A Rapid Gas Chromatographic Method for the Determination of BHA and BHT in Vegetable Oils

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Abstract

A gas liquid chromatographic (GLC) technique has been developed which requires about 20 min for the determination of BHA and BHT in vegetable oils. This method involves the addition of an internal standard to a weighed portion of the oil, dilution of the mixture with carbon disulfide, and injection into the Gas Chromatograph. BHA and BHT are isolated from the nonvolatile vegetable oil by using a short precolumn located in the sample port block of the gas chromatograph. Up to 35 consecutive sample injections per day have been made on the same precolumn with no appreciable effect on the accuracy of the determination. The precolumn is cleaned at the end of each day's operation. The clean precolumn is allowed to equilibrate to sample port block temperature overnight for the following day's analysis. Identification of BHA and BHT can be confirmed with a second GLC column which reverses the elution order of these compounds. Soybean, cottonseed, corn and peanut oils fortified with 20, 60 and 100 ppm each of BHA and BHT showed a recovery range of 97% to 104%.

Introduction

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are frequently added to oils and fats to retard oxidation.

Current methods for BHA and BHT in vegetable oils, fats and fatty foods include extraction of the antioxidant from the product followed by gas chromatographic analysis of the extracts (1–4) or colorimetric determination from their steam distillates (5,6). Product extraction followed by thin layer chromatography (TLC) offers still another approach to the determination of BHA and BHT in oils (7). All of these procedures require several hours of analytical time.

A gas liquid chromatographic (GLC) procedure has been developed which requires about 20 min and exhibits good accuracy and reproducibility.

Experimental Procedure

Apparatus

A Hewlett-Packard F and M Model 5750 Gas Chromatograph equipped with dual flame ionization detectors was used.

GLC Column Preparation

Column packing materials were prepared using the procedure of Burke and Holswade (8). Packed columns were conditioned for 72 hr with 50 ml/min helium flow through the system. Columns packed

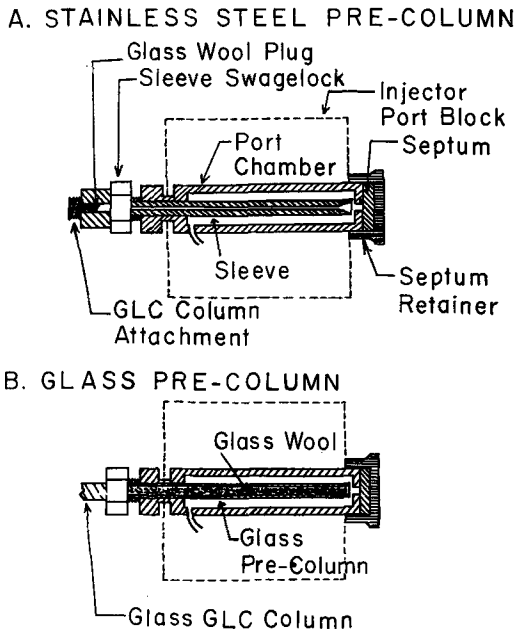

FIG. 1. Diagram of precolumns. Part A: stainless steel precolumn. The stainless steel sleeve is inserted into the port chamber and is secured with Swagelok fittings. Part B: glass precolumn. The first 5 in. of the GLC column (precolumn) inserts into the port chamber. The column is secured with a Swagelok nut and Hewlett-Packard "0" rings.

