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## Note

# Separation of butylated hydroxyanisole isomers by glass capillary gas chromatography

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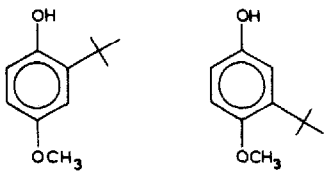
Butylated hydroxyanisole (BHA) is an antioxidant widely used in the food industry to prevent food from oxidative degradation. In addition, BHA has been found as an effective inhibitor of carcinogen-induced neoplasia[1]. Commercially available BHA is a mixture of two positional isomers (Fig. 1): 3-(1,1-dimethylethyl)-4-hydroxyanisole (3-BHA, I, major isomer) and 2-(1,1-dimethylethyl)-4-hydroxyanisole (2-BHA, II, minor isomer). These isomers have been found to have different antioxidant and antineoplastic capacities[2,3]. The available gas chromatographic[4–6] methods for the separation of BHA isomers require derivatization. In continuation of my studies with BHA[7], I have developed a glass capillary gas chromatographic method which is sensitive and does not require any derivatization.



I II

Fig. 1. Structure of BHA isomers.

## EXPERIMENTAL

### *Chemicals*

3-BHA was purified by crystallization with *n*-hexane from the commercially available BHA purchased from Sigma (St. Louis, MO, U.S.A.). 2-BHA was synthesized according to Lam *et al.*[3] *n*-Hexane used was glass distilled, pesticide grade, obtained from Burdicks & Jackson (Muskegon, MI, U.S.A.).

### *Sample preparation*

Stock solutions of 1 mg/ml of 2- and 3-BHA were prepared in *n*-hexane and were further diluted to 5 ng/ml prior to use.

### *Gas chromatography*

Gas chromatography was performed on a Varian Model 3700 equipped with

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a flame ionization detector, a Varian CDS III chromatography data system, and a fused-silica capillary column (30 m × 0.2 mm) coated with DB-1 phase (1 μm film thickness, J & W Scientific, Rancho Cordra, CA, U.S.A.). Nitrogen was used as a carrier gas at a flow-rate of 1 ml/min and the make-up flow-rate was 20 ml/min. Sample split ratio was 1:100. Other conditions were: injector temperature 250°C, detector temperature 350°C, oven temperature 120°C, and sensitivity $16 \times 10^{-12}$. Temperature programming was also used as described in Table I. For each run 2 μl of sample were injected.

## RESULTS AND DISCUSSION

Various conditions used for the separation of 2- and 3-BHA on glass capillary columns are summarized in Table I. Out of three different temperature programming settings, from 75°C to 250°C at a rate of 1°C/min, the isomers were resolved 1 min

TABLE I

GC OF BHA ISOMERS UNDER DIFFERENT CONDITIONS

| *Conditions* | *Retention time (min)* | |
|---|---|---|
| | *2-BHA* | *3-BHA* |
| 60°C 1°C/min 250°C | 67.0 | 67.8 |
| 75°C 1°C/min 250°C | 52.6 | 53.6 |
| 100°C 1°C/min 250°C | 31.4 | 32.0 |
| 120°C isothermal | 26.2 | 27.2 |

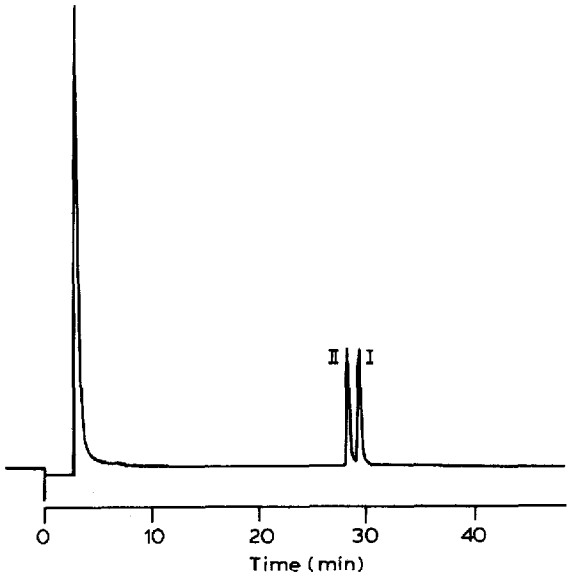


Fig. 2. Gas chromatogram of 2-BHA (II) and 3-BHA (I) under isothermal conditions. Column oven temperature 120°C.

apart in about 54 min. A similar separation was also achieved in a reduced analysis time (*ca.* 28 min) by using isothermal conditions at 120°C (Fig. 2). Vaessen has recently achieved similar separations on a SE-52 coated column[8]. The detection limit under isothermal conditions, at a split ratio of 1:100 and sensitivity setting of $16 \times 10^{-12}$ was about 10 ng. The detection limit could be lowered further either by increasing the sensitivity of detection or reducing the split ratio.

Prior gas chromatographic methods for separation of BHA isomers required derivatization either to corresponding silyl[4] or fluoro[5,6] derivatives. Our method does not require any derivatization and could be used for qualitative as well as quantitative analysis.

ACKNOWLEDGEMENT

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