A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THE ANTIOXIDANTS BHA, BHT AND ETHOXYQUIN IN AQUEOUS AND IN HYDROCARBON SOLUBLE SAMPLES

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A gas–liquid partition chromatographic (GLPC) method for the separation and determination of the antioxidants BHA [2(and 3)-*tert.*-butyl-4-hydroxyanisole], BHT (3,5-di-*tert.*-butyl-4-hydroxytoluene), and ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) in aqueous and in hydrocarbon-soluble samples is described.

BUTTERY AND STUCKEY[1] described a GLPC method for the determination of BHA and BHT in samples of potato granules. The Apiezon L column used in this method, however, must be "aged" for one week in order for the BHA determination to be satisfactory. In addition, because of the high temperatures needed, it is believed that some of the BHA was decomposed on the column, which greatly reduced its life time. Another drawback that limits the sensitivity of the determination is the base line noise observed when this column is used. The method of JENNINGS, CURRAN AND EDWARDS[2] is able to determine BHT in paperboard. The column used for this determination consisted of a propylene glycol stationary phase on C-22 Firebrick. Although this column gives satisfactory results for the determination of the antioxidant BHT in paper board, it lacks the ability necessary to separate BHT from BHA in food products. The usefulness of this column is also limited by the fact that it is specific for BHT.

Very recently SCHWECKE AND NELSON[3] described a GLPC method for the determination of BHA and BHT in samples of foods, fats, oils, and potato products. The chromatographic column used for this work consisted of a mixture of Silicone Gum SE-30 and Tween 80. Because of the use of Tween 80 the column temperature is limited to a maximum of 150°. The method also requires the need of an internal standard (di-BHA) in order to effect the quantitative determination of the two antioxidants.

The development of the simple, accurate and rapid GLPC method described here was undertaken in order to effect the simultaneous separation of the antioxidants BHA, BHT, and ethoxyquin and to determine their concentration with a sensitivity to the nearest part per million as required in food analysis. The column found to be most satisfactory to effect the separation and determination of mixtures of these antioxidants consists of a 20 weight % Silicone Gum SE-30 (General Electric Co.) on 60/80 mesh solid support of either Firebrick (Johns-Manville Co.) or Chromosorb W (Wilkens Instrument and Research, Inc.). When Firebrick was used the most effect-

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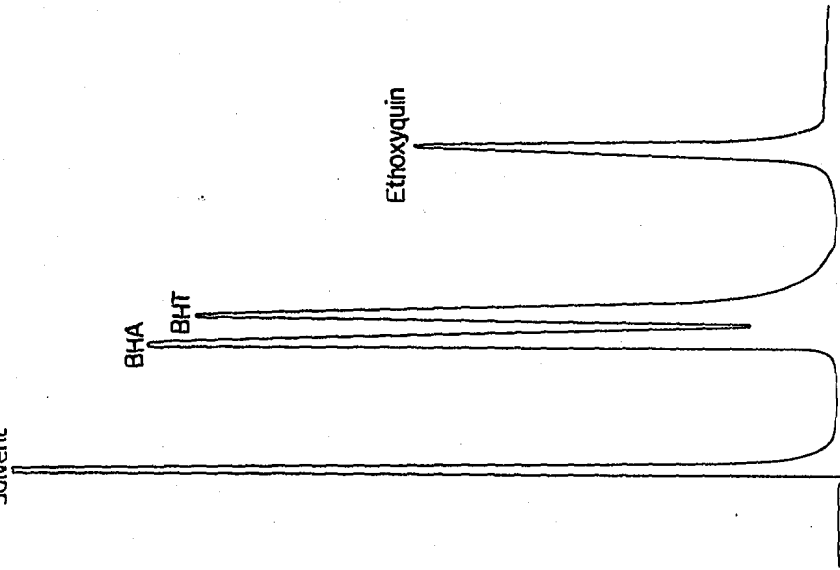

Fig. 3. Chromatogram of a mixture of three antioxidants. Sample: 0.2% BHA, 0.2% BHT and 0.2% ethoxyquin. Temperature: column, 156°; injector, 186°; detector, 230°; programming, 6°/min starting at the 7th min. Flow rate: 185 ml/min.

