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## Note

# Derivatization of butylated hydroxytoluene for gas chromatography with electron-capture detection and high-performance liquid chromatography with ultraviolet detection

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Butylated hydroxytoluene (BHT) is a phenolic antioxidant used in foods, feeds, and petrochemical products. Recently, a number of important toxicological effects of BHT have been reported[1]. Moreover, BHT tends to accumulate in animal[2] and human[3,4] tissues during repeated exposure. In studies on the distribution and fate of BHT in the environment and in animals, it is necessary to measure accurately micro-amounts of BHT. The hydroxy group of BHT is highly sterically hindered and is therefore very difficult to derivatize. Probably because of this, little information has been reported on the direct derivatization of BHT for electron-capture detection (ECD) in gas chromatography (GC). In an earlier paper[5] we described a method for preparing an ECD-sensitive derivative from BHT in two stages. BHT was first dealkylated to 2-*tert.*-butyl-4-methylphenol, which was then derivatized with 1-fluoro-2,4-dinitrobenzene to yield the 2,4-dinitrophenyl ether, which was sensitive to ECD. However, this method is tedious and time-consuming.

The present paper describes a new and simple method for the determination of BHT. The method involves the derivatization of BHT to a cyclohexadienone derivative suitable for both GC with ECD and high-performance liquid chromatography (HPLC) with UV detection.

## EXPERIMENTAL

### *Chemicals*

BHT was purchased from Wako (Osaka, Japan) and recrystallized from ethanol. 2,6-Di-*tert.*-butyl-4-methyl-4-methoxy-2,5-cyclohexadienone (BMMC) was synthesized from BHT according to the method of Coppinger and Campbell[6], and recrystallized from methanol (m.p., 92–94°C). Other reagents were of the highest purity available. All solvents were of pesticide grade.

### *Derivatization*

Bromine (2.5 $\mu$l) was added to BHT (> 1 $\mu$g) in methanol (1 ml) at room

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temperature in a glass-stoppered test-tube. After the reaction mixture had been allowed to stand for 10 min, aqueous 0.5% sodium hydrogen sulphite (5 ml) and hexane (1 ml) were added. The tube was shaken for *ca.* 1 min, and suitable aliquots of the organic phase were analysed by GC with ECD or HPLC with UV detection. The percentage yield of BMMC was obtained by GC–ECD from a standard curve prepared from known amounts of the synthetic derivative.

*Instruments*

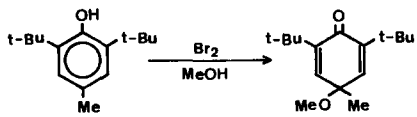
GC analysis was performed on a Hitachi Model 164 gas chromatograph equipped with a $^{63}$Ni electron-capture detector and a 2 m × 3 mm I.D. glass column of 2% OV-1 on Chromosorb W. The oven temperature was 150°C and the flow-rate of nitrogen as carrier gas was 60 ml/min.

HPLC analysis was performed on a Hitachi Model 655 liquid chromatograph equipped with a Waters "Z-module" and a 10 × 0.8 cm I.D. Radial-Pak μPorasil cartridge. The solvent used was hexane–2-propanol (99:1), and the flow-rate was maintained at 1.0 ml/min. The effluent was monitored with a Hitachi variable-wavelength UV monitor at 236 nm.

GC–mass spectrometry (GC–MS) was performed on a JEOL Model JMS D-300 double-focusing mass spectrometer equipped with a 1.5 m × 2 mm I.D. glass column of 2% OV-1 on Chromosorb W. The oven temperature was 140°C and the flow-rate of helium as carrier gas was 40 ml/min. Electron impact spectra were recorded at a resolution 500 or 5000 with an ionizing energy of 70 eV and an ionizing current of 300 μA.

## RESULTS AND DISCUSSION

Coppinger and Campbell[6] have reported that BHT quantitatively reacts with bromine in methanol to form BMMC (Fig. 1). When examined by GC–MS, the product from the derivatization procedure gave molecular and fragment ions consistent with an authentic sample of BMMC: *m/e* 250 ($M^+$, 4% relative intensity); 235 (M − $CH_3$, 24); 194 (MH − *tert.*-butyl, 87); and 179 (MH − $CH_3$ − *tert.*-butyl, 100).




