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Analytical Methods Section

TECHNICAL NOTE

New spectrophotometric methods for the determination of di-*t*-butyl hydroquinone

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Three simple spectrophotometric methods have been developed for the determination of di-*t*-butyl hydroquinone in commercial samples and oils based on the formation of coloured species with Fe(III)-2,4,6-tripyridyl-*S*-azine (TPTZ) or triphenyl tetrazolium chloride (TTC) or IO$_4^-$/Mo(VI)/I$^-$/metol-sulphanilamide (SA).

INTRODUCTION

Di-*t*-butyl hydroquinone (TBHQ) is an antioxidant permitted to be added to edible oils in a concentration of 0·02% either individually or in combination as stated in the Prevention of Food Adulteration Act. India (PFA Act 1954, Rules 1955 as amended up-to 1984). Very few visible spectrophotometric methods have been reported for the estimation of TBHQ (Prasad *et al.*, 1988). The authors have now for the first time developed three simple, rapid, sensitive and accurate methods for the determination of TBHQ at the microgram level, utilizing the reagents Fe(III)-2,4,6-tripyridyl-*S*-azine (TPTZ), triphenyl tetrazolium chloride (TTC) and IO$_4^-$/Mo(VI)/I$^-$/metol-sulphanilamide (SA). The reducing property exhibited by TBHQ forms the basis of all the three methods. The oxidant in its reduced form (after reaction with TBHQ) is either self-coloured as in method B (Formazan from TTC; Lorentz & Hoffmeister, 1967) or forms a coloured complex with another reagent as in method A (Fe(II) with TPTZ after masking excess of Fe(III) with phosphoric acid; Tsen, 1961) and method C (IO$_3^-$ (via I$_2$ after the addition of I$^-$) from IO$_4^-$ with metol-SA after masking the excess of IO$_4^-$ with Mo(VI); Krishna *et al.*, 1980; Nishi & Tounsend, 1968). These methods are sensitive and are useful in purity assays as well as in the estimation of TBHQ in oils.

MATERIALS AND METHODS

Spectral absorbance measurements were made with a Systronics model 105 (MKI) spectrophotometer. A Systronics pH meter was used for all pH measurements (Naroda, Ahmedabad, India).

All the solutions were prepared in double distilled water or GR methanol using GR or CP grade chemicals. Aqueous solutions of TTC (0·3%), sodium hydroxide (1 M), sodium metaperiodate (0·1%) sodium molybdate (2%), potassium iodide (2%), metol (0·3%), SA (0·4%), potassium acid phthalate buffer (pH 3·0) and TBHQ (50 and 200 μg ml^{-1}, respectively), and methanolic solutions of TPTZ (0·312%), ferric chloride (0·27%) and ortho-phosphoric acid (2 × 10^{-2} M) were prepared.

Preparation of standard curves

Method A (Fe(III)-TPTZ)

To each of a series of 25 ml graduated tubes containing 5–100 μg of TBHQ, 0·5 ml of ferric chloride and 1 ml of TPTZ were added successively, the volume was

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brought to 8 ml with distilled water and kept in a boiling water bath for 15 min. The tubes were removed from the bath, cooled and 1 ml ortho-phosphoric acid solution was added, mixed well and diluted to the mark with distilled water. The absorbance of the violet-coloured complex was measured at 590 nm after 2 min and before 60 min against a reagent blank. The amount of TBHQ was computed from its calibration graph.

Method B (TTC-NaOH)

TTC (2 ml) was added to a series of 60 ml Pyrex separating funnels containing 25–300 μg of TBHQ and kept aside for 5 min. Then 1 ml of 1 M NaOH and 10 ml of *n*-butyl alcohol were added and kept aside for 10 min with occasional shaking. The absorbance of the *n*-butyl alcohol layer was measured at 480 nm within 60 min against a reagent blank. The quantity of TBHQ was computed from the standard graph.

