Determination of BHA, BHT and TBHQ in Edible Fats and Oils

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ABSTRACT

A method is described for the rapid analysis of the phenolic antioxidants BHA, BHT and TBHQ in edible fats and oils. The procedure is based on gel permeation chromatography with UV detection, and requires no preliminary isolation of the antioxidants from the matrix. Detection limits with the instrumentation used in this study were <1 ppm for BHA and BHT, and ca. 20 ppm for TBHQ.

INTRODUCTION

The widespread use of phenolic antioxidants in food products has stimulated the development of numerous techniques for their determination. Generally, these techniques have relied on the isolation of the antioxidants from the food matrix by solvent extraction and/or distillation, followed by analysis of the isolated material with either spectrophotometry or gas chromatography. With the exception of the procedure of Hartman and Rose (1), the reported procedures are time consuming and, in some instances, lack sensitivity. Earlier attempts to apply high performance liquid chromatography (HPLC) to the analysis of phenolic antioxidants, including work in our laboratory (2), met with little success when applied to foods.

In recent years, gel permeation chromatography (GPC) has been used with increasing frequency for isolation of low molecular weight compounds from lipid matrices. GPC has also been used for quantitative analysis of mixtures of low molecular weight compounds where sufficient differences exist in solvated molecular size to permit separation. A number of studies involving GPC of phenolic compounds has been reported in the recent literature (3-8). Of these, two (3,7) consisted of investigations of substituent effects on the solvation and elution behavior of phenols, two (4,6) involved analysis of polyolefin samples, and one (8) detailed an 18 hour preparative separation of BHT (2,6-di-*tert*-butyl-4-methylphenol) and the isomers of BHA (2- and 3-*tert*-butyl-4-methoxyphenol).

Pokorny, et al. (5) reported an application of GPC to the analysis of antioxidants in edible fats and oils. Their procedure required a series of time-consuming steps including multiple extractions, removal of fatty acids, and evaporation of the extracting solvent in vacuo. The GPC analysis required over 2 hr for the elution of BHA, which was the component most retained by their chromatographic system. In contrast, the procedure detailed below is rapid, requiring less than 35 minutes for the elution of all sample components, and involves no isolation steps for edible fat and oil products.

EXPERIMENTAL PROCEDURE

Apparatus

A Waters Associates Model ALC/GPC 201 liquid chromatograph equipped with a Waters Model U6K injector was used. The injector was fitted with a 0.25 ml sampling loop. The detector was a Waters Model 440 UV detector operated at a wavelength of 280 nm and a sensitivity of 0.05 AUFS.

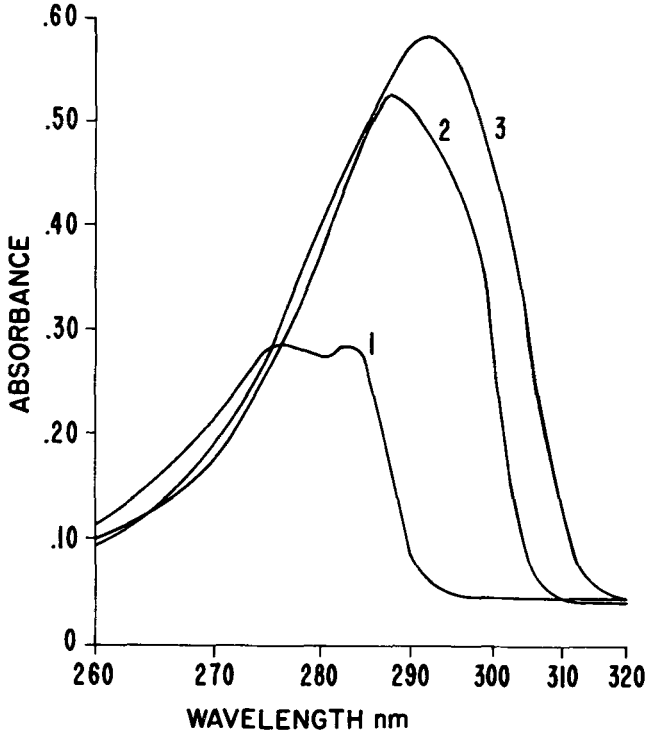

FIG. 1. Ultraviolet absorption spectra of 25 ppm solutions of BHT, BHA and TBHQ: 1 = BHT; 2 = BHA; 3 = TBHQ.