BHT BMMC

Fig. 1. Formation of BMMC from BHT.

The percentage conversions (mean ± S.D.) of BHT to BMMC at BHT concentrations of 1 and 10 μg/ml were 90.1 ± 3.7 and 97.0 ± 2.0%, respectively, for six determinations. Changes in the amount of bromine (2.5–10 μl) did not affect the yield of BMMC. However, a minimal amount of bromine is preferred because commercially available bromine reagents resulted in interfering peaks in the region of the HPLC chromatogram where BMMC peak occurred. No difference in BMMC yield

was found when the reaction time was altered (5–30 min). When the reaction between BHT and bromine was carried out in methanol containing more than 5% of water, the yield of BMMC was lowered to a certain extent as a result of a side reaction. The product of the side reaction was characterized by HPLC and GC–MS, and by comparison with an authentic sample[7], as 2,6-di-*tert.*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone. The BMMC derivative was stable in hexane solution for at least 5 days.

The GC chromatogram of BMMC derivative is shown in Fig. 2. The retention time of BMMC was 3.5 min on the 2% OV-1 column under the conditions employed. The ECD response of BMMC was very high and the detection limit, based on twice the noise level, was *ca.* 5 pg. The peak area response was linearly related to the amount in the range 0.01–0.1 ng. A comparable ECD response has also been reported for 2,6-di-*tert.*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone, an analogue of BMMC[8]. In addition, various conjugated carbonyl compounds such as quinone, 1,2-diketone, and pyruvate esters have been determined by GC–ECD at nanogram levels[9].

Fig. 3 illustrates a typical HPLC chromatogram for a hexane extract obtained after the derivatization procedure. The HPLC eluate was monitored by UV absorbance at 236 nm ($\lambda_{max}$ for BMMC). BMMC eluted at 4.1 min from the μPorasil column at a flow-rate of 1 ml/min and gave a narrow symmetrical peak. Calibration curves constructed from the peak heights for BMMC were linear, with a zero intercept for injections of up to 5 μg. The minimum detectable amount of BMMC was *ca.* 0.5 ng at 0.005 a.u.f.s. However, the practical limit of detection of the HPLC method was somewhat higher because of the relatively high reagent blanks originating from impurities in commercial bromine.

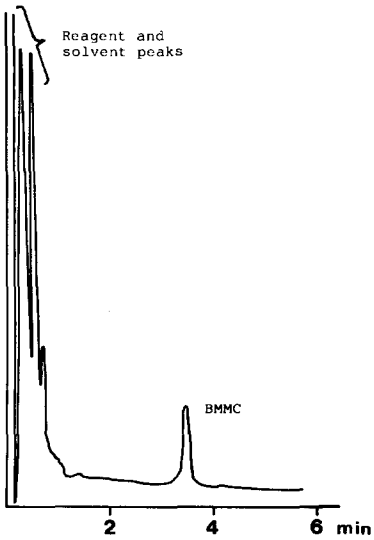



Fig. 2. GC chromatogram of BMMC derived from 0.1 ng of BHT. Chromatographic conditions are described in the text.

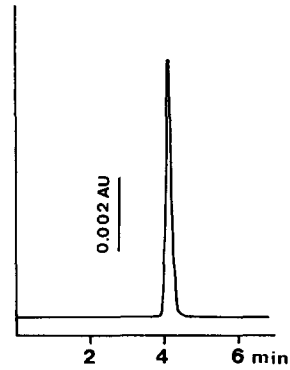



Fig. 3. HPLC chromatogram of BMMC derived from 50 ng of BHT. Chromatographic conditions are described in the text.

In conclusion, the method described is simple and rapid and could be used for sensitive determination of BHT or as a confirmation technique. Applications of this technique to samples of biological origin are currently in progress.

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