Quantitative Determination of BHT in Soap Products by Capillary Gas Chromatography

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ABSTRACT

A simple and rapid capillary gas chromatography (GC) method is described for the quantitative determination of 2,6-di-*tert*-butyl-4-methylphenol (BHT) antioxidant in soap bars, fatty acids, and related intermediates. The procedure involves blending the sample with dimethylformamide in the presence of 2,4-di-*tert*-butylphenol (DTBP) internal standard, filtering the mixture, silylating an aliquot with BSTFA (bis-trimethylsilyltrifluoroacetamide) and quantifying by capillary GC using flame ionization detection. The silyl derivatization and nonpolar capillary column (12 m, methyl silicone, fused silica) provided resolution of BHT from certain fragrance component interferences. The method has a detection limit of approximately 10 ppm. Soaps fortified with BHT showed recoveries of 97.1 ± 3.7% at the 200 ppm level and 92.3 ± 2.2% when spiked at the 75 ppm level. The effect of bar soap storage time on BHT content is also demonstrated.

INTRODUCTION

Although analytical methods including colorimetric (1-3), thin layer chromatography (TLC) (4,5), gas chromatography (GC) (6-8), and high pressure liquid chromatography (HPLC) (9-13) have been reported in the literature for the analysis of 2,6-di-*tert*-butyl-4-methylphenol (BHT) (and certain other antioxidants) in such substances as fats, oils, foodstuffs, packaging materials, etc., the recent publication of Sedea and Toninelli (14) was the only one found which dealt specifically with soap analysis. Their method was a standard addition, packed column GC procedure of the soap sample dissolved in methanol. The authors stated the reason for the standard addition approach (a tedious time-consuming technique) was the need to compensate for perfume interference.

Recent advances in capillary GC technology, however, have greatly enhanced capabilities for resolving complex volatile mixtures including flavors, fragrances, etc. (15-17). Furthermore, the use of derivatization reactions including trimethylsilylation as described by Wyatt (18) for shifting the GC retention times of phenolic antioxidants have provided additional means for the resolution of complex systems.

In this paper we report on a combined silyl derivatization capillary GC method for determining BHT in soap products including bars, neat soap, pellets, and fatty acids. The use of an internal standard (i.e., 2,4-di-*tert*-butylphenol) to enhance the reliability and the ease of quantitation of the method is also included.

EXPERIMENTAL

Instruments and Conditions

Analyses were performed on a Hewlett Packard Model 5840A gas chromatograph equipped with a capillary inlet system and a flame ionization detector. The column was a 12 m × 0.2 mm id methyl silicone fluid coated fused-silica capillary column supplied by Hewlett Packard (no. 19091-60010). A glass split injection system (200:1) was employed with helium carrier gas head pressure of 7.5 psi and helium make-up gas.

The column temperature was held at 100 C for 2 min and then programmed to 144 C at 2 C/min. At this point (i.e., after 24 min), the program rate was increased to 30 C/min and the temperature was allowed to reach 240 C where it was held for an additional 5 min in an effort to clean out the system for the next injection. The inlet temperature was 200 C and the detector temperature was 325 C. A slope sensitivity of 0.01 and an attenuation of 2^{-1} were employed.

Under these GC conditions, the retention times were 15.5 min for silylated DTBP and 22.4 min for silylated BHT.

Reagents and Solutions

*BHT standard (2,6-di-*tert*-butyl-4-methylphenol).* 99% purity, Aldrich Chemical Co.

*DTBP internal standard (2,4-di-*tert*-butylphenol).* 99% purity, Aldrich Chemical Co.

DMF (N,N-dimethylformamide). ACS reagent grade, Sargent-Welch Scientific Co.

BSTFA (bis-trimethylsilyltrifluoroacetamide). Silylation grade, Regis Chemical Co.

BHT standard stock solution. About 400 mg BHT was accurately weighed (±0.1 mg) into a 100 mL volumetric flask, dissolved and diluted to the mark with DMF.

