Determination of Ethoxyquin by Ultraviolet Spectrophotometry

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An ultraviolet spectrophotometric method for the determination of the antioxidant ethoxyquin when present in samples of food or feed is presented here. The determination of ethoxyquin in samples not containing butylated hydroxyanisole (BHA) is carried out by first dissolving the sample in a suitable hydrocarbon. All vitamins, oils, fats, and butylated hydroxytoluene (BHT) present in the sample are dissolved in this solvent along with ethoxyquin. To this hydrocarbon, 0.5*N* HCl solution is added in a 1:1 ratio by volume, thoroughly shaken, and allowed to stand for a few minutes. The aqueous layer containing the ethoxyquin is then removed, and the per cent ethoxyquin in this layer is quantitatively determined by measuring its ultraviolet absorbance. If butylated hydroxyanisole is present, the sample must first be dissolved in ethanol solution and then saponified with KOH before treatment with the hydrocarbon. The per cent recovery of ethoxyquin from various samples determined by the above methods was found to be 98 ● 1%.

Much attention has been given recently to the development of analytical procedures for the determination of the common antioxidants present in food products and feeds. In spite of these many efforts, there still exists a need for the development of a rapid accurate method for the quantitative determination of ethoxyquin (1,2 dihydro-6-ethoxy 2,2,4-trimethylquinoline). The procedure described here, based on the fact that ethoxyquin absorbs ultraviolet radiation strongly in the region of 280 to 320 mμ, provides such a method.

Ethoxyquin was first determined quantitatively by Bickoff and coworkers (*1*), who took advantage of its fluorescence—a physical property associated with many of the common antioxidants. Unfortunately, the fluorescence changes with time (*3*) making accurate analysis of stored samples extremely difficult. Gordon *et al.* (*2, 4*), by means of a modification of the Bickoff method involving an extraction of ethoxyquin with methanol instead of the ether, were able in certain special cases to obtain satisfactory results. This method, however, has certain very definite limitations. First, a freshly distilled standard is not available for every run, and the fluorescence of the stored material differs with the storage conditions. Secondly, the extracted ethoxyquin solution must be completely free of any other fluorescent material. The accuracy of the determination by this method, then, depends on fluorescence of the standard material and on the reliability of reproducing from commercial-grade ethoxyquin a standard ethoxyquin calibration curve.

The method described here eliminates the difficulties mentioned above and makes possible the quantitative determination of ethoxyquin with little regard to its storage time. The procedure can be used even when ethoxyquin is present together with other antioxidants in complicated formulations.

Reagents and Apparatus

Standard ethoxyquin solution was prepared by dissolving 100 mg. weighed to the nearest 0.1 mg. of freshly synthesized ethoxyquin with 10 ml. of ethanol in a 1-liter volumetric flask and made up to the volume with 0.5*N* HCl.

Concentrated ethoxyquin stock solution was prepared by dissolving 100 mg. weighed to the nearest 0.1 mg. of freshly synthesized ethoxyquin in a 1 liter of heptane.

Concentrated KOH solution was made by dissolving 40 grams of KOH in 100 ml. of water.

50% Ethanol solution was made by diluting USP, 190-proof ethanol with distilled water in a 1 to 1 ratio by volume.

Heptane was reagent grade, redistilled, with a b.p. at 99° C.

Isopropanol was spectronic grade.

Ultraviolet spectrophotometer, Beckman model DU, with 10-mm. quartz cell was used.

Table I. Values of Absorptivity of Ethoxyquin Samples Stored for Various Time Periods

Sample of Ethoxyquin	Storage Time in Months	Absorptivity (a) 1st chemist	Absorptivity (a) 2nd chemist
Freshly synthesized at Nopco	0	80.8	...
	10	80.9	80.92
	14	80.8	80.93
Commercial ethoxyquin lot 212	2	81.0	...
	14	81.2	80.97
Commercial ethoxyquin lot W-145	2.5	80.8	80.92
	14.5	80.8	80.93
Unidentified sources	A 12+	80.8	...
	B 12+	80.9	...
	C 12+	80.9	...
	D 12+	81.1	...

Table II. Per Cent Recovery of Ethoxyquin by Partition Extraction with 0.5*N* HCl

Mg. Ethoxyquin in Heptane Solution	Mg. Obtained in Combined HCl Extract	Recovered, %
100.00	98.57	98.57
10.00	9.882	98.82
5.00	4.922	98.43
2.00	1.965	98.26
1.00	0.981	98.16
0.80	0.782	97.8
0.50	0.489	97.8
0.20	0.194	97.0

