A Rapid Method for Analysis of *tert*-Butyl Hydroquinone (TBHQ) in Ethyl Esters of Fish Oil

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A rapid, quantitative gas chromatographic method is described for quantitating the phenolic antioxidant, *tert*-butyl hydroquinone (TBHQ), in fish oil ethyl esters. The procedure entails silyl derivatization of TBHQ in an acetonitrile solution of ethyl esters followed by capillary gas chromatography (GC) analysis with an internal standard method of quantitation. Average recoveries of spiked samples were 98% at the legal limit of .02% (200 μg/g). The method can accurately determine as little as 20 μg/g of TBHQ in ethyl esters of fish oil. The technique has been applied to ethyl esters of vegetable oils with equal success.

KEY WORDS: Fish oil ethyl esters, phenolic antioxidant, silyl derivatization, *tert*-butyl hydroquinone.

Phenolic antioxidants are frequently added to edible oils to minimize oxidative deterioration. Tertiary butyl hydroquinone (TBHQ) has been shown to be the most effective antioxidant for stabilizing edible oils (1). The maximum legal level established by the U.S. Food and Drug Administration for synthetic antioxidants in food products is 0.02% of the oil content of the food (2), based on tests of acute and chronic toxicity (3). Thus, quantitative determination of antioxidant levels is important for both quality control and regulatory purposes.

Fish oils are highly unsaturated triglyceride oils containing up to forty percent by weight of the long-chain omega-3 fatty acids, including eicosapentaenoic acid (EPA,20:5n-3) and docosahexaenoic acid (DHA,22:6n-3). Fish oils and, particularly, their omega-3 fatty acids are of interest as therapeutic substances, nutritional supplements and as food additives, due to their effects in ameliorating heart disease, arthritis, immune diseases, and their apparent requirement for brain development (4–6). Transesterification followed by urea adduction is a processing technique used to increase the percentage of the highly unsaturated omega-3 fatty acids in fish oil (7). The highly unsaturated ethyl ester derivatives are most susceptible to oxidation. Addition of TBHQ to omega-3 ethyl ester concentrates produced from fish oils has been shown to adequately maintain their storage stability (8).

Numerous methods for analysis of TBHQ and other phenolic antioxidants in triglyceride oils by gas chromatography (GC) or high-performance liquid chromatography (HPLC) (9–11) have been published. Most of these methods rely on separation of the analyte from the oil matrix by solvent extraction (12–16) or by distillation (17–19). Unlike the triglyceride oils, fish oil ethyl esters cannot be separated from TBHQ by either of these methods, because their solubility and volatilization temperatures are not sufficiently different. However, with the advent of wall-coated open tubular (WCOT) capillary GC columns, TBHQ in ethyl esters can be analyzed as the silyl derivative in an acetonitrile solution of the esters. Quantitation is achieved by use of an internal standard. Derivatization of the sample is necessary to stabilize the analyte in the hydroquinone form, as reported by Wyatt (14).

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EXPERIMENTAL PROCEDURES

Instrumentation. A Hewlett-Packard Model 5840 GC (Palo Alto, CA) equipped with a flame ionization detector and a megabore DB-5 WCOT column, 30 m length, 0.53 mm i.d. (J & W Scientific, Folsom, CA), was used for the analysis. The column was installed in a standard injection port with appropriate fittings to accommodate a megabore column. Helium was used as carrier gas, with a flow rate of 30 mL/min. The following GC conditions were used: column, 150°C; injection port, 250°C; detector, 250°C; attenuation, 2^3. Sample analysis was carried out isothermally at 150°C for 15 min, followed by a 10-min linear ramp to a bake-out temperature of 250°C. This temperature was maintained for 15 min to elute the residual ethyl esters. Linearity of the GC response was determined by analyzing standards containing 20, 50, 100, 150, or 200 μg/mL TBHQ (Eastman, Rochester, NY) and 200 μg/mL propyl paraben (Sigma, St. Louis, MO) as internal standard, which had been carried through the derivatization procedure. Once linearity was established, the GC was calibrated with a single calibration standard containing 200 μg/mL TBHQ and 200 μg/mL propyl paraben for sample analysis. A 1-μL aliquot of the standard or sample was manually injected.

Standard preparation. The calibration standard used to establish the GC response factor for TBHQ was made by combining in a 16 × 125-mm test tube: 1 mL propyl paraben stock (200 μg/mL in acetonitrile); 1 mL TBHQ (200 μg/mL in acetonitrile); 1 mL of acetonitrile and 250 μL of the silating reagent N,O-*bis*-(trimethylsilyl) trifluoroacetamide (BSTFA) (Pierce, Rockford, IL). The standard solution was mixed on a vortex for 1 min and allowed to stand at room temperature for 15 min. A 1-μL aliquot of the standard was then injected into the GC.

Sample preparation. Ethyl esters of menhaden, safflower, and olive oils were produced at the National Marine Fisheries Service (NMFS) Charleston Laboratory experimental oil-processing facility by transesterification. Purified ethyl esters of EPA and DHA were further processed by supercritical CO_2 extraction followed by HPLC separation. For analysis, 0.1 g ethyl ester was weighed to four places into a 16 × 125-mm culture tube. A 1.0-mL aliquot of acetonitrile and 100 μL of propyl paraben stock (200 μg/mL) were added. The sample was derivatized by the addition of 250 μL BSTFA. The sample was mixed on a vortex for 1 min and allowed to stand at room temperature for 15 min to complete derivatization.