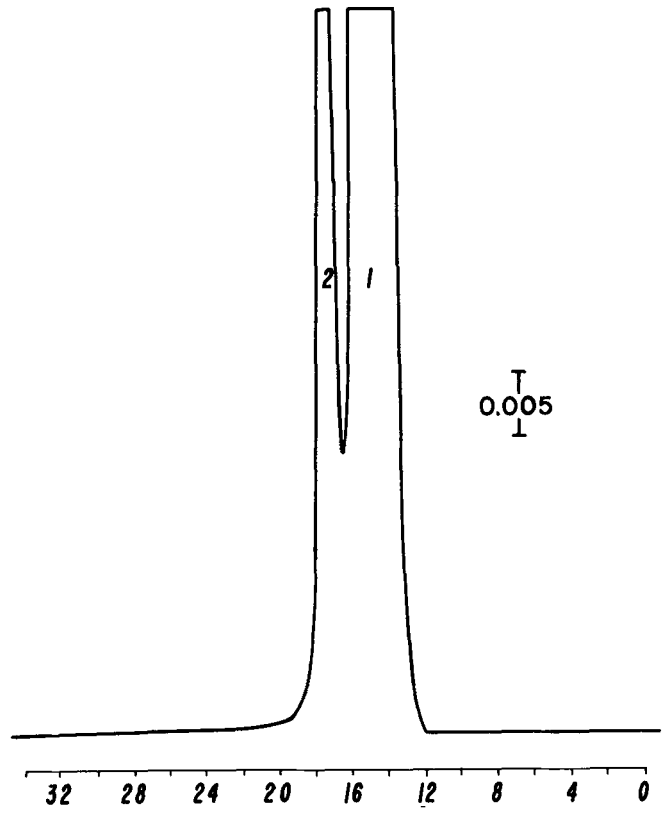

FIG. 2. Chromatogram of a shortening without added antioxidants: 1 = triglycerides and diglycerides; 2 = monoglycerides.

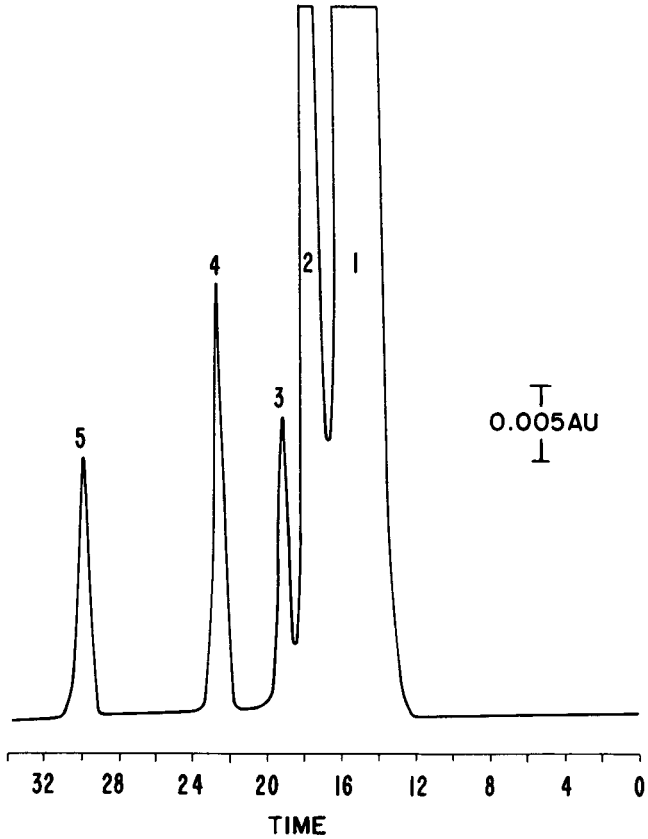

FIG. 3. Chromatogram of a shortening containing 100 ppm of each antioxidant: 1 = triglycerides and diglycerides; 2 = monoglycerides; 3 = BHT; 4 = BHA; 5 = TBHQ.

This wavelength was selected as the most appropriate for monitoring mixtures of the antioxidants of interest (Fig. 1).

Chromatographic Columns

The chromatographic system consisted of two 500Å and three 100Å μStyragel columns, manufactured by Waters Associates (Milford, MA). The columns were connected in series with the 500Å columns placed in the system immediately following the injector and preceding the 100Å columns.

Reference Materials

BHA, BHT and TBHQ (2-*tert*-butylhydroquinone) were Eastman Tenox grade.

Chromatographic Conditions

Reagent grade chloroform that had been filtered through a 0.5 micron Millipore filter was used as the mobile phase. The flow rate was 2 ml/min. The entire chromatographic system was operated at ambient temperature. The detector reference cell contained air.