DTBP internal standard stock solution. About 400 mg DTBP was accurately weighed (±0.1 mg) into a 100 mL volumetric flask, dissolved and diluted to the mark with DMF.

BHT-DTBP standard mixture. 250 µL BHT standard stock solution and 250 µL DTBP internal standard stock solution were transferred by means of 500 µL syringes to a 100 mL volumetric flask and diluted to volume with DMF.

BHT-DTBP standard silylated mixture. 1.0 mL of BHT-DTBP standard mixture was pipeted into a septum vial, 500 µL of BSTFA was added, the vial was capped and its contents mixed. [The septum vials had 3.5 mL capacity, were obtained from Pierce Chemical Company (no. 13019) and contained teflon-silicone discs (no. 12712) in the screw caps.]

The standard stock solutions were prepared weekly and stored in a refrigerator. The diluted standard mixtures were prepared fresh for each day's analysis.

Assay Procedure and Calculation

Ten g of finely divided soap product was accurately weighed (to the nearest 0.01 g) into a Waring blender jar, 200 mL of DMF was added, followed by 500 µL of DTBP internal standard stock solution, and the mixture was thoroughly blended for 5 min. A portion of the mixture was filtered by gravity through Whatman no. 41 paper into a beaker. One mL of filtrate was pipeted into a septum vial, 500 µL of BSTFA reagent was added, the vial was capped and shaken well. Five µL of sample was injected into the GC and compared with 5 µL injections of the BHT-DTBP standard silylated mixture. (Unknown samples were first run without internal standard addition to show that their chromatograms were uninterfered with in the DTBP retention

region.)

Based upon the GC response factor (R) determined from the standard silylated mixture (which was prepared with the same internal standard stock solution used to spike the soap samples), BHT content (ppm) was calculated using the following equations:

$$R = \frac{W_{BHT} \times P_{DTBP}}{W_{DTBP} \times P_{BHT}} \quad [1]$$

where W_{BHT} and W_{DTBP} are the weights of BHT and DTBP, respectively, in the standard mixture, and P_{DTBP} and P_{BHT} are the GC peak heights of these standard components.

$$\text{ppm BHT} = \frac{R \times W_{IS} \times P_{BHT}}{W_{sample} \times P_{DTBP}} \quad [2]$$

where R is the response factor, P_{BHT} and P_{DTBP} are the sample peak heights of BHT and DTBP, respectively, W_{sample} is the sample weight in g (e.g. 10g), and W_{IS} is the weight of internal standard in μg (e.g. 2000μg) added to the sample.

RESULTS AND DISCUSSION

Sample Preparation

The initial attempt at sample preparation involved a petroleum ether extraction of the soap dissolved in an alcohol/water mixture. Since this resulted in gross GC interferences, another procedure based upon the steam volatility properties of BHT (19,20) was attempted in an effort to achieve a cleaner extract. This next procedure involved a simultaneous distillation/extraction technique using special glassware (previously employed in our laboratory for trace germicide determinations) (21). The soap sample was dissolved in water, reacted with a precipitating agent of barium chloride-calcium chloride and then distilled/extracted with hexane. The hexane extract was then concentrated to a small volume and run by GC.

Although the distillation/extraction technique gave satisfactory BHT recoveries, it had no advantages (most of the volatile fragrance components were still present) and several disadvantages (slower and more complicated) compared to the DMF blending/filtration approach (22) finally adopted.

In addition, by using the GC instrument at a very sensitive attenuation setting (e.g. 2^{-1}), the DMF soap extract could be assayed without further concentration. This avoided the possibility of BHT loss due to volatilization, oxidation, etc., which had been evident (but variable) in previous treatments involving evaporative concentration of BHT solutions. This was especially apparent in experiments in which the sample was evaporated to dryness (even at room temperature under nitrogen) before being redissolved in a known amount of fresh solvent.

