Determination of Butylated Hydroxyanisole, Butylated Hydroxytoluene, and Ethoxyquin in Hydrocarbon-Soluble Samples

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A rapid systematic method for the analysis of BHT and BHA in the presence or absence of ethoxyquin, based on solvent extraction and column chromatographic procedures, is described. The sample of food or feed containing the antioxidants is first dissolved in a suitable hydrocarbon. A sample of this solution is thoroughly shaken with 1*N* HCl. Ethoxyquin if present is found in the aqueous phase which is separated off and determined by ultraviolet spectrophotometry. The solvent phase contains mostly BHA, BHT, and other organic ingredients. The BHA is then brought into a 70% ethanol solution, and determined either colorimetrically or by ultraviolet absorption spectrophotometry. Another sample of the original hydrocarbon solution is passed through a Florisil chromatographic column. All antioxidants except BHT will remain absorbed on the column. The BHT in the eluted solution is then determined by ultraviolet spectrophotometry.

Quantitative determinations of BHA (2- or 3-*tert*-butyl-4-hydroxyanisole) and BHT (3,5-di-*tert*-butyl-4-hydroxytoluene), when present together and in mixtures along with ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline), pose a difficult analytical problem because of the interference of these antioxidants with each other and with other chemical constituents usually found in complicated food and feed formulations. Although there are many methods cited in the literature (*7–10*) for the determination of these antioxidants, the procedures, in general, are long and tedious, do not permit the separate determination of these compounds when present together, and are usually only applicable for a specific case.

The object of this paper is to describe a systematic procedure for the determination of BHA, BHT, and ethoxyquin when present alone or in combination in hydrocarbon-soluble samples. (The procedure for the determination of these antioxidants when present in water-soluble samples is at present in preparation.) This procedure employs, with necessary modification, several of the methods already described in the literature along with several innovations. In arriving at this procedure, speed as well as accuracy has been the object of primary consideration.

Reagents

The reagents used as solvents for extraction of BHT were first purified by passing them through a Florisil column and discarding the first two 25-ml. portions effluxed. The ultraviolet absorptions of these solvents were then checked.

Isoöctane, redistilled, reagent grade.

Petroleum ether, b.p. 40° to 60° C.

Skellysolve B, redistilled.

BHA, twice recrystallized from petroleum ether, m.p. 59° to 60° C.

BHT, (Nopco Food Grade). Purity 99% (min.), m.p. 69° to 70° C.

Ethoxyquin, (freshly prepared by the authors' research laboratory for an arbitrary standard) and commercial samples supplied from two sources.

Apparatus

Spectrophotometer, Beckman ultraviolet model DU.

Colorimeter, Beckman DU model B or Evelyn colorimeter.

Materials

Glass wool.

Florex XXS (calcinated), 60- to 100-mesh, obtained from Floridin Co. The water content of each 1-pound batch of this material is approximately 6 to 7% as determined by the manufacturer.

Florisil, 60- to 100-mesh, Floridin Co., Tallahassee, Fla.

Preparation of Columns

Into a 25-cm. long, 20-mm. diameter chromatographic column, insert a small plug of glass wool and add with gentle tapping about 20 grams of Florisil which has been prewashed with the solvent before use. Then wash with two 15-ml. portions of solvent.

Experimental

A solid or liquid sample, containing BHA, BHT, and ethoxyquin weighed to the nearest 0.15 mg., is dissolved in 100 ml. of heptane. Other hydrocarbons, such as hexane (redistilled, reagent grade), cyclohexane (redistilled, reagent grade), isoöctane, Skellysolve B, and petroleum ether, can also be used in place of heptane. These solvents have been tried and were found to give similar results. The sample hydrocarbon mixture is shaken for a few minutes to ensure that the sample is completely dissolved into the hydrocarbon.