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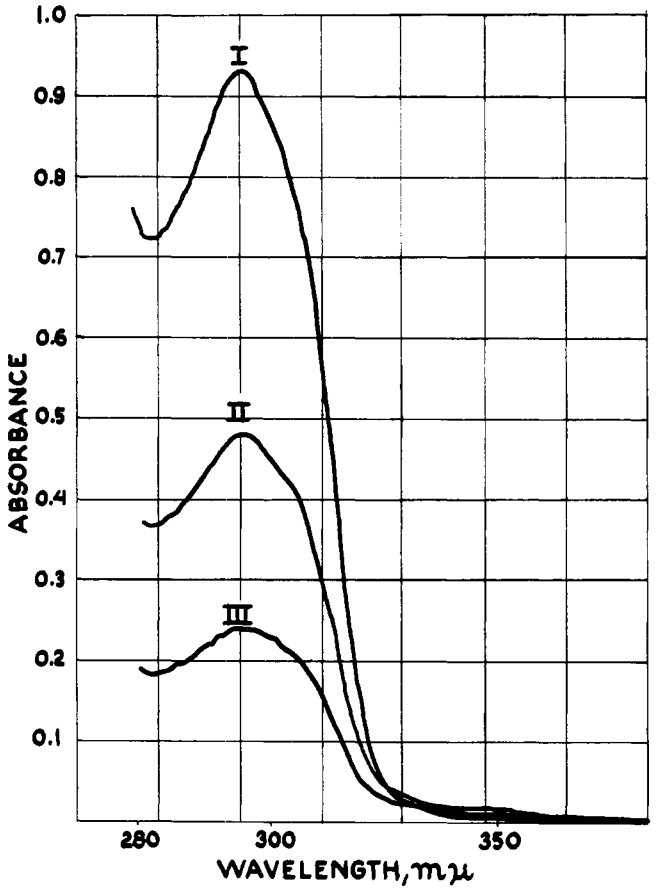

Figure 1. Absorbance vs. wave length of standard ethoxyquin solution(s)

(I) 0.0112 mg./ml.; (II) 0.0056 mg./ml.; (III) 0.0028 mg./ml.

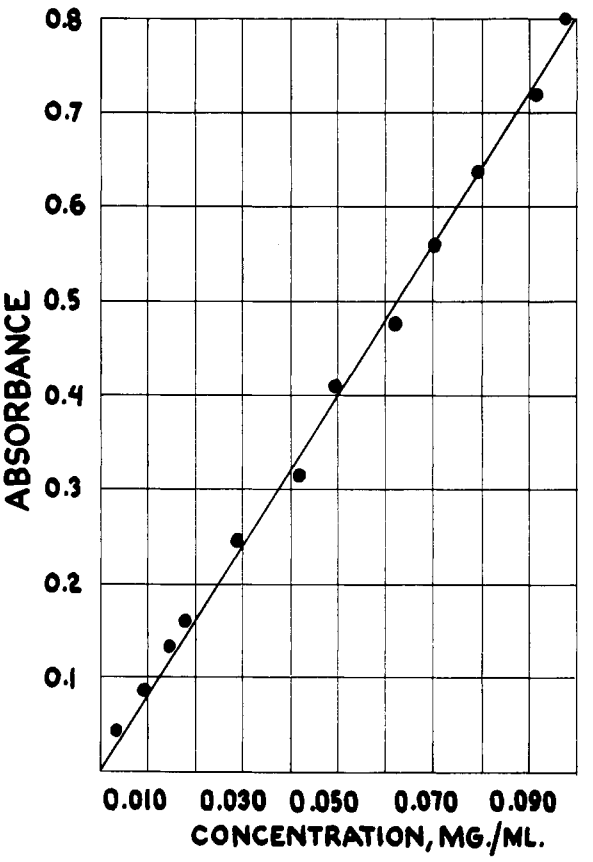

Figure 2. Absorbance at 296 mμ vs. concentration of ethoxyquin known solution

Table III. Study of Background Ultraviolet Readings of Various Materials Usually Present in Samples along with Ethoxyquin

HCl Extract of Sample[a]	$A_{296m\mu}$[b]
BHA, 0.050 gram	0.228
0.010 gram	0.062
0.005 gram	0.058
(saponified sample)	0.002
BHT	0.000
Vitamin A	0.003
Vitamin D	0.000
Vitamin E	0.002
Propyl gallate (satd. solution)	0.002

[a] Except where specified, all readings were taken of a 1% solution in heptane.
[b] These readings represent the average value of three duplicate runs.

Table IV. Determination of Ethoxyquin in Various Commercial Samples

Samples	*Mg. Ethoxyquin Calcd. Based on 100% Claim*[a]	*Run*	*Ethoxyquin Obtained, Mg.*
Santoquin, 66.6%	6.66	I	6.70
		II	6.71
Commercial Sample, 70%	7.82	I	7.69
		II	7.71
Commercial Sample, 50%	10.00	I	10.02
		II	10.04
		III	10.04
NOPCO Feed Premix L[a]	3.40	I	3.28
		II	3.24
		III	3.29
		IV	3.28
NOPCO Feed Premix[a]	0.49	I	0.475
		II	0.479
Synthetic Feed Sample[a]	0.502	I	0.487
		II	0.485

[a] These samples contain BHA and BHT in combination with ethoxyquin and a prior saponification was required.