Derivative Formation

In an effort to improve the resolution of BHT from interfering peaks, silyl derivative formation using a reagent of BSTFA containing 1% TMCS (trimethylchlorosilane) was attempted. Recent work by Wyatt (18) for determining a series of antioxidants extracted from vegetable oil indicated that BHT required heat treatment (80 C for 20 min) for silylation to occur using BSTFA in a mixed solvent system of acetonitrile-DMF.

Studies in our laboratory regarding the reaction of BHT with BSTFA under various solvent conditions indicated no reaction in acetone, hexane, or pyridine, partial reaction in acetonitrile (which increased with heating), and essentially instantaneous and complete reaction in DMF at room temperature. Furthermore, it was found that the reaction in DMF was complete with or without the presence of TMCS in the BSTFA reagent. These findings are essentially in agreement with those of Friedman and coworkers (23), who previously described the unique properties of DMF in the silylation of certain types of hindered phenols.

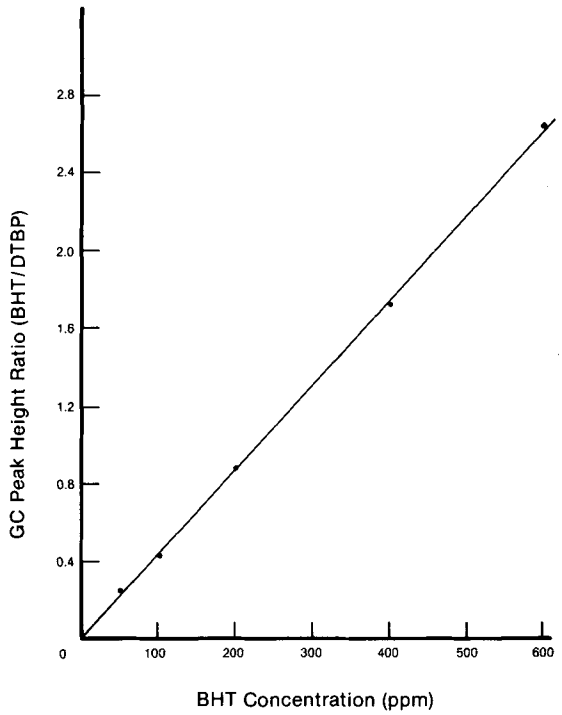


FIG. 1. Ratio of peak heights of silylated BHT to silylated DTBP (internal standard) versus BHT equivalent concentration (ppm). DTBP content held constant at a level equivalent to 200 ppm in a 10 g sample.

TABLE I

Recoveries of BHT from Spiked Placebo Bars[a]

BHT added (ppm)	BHT found (ppm)	Recovery (%)
75	71.6	99.5
75	68.5	91.3
75	67.1	89.5
75	69.2	92.3
75	69.8	93.1
200	185	92.5
200	198	99.0
200	195	97.5
200	189	94.5
200	204	102

Average ± standard deviation: 97.1 ± 3.7%.

[a]Spiking was done by adding aliquots of a standard solution of BHT in DMF to samples in the blender jars.

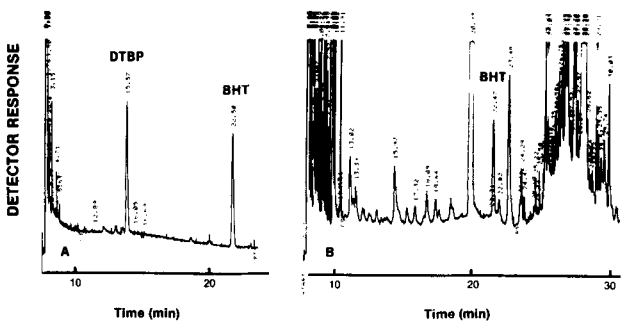
In addition, silylation had a profound affect upon the GC retention time (rt) of BHT, increasing the rt from 13.5 min to 22.4 min under the conditions employed. Although there were also some effects on the retentions of certain other components of the soap sample, silylated BHT was completely resolved from interferences and could be quantitatively determined in a variety of commercial bar soaps.

