spots on a pink background of brominated fluorescein. This method can detect less than 2 γ but requires careful adjustment of reagent concentrations and two successive sprayings.

A simpler detector solution for many sulfur-containing phosphate ester insecticides is a 0.5% solution of 2,6-dibromo - *N* - chloro - *p* - quinoneimine (DCQ) in cyclohexane. The chromatogram is sprayed and then heated in an oven at 110° C. for 7 minutes. Spot colors range from yellow to brownish red, and as little as 1 γ of some insecticides can be detected. Table I lists the limits of detection of spots of various sulfur-containing phosphate ester insecticides on paper strips (coated with either silicone or β-methoxypropionitrile for chromatography).

2,6 - Dibromo - *N* - chloro - *p* - quinoneimine is a sensitive color reagent for phenols in dilute solution (*3*, *5*). It has been used as a color reagent for uric acid (*4*), and a 1% solution in 95% ethyl alcohol has been used as a spot detector for uric acid and creatinine on paper chromatograms (*1*). Colors ranging from yellow to brown were observed with spots of tryptamine, indole-3-acetic acid, and other compounds on paper chromatograms (*1*). In this laboratory, 2,6 - dibromo - *N* - chloro - *p* - quinoneimine has been used to detect glutathione and cysteine on paper chromatograms, and with it various other reducing agents—such as catechol, iodide, and thiosulfate—have been observed to give yellow to brown spots, even at room temperature. Gibbs (*5*) had shown that this spot detector reacts with reducing agents.

On paper chromatograms, however, very few substances are likely to be present that will interfere with the detection of the sulfur-containing phosphate ester insecticides. Insecticides having the phosphorus-sulfur linkage give a reddish color. The carbon-sulfur-carbon linkage gives a yellowish color. The phosphorus-sulfur-carbon linkage apparently does not give color, but the only example of this type tested was the *S*-ethyl isomer of parathion (isoparathion). Phosphate ester insecticides that contain no sulfur give no color. Metabolites of the sulfur-containing insecticides may also fail to give color if the sulfur is oxidized—e.g., from a thioether to a sulfoxide.

Literature Cited

(1) Berry, H. K., Sutton, H. E., Cain, L., Berry, J. S., University of Texas Publication, No. **5109,** 33 (1951).
(2) Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **37,** 984 (1954).
(3) Ettinger, M. B., Ruchhoft, C. C., *Anal. Chem.* **20,** 1191 (1948).
(4) Fearon, W. R., *Biochem. J.* **38,** 399 (1944).
(5) Gibbs, H. D., *J. Biol. Chem.* **72,** 649 (1927).
(6) March, R. B., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 732 (1954).

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FOOD ANTIOXIDANT ANALYSIS

Infrared Analysis of Commercial Butylated Hydroxyanisole

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Butylated hydroxyanisole (BHA) is a widely used food antioxidant consisting of a mixture of 2- and 3-*tert*-butyl-4-hydroxyanisole. The 3-*tert*-butyl-4-hydroxyanisole isomer is reported to be a more effective antioxidant than the 2-*tert*-butyl-4-hydroxyanisole isomer, so a means of controlling the isomeric composition of the commercial product is important. An infrared method has been developed which allows the isomers to be determined with a standard deviation of approximately ±1%. The method is rapid and requires very little recalibration and has been used for several years as a convenient and effective quality control measure on commercial butylated hydroxyanisole.

COMMERCIAL BUTYLATED HYDROXYANISOLE (BHA), a widely used food antioxidant, is essentially a mixture of 2-*tert*-butyl-4-hydroxyanisole (2-BHA) and 3-*tert*-butyl-4-hydroxyanisole (3-BHA). As it has been reported that 3-*tert*-butyl-4-hydroxyanisole is a more effective antioxidant when added to lard than 2-*tert*-butyl-4-hydroxyanisole (*1*, *4*), it is important that the isomeric composition of commercial butylated hydroxyanisole be known.

