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Note

Rapid determination of isomer ratios of butylated hydroxyanisole by high-performance liquid chromatography

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Butylated hydroxyanisole (BHA) is widely used as an antioxidant in food and cosmetic products. Commercially available BHA consists of two isomers (Fig. 1): 3-*tert.*-butyl-4-hydroxyanisole (3-BHA, major isomer, >85%) and 2-*tert.*-butyl-4-hydroxyanisole (2-BHA, minor isomer, <15%). 3-BHA has the stronger antioxidant properties[1].

BHA is both an efficient inhibitor and promoter of chemically induced carcinogenesis[2,3]. 2-BHA is the more effective inhibitor of benzo[*a*]pyrene-induced forestomach neoplasia in mice[4]. Further, BHA causes forestomach lesions, hyperplasia, papillomas and carcinomas in rodents after feeding of high levels in the diet[3,5]. This carcinogenic action of BHA is largely attributable to 3-BHA, the main component; 2-BHA appears to be much less effective[5].

Therefore, in experiments with BHA its isomer ratio should be known. Also, current pharmacopoeias require that the levels of 2- and 3-BHA in a batch be determined. We have previously reported a sensitive high-performance liquid chromatography (HPLC) method for measuring plasma BHA concentrations[6]. That method made no distinction between 2- and 3-BHA, however, as both analytes had the same retention time. We now report a rapid and simple method for the determination of the isomer ratio in BHA batches, applying normal-phase HPLC and UV or fluorescence detection, which is convenient with respect to simplicity, speed, column maintainance, precision and cost in comparison with other methods involving thin-layer chromatography[7,8], gas chromatography[8,9], HPLC[8,10,11], column chromatography[12] or non-chromatographic methods[8,13].

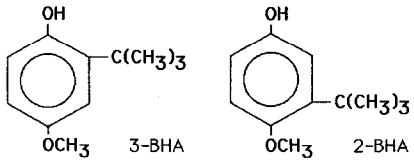

Fig. 1. Structures of 3-BHA (major isomer, >85%) and 2-BHA (minor isomer, <15%).

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EXPERIMENTAL

Reference standards were a gift from Rhône-Poulenc (Dagenham, U.K.) and contained 85.3, 88.8, 91.5 and 96.2% of 3-BHA respectively (determined by infrared spectroscopy[14]). Two batches of food-grade BHA were a gift from J. Dekker (Wormerveer, The Netherlands) and one batch of BHA was purchased from Sigma (St. Louis, MO, U.S.A.). *n*-Heptane (Fisons, Loughborough, U.K.) and ethanol (Merck, Darmstadt, F.R.G.) were of HPLC grade.

Solutions of 100 μg/ml of 2- and 3-BHA were prepared in *n*-heptane and 20-μl samples were injected on to a LiChrosorb Si 60-5 column (250 × 4.6 mm I.D.) (Chrompack, Middelburg, The Netherlands). The mobile phase was *n*-heptane–ethanol (400:5, v/v) at a flow-rate of 2.5 ml/min. The effluent was monitored by a Kratos Spectroflow 980 programmable fluorescence detector set at 290 nm with a cut-off filter at 320 nm in series with a Kratos Spectroflow 783 programmable UV detector set at 290 nm. For the determination of isomer ratios, peak heights were measured in addition to peak areas (by cutting and weighing).

RESULTS AND DISCUSSION

Preliminary experiments indicated that a mixture of 2- and 3-BHA can be separated on a LiChrosorb 5 RP-18 column (150 × 4.6 mm I.D.) and on a LiChrosorb RP-18 10-cm cartridge column when eluted with methanol–water–acetic acid (40:59:1, v/v/v) or an eluent of comparable strength with acetonitrile instead of methanol. These methods resulted in long retention times (up to 1 h), however, and produced very asymmetric peaks, 3-BHA eluting before 2-BHA.

Fast elution of separated isomers can be achieved, however, on a normal-phase LiChrosorb Si 60-5 column. Fig. 2 shows the effect of increasing concentrations of ethanol in *n*-heptane on the capacity factors (k') of the BHA isomers. Ethanol (1.25%) produced a fast and complete separation of isomers, with almost symmetrical peaks, within 11 min; 3-BHA and 2-BHA elute after 8.9 and 10.3 min, respectively. The limit of detection was about 0.5 ng for either isomer. Typical chromatograms are shown in Fig. 3.