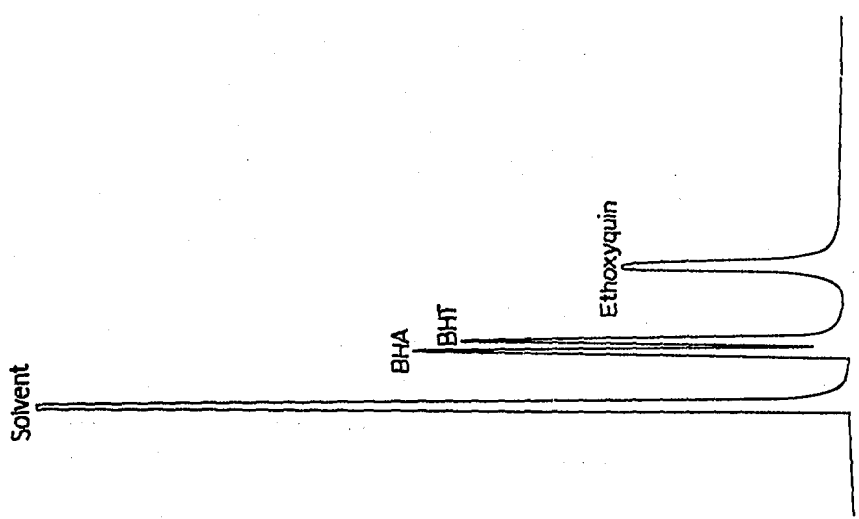

Fig. 2. Isothermal chromatogram of BHT, BHA and ethoxyquin at 188°. Column: 5 ft.; 20% Silicone Gum SE-30 on 60/80 mesh Firebrick. Sample: 0.1% BHA, 0.09% BHT and 0.12% ethoxyquin in heptane. Temperature: column, 188°; injector, 185°; detector, 230°. Flow rate: 185 ml/min.

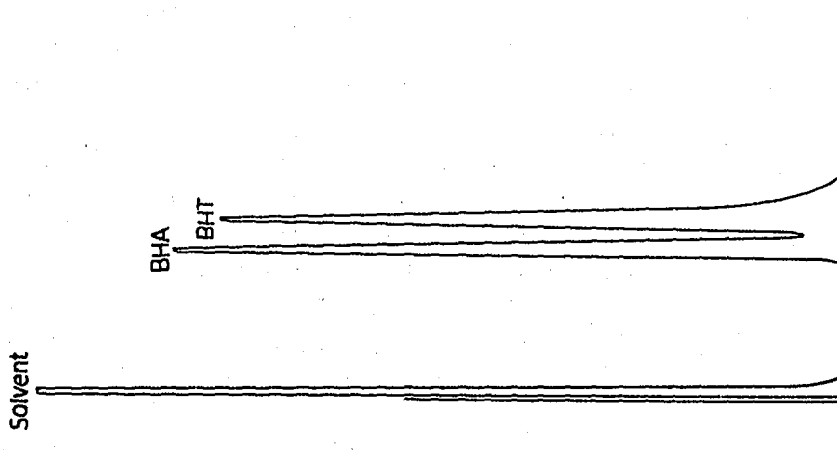

Fig. 1. Isothermal chromatogram of a mixture of BHA and BHT. Column: 5 ft.; 20% Silicone Gum SE-30 on 60/80 mesh Firebrick. Sample: 0.2% BHA, 0.2% BHT in heptane. Temperature: column, 152°; injector, 186°; detector, 230°. Flow rate: 185 ml/min.

ive column length was found to be 5 ft. Helium was used as a carrier gas at various flow rates as indicated in the sample chromatograms. A 5-ft. Firebrick column operated at a column temperature of 150° was found to give the most satisfactory separation of BHA and BHT as shown in Fig. 1. For the simultaneous separation and determination of BHA, BHT, and ethoxyquin, however, a column temperature of 190° was used. An example chromatogram of this separation and determination is shown in Fig. 2. The conditions used to effect the separation of these antioxidants using a 5-ft. Firebrick column by a temperature programming procedure is indicated in the example chromatogram shown in Fig. 3.

