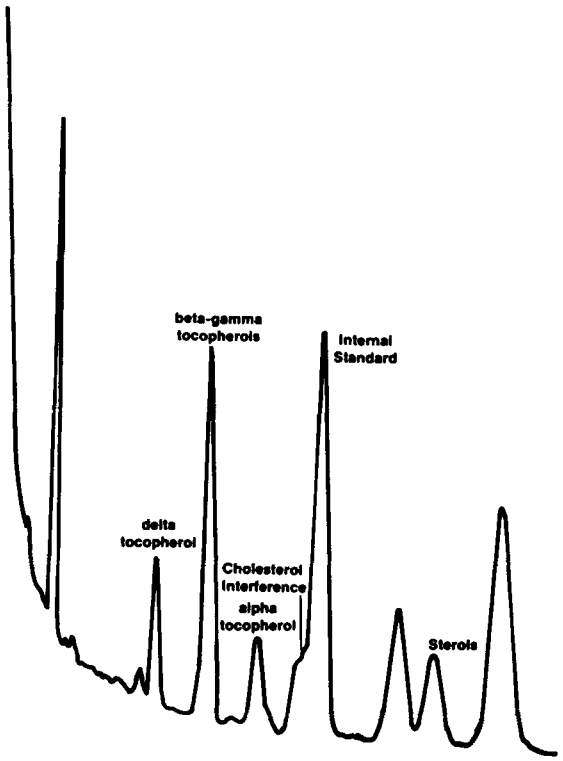

FIG. 1. Packed column chromatogram of a deodorizer distillate after saponification with cholesterol interference under the internal standard.

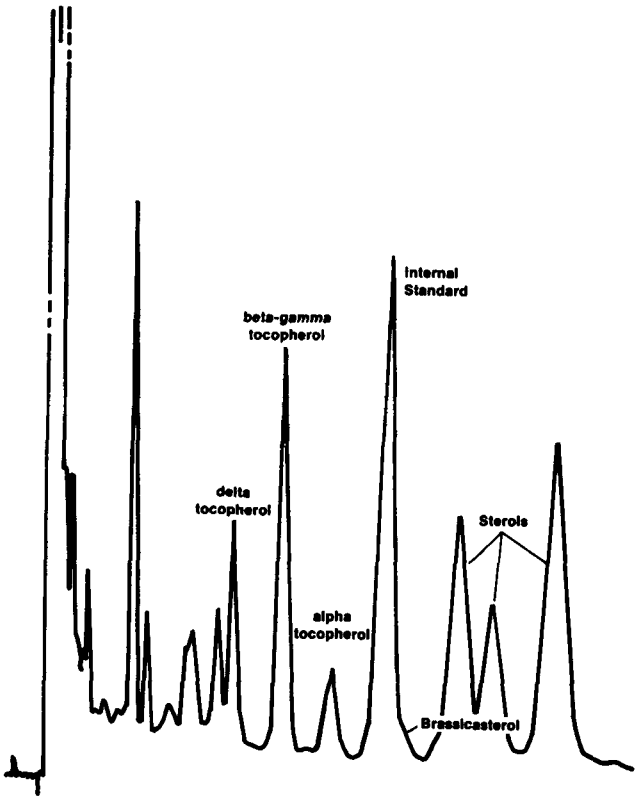

FIG. 2. Packed column chromatogram of a deodorizer distillate containing brassicasterol, an interference to the internal standard.

Preparation of samples. Samples should be heated on a steam bath and shaken well before sampling. Accurately weigh 180–250 mg of sample into a 50-ml Erlenmeyer flask. Add one ml of pyridine and 2 ml of Sylon BFT. Heat contents of the flask to 50°C for 5–10 min. Add 4.00 ml of internal standard solution and 15 ml of chloroform. Inject one-μl aliquots into the gas chromatograph.

Gas chromatography conditions. Temperature program: 140–300°C at 10°/min, hold 10 min, then 300–320°C at 5°/min, hold 10 min. Injector temperature, 260°C; detector temperature, 345°C; splitter flow, 150 cc/min; column flow, 2 cc/min; carrier gas, helium; peak width, 0.08; attenuation, 2↑1.

RESULTS AND DISCUSSION

Comparison of packed and capillary chromatograms. Figures 1 and 2 show chromatograms of deodorizer distillates, using the current packed column method, which contain cholesterol and brassicasterol, both of which are interferences with the internal standard peak. Interferences, of unknown composition, have been observed around all tocopherol peaks, even after saponification. Figure 3 shows a capillary chromatogram of a deodorizer distillate containing cholesterol, an interference when using the packed column method. Note in the capillary chromatogram, cholesterol is well resolved from the peaks of interest. As of this writing, no interferences have been observed with any of the peaks of interest using the capillary method.