A 25.0-ml. sample of this hydrocarbon solution is then passed through the Florisil-packed chromatographic column. All antioxidants and other interfering substances with the exception of BHT are retained on the column. Additional solvent is passed through the column so that the eluent will total about 180 ml. The eluate is collected in a 200-ml. volumetric flask and diluted to this volume with solvent. BHT can then be determined from this solution. The ultraviolet absorbance is read at 272, 277, 280, 283.5, and 287 mμ; an inverted w-shaped spectrum, as shown in Figure 1, which is a qualitative test specifically known for this antioxidant indicates the presence of BHT. A linear relationship is shown in Figure 2 of absorbance *vs.* BHT concentration over the range from 0.02 to 0.12 mg. per ml. (corr.). Although either of these wavelengths can be used, calculations using the absorbance at 283.5 mμ were used.

The weight of sample taken depends on its antioxidant content. This method gives good results when about 0.05 mg. per ml. of BHT is present in the sample. The amount of BHA can be as little as half of the amount of BHT in the sample; however, further dilution of the

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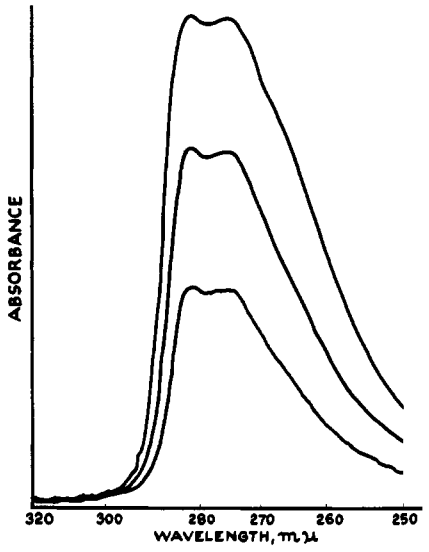

Figure 1. Absorbance *vs.* wavelength of various concentrations of BHT

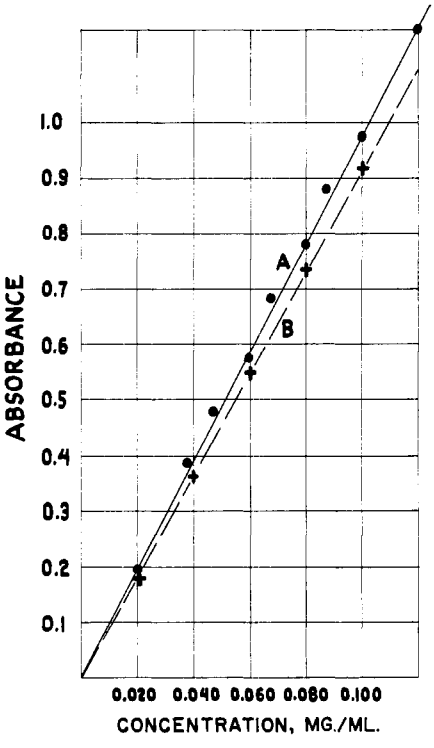

Figure 2. Absorbance at 283.5 mμ (*A*) and 277 mμ (*B*) *vs.* concentration of BHT known solution

alcohol extract may be used for the ultraviolet absorption readings.

A 35-ml. sample of the heptane solution is used for the determination of ethoxyquin and BHA. The heptane solution is first shaken with about 15 ml. of 1*N* HCl in a 100-ml. separatory funnel. The ethoxyquin present in the sample is retained in the HCl phase, which is separated and used for the determination of ethoxyquin by the ultraviolet spectrophotometric method previously described (*4*).

To ensure that the extraction of the ethoxyquin by the HCl solution is complete, the above procedure is usually repeated three times. The extracts are then combined and used for the determination.

The heptane solution remaining in the separatory funnel is then washed several times with distilled water to remove any residual acid left in this phase. The BHA present is then extracted by treatment with three separate portions of 25 ml. each and a fourth portion of 15 ml of a 70% ethanol solution (USP, 190 proof, 700 ml. of ethanol made up to 1 liter with distilled water) made up to 100.0 ml. with ethanol. The BHA present is then determined spectrophotometrically. A linear plot of BHA concentrations *vs.* absorbance is shown in Figure 3.

