Technical

Simultaneous Analysis of BHA, TBHQ, BHT and Propyl Gallate by Gas Chromatography as Extracted from Refined Vegetable Oil

D.M. WYATT, Technical Service and Development Division, Eastman Chemical Products, Inc., Kingsport, TN 37662

ABSTRACT

A method has been developed for the simultaneous gas chromatographic (GC) analysis of 4 phenolic antioxidants extracted from refined cottonseed oil. The antioxidants in the study were butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), and propyl gallate (PG). The method involves extraction with acetonitrile, followed by silyl derivatization prior to GC injection. The method was applied to a study designed to measure the percentage of recovery of each antioxidant at the 100 ppm level over a two-week time span. Statistical treatment of the results indicate there is no significant time trend in any of the 4 antioxidants. The percentage recoveries range from 84% to 108%. The standard deviations for the analysis range from 8 ppm to 10.2 ppm.

INTRODUCTION

There is a need in the marketplace for a general analytical technique for determination of the four phenolic antioxidants, BHA, TBHQ, BHT and PG in refined vegetable oils. Work in this and other laboratories has shown that gas chromatography offers an attractive alternative to conventional UV or colorimetric methods.

Page and Kennedy (1) developed an electron capture gas liquid chromatography (GLC) technique for direct injection of the oil for analysis of BHA, TBHQ and PG, but did not include BHT. Kline et al. (2) developed 2 separate procedures which included BHA, BHT and TBHQ by one technique, but used the second procedure for a group which included PG. Hartman and Rose (3) described a direct injection procedure for only BHA and BHT, as did Senten et al. (4). Stoddard (5) described a technique for extracting these 4 antioxidants, but the procedure required a Florisil clean-up procedure for the BHT. None of these references included 2 essential requirements for our needs: (a) a gas chromatographic method for simultaneous analysis of the 4 antioxidants and/or (b) an investigation of the effect of time on the percentage recovery. Time has been shown to be an important factor in the development of an analytical technique (6). Our objective was to develop a gas chromatographic method which fulfilled these 2 needs.

Previous work in the Eastman Laboratories (7) had indicated that a simple acetonitrile extraction followed by derivatization prior to GLC analysis was sufficient for analyzing TBHQ in refined vegetable oil. We hoped to use this basic procedure and extend it to the simultaneous analysis of all 4 antioxidants.

EXPERIMENTAL

The GLC work was done on a Perkin-Elmer 900 flame ionization GC with a glass-lined injection port. Conditions are described in Table I. Chromatographic data were processed by a Hewlett Packard 3353 data system. The auto pipette was made by Eppendorf. All solvents used were reagent grade or better. The antioxidants were all food grade purity obtained from DPI, Rochester, NY.

PROCEDURE

Basic Design

A study was designed to include (a) any error due to the effects of time on the recovery of the antioxidants, (b) the error due to the extraction, and (c) the error due to the chromatographic analysis. Six identical oil samples were spiked with ca. 100 ppm of each antioxidant on day 0. Two of the samples were extracted on that day, 2 were extracted 7 days later, and the final two 15 days later. Each extracted sample was analyzed 4 times on the gas chromatograph. Thus, over a 2-week period (time error) there were 6 extractions (extraction error) and 24 chromatograms (analytical error). A single concentration (100 ppm) was used in this study rather than a range of concentrations. A previous study on TBHQ (7) had covered the concentration range from 50-150 ppm.

Preparation of Samples

To prepare these oil samples, a stock standard was made containing the following weights of each antioxidant in 50 mL of ethyl acetate (EtOAc): (a) 0.05625 g BHT, (b) 0.05018 g BHA, (c) 0.05171 g TBHQ, and (d) 0.05237 g PG. One-mL volumetric vials were filled with 1 g of refined cottonseed oil. These samples were weighed to 5 decimal places. To each oil sample was added 0.1 mL of the above

TABLE I

GLC Conditions

Column	10% GE-Versilube F-50 on 100/120 Gas Chrom Q
Column temperature	150 C + 12 C/m to 210, ISO-12 min
Injection temperature	220 C
Detector temperature	240 C
Column length	6 ft
Column size	1/8-in. od
Column material	Nickel
Carrier gas	N_2, 25 mL/min
H_2 flow	30 mL/min
Air flow	320 mL/min
Detector output	5 meV FSD
Sensitivity	5×10^{-12} Amps
Range	X 1
Recorder attenuation	X 256

stock standard, using a 0.1 mL automatic pipette. These spiked samples were then shaken lightly. EtOAc was used because it was miscible with the oil and allowed good dispersion of the antioxidant. After mixing, each spiked sample was put under a light stream of N_2 until there was no noticeable EtOAc odor. The samples were then capped and stored at normal room temperature and light to await extraction. The exact concentration of each antioxidant is shown at the bottom of Table II.