Accuracy and precision. Accuracy of this assay was determined by spiking known concentrations of α-tocopherol over a range of 12–73 mg/g into a sample initially containing eight mg/g of α-tocopherol. Average recovery of the spiked amount was 99.2%.

Precision of the assay was determined by repetitive assays of a sample containing 100 mg/g total tocopherols. The assays were performed by one operator using one instrument over a period of 10 days for a total of 10 assays. Relative standard deviation of the total tocopherols was found to be ± 1.1%.

Comparison of results using capillary GC vs packed column GC. Table 1 shows results of nine deodorizer distillates assayed using both capillary GC and packed column GC. Note that after saponification, many samples using packed column chromatography show an increase in tocopherol content. Such an increase is due to the presence of tocopheryl esters. Because saponifi-

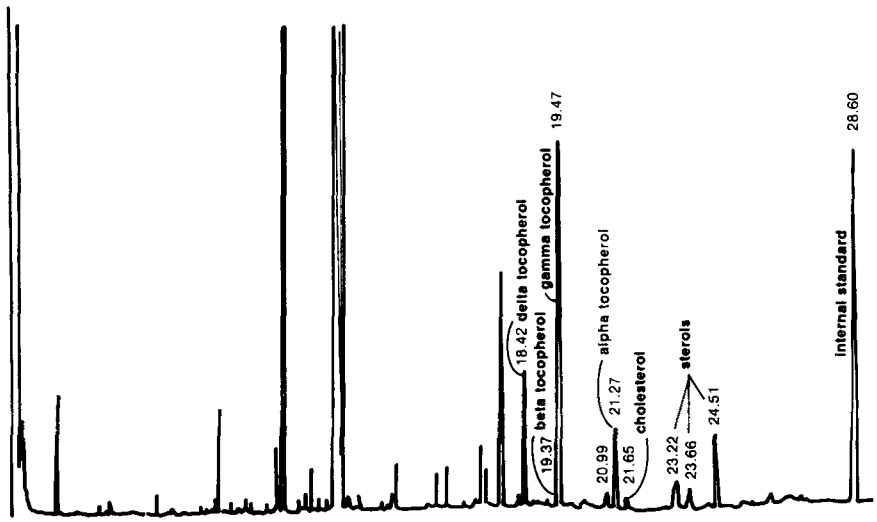

FIG. 3. Typical capillary chromatogram of a deodorizer distillate, containing cholesterol and internal standard.

TABLE 1

Comparison of Results via Packed Column Chromatography vs Capillary Chromatography

Sample #	1			2			3		
	Packed column		Capillary	Packed column		Capillary	Packed column		Capillary
	U	S		U	S		U	S	
δ	15	14	14	14	13	13	19	20	20
β			1			1			1
γ	47	49	46	49	50	50	47	48	45
α	15	10	10	11	10	9	7	7	6
total	77	73	71	74	73	73	73	75	72

Sample #	4			5			6		
	Packed column		Capillary	Packed column		Capillary	Packed column		Capillary
	U	S		U	S		U	S	
δ	27	28	26	34	32	33	10	10	9
β			1			3			1
γ	63	66	65	83	82	81	25	27	25
α	10	8	10	20	16	18	5	4	4
total	100	102	102	137	130	135	40	41	39

Sample #	7			8			9		
	Packed column		Capillary	Packed column		Capillary	Packed column		Capillary
	U	S		U	S		U	S	
δ	33	36	31	15	16	14	10	11	10
β			2			2			1
γ	83	93	80	44	50	42	32	37	33
α	12	12	11	8	5	8	9	11	9
total	128	141	124	67	71	66	51	59	53

U, unsaponified; S, saponified.

TABLE 2

Summary of Advantages of Using Capillary Gas Chromatography for the Assay of Deodorizer Distillate vs Packed Column Chromatography

Item	Capillary	Packed column
Sample preparation	5–10 min	2–3 hr
Chromatography	32 min, temp programmed	40 min, isothermal
Resolution	98% resolution of β-γ isomers	No resolution between β and γ isomers
Interferences	None	Possible interference with all tocopherols and internal standard
Accuracy	99%+	Unknown
Precision	± 1.1% RSD[a]	± 3.2% RSD[a]
Total assay time	Ca. 40 min	Ca. 4 hr

[a]Relative standard deviation.

cation is unnecessary when using capillary GC, the presence of tocopheryl esters has no effect on the proposed method. For samples without tocopheryl esters, results by capillary and packed column GC agree very well.

Table 2 summarizes the advantages of analyzing deodorizer distillate by capillary chromatography vs packed column chromatography. While this method has been tested only on deodorizer distillate derived from soybean oil, it should work well for distillates derived from other vegetable oils, with little or no modifications.

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