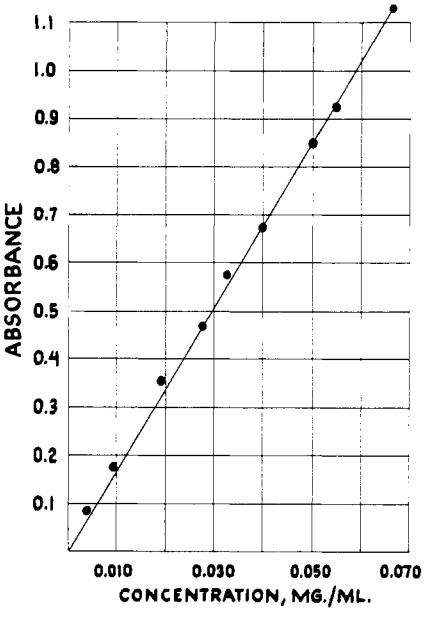

Figure 3. Absorbance at 292 mμ *vs.* concentration of BHA solution

Results and Discussion

To ascertain the accuracy of analysis for BHA and BHT together with, and in the absence of, ethoxyquin, triplicate runs were performed on the standard antioxidant solution. The average percent deviation for the determinations of BHA and BHT was found to be ±1.0% for the standard mixture not containing ethoxyquin. When ethoxyquin was present at the same concentration of BHA, the percent accuracy for the determination of BHA was found to be higher than 97%, and no effect was observed on the BHT determination. The results of this study are given in Table I and Table III. The column chromatographic method used here for the separation and determination of BHT is much simpler than that described by Phillips and Hinkel (*9*). The greatest advantage of the Florisil column is that it reduces the time required for complete column separation from 4 to 5 hours to about 25 minutes. Also, the activation and deactivation appear to be less critical for this column than might be expected. The object of this activation-deactivation is to maintain the water content of the column material at about 6% by weight. A number of experiments gave good results with this column even without preliminary treatment. However, if a large amount of vitamin A is present, a previous deactivation of the Florisil column having a 6 to 7% water content is recommended.

Another advantage of this column is that any of the solvents enumerated above can be used for the extraction of the BHT. However, for samples con-

Table I. Percent Recovery of BHT and BHA in Mixture Samples after Column and Solvent Partition Extraction, Respectively

BHT, Mg./Ml.			BHA, Mg./Ml.		
Actual	Found[a]	Recovery, %	Actual	Found[a]	Recovery, %
0.0050	0.0049	98.0	0.0020	0.0019	98.5
0.0080	0.0078	98.8	0.0050	0.0049	98.0
0.0100	0.0098	98.0	0.0104	0.0103	96.7
0.0200	0.0196	98.0	0.0075	0.0073	97.3
0.0502	0.0499	99.4	0.0150	0.0145	96.7
0.1050	0.1030	98.1	0.0200	0.0193	96.5
0.1500	0.1480	98.7	0.0500	0.0493	98.6

[a] Average of triplicate runs.

Table II. Determination of BHA and BHT of Known Solutions

BHA, Mg./Ml.		BHT, Mg./Ml.	
Actual	Found	Actual	Found
0.0020	0.0019	0.0050	0.0048
	0.0020		0.0049
	0.0019		0.0047
0.0050	0.0048	0.0100	0.0098
	0.0049		0.0098
	0.0047		0.0097
0.0104	0.0104	0.0200	0.0198
	0.0102		0.0195
	0.0103		0.0196
0.0200	0.0193	0.0502	0.0494
	0.0196		0.0492
	0.0194		0.0496
0.0500	0.0492	0.1050	0.1030
	0.0494		0.1040
	0.0493		0.1035

