Journal of Chromatography, 262 (1983) 393–396
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,783

Note

High-performance liquid chromatographic separation of the isomers of butylated hydroxyanisole

G. A. S. ANSARI
Chemical Pathology Laboratory and Division of Biochemistry, The University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)
(Received February 7th, 1983)

Butylated hydroxyanisole (BHA) is a widely used antioxidant in the food industry. Besides preventing food from oxidative degradation, BHA is also an effective inhibitor of carcinogen-induced neoplasia[1]. The commercially available BHA is a mixture of two positional isomers (see Fig. 1), 3-(1,1-dimethylethyl)-4-hydroxyanisole (3-BHA, major isomer, I) and, 2-(1,1-dimethylethyl)-4-hydroxyanisole (2-BHA, minor isomer, II) in an approximately 85:15 ratio[2]. These isomers have similar oxidation potentials with different antioxidant capacities. 3-BHA is approximately 2.4 times more effective as an antioxidant than 2-BHA[3]. When subjected to various oxidizing agents (I) and (II) produced different oxidation products due to the difference in the position of *tert.*-butyl group[4]. 2-BHA is twice as effective as 3-BHA in inhibiting forestomach neoplasia in mice induced by benzo(*a*)pyrene[2,5]. Thus antitumor activity and antioxidant capacity do not correspond to each other. Because of the differences in the function of 3- and 2-BHA, a method was needed to evaluate the isomeric purity of the BHA preparations. However, the available methods for the separation of the BHA isomers using thin-layer[6], column[7] and gas chromatography[8–11], either lack sensitivity or require derivatization. This report describes an isocratic high-performance liquid chromatographic (HPLC) method for separation of BHA isomers. This method detects <2 ng of the isomers and does not require a prior derivatization.

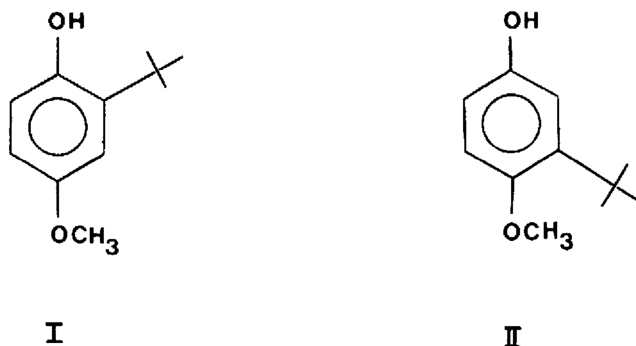

Fig. 1. Structures of BHA isomers. I = 3-BHA; II = 2-BHA.

0021-9673/83/$03.00 © 1983 Elsevier Science Publishers B.V.

EXPERIMENTAL

Chemicals

3-BHA was crystallized with *n*-hexane from 3(2)-BHA purchased from Sigma (St. Louis, MO, U.S.A.). 2-BHA was a gift from Dr. L. K. T. Lam, University of Minnesota, Minneapolis, MN, USA. All solvents used were high purity grade obtained from Burdick and 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 yield final concentrations of 10 ng/ml and 1 ng/ml.

High-performance liquid chromatography

HPLC was conducted on a Beckman gradient liquid chromatograph Model 334 with a Pirkle Type I-A (5-μm particle size, 25 cm × 4.6 mm I.D.) column purchased from Regis (Morton Grove, IL, U.S.A.). The column effluent was monitored at 288 nm with a Beckman Model 165 variable-wavelength detector. Pumps A and B contained *n*-hexane and 2-propanol, respectively. A 20-μl volume of sample was injected in each run and the flow-rate was 1.0 ml/min.

RESULTS AND DISCUSSION

Preliminary studies indicated that the BHA isomers could not be resolved on a μPorasil column using hexane or on a μBondapak C_{18} column using methanol–water (9:1) or acetonitrile–water (9:1) as a solvent. Resolution of these isomers, however, could be achieved on a Pirkle Type I-A using a binary solvent containing hexane and 2-propanol. The Pirkle Type I-A column has a spherical 5-μm γ-aminopropyl packing which has been modified by treatment with the N-3,5-dinitrobenzoyl derivative of D-phenylglycine and used for the separation of optical isomers[12–14]. This is perhaps the first reported use of Pirkle Type I-A column for separation of positional isomers.