Mahon and Chapman (*2*) described a colorimetric method for estimating the proportion of the isomers in commercial butylated hydroxyanisole, as well as in that which is removed from fats and antioxidant preparations. The method is based upon the fact that when 3-*tert*-butyl-4-hydroxyanisole reacts with 2,6-dichloroquinonechloroimide–borax reagent, it produces 5.2 times as much absorbance at 620 mμ as does 2-*tert*-butyl-4-hydroxyanisole, whereas the latter isomer, on reaction with ferric chloride-1,1′-bipyridine reagent, produces 1.1 times as much absorbance at 515 mμ as does the former isomer. Otis (*3*) has used an infrared method to analyze crude butylated hydroxyanisole for the two isomers and for one or more impurities which were present in these samples. In order to handle this complex system, simultaneous equations and absorbance values taken at three or four selected wave lengths were used.

As part of the quality control of the commercial antioxidant being produced on a plant scale, a simple and rapid method for the determination of the isomer ratio was desired. As the product at this point was essentially pure butylated hydroxyanisole, it appeared that a simplified version of the infrared method might give the desired results. In the current investigation, the ratio of the absorbances at 10.74 and 10.92 microns of a carbon disulfide solution of butylated hydroxyanisole provided a reliable value for the isomeric composition of the commercial product.

Apparatus and Materials

Spectrophotometer. A Baird double-beam recording spectrophotometer equipped with a scattered light filter and a 2X slit mechanism was employed. Cells of approximately 0.2 and 0.4 mm. were used with a 1.3-cm. rock salt plate in the reference beam.

2- and 3-*tert*-Butyl-4-hydroxyanisole. Samples of each of the pure isomers were available from earlier development work on the preparation of butylated hydroxyanisole. The melting point of the

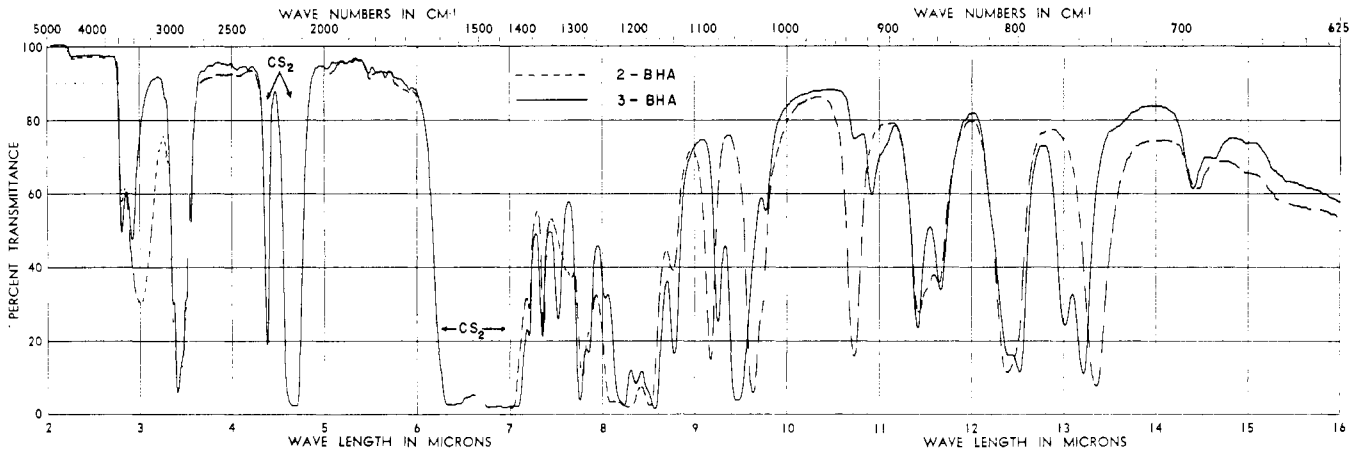

Figure 1. Spectra of butylated hydroxyanisole isomers

0.2-mm. cell, 80 mg. per ml. in CS_2

2-*tert*-butyl-4-hydroxyanisole isomer was 65° C. and that of the 3-*tert*-butyl-4-hydroxyanisole isomer 64° C. The infrared spectra of the samples showed that they were essentially free of the impurities normally found in the crude product.