When Chromosorb W is used as the solid support, higher weight % of Silicone Gum SE-30 or a longer column is required for the separation of BHA and BHT. In this study a 10-ft. column coated with 20 weight % of the Silicone Gum was used. The Chromosorb W column gives a clear separation of individual components and can be used for trace analysis. Figs. 4 and 5 show the difference in the detector response for the two different columns when the chromatograph was operated under identical conditions. Fig. 6 is a sample chromatogram showing the optimum conditions necessary for the simultaneous separation and determination of BHA, BHT, and ethoxyquin when a 10-ft. Chromosorb W column was used.

Purity determination of BHA, BHT, and ethoxyquin commercial samples obtained according to the conditions given in Figs. 1 and 2, are compared with the results

TABLE I

PURITY DETERMINATION OF COMMERCIAL ANTIOXIDANTS

	Purity (%)							
Sample	*BHA*		*Sample*	*BHT*		*Sample*	*Ethoxyquin*	
	*GLPC**	*Chemical***		*GLPC**	*Chemical***		*GLPC****	*Chemical***
A	99.5	99.4	D	99.4	99.2	G	98.6	98.5
	99.2	99.6		99.2	99 1		98.2	98.4
	99.7	99.5		99.5	99.0		98.0	98.2
B	99.1	99.0	E	99.5	99.3	H	97.5	97.3
	99.4	99.2		99.2	99.4		97.2	97.0
	99.6	99.1		99.7	99.2		96.9	97.1
C	98.7	98.6	F	98.2	98.4	I	97.2	97.4
	98.8	98.7		98.6	98.3		97.6	97.6
	98.4	98.5		98.7	98.6		97.5	97.7

* GLPC method shown in Fig. 1.
** Ultraviolet spectrophotometric methods.
*** GLPC method as shown in Fig. 2.

obtained by ultraviolet spectrophotometric methods[4,5]. The results are tabulated in Table I. Using the conditions for the simultaneous separation as shown in Figs. 2, 3 and 6, the % recovery of the antioxidants BHA, BHT, and ethoxyquin in several commercial samples are compared and given in Table II.

Fig. 4. Chromatogram of BHA and BHT using a long Firebrick column. Column: 10 ft.; 10% SE-30 on 60/80 mesh Firebrick. Sample: 0.15% BHA and 0.15% BHT in 30 ml of heptane. Temperature: Column, 160°; injector, 186°; detector, 230°. Flow rate: 180 ml/min.