Detector Calibration

Calibration was accomplished by injecting standards consisting of various concentrations of the antioxidants in chloroform. The concentrations used were 0.2, 0.5, 1, 2, 5, 10, and 20 ppm of each component. The injection volume used was 0.25 ml. Calibration curves were prepared by plotting chromatographic peak areas vs. concentration.

Assay Procedure

Ten g of melted and thoroughly mixed fat or oil were weighed into a 100 ml volumetric flask and diluted to volume with chloroform. The sample solutions were stored in darkness until analysis. Immediately prior to analysis, an aliquot was filtered through a Swinney type filter containing a 0.5 micron Millipore filter element. A 0.25 ml aliquot of the filtered sample solution was then injected onto the columns.

The chromatographic peak areas for each sample were measured and the quantities of BHA, BHT, and TBHQ present were obtained from the appropriate calibration curve.

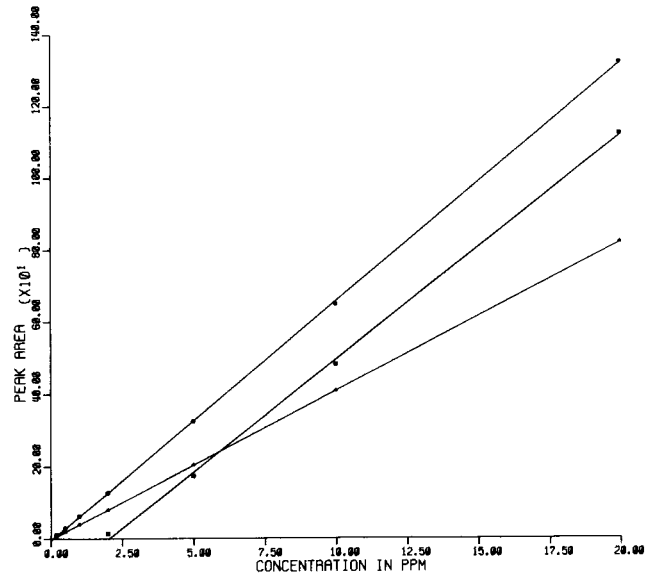

FIG. 4. Calibration curves for antioxidants in chloroform: •BHA; ▪TBHQ; ▲BHT.

TABLE I

Recovery of Antioxidants Added to Shortening

ppm Added	ppm Recovered BHA	BHT	TBHQ
200	197.6	195.3	199.4
100	97.8	97.3	97.3
50	50.0	48.8	48.0
20	20.0	19.1	23
10	10.3	9.4	--
5	5.2	5.2	--
2	2.0	2.5	--
$\bar{d}$	0.7	1.5	2.1

Method Accuracy and Precision

Soybean oil and a shortening (partially hydrogenated soybean oil with added mono- and diglyceride emulsifiers) were fortified at levels of 0, 2, 5, 10, 20, 50, 100 and 200 ppm each of BHA, BHT and TBHQ. These samples were analyzed using the aforementioned procedure, and recovery data for the added antioxidants were obtained. Linear regression analysis of the data was used to provide an estimate of precision.

RESULTS

Figure 2 shows the chromatogram obtained for a partially hydrogenated soybean oil shortening that did not contain added antioxidants. Figure 3 shows the chromatogram of the same shortening after it was fortified with BHA, BHT and TBHQ at levels of 100 ppm each. The resolution of the antioxidants from one another and the oil matrix is satisfactory. Figure 4 shows the calibration curves obtained for the antioxidants in chloroform. Tables I and II present recovery data for a shortening and a soybean oil.

TABLE II

Recovery of Antioxidants Added to Soybean Oil

ppm Added	ppm Recovered BHA	BHT	TBHQ
200	196.6	198.7	191.4
100	99.1	98.3	96.3
50	49.7	50.3	46.6
20	20.0	19.3	23
10	9.9	10.0	---
5	5.2	7.0	---
2	2.4	3.0	---
$\bar{d}$	0.8	1.0	4.7

TABLE III

Summary of Linear Regression Analysis of Concentration vs. Peak Area for Antioxidants in Various Matrices

	Slopes BHA	BHT	TBHQ
Chloroform	0.15140	0.24366	0.16094
Shortening	0.15370	0.24980	0.16295
Soybean oil	0.15416	0.24677	0.17023
Average	0.15309	0.24674	0.16471
S.D.	0.00148	0.00307	0.00489
Rel S.D.	0.97%	1.24%	2.97%

Average deviations, $\bar{d}$, are also given. Linear regression analysis of concentration vs. peak area for the standards and fortified samples gave the slopes shown in Table III. These data demonstrate the excellent accuracy and reproducibility of the procedure.