RESULTS AND DISCUSSION

Linearity of the GC response was determined by analyzing 20, 50, 100, 150 and 200 μg/mL TBHQ standards in

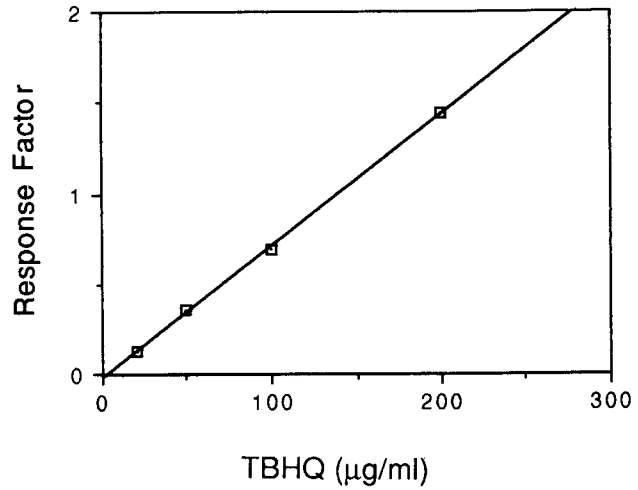

FIG. 1. GC response factors for TBHQ as compared to the internal standard, propyl paraben. Response factors were calculated as the area of TBHQ divided by the area of 200 µg/mL propyl paraben.

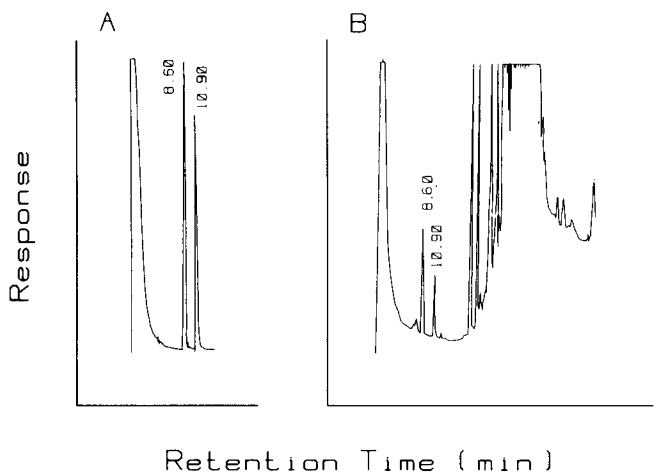

FIG. 2. Representative chromatograms of TBHQ analysis by direct injection of silyl-derivatized TBHQ (a) 200 µg/mL TBHQ standard (r.t. = 8.68) with 200 µg/mL internal standard propyl paraben (r.t. = 10.90), (b) an ethyl ester sample containing 200 µg/g TBHQ (r.t. = 8.68) and 200 µg/mL internal standard (r.t. = 10.90). Ester matrix components begin at r.t. = 20 and rapidly elute from the column, allowing completion of the analysis in 40 min.

acetonitrile in the presence of 200 µg/mL propyl paraben internal standard and carried through the derivatization procedure (Fig. 1). Response factors were found to be similar to those determined by Austin and Wyatt (13) using packed-column analysis.

Chromatographic separation of the TBHQ from 0.1 g fish oil ethyl esters was successfully accomplished by this procedure because the retention times of TBHQ and the internal standard are substantially shorter than those of the ester components. A typical chromatogram obtained from the analysis of TBHQ is shown in Figure 2, with the peaks of interest appearing at 8.68 and 10.90 min, respectively, for TBHQ and propyl paraben.

TABLE 1

Recovery of Added TBHQ in Ethyl Esters of Fish Oil

Spike level µg/g	% Recovery of added TBHQ	
	Mean	Range
200	98 (n = 10)	80-116
100	94 (n = 2)	93-95
60	99 (n = 2)	93-105
40	107.5 (n = 2)	106-109
20	103 (n = 3)	90-118

In spike recovery experiments (Table 1), a mean recovery of 98% of added TBHQ was obtained at the legal limit of .02%. The detection limit of the method was determined by analyzing fish oil ethyl esters that had been spiked with TBHQ at 20, 40, 60, 100, or 200 µg/g. Precision and recoveries were adequate above 20 µg/g, which we determined to be the limit of detection of the procedure. Below 20 µg/g interference from short-chain oxidation components in the ethyl esters resulted in variable recoveries of the spiked analyte. However, in purified ethyl esters of EPA and DHA we could detect amounts in spiked samples as low as 5 µg/g with good precision (recovery 103%, range 95-113). The method has been applied with equal success to analysis of TBHQ in ethyl esters of vegetable oils.

Previously published GC methods for quantitation of derivatized TBHQ (13,14) employed packed-column analysis, which is not amenable to the injection of ethyl esters at the concentrations present in this method; therefore, a means of removing the ester matrix would be needed. The similarity in solubility and volatilization temperature of these ethyl esters and TBHQ precluded solvent extraction and distillation methods for removal of the ethyl ester sample matrix. Research in this laboratory and by other investigators (9) demonstrated loss of TBHQ during the evaporation steps required by many of the procedures for separation of TBHQ from the sample matrix; thus, the percent recoveries of TBHQ were reduced to unacceptable levels. Analysis of the derivatized TBHQ in an acetonitrile solution of the ethyl esters on a bonded megabore capillary column allowed us to overcome this major obstacle. The bake-off step following elution of the components of interest provided for the rapid removal of ester components, thus keeping the GC analysis time to a minimum. The bonded megabore capillary column chosen for the analysis showed no loss in performance due to injection of the ethyl ester matrix. The method should also be amenable to analysis on standard 0.25 mm i.d. capillary columns.

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