TABLE II

Fatty Acid Composition of Oils Determined by Gas Liquid Chromatography

Sample	Component acids (% by wt)										
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	21:0	22:0	24:0
A	0.1	1.3	0.2	0.3	8.5	87.8	1.4	trace	0.1	0.2	0.1
B	trace	1.6	0.2	0.5	9.1	87.2	1.4	trace	trace	trace	–

dence of conjugated unsaturation or of unusual functional groups.

Fatty acid compositions of the oils, based on GLC analyses of their methyl esters, are shown in Table II. Linoleic acid was present in extremely high levels (87.8% and 87.2%, respectively), and together with oleic and linolenic acids the 18-carbon unsaturated acids amounted to 97.7% of the total acids in individual oils. Probably this concentration of linoleic acid (87.8%) is one of the highest so far reported in any seed oil.

ACKNOWLEDGMENT

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REFERENCE

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A Rapid Method for Analysis of Refined Vegetable Oils for TBHQ by Gas Chromatography[1]

RICHARD E. AUSTIN and **DAVID M. WYATT**, Eastman Chemical Products, Inc., Kingsport, TN 37662

ABSTRACT

A simple and rapid extraction technique using acetonitrile solvent has been used to extract TBHQ antioxidant from refined soybean and cottonseed oils. The extracts have been analyzed by a gas chromatographic technique, using silyl derivatization, to quantitate the TBHQ content of the oil. The method has been calibrated using 14 data points for linear regression, showing a coefficient of determination of 0.9587. Average recoveries over a 14-day period range from 102 to 106.6% at the 50-150 ppm levels. The overall test standard deviation, which includes the effects of time, is 5.9.

INTRODUCTION

Gas chromatographic procedures have been proposed to analyze for tertiarybutylhydroquinone (TBHQ) antioxidant in food oils. These procedures employ direct injection of the diluted oil sample (1) or time-consuming extractions, followed by concentration steps which treat the antioxidant severely by drying under vacuum (1,2). While we have sometimes used the direct gas chromatographic injection approach successfully, we also have experienced severe difficulty with the breakdown of TBHQ in the injection port of the chromatograph (D.M. Wyatt, unpublished results).

In the method described in this paper, use of the N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) derivative of TBHQ conveniently bypasses this problem. There also is no need for tedious extraction methods to remove TBHQ from food oils. A simple agitation of the oil with acetonitrile, followed by slight concentration of an aliquot of the acetonitrile layer, are sufficient for acceptable TBHQ recoveries. Published methods for TBHQ analysis generally have not included the effect of time on analytical precision; this aspect has been included in our method.

[1]Presented at the JOCS/AOCS Annual Meeting, 1979, San Francisco.

METHODS

Gas Chromatographic Conditions

The column used was a 6 ft, 1/4 in. od glass column packed with 10% Versilube F-50 on 100-200 mesh Gas Chrom Q. The chromatograph was operated with a column temperature of 190 C, injection temperature 200 C, and flame ionization detector temperature 240 C. The carrier gas was nitrogen at a flow rate of 53 ml/min, and the hydrogen and air flow rates to the detector were set for optimal response for the specified chromatographic conditions. The injection port was glass-lined. The injected volume was 1.5 μl. The detector output was 5 mV, FSD with electrometer sensitivity of 5×10^{-12} amps.

Calibration

Separate acetonitrile stock solutions of TBHQ and the internal standard, propylparahydroxy benzoate (propyl paraben), were accurately prepared and diluted to give a final concentration of 102 μg/ml for TBHQ and 156 μg/ml for the propyl paraben. This allowed convenient combination of either 0.5, 1.0, or 1.5 ml of TBHQ standard solution to 1 ml of propyl paraben standard solution, thus approximating the concentration range of interest, 50-150 μg TBHQ, with the amount of internal standard being held constant at 156 μg in any combination. One ml of BSTFA was added to each solution and the combined solution was concentrated by evaporation under a nitrogen stream to ca. 1 ml just

prior to gas chromatographic injection. Fourteen data points were obtained using this procedure and chromatographic conditions. A least squares calibration was calculated based on the ratio of the TBHQ chromatographic response to the internal standard response and the known weight of the TBHQ and the internal standard. These data are summarized in Table I.

Experimental Design

Samples of soybean and cottonseed oils, containing no antioxidants, were chosen for method evaluation. Fifty-gram portions of each oil were treated to contain 50.1, 100.2 and 150.3 ppm TBHQ. The treatment consisted of adding an appropriate aliquot to the oil from a TBHQ stock solution in ethyl acetate. The oil was warmed slightly (under an exhaust hood) to evaporate the solvent, and was mixed with agitation.

The experimental design required duplicate determinations for TBHQ on each oil sample at 0, 7 and 14 days, day 0 being the day of sample preparation. The analysis procedure consisted of adding 2 ml of acetonitrile containing a known concentration of internal standard (83 μg/ml) to a 3-ml volumetric flask. The oil sample was added to the mark with a dropping pipette. The flask was stoppered, shaken for ca. 30 sec, and allowed to settle; and an approximate 1.5-ml aliquot of the upper acetonitrile layer was withdrawn with a syringe. This aliquot was transferred to a 4-dram vial and 1 ml of BSTFA solution was added. The vial contents were then concentrated to ca. 1 ml under a nitrogen stream and 1.5 μl was injected into the chromatograph, using the previously listed conditions. A representative chromatogram is shown in Figure 1. It should be noted that there is no need for rigid adherence to precisely the same volumetric containers used here; however, precise measurement is critical and the appropriate ratios should be maintained.