Method C (IO_4^-/Mo(VI)/I^-/metol-SA)

Buffer solution (pH 3·0, 15 ml) and sodium metaperiodate (1 ml) were added to a series of 25 ml graduated tubes containing 50–600 μg of TBHQ and kept for 15 min. Then 1 ml of sodium molybdate was added, and after 10 min 2 ml of potassium iodide was added. After a further 5 min, 1 ml of metol and 1 ml of sulphanilamide were added successively with an interval of 2 min. The total volume was made up to the mark with distilled water in each case. The absorbances were measured at 520 nm during the stability period (10–30 min) against a reagent blank prepared under similar conditions omitting TBHQ. The amount of TBHQ was computed from its calibration curve.

Determination of TBHQ in oils

Ten grams of oil containing TBHQ were initially dissolved in 100 ml of petroleum ether and extracted with four 20 ml portions of acetonitrile. The combined acetonitrile extract was evaporated to dryness and the residue was then dissolved in an appropriate volume of aqueous methanol (1:1, v/v). The recommended procedure was then followed for estimation of TBHQ.

Table 1. Optical characteristics, precision and accuracy of proposed methods for TBHQ

	Method A	Method B	Method C
Beer's law limits (μg ml^{-1})	9·2–4·0	2·5–30·0	2·0–24·0
Molar absorptivity (l mole^{-1} cm^{-1})	4·44 × 10^4	4·84 × 10^3	5·55 × 10^3
Regression equation A			
Slope (b)	0·1988	0·0216	0·0249
Intercept (a)	0·0038	0·0049	0·0033
Correlation coefficient	0·9999	0·9999	0·9997
% RSD	1·05	0·93	1·28
% Range of error + (confidence limits)			
0·05 level	1·07	0·95	1·31
0·01 level	1·74	1·54	2·12

* From six determinations

Table 2. Recovery of TBHQ from oils by the proposed and reference methods

Sample	Amount of TBHQ added (mg)	Per cent recovery			
		Proposed methods			Reference method
		A	B	C	
Coconut oil	10	99·8	99·7	99·8	98·20
Sunflower oil	10	98·8	99·6	99·8	98·60
Groundnut oil	10	99·2	98·9	99·3	99·10
Cotton seed oil	10	99·4	99·4	99·1	98·90

RESULTS AND DISCUSSION

A study of the effect of concentration of reagents and other parameters in each method with respect to maximum sensitivity and stability, minimum blank and obedience to Beer's law led to the procedures. The optical characteristics such as Beer's law limits, molar absorptivity and regression data obtained from linear least squares treatment such as slope, intercept and correlation coefficient are given in Table 1. The per cent relative standard deviation and per cent range of error obtained from six replicate samples are also given in Table 1.

Comparison of the values of the recovery experiments of TBHQ in various oils with those from methods proposed and reported by Prasad *et al.* (1988) reveals good recovery and accuracy (Table 2).

The proposed method is sensitive and reasonably accurate. Since the three proposed methods are based on the reducing properties of TBHQ, other antioxidants with similar reducing properties such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) interfere with the determination of TBHQ by the proposed methods. The close similarity in the solubility properties of these antioxidants and TBHQ frustrated the attempts to remove the interferences by selective solvent extraction procedures. Preliminary separation by TLC is therefore necessary for the estimation of TBHQ in such samples. Good separation of PG and BHA from TBHQ was achieved using Silica Gel G–Kieselguhr (2 : 1) impregnated with EDTA as stationary phase and a mixture of chloroform/xylene/methanol (8 : 2 : 1, v/v/v) as the mobile phase. The R_f values are found to be 0·37, 0·59 and 0·91 for TBHQ, PG and BHA, respectively, in the above system. Separation of BHA, BHT and TBHQ using Silica Gel as stationary phase and *n*-hexane/acetic acid (6 : 1, v/v) as the solvent has also been reported (Nakazato *et al.*, 1980).

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