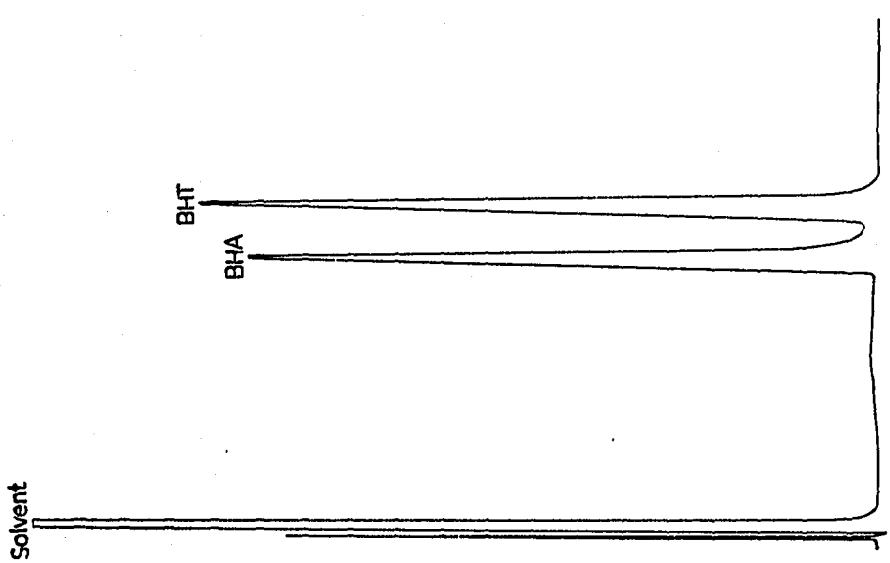

Fig. 5. Chromatogram of BHA and BHT using a Chromosorb column. Column: 10 ft.; 20% SE-30 60/80 mesh Chromosorb W. Sample: 0.15% BHA and 0.15% BHT in heptane. Temperature: column, 160°; injector, 186°; detector, 230°. Flow rate: 180 ml/min.

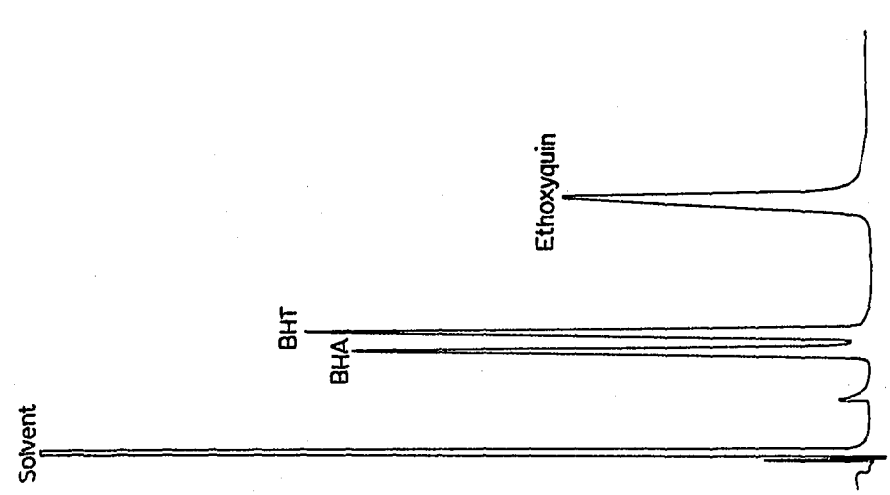

Fig. 6. Isothermal chromatogram of trace antioxidants. Column: 10 ft.; 20% SE-30 on 60/80 mesh Chromosorb W. Sample: 0.15% BHA, 0.15% BHT and 0.25% ethoxyquin in heptane. Temperature: column, 185°; injector, 185°; detector, 240°. Flow rate: 175 ml/min.

TABLE II

COMPARISON OF THE SIMULTANEOUS DETERMINATION OF BHT, BHA, AND ETHOXYQUIN ON VARIOUS OPERATION CONDITIONS (mg/ml)

Sample		*BHA*	*BHT*	*Ethoxyquin*
1	Actual	0.0010	0.0010	0.0020
	Found a*	0.0009_8	0.0009_9	0.0019_7
	b	0.0009_6	0.0009_6	0.0019_4
	c	0.0010_1	0.0009_9	0.0019_6
2	Actual	0.0050	0.0050	0.0050
	Found a	0.0049	0.0050	0.0049
	b	0.0048	0.0049	0.0050
	c	0.0050	0.0050	0.0049
3	Actual	0.0125	0.0125	0.0125
	Found a	0.0123	0.0124	0.0150
	b	0.0125	0.0124	0.0148
	c	0.0125	0.0125	0.0149
4	Actual	0.250	0.250	0.300
	Found a	0.251	0.249	0.299
	b	0.248	0.251	0.297
	c	0.249	0.252	0.296

* a: conditions shown in Fig. 1 (BHA and BHT) and Fig. 2 (ethoxyquin); b: conditions shown in Fig. 3; c: conditions shown in Fig. 6. All results are averages of duplicate determinations.