It should be pointed out that BHT can be quantified with or without silyl treatment in nonperfume containing samples such as soap pellets. Although neat soap and fatty acid samples also contain no perfume additives, it is recommended that fatty acid samples be silylated but that neat soap, due to its high (about 30%) water content (which reacts with BSTFA), be determined without silylation treatment.

TABLE II

Reproducibility of Method for BHT in Bar Soap

	Bar A (prepared with 190 ppm) BHT found (ppm)	Bar B (prepared with 150 ppm) BHT found (ppm)
	180	149
	187	140
	182	137
	190	148
Average ± standard deviation:	184.8 ± 4.6	143.5 ± 5.9
Percent relative standard deviation	2.5%	4.1%



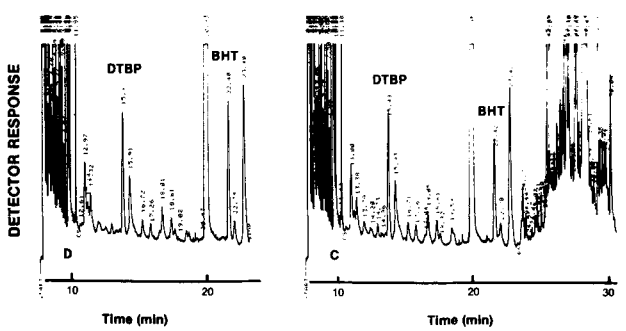


FIG. 2. Capillary gas chromatograms of: A. Silylated standard mixture of DTBP and BHT (200 ppm each); B. Silylated soap extract (as is); C. Silylated soap extract prespiked with DTBP internal standard (200 ppm); and D. Silylated soap extract prespiked with DTBP (200 ppm) and additional BHT (75 ppm).

Internal Standard

A series of potential internal standards (all containing phenolic groups) were investigated in an effort to find one (or more) whose retention characteristics would be suitable for use in bar soap formulations of interest. The compounds and their relative retention times compared to BHT (run under our GC conditions following silylation) are listed as follows: 2-*tert*-butylphenol (0.30), 4-*tert*-butylphenol (0.34), methyl paraben (0.55), 3,5-di-*tert*-butylphenol (0.63), 2-butyl-4-hydroxyanisole (0.66), 2,4-di-*tert*-butylphenol (DTBP) (0.68), *tert*-butylhydroquinone (0.84), 2,6-di-*tert*-butylphenol (0.87), propyl paraben (0.90), and butyl paraben (1.13).

Of this group, DTBP seemed most suitable and possessed the basic internal standard requirements (11,24): (a) It did not elute with a component of the sample; (b) It eluted relatively close to BHT; (c) Its response factor was very similar to that of BHT.

Subsequently, a calibration curve prepared by plotting the peak height ratio of silylated BHT to silylated DTBP (using a constant amount of DTBP comparable to 200 ppm in a 10-g sample) vs BHT concentration was shown to be linear in the range of 0 to 600 ppm BHT (see Fig. 1).

TABLE III

Effect of Bar Soap Aging on BHT Content

Age (months)	BHT found (ppm)[a]
1	148
3	144
6	130
12	103
18	85

[a]The initial BHT content was 150 ppm.

Accuracy and Precision

BHT methodology validation studies were conducted in which placebo soap samples (containing all ingredients but BHT) were intentionally fortified with known levels of BHT. The samples, which showed no peaks at the BHT or DTBP retention regions, were spiked by adding known amounts of BHT standard stock solution (i.e. 187 μL for the 75 ppm level and 500 μL for the 200 ppm level) to 10 g of placebo in a Waring blender jar along with 500 μL of DTBP prior to blending with 200 mL of DMF. Five replicate samples showed recoveries of 97.1 ± 3.7% at the 200 ppm BHT level and 92.3 ± 2.2% when spiked at the 75 ppm level (see Table I). Although these data were calculated using the internal standard method, BHT external standard calculations gave similar results (indicating essentially no antioxidant loss during sample preparation or analysis).