Procedure

Weigh 1.0 gram of the sample into a 10-ml. volumetric flask. Dissolve the sample in carbon disulfide, dilute to the mark with this solvent, and mix the solution thoroughly. Fill a 0.4-mm. liquid-sample cell with the solution and measure the absorption spectrum from 10.2 to 11.3 microns using a 1.3-cm. rock salt plate in the reference beam, normal scan speed, and 2X slits (if the transmittance at 10.74 microns is less than 20%, repeat the measurement using a 0.2-mm. cell). After cleaning the cell, fill it with carbon disulfide and measure the absorption from 10.2 to 11.3 microns. Correct the absorbances of the sample at 10.74 and 10.92 microns by subtracting the absorbances of the pure solvent at these wave lengths. Divide the corrected absorbance at 10.74 microns by that at 10.92 microns and refer the quotient to a previously prepared calibration line to find the isomer composition of the sample.

Results

Spectra. The infrared spectra of carbon disulfide solutions of the two pure isomers are shown in Figure 1. The bands at 4.4 and 4.7 microns, and the broad band between 6.2 and 7.0 microns are results of the solvent. The 3-*tert*-butyl-4-hydroxyanisole is characterized by strong bands at 9.45, 13.02, and 13.22 microns while the 2-*tert*-butyl-4-hydroxyanisole isomer shows distinctive bands at 9.65, 10.74, and 13.35 microns.

A method of analysis appeared to be possible by developing a set of simultaneous equations from the absorbances at two of these wave lengths such as 9.45 and 10.72 microns. For a routine control test, however, it was thought that a procedure based upon an absorbance ratio would be more satisfactory, as the calibration data would be less affected by small changes in cell thickness or by imperfections in the cell windows as a result of continuous routine use. It was anticipated that many of the samples to be tested would be over 90% 3-*tert*-butyl-4-hydroxyanisole, so a pair of wave lengths was sought at which the absorbance ratio would change rapidly with increasing 2-*tert*-butyl-4-hydroxyanisole isomer content. On the other hand, it seemed desirable to choose one wave length at which the absorbance did not vary greatly with the isomer ratio in order to avoid working with a highly curved calibration line. While such a restriction would lower the sensitivity of the determination, the increased precision which it would permit was thought to be a more important consideration. The ratio between the absorbance at 10.74 microns and that at 10.92 microns was used.

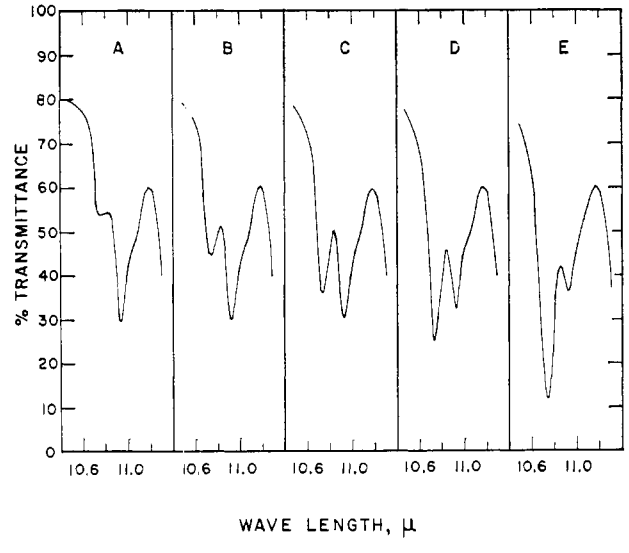

Figure 2. Spectra of isomeric mixtures of butylated hydroxyanisole

0.4-mm. cell, 100 mg. per ml. in CS_2
% 3-BHA: *A*, 100; *B*, 95; *C*, 90; *D*, 80; *E*, 60