Extraction

To a one milliliter volumetric flask containing one gram of a spiked oil was added one milliliter CH_3CN which has been stored over a molecular sieve. To this was added 0.1 mL of a solution containing the internal standard, 0.05724 g butyl parahydroxybenzoate (butyl paraben) in 50 mL CH_3CH. The mixture was shaken lightly by hand for 10-15 sec. The CH_3CN layer rose to the top after a few minutes of standing. This top layer was drawn off using a long-nose disposable pipette and transferred to a 4-dram vial with a polyethylene-lined cap. This extraction procedure was repeated 5 more times using 1 mL of CH_3CN each time. No more internal standard was added.

Derivatization

The vial should now contain ca. 6 mL of CH_3CN and extracted components. To this was added 2 mL DMF (dimethyl formamide) and 1.5 mL BSTFA, [N, 0-bis-(trimethylsilyl)-trifluoroacetamide]. The vial should now be ca. 3/4 full. It was heated in a hot oil bath at ca. 80-90 C for 20 min. This completed the derivatization.

Chromatographic Analysis

The derivatized extract was removed from the oil bath and allowed to cool for several minutes. The solution was then concentrated under N_2 to ca. 1 mL. This final volume will vary according to individual column efficiency, but for our column, this volume allowed sufficient concentration to give baseline recovery from the solvent peak prior to elution of the first antioxidant. The volume of extract injected was 0.6 μL. Table I gives the GLC conditions. Figure 1 shows a chromatogram from one of the extracts from day 0.

A chromatographic standard was made from the stock solution used to spike the oil samples. A 0.1-mL aliquot of the stock standard and 0.1 mL of the internal standard solution were added to 6 mL of CH_3CN. DMF and BSTFA were added as in the unknowns, and the final solution was heated and concentrated as previously described. This standard was used to develop response factors for each individual antioxidant. A fresh standard was prepared for each day's analysis. These response factors were used to calculate concentrations for the chromatograms of the oil extracts.

DISCUSSION

Results of Designed Study

Table II shows a summary of all analyses for the designed study and the actual concentrations of each antioxidant added on day 0 of the study. These results are expressed as percentage recoveries in Table III.

Table IV shows the standard deviation for each antioxidant. These standard deviations reflect the contributions to precision due to (a) time, (b) extraction, and (c) analytical technique as discussed previously. The standard deviations represent the expected precision for a single extraction followed by a single GLC analysis at the 100 ppm level for each antioxidant.

TABLE II

GLC Results: Experimentally Determined Concentration (ppm)*

	Duplicate extractions							
	BHA		TBHQ		BHT		PG	
Day	A	B	A	B	A	B	A	B
0	94	98	97	96	82	88	100	88
	101	105	115	103	121	93	109	97
	107	115	112	111	96	103	117	87
	123	115	95	110	109	103	102	89
7	105	89	89	90	105	93	94	88
	111	111	93	104	106	122	113	101
	108	108	98	102	110	106	113	110
	97	104	87	91	101	101	95	96
15	110	99	96	86	106	95	97	93
	111	92	96	81	104	93	99	82
	103	101	94	87	103	96	96	93
	108	98	94	86	105	96	97	93

*Actual concentrations added on day 0 were: (a) BHA, 100 ppm; (b) TBHQ, 113 ppm; (c) BHT, 113 ppm; (d) PG, 105 ppm.

Based on results from the statistical study, no significant time trend was observed over the 15-day period. BHA and TBHQ each showed a consistent decrease in the average recovery from one time frame to the next, but this decrease was determined not to be significant.

Extraction

Acetonitrile was chosen as the extracting solvent because the antioxidants have a high affinity for this solvent, it is immiscible with the oil, and the derivatization can be effectively carried out in it. A very small amount of the oil was carried over into the acetonitrile but did not have any effect on the column throughout the study. This has also been observed by other workers (4).

The number of extractions with acetonitrile deserves some comment. We have effectively extracted greater than 90% TBHQ from cottonseed oil by a single extraction (7). During the development of this project, it was noted that BHT was the most difficult antioxidant to extract. Senten et al. (4) noted that 30-35% of the BHT was extracted by each CH_3CN aliquot whereas as much as 65-70% of the BHA can be extracted. Phipps found that as many as 10 extractions were required to recover 99% of BHT with acetonitrile (8). We found that 6 extractions gave sufficient recoveries for this study. We feel fewer extractions would be necessary if BHT was not present, but the exact number of extractions required for each particular antioxidant was not investigated.