Additional accuracy and precision data were obtained by running quadruplicate determinations on fresh production samples of two bar soaps having somewhat different compositions. Bar A, initially prepared with 190 ppm of BHT, showed analysis results of 184.8 ± 4.6 ppm, whereas, bar B, formulated with 150 ppm showed 143.5 ± 5.9 ppm of BHT. The individual values along with their relative standard deviations are given in Table II.

The detection limit of the method was estimated to be approximately 10 ppm based upon the calibration data and using the generally accepted definition (25,26) as being the minimum sought-for ingredient which can produce a signal twice as great as the noise level. If required, a more sensitive detection limit can be achieved by adjusting the sample size and/or DMF volume.

Typical capillary GC curves for silylated standards and soap extracts are shown in Figure 2.

Bar Soap Aging

Several samples of commercial and experimental bar soaps containing BHT were analyzed at various time periods in order to monitor the effect of aging on BHT content. As indicated in Table III, bar soap C, initially prepared with 150 ppm of BHT, although showing a fairly steady decrease of antioxidant content with time, still contained 85 ppm of BHT after 18 months aging at ambient temperature. However, because additional aging experiments have indicated that BHT stability is apparently quite dependent upon certain sample composition and storage factors, the monitoring of BHT levels for both formulation and storage studies will be of continuing interest in our laboratory.

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Synergism in Binary Mixtures of Surfactants: II. Some Experimental Data

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ABSTRACT

The conditions derived previously for three types of synergism in aqueous binary mixtures of surfactants—mixed micelle formation, surface tension reduction efficiency, and surface tension reduction effectiveness—are reviewed and verified by use of experimental data from the chemical literature. They involve the experimentally determined parameters, β and β^M, related to the interaction between the two surfactants in the mixed monolayer at the aqueous solution/air interface and in the mixed micelle, respectively. The experimental data needed to determine whether a binary surfactant system is capable of synergism in these respects are: (a) the surface tension/log concentration curves of the individual surfactants in the vicinity of their critical micelle concentrations (cmc); (b) the cmc of at least one mixture of the two surfactants; and (c) the solution phase concentration of at least one mixture of the two surfactants needed to produce a surface tension attainable by both individual surfactants. From the available data, some tentative generalizations regarding the effect of chemical structure and the molecular environment of the values of β and β^M have been made.

For the past few years, we have been studying the interfacial properties of aqueous solutions containing two surfactants and the degree of molecular interaction between the surface-active components. Recently (1), we have derived equations showing the conditions necessary for synergism in these systems. To date, we have investigated synergism in three areas: (a) mixed micelle formation; (b) surface tension reduction efficiency; and (c) surface tension reduction effectiveness.

The relationships derived all involve a molecular interaction parameter, β, that is determined experimentally. The basic equations for determining the value of β are:

$$\frac{X^2 \ln \frac{C_{12}\alpha}{C_1 X}}{(1-X)^2 \ln \frac{C_{12}(1-\alpha)}{C_2(1-X)}} = 1 \qquad [1]$$

$$\beta = \frac{\ln \frac{C_{12}}{C_1 X}}{(1-X)^2} \qquad [2]$$

where C_1, C_2, and C_{12} are the solution phase concentrations of surfactants 1 and 2 and their mixture, respectively, required to produce a given effect; α is the mole fraction of surfactant 1 in the total mixed surfactant in the solution phase; and X is its mole fraction in the total mixed surfactant in the surface phase. In these equations, the only quantities that must be measured experimentally are C_1, C_2, and C_{12}. Equation 1 is solved numerically for the value of X and the value of β is then obtained from Equation 2.

Synergism in Mixed Micelle Formation

Synergism in this respect is present when the critical micelle concentration (cmc) of any mixture is lower than those of both surfactants in the mixture. Here, the experimental

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