Table I summarizes the effect of 2-propanol concentration in hexane on the separation of BHA isomers on Pirkle Type I-A column. Column effluent was monitored at 288 nm with a detection limit between 1–2 ng at 0.01 AUFS. These isomers

TABLE I

HPLC RETENTION TIME FOR 2- AND 3-BHA

2-Propanol in hexane (%)	*Retention time (min)*	
	2-BHA	*3-BHA*
0	—	—
1	96.0	47.8
2	30.4	21.6
3	17.2	13.3
5	10.7	9.0
7	8.2	7.0
10	6.8	6.0

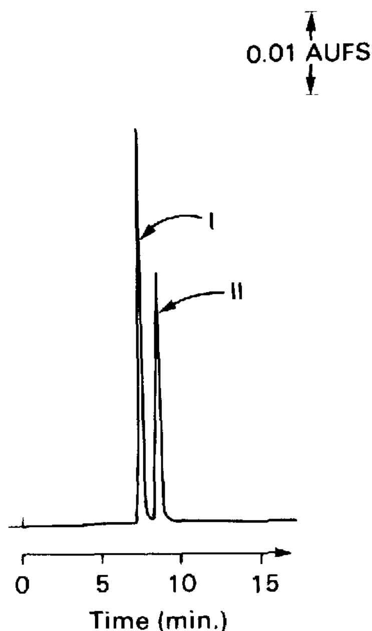

Fig. 2. HPLC chromatogram of 2-BHA (II) and 3-HBA (I) on Pirkle Type 1-A column using 7% 2-propanol in hexane.

could not be eluted from the column when hexane alone was used as a mobile phase. On addition of 1% 2-propanol in hexane, 3-BHA and 2-BHA can be eluted from the column in 100 min with excellent resolution but the peaks were broad and unsymmetrical. Further increases in the 2-propanol concentration up to 10% reduced the analysis time to 7 min. Concentrations above 10% were not used because the chiral phase of the column is ionically bound and could be washed off with high polarity

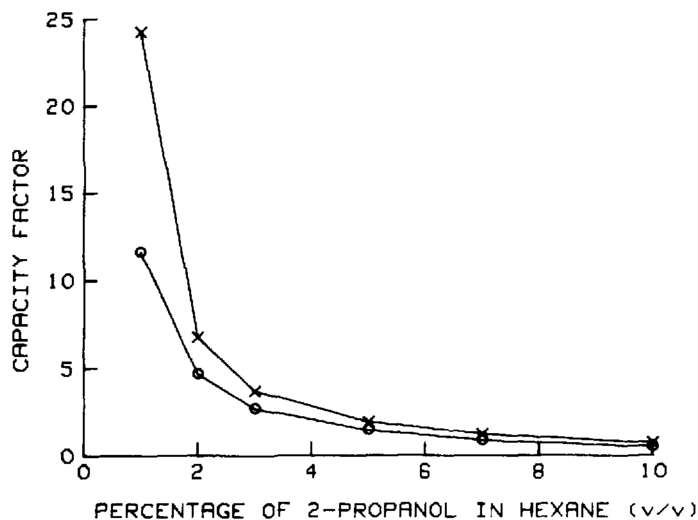

Fig. 3. Effect of concentration of 2-propanol in hexane on elution profile of 2-BHA (×) and 3-BHA (O).

solvents. A representative chromatogram with 7% 2-propanol in hexane is shown in Fig. 2.

Effect of 2-propanol concentration on the capacity factor of BHA isomers is shown in Fig. 3. There was a drastic drop in the capacity factor between 1 to 2% 2-propanol and then it decreased slowly with the increase of 2-propanol.

In conclusion, 3-BHA and 2-BHA can readily be separated without derivatization on a Pirkle Type I-A column using 2-propanol (1–7%) in hexane. Interaction of the phenolic group of BHA with the stationary phase could be responsible for the separation of these isomers. The phenolic group of 3-BHA is sterically hindered by an *o-tert.*-butyl group and therefore could not interact effectively with the stationary phase which results in rapid elution.

ACKNOWLEDGEMENTS

This study was supported by Institutional Research Grant IN112D, from the American Cancer Society. I thank L. Alice Hu for technical assistance and Mary Treinen Moslen for constructive criticism of the manuscript.

REFERENCES

1 L. W. Wattenberg, in J. Slaga (Editor), *Carcinogenesis, Vol. 5, Modifiers of Chemical Carcinogenesis*, Raven Press, New York, 1980, Ch. 4.
2 L. W. Wattenberg, J. B. Coccia and L. K. T. Lam, *Cancer Res.*, 40 (1980) 2820.
3 G. E. Penketh, *J. Appl. Chem.*, 7 (1957) 512.
4 F. R. Hewgill, *J. Chem. Soc.*, (1962) 4987.
5 L. K. T. Lam, R. P. Pai and L. W. Wattenberg, *J. Med. Chem.*, 22 (1979) 569.
6 M. R. Sahasrabudhe, *J. Ass. Offic. Anal. Chem.*, 47 (1964) 888.
7 P. Kauffman, *J. Chromatogr.*, 132 (1977) 356.
8 B. D. Page and B. P. C. Kennedy, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 1208.
9 E. S. Stoddard, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 1081.
10 E. Bailey, L. Della Corte, P. B. Farmer and A. J. Gray, *J. Chromatogr.*, 225 (1981) 83.
11 G. A. S. Ansari, unpublished results.
12 W. H. Pirkle and D. W. House, *J. Org. Chem.*, 44 (1979) 1957.
13 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 46 (1981) 2935.
14 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143.