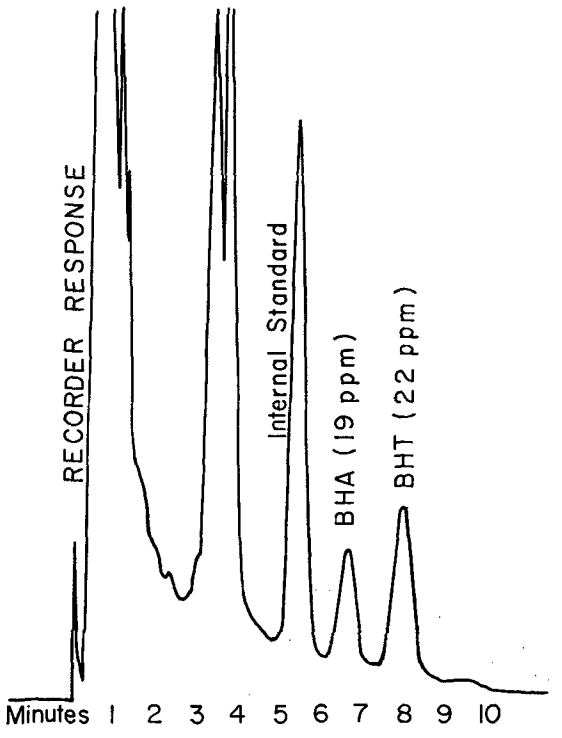

FIG. 2. A 6 ft × 4.5 mm i.d. aluminum column packed with 10% DC 200 on 80–100 mesh Gas Chrom Q, column: 160 C; detector: 250 C; injector port: 250 C; flow-rate: 50 ml/min helium; sensitivity: 4×10^{-13} amp. full scale deflection; sample: cottonseed oil fortified with BHA and BHT; sample size injected: 6.0 μl containing 0.100 g oil/ml.

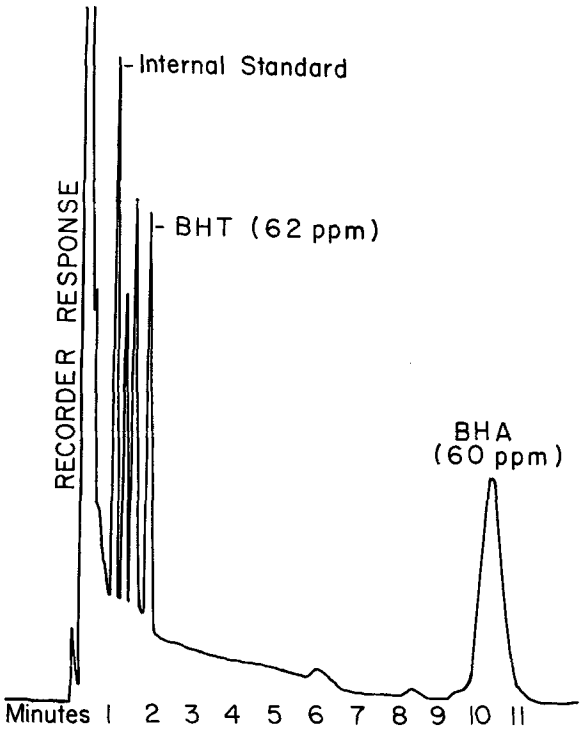

FIG. 3. A 4 ft × 4.5 mm i.d. aluminum column packed with 10% Carbowax 20 M on 80–100 mesh Gas Chrom Q, column: 190 C; remaining conditions same as Figure 2; sample: cottonseed oil fortified with BHA and BHT; sample size injected: 5.0 μl containing 0.100 g oil/ml.

with 10% DC 200 or 10% Carbowax 20 M were conditioned at 250 C and 200 C, respectively.

GLC Precolumn Preparation

Two GLC precolumn variations were tested: First, a 6 ft × 4.5 mm i.d. (¼ in. o.d.) aluminum column packed with 10% DC 200 (12,500 cst.) on 80 to 100 mesh Gas Chrom Q. The GLC column was attached to a 1/16 in. i.d. stainless steel sleeve placed in the injector port block of the gas chromatograph. (This sleeve is a standard injector port block sleeve for the Hewlett Packard F and M Model 5750 Gas Chromatograph.) A small, loosely packed wad of siliconized glass wool (about ¼ in. in length) was placed in the outlet end of the injector port sleeve. When the sample aliquot was injected into the gas chromatograph, the nonvolatile oil remained in the stainless steel sleeve. The sleeve was removed from the gas chromatograph at the end of each day's analysis. The interior walls were washed with chloroform and the siliconized glass wool plug was replaced with a new plug. The sleeve was then installed in the gas chromatograph and allowed to equilibrate at operating temperature overnight (Fig. 1, Part A). Second, a 6 ft × 4 mm i.d. glass column packed with 10% DC 200 (12,500 cst.) on 80 to 100 mesh Gas Chrom Q. The first 4 in. of the column were packed with siliconized glass wool (Analab JU6). An on-column injection was used to introduce the sample into the gas chromatograph. The nonvolatile oil remained in the siliconized glass wool precolumn. At the end of each day's analysis, the glass wool containing the oil was replaced with new siliconized glass wool and the column was allowed to equilibrate overnight at operating temperature (Fig. 1, Part B).