EXPERIMENTAL

Instruments

An Aerograph 350-B chromatograph (Wilkens Instrument, Walnut Creek, Calif., U.S.A.) provided with dual thermal conductivity detector cells was used for our initial investigation. An Aerograph 600-B provided with a flame ionization detector was used for the trace analysis.

Carrier gas

Helium, Matheson's 1A.

GLPC columns

The standard procedure of packing the columns was used. The Silicone Gum SE-30 was first dissolved in chloroform. A slurry of this solution with the solid support (Chromosorb W or Firebrick) was made and then dried in a vacuum oven. The actual weight % of the substrate was calculated on the dried weight basis. The column material was then packed, according to the usual procedure, into 1/4 in. and 1/8 in. copper tubing.

Materials

All reagents used were reagent grade and obtained from Eastman Chemical Products, Inc., Kingsport, Tenn., U.S.A. The standard antioxidants BHA, BHT, and ethoxyquin used in the initial investigation were freshly synthesized by Nopco Chemical Company, Organic Chemical Division, Harrison, N.J., U.S.A.

TABLE III

DETERMINATION OF ANTIOXIDANTS IN COMMERCIAL PRODUCTS (mg/g)

	BHA		*BHT*		*Ethoxyquin*	
	Claim	*Found*	*Claim*	*Found*	*Claim*	*Found*
Vitamin A oil	2.50	2.42	2.50	2.48	—	—
		2.36		2.44		—
		2.39		2.47		—
Vitamin A oil	5.00	4.88	5.00	4.86	—	—
		4.92		4.92		—
		4.87		4.90		—
Multiple vitamin mixture A	0.25	0.235	0.25	0.238	—	—
		0.237		0.240		—
		0.234		0.235		—
Multiple vitamin mixture B	1.00	0.975	1.00	0.980	1.00	0.965
		0.978		0.985		0.968
		0.977		0.983		0.971
Feed premix, Nopco L	—	—	3.50	3.42	3.50	3.44
		—		3.40		3.38
		—		3.39		3.36
Feed premix, Nopco V	2.28	2.24	2.85	2.78	2.65	2.61
		2.25		2.76		2.59
		2.26		2.78		2.60
Feed, synthetic (I)	100 p.p.m.	98.8	100 p.p.m.	99.2	100 p.p.m.	98.4
		99.0		99.4		98.6
		99.1		99.2		98.2
Feed, synthetic (II)	50 p.p.m.	48.5	50 p.p.m.	49.0	50 p.p.m.	48.5
		48.7		48.8		48.2
		48.8		48.7		48.0

PROCEDURE

Hydrocarbon-soluble samples

The sample, (fat, oil, etc.) was dissolved in a suitable low boiling point hydrocarbon. When the antioxidants were present only in trace amounts the solution was concentrated over a steam bath. No appreciable loss of antioxidants was observed due to this concentration process. Materials usually found in samples of this kind such as vitamins A, D, B_{12} and niacin, calcium *d*-pantothenate, riboflavin, etc. were found not to interfere.

Water-soluble samples

For water-soluble samples not containing proteins, direct injections of the solution into the chromatograph provided with a flame-ionization detector was found to give satisfactory results. No prior concentration of the solution was necessary and the usual ingredients found in this class of samples does not interfere with the determination.

For water-soluble samples containing proteins such as gelatin products, direct

injection of the aqueous solution was found to give rise to base line noise after several applications. For this class of samples it was found advantageous to reflux the sample with methanol, and use the methanol solution for the GLPC determination.

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SUMMARY

A gas–liquid partition chromatographic (GLPC) method for the simultaneous separation and determination, to the nearest part per million, of the antioxidants BHA, BHT, and ethoxyquin is described. The columns used to effect the separation and determination of these antioxidants consist of Silicone Gum SE-30 on either Chromosorb W or Firebrick. The relative merit of these two solid supports is indicated. The method was then applied to the determination of these antioxidants in water- and hydrocarbon-soluble commercial samples. The data obtained from the GLPC method is compared with that obtained from ultraviolet spectrophotometric studies.

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