As indicated in Figure 4, the calibration curve for TBHQ does not pass through the origin. This is the result of a reproducible loss of about 0.5 μg of TBHQ on passing through the chromatographic system. Alteration of the mobile phase by the addition of tetrahydrofuran, which solvates TBHQ more strongly than chloroform, led to loss of resolution of the antioxidants from one another, and did not improve the recovery of TBHQ. Although the observed loss remains unexplained and requires further research to establish its origin, it is reproducible and does not hinder the analysis of samples containing more than 20 ppm TBHQ.

DISCUSSION

We have described a procedure, based on gel permeation chromatography, for the rapid analysis of BHA, BHT and TBHQ in edible fats and oils. It should be pointed out that this procedure can also be applied to other matrices. In many cases, a relatively simple chloroform extraction, followed by filtration and injection of the extract, provides a reliable analysis. For example, we have used this approach in our laboratory for analysis of antioxidant premixes, spice formulations and certain types of stabilized sausage products. It seems reasonable to expect that a wide variety of food products, previously analyzed only with difficulty, might be analyzed with relative ease using the GPC approach.

In addition, the basic concept of utilizing GPC for the analysis of small molecules is clearly not limited to the three compounds studied in this work. For example, both methyl and propyl p-hydroxybenzoic acid are used as preservatives in a wide range of products. These compounds can be separated from one another and from BHA, BHT and TBHQ using the same set of columns described in this study. Figure 5 illustrates the separation of all five compounds. It should be mentioned that while we did not use an internal standard in the analysis of BHA, BHT and TBHQ, either of the parabens would be a logical choice if use of an internal standard is desired.

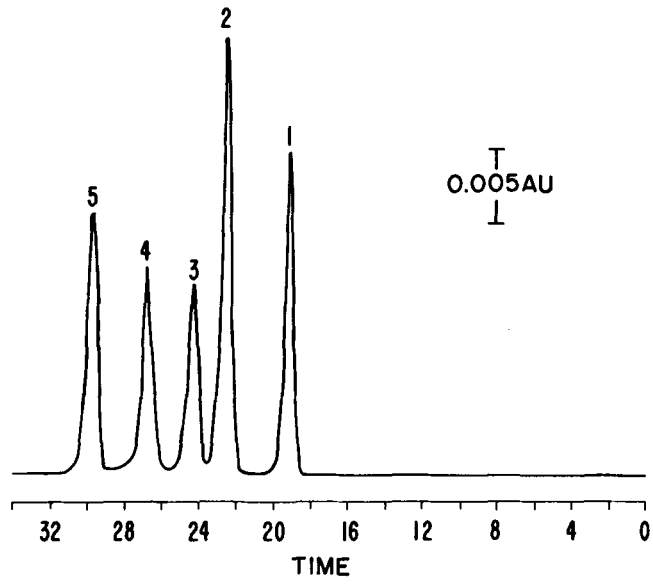

FIG. 5. Chromatogram of 10 ppm solutions of phenolic antioxidants in chloroform: 1 = BHT; 2 = BHA; 3 = propyl-p-hydroxybenzoate; 4 = methyl-p-hydroxybenzoate; 5 = TBHQ.

We believe that GPC has great potential in the analysis of food additives, particularly phenolic preservatives, and we anticipate greatly increased utilization of this technique in the future.

ACKNOWLEDGMENT

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REFERENCES

1. Hartman, K.T., and L.C. Rose, AOCS 47:7 (1970).
2. Bowers, R.H., "Application of High Speed Liquid Chromatography to Antioxidant Determinations," 47th Annual Meeting of the American Oil Chemists' Society, Paper No. 84, 1973.
3. Yochikawa, T., K. Kimura, and S. Fujimura, J. Appl. Polym. Sci. 15:2513 (1971).
4. Coupek, J., S. Pokorny, J. Protivova, J. Holcik, M. Karvas, and J. Pospisil, J. Chromatogr. 65:279 (1972).
5. Pokorny, S., J. Coupek, and J. Pokorny, Ibid. 71:576 (1972).
6. Protivova, J., J. Pospisil, and L. Zikmund, J. Polym. Sci., Polym. Symp., 40, 233 (1973).
7. Coupek, J., S. Pokorny, and J. Pospisil, J. Chromatogr. 95:103 (1974).
8. Kauffman, P., Ibid. 132:356 (1977).

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