TABLE I

Recovery of BHA and BHT From Oils

Oil	Anti-oxidant added, ppm	Antioxidant Recovered: On aluminum column, %	Antioxidant Recovered: On glass column, %
BHA as Antioxidant			
Soybean	20.0	101.4	101.1
	59.9	104.4	102.6
	99.8	102.1	100.5
Cottonseed	20.0	97.4	98.3
	59.9	97.4	99.2
	99.8	98.0	100.6
Corn	20.0	100.4	98.7
	59.9	100.9	102.8
	99.8	101.3	101.0
Peanut	20.0	103.5	97.8
	59.9	103.4	100.8
	99.8	103.2	98.8
Average (12)		101.1	100.2
Std. Deviation		2.33	1.56
BHT as Antioxidant			
Soybean	20.7	99.2	101.8
	62.2	101.1	101.4
	103.7	100.2	99.4
Cottonseed	20.7	103.8	101.6
	62.2	100.8	102.4
	103.7	99.3	100.0
Corn	20.7	99.1	100.4
	62.2	98.4	98.8
	103.7	100.4	101.8
Peanut	20.7	101.9	98.4
	62.2	102.7	100.6
	103.7	99.9	99.6
Average (12)		100.6	100.5
Std. Deviation		1.53	1.24

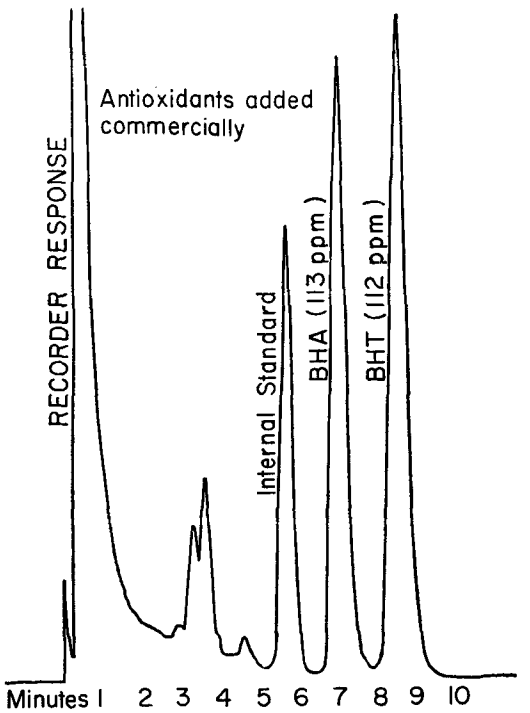

FIG. 4. A 6 ft × 4 mm i.d. glass column packed with 10% DC 200 on 80–100 mesh GAS Chrom Q, column: 155 C; remaining conditions same as Figure 2; sample: special commercially prepared oil; sample size injected: 5.0 μl containing 0.100 g oil/ml.

TABLE II

Reproducibility Data for Method Tests

Oil	Aluminum (GLC) column ppm antioxidants found			Glass (GLC) column ppm antioxidants found		
	BHA	BHT	Total	BHA	BHT	Total
Special[a]	114.4	113.9	228.3	114.5	111.4	225.9
commercially	115.1	114.1	229.2	113.4	111.5	224.9
prepared oil	113.5	111.1	224.6	115.7	112.6	228.3
Average (3 tests) total antioxidants			227.4 ppm			226.4 ppm
Reproducibility range (total antioxidants)				98.8% to 100.8%		99.3% to 100.8%
Standard deviation				1.99		1.43
Peanut oil[a]	86.4	69.3	155.7	81.5	68.3	149.8
	85.7	67.1	152.8	83.2	69.2	152.4
	86.8	69.4	156.2	83.7	67.3	151.0
Average (3 tests) total antioxidants			154.9 ppm			151.1 ppm
Reproducibility range (total antioxidants)				98.6% to 100.8%		99.1% to 100.9%
Standard deviation				1.50		1.06

[a] Commercial oils containing BHA and BHT at levels intended to be 0.02% for the specially prepared oil and 0.015% for the peanut oil.