Table III. Determination of BHA, BHT, and Ethoxyquin of Known Solutions

BHA, Mg./Ml.		BHT, Mg./Ml.		Ethoxyquin, Mg./Ml.	
Actual	Found	Actual	Found	Actual	Found
0.0025	0.0023	0.0050	0.0048	0.0025	0.0025
	0.0022		0.0049		0.0024
	0.0022		0.0047		0.0024
0.0050	0.0047	0.0100	0.0098	0.0050	0.0048
	0.0048		0.0098		0.0049
	0.0046		0.0097		0.0047
0.0150	0.0146	0.0200	0.0198	0.0100	0.0098
	0.0145		0.0195		0.0099
	0.0147		0.0196		0.0097
0.0250	0.0242	0.0502	0.0494	0.0250	0.0243
	0.0243		0.0492		0.0242
	0.0244		0.0496		0.0245
0.0500	0.0488	0.1050	0.1030	0.0500	0.0494
	0.0490		0.1040		0.0493
	0.0491		0.1030		0.0492

Table IV. Determination of BHA, BHT, and Ethoxyquin in Commercial Samples

	BHA, Mg.		BHT, Mg.		Ethoxyquin, Mg.	
Sample[a]	Theory	Found	Theory	Found	100% Claim	Found
Vit. A, oil A	4.20	4.12	4.20	4.14	...	...
		4.10		4.12		
		4.10		4.15		
oil B	6.80	6.72	7.50	7.38	...	...
		6.70		7.42		
		6.68		7.44		
oil C	3.50	3.38	3.50	3.42	...	...
		3.41		3.44		
		3.40		3.44		
Multiple vit., premix I	12.00	11.88	18.00	17.75	...	...
		11.73		17.72		
		11.72		17.78		
Multiple vit., premix II	20.00	19.64	15.00	14.85	25.00	24.62
		19.68		14.87		24.65
		19.63		14.84		24.67
Multiple vit., premix III	5.00	4.82	5.00	4.90	...	...
		4.85		4.88		
		4.86		4.86		
Nopco Feed, premix L	7.50	7.36	7.50	7.41	3.40	3.28
		7.32		7.38		3.29
		7.35		7.40		3.28
Nopco Feed, premix V	2.80	2.68	2.80	2.72	3.20	3.12
		2.65		2.72		3.11
		2.66		2.70		3.13

[a] The first three samples are vitamin A (palmitate or acetate) products. The vitamin and feed premixes contain all or most of the listed ingredients: vitamins A and D, niacin, vitamin B_{12}, *d*-calcium pantothenate, riboflavin, BHT, BHA, and ethoxyquin either in wax or ethyl-cellulose coating.

taining a low concentration of BHT, Skellysolve B or petroleum ether is recommended, since an evaporation procedure at a reduced pressure may be necessary. For common samples, hexane or heptane were found to give good results, and these were the solvents used in obtaining the data presented in this paper.

Florex XXS can also be used effectively as a column filter. Unfortunately, when the column was prepared for some individual batches, the effluents were found to give high ultraviolet absorption readings. This difficulty can be remedied by washing the column thoroughly with solvent before using, but the procedure is tedious.

Table II shows the actual recovery of BHA and BHT from known standard solutions of these two antioxidants when present alone. Table III gives the recovery of BHA, BHT, and ethoxyquin from known solutions of varying concentrations of these antioxidants. When traces of BHT, tocopherol, vitamin additives, and other commonly interfering ingredients were brought into the alcoholic solution, no significant change of the ultraviolet absorption was observed. Thus, this procedure may prove to be specific for the separation and determination of BHA, and can be used to determine this antioxidant in commercial products. This method then would enable one to determine the concentration of BHA directly without the tedious steam distillation process described by Anglin, Mahon, and Chapman (*7*).

Table IV gives the results for the determination of BHA, BHT, and ethoxyquin in a number of commercially available samples. (Samples used for the evaluations were freshly prepared.) Where the concentrations of antioxidants are known, they are indicated in the table. The average percent recovery of BHA from these various samples was found to be 97 ± 2%.

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