The method of calculation used the least squares calibration previously discussed through the standard equation $Y = mX + b$, where the linear regression has yielded slope (m) and Y-intercept (b). The experimentally determined variable (X) is the ratio of TBHQ/internal standard chromatographic areas determined by the analysis procedure.

TABLE I

Linear Regression Calibration for Gas Chromatographic Measurement of TBHQ/Propyl Paraben (Internal Standard)

Wt. TBHQ in solution (μg)	Wt. int. std in solution (μg)	Ratio TBHQ area/ int. std. area	Ratio wt. TBHQ/ wt. int. std.
51	156	0.7229	0.3269
51	156	.7257	.3269
102	156	.9125	.6538
102	156	.9648	.6538
153	156	1.5138	.9808
153	156	1.4511	.9808
51	156	.7102	.3269
153	156	1.4528	.9808
51	156	.4717	.3269
102	156	.9111	.6538
152	156	1.4261	.9744
102	156	.9176	.6538
102	156	.9757	.6538
152	156	1.4096	.9744

Y-Intercept = −0.0442.
Slope = 0.6985.
Coefficient of determination = 0.9587.

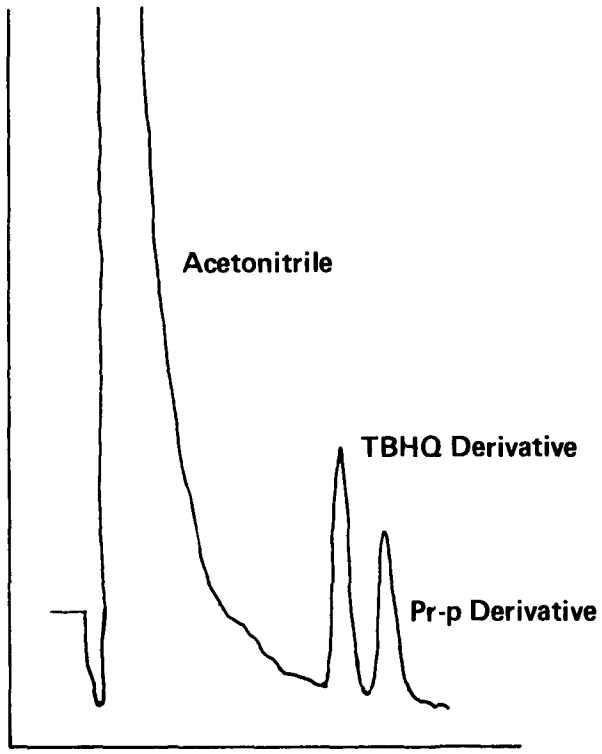

FIG. 1. Representative chromatogram of cottonseed oil extract 150 ppm TBHQ level. Retention times for TBHQ and internal standard are 11.3 and 13.2 min.

The weight ratio of TBHQ/internal standard (Y) was then calculated. Since the weight of the internal standard is known, the weight of TBHQ can be determined. The determined weight of TBHQ was then related to sample weight to obtain concentration in the oil. The weight of oil used by this procedure was experimentally determined to be 0.95024 g. Chromatographic areas were obtained using a Hewlett-Packard 3353A Automation System.

RESULTS AND DISCUSSION

The results of the designed experiment are presented in Table II. The data pair for soybean oil, 100 ppm level day 14, as well as the data pair for cottonseed oil, 150 ppm

TABLE II

Analytical Recovery of TBHQ in Refined Vegetable Oils (ppm)

Sample	Theoretical (ppm)	Analytical recovery Day 0	Day 7	Day 14
COT-50	50.1	48.6	52.1	52.2
		56.4	46.2	43.6
COT-100	100.2	116.0	104.1	107.5
		109.8	103.6	107.1
COT-150	150.3	153.7	174.6[a]	157.5
		149.6	130.3[a]	153.5
SOY-50	50.1	62.1	52.6	56.7
		59.9	54.9	52.0
SOY-100	100.2	102.8	107.2	129.2[a]
		103.4	106.3	151.5[a]
SOY-150	150.3	150.4	163.9	158.0
		137.2	159.0	152.7

[a]Statistical outlines as noted in Results and Discussion. Values were not included in the analysis of variance (Table III).

TABLE III

Analysis of Variance

Source	SS	DF	MS	F	$F_{.05}$
Inhib. level	55508.7	2	27754.4	558.4	9.6
Oils	149.0	3	49.7	—	3.7
Long time	522.5	10	52.3	2.9	2.5
Short time	292.5	16	18.3		
		31			

$$\frac{52.3 - 18.3}{2} = 17.0$$

$$18.3 + 17 = 35.3 = s_T^2$$

$$5.9 = s_T \text{ (Test Precision)}$$

level day 7, show significant differences and do not appear in line with the other data. Examination of the associated chromatograms for these sets showed unusual problems with solvent tailing resulting in different baseline treatment by the automation system. A possible explanation for this tailing could be excess oil in the analytical aliquot; nevertheless, both sets were proven to be statistical outliers and were not included in the statistical evaluation which is shown in Table III.

In the discussion of the statistical treatment, it should be noted that there is a significant difference between short-time and long-time variance, the short-time variance being defined as the chromatographic measurement itself. This is expected since the long-time variance includes the extraction manipulation and effects of time. While time effect almost always causes precision to suffer, it must always be included to completely define the precision of a test method.

With average recoveries per TBHQ level ranging from 102 to 106.6% and an overall test standard deviation of 5.9, we feel that a satisfactory method for analysis of food oils for TBHQ has been developed.

REFERENCES

1. Kline, D.A., F.J. Joe and T. Fazio, JAOAC 61:513 (1978).
2. Stoddard, E.E., Ibid. 55:1081 (1972).

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