Experimental

Ultraviolet spectrophotometry has been chosen for the quantitative determination. The ultraviolet absorption spectrum for the standard ethoxyquin solution shows absorption in the range from 280 to 320 mμ with a maximum (corrected) at 296 mμ as indicated in Figure 1. The absorptivity (a) was found to be 80.9, and the $\lambda_{min.}/\lambda_{max.}$ was calculated to be 0.836.

The absorptivity of ethoxyquin in dilute HCl solution was found to be practically constant with little regard to its storage time. The absorptivity of ethoxyquin solutions at 296 mμ from different sources and lot batches is given in Table I. All of these samples had been stored in clear glass bottles at room temperature.

Suitable aliquots were then prepared from the concentrated ethoxyquin stock solution by dilution with 0.5N HCl. These aliquots were then used for the ultraviolet absorption studies. The

curve obtained by plotting the absorbance at 296 mμ versus the concentration over the range from 0.005 mg. per ml. to 0.1 mg. per ml. gives a straight line relationship as shown in Figure 2.

Determination of Ethoxyquin in Samples Not Containing BHT. When the amount of ethoxyquin is to be determined in a solid, a sample weighed to the nearest 0.1 mg. containing about 4 to 15 mg. of ethoxyquin is placed in a 250-ml. conical flask and refluxed with 40 ml. of suitable hydrocarbon such as heptane for 20 to 30 minutes. Any of the solvents—heptane, hexane, or petroleum ether—can be used. All fats, oils, vitamins, and BHT will be dissolved in the hydrocarbon along with the ethoxyquin. This hydrocarbon solution is then transferred into a separating funnel and thoroughly shaken with an equal volume of 0.5*N* HCl. The lower aqueous layer containing the dissolved ethoxyquin is removed. The concentrations of ethoxyquin present in this layer is then determined by ultraviolet spectrophotometry.

If the original sample is an oily liquid, refluxing is not necessary. A suitable amount of an accurately weighed sample containing 4 to 15 mg. of ethoxyquin is added to 40 ml. of hydrocarbon, vigorously stirred, and/or slightly heated. Treatment with the 0.5*N* HCl solution as above is all that is required for the determination of ethoxyquin present in the sample.

Determination of Ethoxyquin in Samples Containing BHA. If the sample, either solid or liquid, contains BHA, the following procedure must be used. A sample containing from 4 to 15 mg. of ethoxyquin is first refluxed for 20 minutes with 50 ml. of 50% ethanol solution. To this solution, 10 ml. of concentrated KOH solution is added, and refluxing is continued for another 10 minutes. The KOH causes complete saponification of all the fatty materials and of the BHA present in the sample. Ethoxyquin is then extracted from the above solution by treatments with three separate portions of 35, 35, and 25 ml. of heptane. Other solvents such as hexane or petroleum ether can also be used here. Since BHA is present in the alcoholic layer as a potassium salt, it will not be extracted by the hydrocarbon solvent. These heptane solutions are then combined in a 250-ml. separatory funnel and washed several times with distilled water until all of the excess KOH has been removed as indicated by a negative phenolphthalein test. The ethoxyquin is then extracted from the heptane solution by treating it four separate times with 45 ml. each of 0.5*N* HCl. As above, the aqueous layer is separated from the hydrocarbon layer and the percentage of ethoxyquin in this composite aqueous solution is determined by ultraviolet spectrophotometry.

Results

To determine the per cent recovery of ethoxyquin by the HCl extraction procedure, a series of ethoxyquin solutions of known concentrations was prepared. From this investigation, the per cent recovery of the ethoxyquin by the HCl partition extraction was found to be 98 ± 1%. The data obtained are recorded in Table II.

Table III shows the background ultraviolet absorption readings at 296 mμ of various substances usually present in samples along with ethoxyquin. This study shows that the presence of BHT, propyl gallate, and the vitamins A, D, and E should have no effect on the result for the determination of ethoxyquin by this method. The presence of BHA, however, before saponification is shown to give a high absorption reading at 296 mμ. However, if the sample has been saponified by the procedure outlined above, the presence of BHA will have only a negligible effect on the result for the determination of ethoxyquin by this method.

The method was then applied to determine the percentage of ethoxyquin in several commercial samples. Duplicate runs for each determination were performed. The results of these determinations are given in Table IV.

This method was found to be applicable both for samples containing a very low concentration of ethoxyquin as well as for samples containing a high level of ethoxyquin. For low concentrations of ethoxyquin, the hydrocarbon solution is concentrated before the extraction procedure with the HCl is employed. No considerable loss of ethoxyquin was observed on evaporation of the solvent to a small volume.

Attempts were also made to use other dilute acids, namely, sulfuric, acetic, and nitric, in place of the HCl for the partition extraction. These, however, showed no major advantages. As a matter of fact, these dilute acids were found to bring down to the aqueous layer more of the BHA and other organic ingredients from the hydrocarbon solution than the HCl solution. The normality of the HCl solution was found to be not critical, and concentrations in the range of 0.2 to 1*N* were found to give similar results.

On the whole, the procedure presented here offers a simple accurate method for the analysis of ethoxyquin in various samples of food and feeds.

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