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Note

Comparative gas chromatographic behaviour and detection limits of 2,6-di-tert.-butyl-4-methylphenol, 3-tert.-butyl-4-hydroxyanisole (BHA), and the trifluoroacetate of BHA

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The phenolic antioxidants BHA (2- and 3-*tert.*-butyl-4-hydroxyanisole) and BHT (2,6-di-*tert.*-butyl-4-methylphenol) have been used extensively in foodstuffs[1,2]. Because of the dynamic conditions[2] associated with their action, any satisfactory analytical approach must cover a wide concentration range extending from normal usage[1,3–6] to the trace level. Gas chromatography (GC) presents an ideal approach for these two extremes of concentration, and both BHA and BHT are amenable to this analytical procedure. Low detection limits can be achieved, and polar and non-polar stationary phases have been successfully applied to quantifying the phenols[7].

A common technique for improving detection limits is that of converting polar compounds into less polar and often more volatile derivatives. For these two compounds, the most widely used derivative has been the trimethylsilyl ether[8–12]. However, Kato *et al.*[13] prepared the trifluoroacetate derivative of BHA and studied its behaviour on various stationary phases using the flame ionization detector (FID).

In addition to a comparison of the GC behaviour of BHT, BHA and its trifluoroacetate, this paper presents detection limits for the three compounds, together with calibration data for the trifluoroacetate using an electron-capture detector (ECD). The derivatization reaction is shown to be simple, quantitative for amounts of BHA ranging from 10–100 μg, and a suitable basis for a quantitative analytical procedure.

EXPERIMENTAL

Synthesis of 2-tert.-butyl-4-methoxyphenyl trifluoroacetate

For the synthesis of 2-*tert.*-butyl-4-methoxyphenyl trifluoroacetate (BHAT) 2.0 g BHA (Koch-Light, Colnbrook, Great Britain) were dissolved in 6 ml hexane and 2.5 g trifluoroacetic anhydride (Eastman-Kodak, Rochester, N.J., U.S.A.) were added. The mixture was sealed in an ampoule and heated at 80° for 1 h. After cooling, the contents of the ampoule were washed with 25 ml 0.1 *M* sodium hydroxide and 2 × 10 ml water, and the organic phase was dried over anhydrous calcium chloride. Removal of the solvent yielded a straw-coloured oil (2.8 g, 90%). [Found: C, 56.7%; H, 5.8%; $C_{13}H_{15}F_3O_3$ requires C, 56.5%; H, 5.5%].

Gas chromatography

The work was performed on a Hewlett-Packard F & M Model 5750 gas chromatograph, fitted with a FID and an ECD. Commercial nitrogen of high purity was used without further purification as carrier gas with the FID. Argon containing 10% methane was employed as carrier gas in the ECD work. The gas flow-rate in both cases was 40 ml/min.

Columns were borosilicate glass coils (1.5 m × 6 mm O.D.) packed with Chromosorb W AW DMCS (80–100 mesh) which had been coated with SE-30 (5 or 10%, w/w). Solutions were prepared in hexane by serial dilution of stock solutions (10 mg/ml for BHA and BHT; 1.0 mg/ml for BHAT). Calibration data were obtained by reaction of 10–100 μg BHA with 0.1 ml trifluoroacetic anhydride in 0.5 ml hexane for 30 min at 80° using PTFE-lined, screw-cap vials (12 cm × 1 cm O.D.). After cooling, dilute alkali (1 ml 0.1 *M* sodium hydroxide) and the internal standard (1.00 μg hexachlorobenzene in 0.5 ml hexane) were added and shaken vigorously. The clear supernatant layer (1 μl) was injected directly onto the column.

RESULTS AND DISCUSSION

Microanalytical, infrared spectroscopic and GC data indicate that the derivative is BHAT.

The infrared spectrum of this compound is shown in Fig. 1a, where the main feature is the appearance of a strong carbonyl stretching absorption at 1800 cm^{-1} resulting from the formation of the ester. In the spectrum of BHA (Fig. 1b) this band is, of course, absent and shows instead an intense band at 3420 cm^{-1} due to the absorption of the hydroxyl group. The hydroxyl absorption in BHT (see Fig. 1c) occurs at a shorter wavelength than in BHA, presumably because in the case of BHT the hydroxyl group is unable to participate in hydrogen bonding as a result of the steric hindrance from the two bulky, adjacent *tert.*-butyl groups.

Relative retention data and detection limits for the three compounds are presented in Table I. With the non-polar stationary phase SE-30, BHA emerges from the column ahead of BHT but the order can be reversed on a more polar phase[9] due to increased interaction with the more accessible hydroxyl group in BHA. Indeed, this interaction has been shown to increase markedly as the polar nature of the stationary phase increases[13]. However, BHAT elutes before BHA and BHT on the non-polar phase SE-30, and, as would be expected, has been found[13] to do so consistently on progressively more polar stationary phases.