Table I gives the results of the determination of isomer ratios of 2- and 3-BHA in

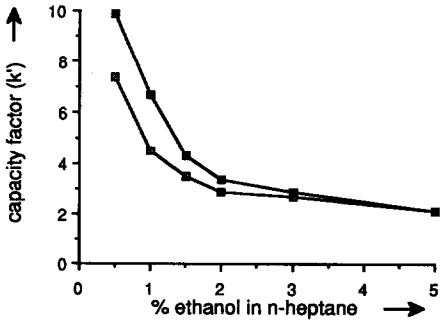

Fig. 2. Effect of increasing concentrations of ethanol in *n*-heptane on the capacity factors (k') of (■) 2-BHA and (⊡) 3-BHA.

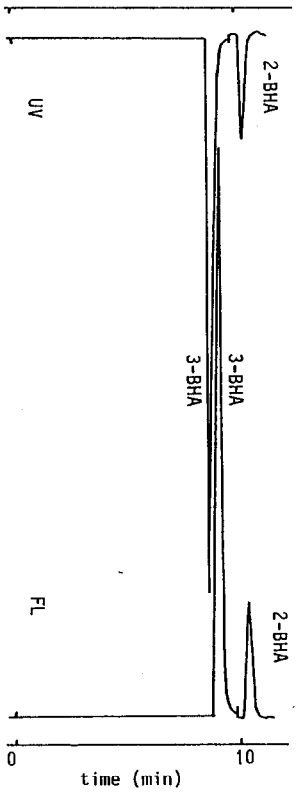

Fig. 3. Typical chromatograms for the separation of 2- and 3-BHA on a LiChrosorb Si 60-5 column using ethanol–*n*-heptane (400:5). For UV detection (up) the range was set at 0.05 a.u.f.s. for 0–9.5 min and at 0.01 a.u.f.s. thereafter; for fluorescence detection (down) the settings were 0.25 and 0.05 a.u.f.s., respectively.

TABLE I

DETERMINATION OF ISOMER RATIOS OF 2-BHA AND 3-BHA IN DIFFERENT BATCHES

Isomer ratios are expressed in terms of the percentage of 3-BHA (mean ± S.D.).

Batch[a]	*Peak-area method*		*Peak-height method*	
	UV detection	*Fluorescence detection*	*UV detection*	*Fluorescence detection*
RP (85.3%)	85.8 ± 0.5	85.4 ± 0.5	87.4 ± 0.2	86.8 ± 0.2
RP (88.8%)	90.0 ± 0.2	89.7 ± 0.2	91.3 ± 0.1	90.8 ± 0.1
RP (91.5%)	91.8 ± 0.3	91.5 ± 0.3	92.9 ± 0.1	92.5 ± 0.1
RP (96.2%)	96.2 ± 0.2	96.0 ± 0.1	96.7 ± 0.1	96.5 ± 0.0
FG I	86.9 ± 0.3	86.5 ± 0.2	88.4 ± 0.1	87.8 ± 0.2
FG II	93.6 ± 0.3	93.0 ± 0.2	94.2 ± 0.1	93.8 ± 0.1
S	95.8 ± 0.2	95.5 ± 0.1	96.3 ± 0.1	96.1 ± 0.1

[a] RP, sample from Rhône-Poulenc containing known isomer ratios; FG, food-grade sample; S, commercially obtained sample.

several batches. Determinations based on peak-area measurement after UV or fluorescence detection gives a reliable index of isomer ratios as the values determined are in close agreement with those already known. Determinations based on peak heights give poorer results. The coefficients of variation (C.V.) for the peak-area and peak-height methods were 0.3% and 0.1% respectively, with no difference between UV and fluorescence detection. The larger C.V. of the peak-area method may be due to the weighing method.

In conclusion, 2- and 3-BHA are easily separated on a LiChrosorb Si 60-5 column using 1.25% ethanol in *n*-heptane as the eluent. The isomer ratios are determined by measuring peak areas based on UV or fluorescence detection.

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