We have also found that 90-95% of the internal standard, butyl paraben, was recovered using this extraction procedure. Assuming normal component loss due to transfer, we felt that this recovery was high enough to lend no bias to the recoveries of the antioxidants. The addition of the internal standard to the first acetonitrile-oil extraction is advantageous in that it eliminates sample loss as a possible source of low recovery.

Need for Derivatization

This method is designed for simultaneous analysis of these 4 antioxidants. In actuality, the only 2 antioxidants which require silyl derivatization are PG and TBHQ. Work in our laboratory indicated underivatized PG will not elute under the described conditions. There is some question in the literature about the need to derivatize TBHQ. Some workers feel that TBHQ can be analyzed effectively either deriv-

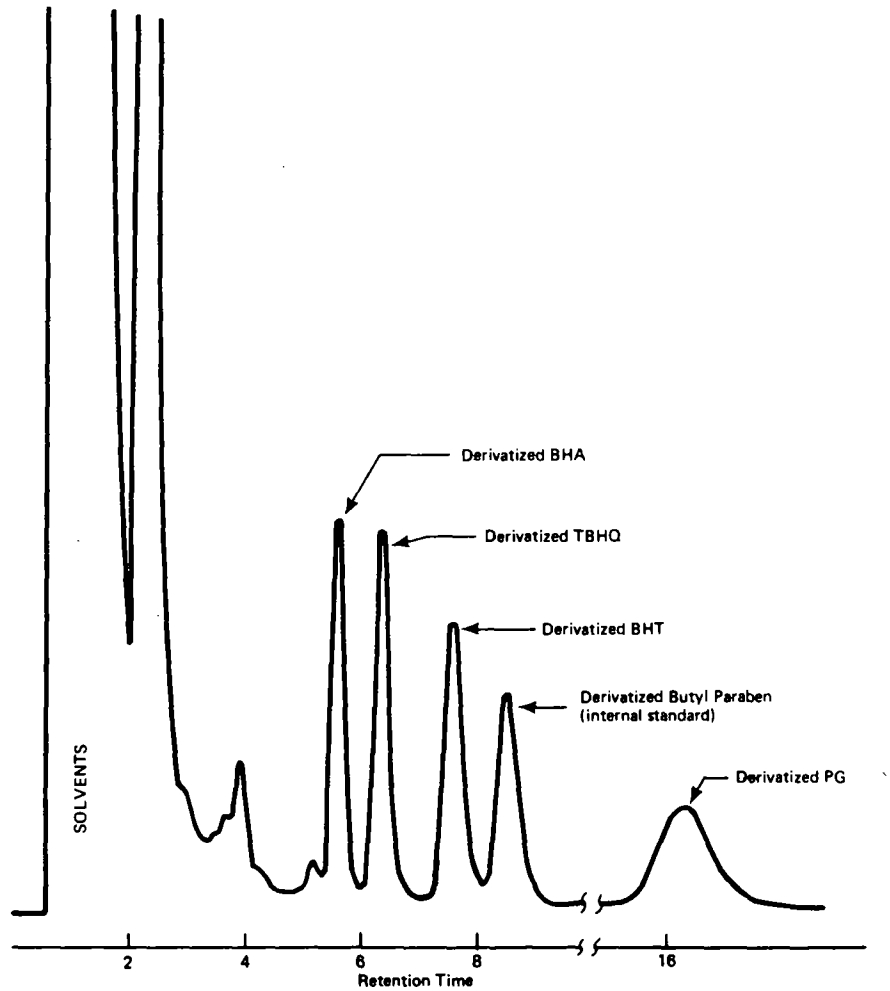

FIG. 1. Typical chromatogram of the 4 antioxidants, ca. 100 ppm each in the refined oil.

atized or underivatized (2). However, work in our laboratory has indicated that some TBHQ may oxidize to its completely oxidized analog if injected without derivatization (7,9).

A study was undertaken to define the effect derivatization would have on the chromatography of these antioxidants. Figure 2a shows a representation of a chromatogram of 100 ppm in oil of BHA, BHT and TBHQ with no derivatization. Sticks are used for clarity and represent exact retention time and approximate relative intensity. Conditions were the same as those in Table I except that the oven temperature was 230 C isothermal. Note also that the internal standard here was propyl parahydroxybenzoate. The BHT and TBHQ are unresolvable under this condition.

Figure 2b represents a chromatogram of a solution containing the same 3 antioxidants to which only BSTFA had been added for derivatization. The retention times of BHA and TBHQ have shifted compared to Figure 2a, indicating derivatization has occurred. However, the retention time of the BHT peak remained unchanged, indicating no derivatization. In this case, the derivatized BHA and the underivatized BHT peaks were unresolvable.