The detection limits for BHA and BHT given in Table I suggest an improvement on established methodology[7] and show values consistent with the higher carbon content of BHT. More important is the lower detection limit of BHAT (by a factor of about 15) achieved with the ECD. Coupled with the enhanced sensitivity of the derivative is its stability in the presence of water and dilute base, together with its long-term stability at low temperature (21 weeks at 2°). Of further possible advantage is the fact that the same derivatization technique was unsuccessful when applied to BHT. This possibility is evidently not excluded by the steric hindrance derived from the two bulky *tert.*-butyl groups since formation of both the methyl[13] and trimethylsilyl[12,13] ethers has been reported. Based on GC evidence, the derivatization procedure described produced only a single compound (BHAT). However, when the ratio of acetyl-

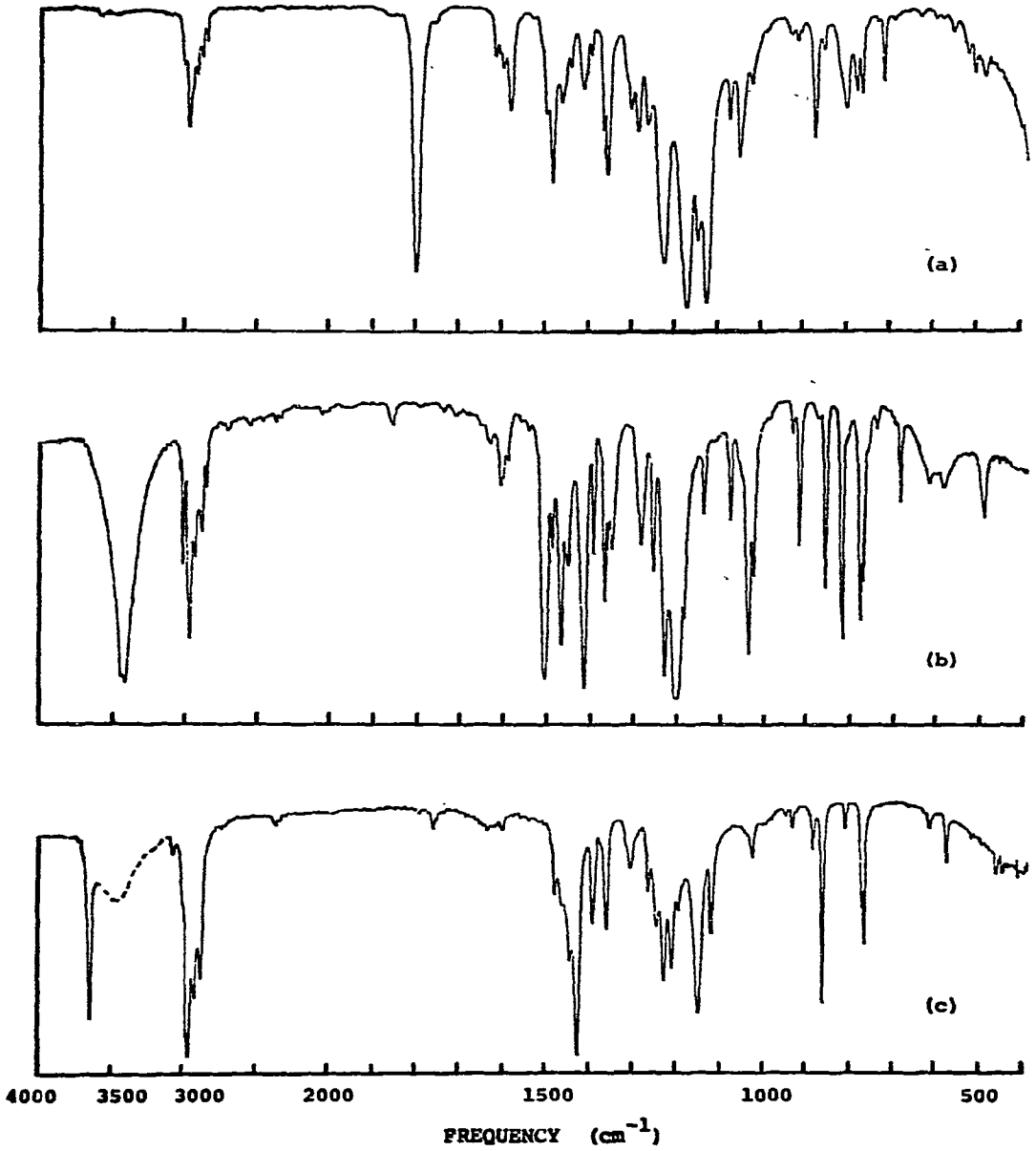

Fig. 1. Infrared spectra of (a) BHAT (thin film), (b) BHA (KBr disc), and (c) BHT (KBr disc). In (c) the broken line shows the hydroxyl band arising from the halide.

ating reagent and BHA concentrations was increased (from 10^3) to 10^4, two additional minor components of longer retention time appeared in the chromatograms. Although the identity of the peaks has not been assigned, reagent contamination was discounted.

The calibration data presented in Table II were obtained in the manner described. The resulting linear calibration plot was coincident with that obtained by

TABLE I

GAS CHROMATOGRAPHIC DATA FOR BHT, BHA AND BHAT

Compound	*Relative retention time**	*Detection limit (ng)***
BHA	1.00	5.0***
BHAT	0.68	0.5§
BHT	1.21	4.5***

* Determined by FID at 175° on a 10% SE-30 column with 20 ng of each compound.
** Detection limits were based on a 3-times noise level criterion.
*** Value determined with column and conditions referred to in the first footnote.
§ Value determined on 5% SE-30 column at 160° with ECD.

TABLE II

CALIBRATION DATA FOR BHAT WITH ECD

	BHA concentration (μg)				
	10	*20*	*40*	*80*	*100*
Peak height ratio*,**	0.05	0.10	0.19	0.42	0.50

* Internal standard hexachlorobenzene (retention time relative to BHAT is 4.1); 5% SE-30 column at 160°.
** Results are the means of three determinations.

working with the corresponding amounts of the previously prepared, pure BHAT, thereby confirming the quantitative conversion of the procedure. It may be noted that an internal standard with an unusually long retention time was chosen so that no peaks would be obscured between the elution of the two compounds.

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