Gas Chromatograph Conditions

Temperatures were the following: column, 160 C; detector, 250 C; and injector port block, 250 C. Gas Flows were: helium (carrier gas), 50 ml/min; hydrogen, 40 ml/min; and compressed air, 450 ml/min. Sensitivity was as follows: range, 10; and attenuator, 2. The chart speed was ½ in./min.

Calibration of Flame Ionization Detector

Five standard solutions were prepared containing 2, 5, 8, 10 and 12 μg/ml each of BHA (Eastman) and BHT (Eastman) in carbon disulfide (Baker's Analyzed Reagent Grade). Each of the standard solutions also contained 6 μg/ml methyl undecanoate (Applied Science 99+ Purity) which served as an internal standard.

Each solution was injected into the gas chromatograph and the flame ionization detector calibrated using the procedure of Sawardeker and Sloneker (9):

$$K = \frac{\text{Peak area for antioxidant/peak area of internal std.}}{\text{Wt of antioxidant/wt of Internal Std.}}$$

Assay Procedure

The oil, 2.50 g, was weighed into a 25 ml glass stoppered volumetric flask, and 5.00 ml of internal standard solution containing 30.0 μg/ml of methyl undecanoate in carbon disulfide were added. The mixture was made to volume with carbon disulfide. Three to seven microliters of the mixture were injected into the gas chromatograph.

The detector calibration technique of Sawardeker and Sloneker (9) was modified to determine the quantities of BHA and BHT present in the oil by converting the calculations to yield ppm of antioxidant as follows:

$$\text{Ppm Antioxidant} = \frac{\text{Wt of Internal Std. } (\mu g) \times \text{peak area of antioxidant}}{\text{Sample wt } (g) \times \text{appropriate K value} \times \text{peak area of Internal Std.}}$$

Method Accuracy and Reproducibility

Soybean, cottonseed, corn and peanut oils were fortified at 0, 20, 60 and 100 ppm each of BHA and BHT.

A commercially prepared oil and peanut oil each containing BHA and BHT were also tested.

Results and Discussion

The GLC columns used for quantitation of BHA and BHT were either glass or aluminum 6 ft columns packed with 10% DC 200 (12,500 cst.) on 80 to 100 mesh Gas Chrom Q and fitted with the appropriate precolumns. Recovery results for BHA and BHT in fortified soybean, cottonseed, corn and peanut oils ranged from 97% to 104% (See Table I). Determination of BHA and BHT in peanut oil and a commercially prepared oil showed a reproducibility range of 98.6% to 100.9% (Table II).

The 6 ft DC 200 columns provided a rapid elution with baseline resolution for the Internal Standard (methyl undecanoate), BHA and BHT (Fig. 2 and 4). Results obtained with both the 6 ft 10% DC 200 glass column (4 mm i.d.) and the 6 ft 10% DC 200 aluminum column (4.5 mm i.d.) showed close agreement, indicating that either precolumn variation was adequate for the determination of BHA and BHT in vegetable oils (Tables I and II). Detector calibration resulted in a linear response over the range of 0 to 120 ppm each of BHA and BHT.

In actual practice BHA and BHT could be detected as low as 2 ppm. However, quantitative accuracy fell off rapidly below the 5 ppm level.

The elution order of BHA and BHT was reversed by using a 4 ft × 4 mm i.d. glass column with a 4 in. siliconized glass wool precolumn or a 4 ft × 4.5 mm i.d. aluminum column with the stainless steel precolumn described. Both columns were packed with 10% Carbowax 20 M on 80 to 100 mesh Gas Chrom Q and operated under the same conditions as the 10% DC 200 columns, except column temperature, which was 190 C. The change in elution order with these columns provided a means of confirming the identification of BHA and BHT (Fig. 2, and 3).

Samples of soybean, cottonseed, corn and peanut oils which contained no BHA and BHT were chromatographed. Resulting chromatograms showed no significant peaks at the elution times of BHA and BHT with either the DC 200 or Carbowax 20 M GLC columns. The lack of interference peaks (compounds) indicates that reliable quantitative and qualitative results can be obtained using the outlined GLC systems. Consecutive injections of oil samples into the

precolumn GLC system showed that the technique provides reasonably long column life. Over 200 sample injections (up to 35 per day) resulted in no detectable differences in column efficiency or method accuracy.

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