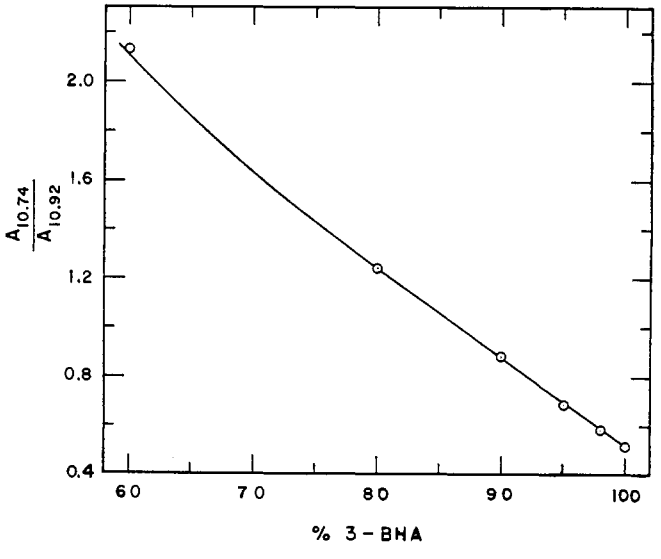

Figure 3. Calibration line for determination of 3-BHA (60 to 100%)

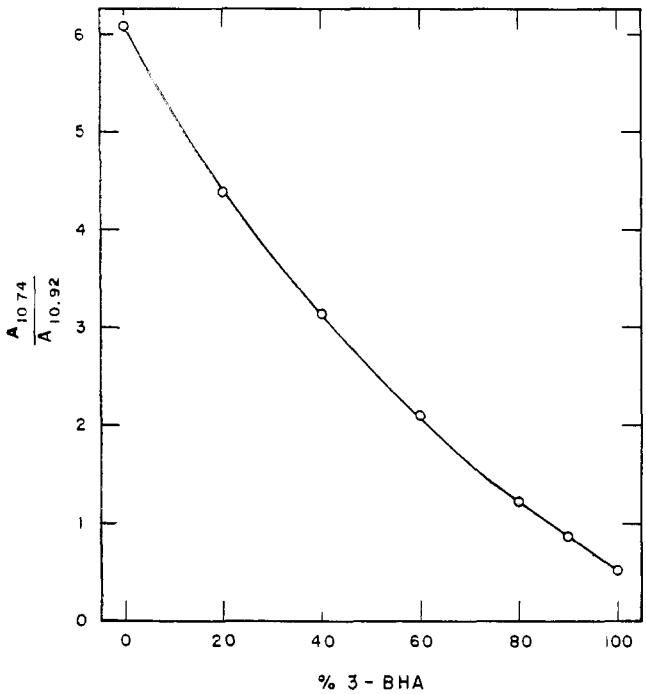

Figure 4. Calibration line for determination of butylated hydroxyanisole isomers

Entire concentration range

Figure 2 shows the spectra from 10.0 to 11.3 microns of a number of synthetic mixtures containing up to 40% of 2-*tert*-butyl-4-hydroxyanisole, these data being obtained with a somewhat thicker cell than the one used to measure the reference spectra of Figure 1. The ratios of the absorbances at 10.74 microns to those at 10.92 microns are plotted against the isomeric compositions of the mixtures in Figure 3. These data show that the ratio is sensitive to a change in isomer content and that the calibration line is essentially linear if the concentration of 3-*tert*-butyl-4-hydroxyanisole is 70% or greater.

