NOTES

SIMPLIFIED DETERMINATION OF *tert*-BUTYLHYDROQUINONE IN VEGETABLE OILS, BUTTER, AND MARGARINE

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A simple and rapid determination of *tert*-butylhydroquinone (TBHQ) in vegetable oils, butter, and margarine by using high-performance liquid chromatography (HPLC) is described. TBHQ was effectively separated, identified, and quantitated on LiChrosorb NH₂ with hexane-ethanol eluent. Detection was done at UV 293nm with 0.04AUFS. *p*-Aminoacetophenone was used as an internal standard. The recovery of TBHQ added to oily foods was between 93.1-99.5% in the range of 100ppm of TBHQ.

INTRODUCTION

tert-butylhydroquinone (TBHQ) is used to prevent the oxidative degradation of fats and oils in the United States and other countries. Although butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), nordihydroguaiaric acid (NDGA), and some other chemicals are permitted as food additives in Japan, TBHQ is not registered as a food additive. However, a rapid and simple method is required for the determination of TBHQ in order to inspect imported foods.

Numerous methods have been published for qualitative and quantitative determination of TBHQ. Recently, the methods by means of high-performance liquid chromatography (HPLC) have been reported¹⁾⁻⁴⁾. The removal of lipid material from foods plays an important part for the determination of TBHQ in oily food going to the interference of lipid. By using normal phase HPLC system, TBHQ is simply separated from lipid. It takes only about 30 min to complete analysis for the determination of TBHQ in oily food.

EXPERIMENTAL

Reagents

TBHQ, hexane, ethanol, anhydrous sodium sulfate, and *p*-aminoacetophenone were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan). The standard solution was prepared by dissolving 20 mg TBHQ in 100 ml hexane-ethanol (85+15, v/v). The internal standard solution (I.S.) was prepared by dissolving 10 mg *p*-

Fig. 1 Liquid chromatograms of oily foods after addition of 0.1 mg TBHQ/g

TBHQ: *tert*-butylhydroquinone

I.S.: internal standard, *p*-aminoacetophenone

Conditions: column, LiChrosorb NH₂, 4 mm i.d. x 25 cm; mobile phase, hexane-ethanol (85+15); flow rate, 1.2 ml/min; sample, injected, 10 μ l; column temp., 40°C; detector, UV 293 nm

volume to 10 ml with hexane-ethanol (85+15).

aminoacetophenone in 200 ml hexane-ethanol (85+15).

Apparatus

A Shimadzu HPLC model LC-2 equipped with a Shimadzu variable visible-ultraviolet detector was used and monitored at 293 nm. The column was a 250 x 4 mm i.d. stainless steel tube packed with LiChrosorb NH₂ (E. Merck, Darmstadt, GFR). As the eluent hexane-ethanol was served at the ratio of 85 to 15 v/v, and a flow rate of 1.2 ml/min was employed. The column was thermostated with an oven at 40°C.

Preparation of test solution in vegetable oils

Test solution was prepared as follows: 2 g of sample was transferred to 10 ml volumetric test tube with addition of 1.0 ml of I.S. Subsequently, it makes

Preparation of test solution in butter and margarine

Test solution was prepared as follows: 2 g of sample was transferred to 200 ml beaker with the addition of 50 ml of hexane-ethanol (99+1) and ca. 10 g of anhydrous sodium sulfate. The solution was mixed by glass rod, and filtered through No.2 filter paper. More 50 ml of hexane-ethanol was added to the residue. The solution was mixed and filtered through the No.2 filter paper. The combined filtrate was evaporated under vacuum and then 1.0 ml of I.S. was added to the concentrate. The solution was diluted to 10 ml with hexane-ethanol (85+15).

Recovery of TBHQ added to oily foods

One ml of TBHQ standard solution was added to 2 g of oily foods. Subsequently, the mixtures were treated according to above procedures. The recovery tests were repeated five times.

RESULTS AND DISCUSSION

To remove lipid material from food, liquid-liquid extraction procedure was usually employed²⁾. However, no cleanup method to separate lipid from TBHQ was required by using gel permeation chromatography¹⁾ and normal phase HPLC system³⁾. Niekerk et al.³⁾ used LiChrosorb SI60 column, and determined directly TBHQ in vege-

Table 1 Recovery of *tert*-butylhydroquinone added to foods *

Foodstuffs	Recovery ** (%)	CV (%)
Salad oil	96.8 ± 0.47	0.49
Sesame oil	99.5 ± 1.18	1.19
Cotton seed oil	96.5 ± 0.38	0.39
Rape seed oil	98.9 ± 1.44	1.46
Coconut oil	96.2 ± 0.73	0.76
Butter	94.0 ± 1.49	1.58
Margarine	93.1 ± 1.35	1.45

* TBHQ was added at 100 ppm level

** Mean ± standard deviation, $n=5$

table oils without any cleanup method. We used LiChrosorb NH_2 column and hexane-ethanol(85+15) for the mobile phase, and *p*-aminoacetophenone as an internal standard. We determined TBHQ in vegetable oils, butter, and margarine.

Hexane-ethanol(99+1) was employed to extract TBHQ in butter and margarine. Although TBHQ does not dissolve in hexane, the solubility of TBHQ is increased by adding ethanol to hexane. Ethanol, however, extracts various more polar components from sample. Polar components are held in normal phase HPLC column and decrease column capacity. Therefore, we controlled the concentration of ethanol at 1%(v/v). But the extract from butter or margarine and vegetable oils were dissolved in the mobile phase of HPLC. TBHQ was eluted with a capacity factor of k' equal 0.78. k' values of BHA and BHT were 0.24 and 0, respectively, and those of PG and NDGA were more than 10. They did not interfere the determination of TBHQ. The method described was tested for analyses of salad oil, colza oil, coconut oil, sesame oil, cotton seed oil, butter, and margarine. Figure 1 shows typical chromatograms of fortified TBHQ in salad oil, sesame oil, and butter. In the case of sesame oil, the determination of TBHQ was interfered with appearance of unknown peak with the same k' value of TBHQ. However, the other foods did not show any peaks at or near $k'=0.78$.

A plot of relative peak height of TBHQ vs I.S. showed linear correlation from 1 to 30 μg TBHQ/ml. The least-squares method was applied to calculate the line of best fit for the data($y=0.084x-0.018$, $r=0.9992$). Table 1 shows the recoveries of TBHQ fortified with 100 ppm TBHQ. Added TBHQ was recovered in the range 93.1-99.5%. The limit of detection for TBHQ in food was 2 ppm.

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Keyword phrases

high performance liquid chromatography of *tert*-butylhydroquinone; *tert*-butylhydroquinone in vegetable oils, butter, and margarine; antioxidant for food.

(Received August 25, 1983)