Figure 2c shows a chromatogram of a standard identical to that in 2b except that 2 mL of DMF and 20 min of heat in an oil bath at 80 C were used to catalyze derivatization. Note that the BHT retention time has now shifted and resolution of all antioxidants is possible. Thus, this combination of DMF and heat is necessary for the derivatization of the sterically hindered hydroxyl group on BHT (10, and L. Scheur, personal communication).

TABLE III

Composite Results

		% Recovery			
		BHA	TBHQ	BHT	PG
Day 0,	A	106	102	90	102
	B	108	102	86	86
Day 7,	A	105	87	105	100
	B	103	91	105	95
Day 15,	A	104	94	104	96
	B	94	84	94	89

TABLE IV

Standard Deviations[a]

Antioxidant	Standard deviation (ppm)
BHA	8.0
TBHQ	10.2
BHT	9.2
PG	8.0

[a]Assume single extraction–single GLC analysis at the 100 ppm level with all 4 antioxidants present.

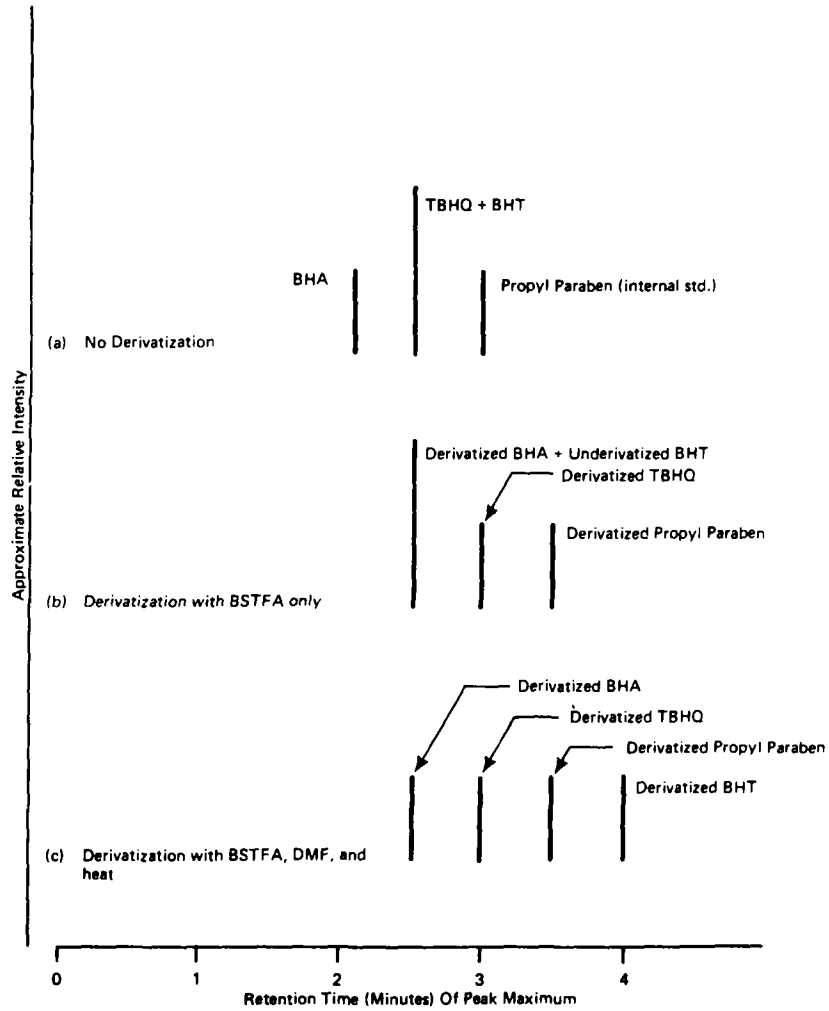

FIG. 2. Effects of derivatization on retention times.

Linearity of Calibration

The calibration for the designed study previously discussed was made by calculating response factors for each antioxidant from a single solution made up at the concentration of the unknowns, i.e., 100 ppm in the oil. If analysis is required over a wider range of concentrations, it would be necessary to calibrate from a linear regression analysis.

A study was undertaken to check the linearity of calibration for these 4 antioxidants using butyl paraben as the internal standard and covering the concentration range 0-180 ppm. The results of the linear regression analyses gave coefficients of determination which were all high, indicating good linearity. An additional internal standard (dibutylphthalate), for which the retention time and peak shape was close to PG, was used for calibrating PG, but this did not result in any improvement in linearity for PG calibration.

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