In a separate experiment, a series of synthetic mixtures covering the entire range of isomeric composition was studied using a 0.2-mm. cell. The ratios of the absorbances at 10.74 and 10.92 microns obtained under this set of conditions are plotted in Figure 4. While the curve deviates from a straight line when the 3-*tert*-butyl-4-hydroxyanisole concentration falls below 70%, the curvature is not excessive. With concentrations of this isomer below 20%, however, it becomes difficult to determine the absorbance at 10.92 microns accurately, as there is no well defined inflection in the spectrum. In this concentration range more accurate analyses can be obtained from the absorption at 13.02 microns (see Discussion). With the samples studied in both 0.2- and 0.4-mm. cells, the absorbance ratios did not vary more than the amount equivalent to 1% 3-*tert*-butyl-4-hydroxyanisole. Thus, a change of up to 100% in cell thickness can safely be tolerated without requiring calibration.

Precision. Each of three samples of butylated hydroxyanisole containing approximately 99, 93, and 80% of the 3-*tert*-butyl-4-hydroxyanisole isomer was analyzed eight times by two laboratory technicians over a 2-month period. The standard deviations for the isomer determinations were 0.4, 0.5, and 1.2%, respectively (Table I).

Comparison with Colorimetric Method. Table II shows the results obtained when three isomeric mixtures of butylated hydroxyanisole were analyzed by both the colorimetric method of Mahon and Chapman and the proposed infrared method. The agreement between the two sets of results is satisfactory.

Discussion

In order to ensure that a uniform sample is taken for analysis, it is sometimes desirable to melt and mix the entire sample of butylated hydroxyanisole. The pure isomers used to prepare the calibration curve and the commercial samples composed of flakes or pellets do not require melting. If a sample has once been melted, however, and allowed to solidify slowly without stirring, the isomeric composition is not uniform throughout the mass This is of particular importance with samples containing appreciable concentrations of both isomers as the melting points of such mixtures are much lower than those of the pure isomers.

In the course of development work on the preparation of butylated hydroxyanisole, it has sometimes been necessary to determine rather small concentrations of 3-*tert*-butyl-4-hydroxyanisole in 2-*tert*-butyl-4-hydroxyanisole. With samples of this type the absorbance at either 9.45 or 13.02 microns provides a very sensitive measure of the 3-*tert*-butyl-4-hydroxyanisole isomer concentration. Working curves prepared from synthetic mixtures at both wave lengths have given entirely satisfactory analyses.

The infrared method described has been used as a routine quality control check for a number of years. Very few recalibrations have been required during that time, and often these amounted to only two or three point checks to establish the validity of the line being used. Dismantling, cleaning, and reassembling the cell has essentially no effect on the calibration line. These considerations and the simplicity of the test highly recommend its use if infrared equipment is available. The initial expense of the equipment required is probably the greatest disadvantage from the standpoint of processors using the antioxidant. This factor could be minimized by using a nonrecording–single-beam instrument which would probably give equally good results.

Literature Cited

(1) Dugan, L. R., Jr., Hoffert, Eugenia, Blumer, G. P., Dabkiewicz, Irene, Kraybill, H. R., *J. Am. Oil Chemists' Soc.* **28,** 493–5 (1951).
(2) Mahon, J. H., Chapman, R. A., *Anal. Chem.* **24,** 534–6 (1952).
(3) Otis, M. V., unpublished data from Research Laboratories, Tennessee Eastman Co.
(4) Rosenwald, R. H., Chenicek, J. A., *J. Am. Oil Chemists' Soc.* **28,** 155 (1951).

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Table I. Precision of Infrared Method

Sample	Av. 3-BHA, %	Range, %	Std. Dev., %	No. of Detns.[a]
1	79.8	77.7–80.6	1.2	8
2	93.4	92.6–93.9	0.5	8
3	99.5	99.0–100.0	0.4	8

[a] Samples run by 2 operators over 2-month period.

Table II. Comparison of Infrared and Colorimetric Methods

	3-BHA, %	
Sample	Infrared	Colorimetric
1	68	71
2	90	88
3	98	95