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Spectrophotometric method for determination of butylated hydroxyanisole in oils

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Abstract

A simple and sensitive spectrophotometric method for the determination of butylated hydroxyanisole was developed. This method is based on oxidative coupling reaction between butylated hydroxyanisole with orcinol in the presence of hydrogen peroxide and enzyme horseradish peroxidase to produce colored product, which is measured spectrophotometrically at 420 nm. The color was stable for 15 minutes. Beer's law was valid within a concentration range of 5-25 µg/ml. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common excipeints. The validity of the method was tested by analyzing butylated hydroxyanisole in oils. Good recoveries were obtained. This method is successfully employed for the determination of butylated hydroxyanisole in oils.

Key words: Butylated hydroxyanisole, orcinol, visible spectrophotometric determination, Beer's law.

INTRODUCTION

Butylated hydroxyanisole is a mixture of the isomeric oraganic compounds, 3-tert-butyl-4-hydroxyanisole and tert-butyl-4-hydroxyanisole. Butylated hydroxyanisole[1] is prepared from tertiary butyl hydroquinone, dimethyl sulphate and sodium hydroxide. It is a waxy solid that exhibits antioxidant properties. Butylated hydroxyl anisole prevents food from becoming rancid and exhibits antioxidant properties in fat containing foods, cosmetic formulation, as a scavenger of free radicles[2] and synergism with acids, butylated hydroxyl toluene, propyl gallate, hydroquinone, methionine, lecithin and thio propionic acid. Butylated hydroxyl anisole can easily applied to food items due to its outstanding solubility in fats and oils. Butylated hydroxyl

anisole adds excellent stability to food products, fats, oils, vitamins and pet foods. It is incompatible with oxidizing agents and ferric salts[3]. Butylated hydroxyl anisole is capable of activating mitogen-activated protein kinases, extra cellular signal-regulated protein kinase 2 and c-jun N-terminal kinase. Butylated hydroxyl anisole prevent loss of 11 beta hydroxlase acivity[4] in cultured bovine adrenocortical cells. Butylated hydroxyanisole stabilizes the petroleum wax coatings of food packaging[5]. Butylated hydroxyanisole blocks the inhibitory effects of tumor necrosis factor ∝ on collagen production in human dermal fibroblasts[6].

The reported methods in the literature for the determination of butylated hydroxyanisole are spectrophotometric methods[7-11], capillary electrophoresis[12], HPLC[13-16], micellar electro kinetic chromatography[17], T format luminescence spectrometer[18] and voltametric methods[19]. Among the various methods available for the determination of butylated hydroxyanisole spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents a new spectrophotometric method for the determination of butylated hydroxyanisole in oils.

MATERIALS AND METHODS

Spectral and absorbance measurements were carried out by using Systronics UV – Visible Double beam spectrophotometer model 2201. Systronics digital pH meter was used to adjust and determine the hydrogen ion concentration (pH) of the solutions. Remi desktop centrifuge with 24,000 rpm for the extraction of horseradish peroxidase (HRP). Homogenizer with a high speed blender 3-4 x 15 sec. for homogenization of Horseradish root.

Materials and Reagents: All materials and reagents were of analytical grade and double distilled water was used. Pure form of butylated hydroxyanisole from Merck
Orcinol solution (0.3% w/v): Prepared by dissolving 300 mg of Orcinol in 100 ml reagent grade distilled water.
Hydrogen peroxide (0.01M): Prepared by dissolving 0.10 ml of 30% H_20_2 in 200ml of reagent grade distilled water just prior to experiments.
Phosphate buffer (0.1M, pH-7.0): Potassium dihydrogen phosphate –di sodium hydrogen phosphate buffer was prepared as follows.

Stock Solutions for buffer:
a. 0.5 M KH_2PO_4 solution: 68.04g of KH_2PO_4 is dissolved in 1 liter of reagent grade distilled water.
b. 0.5 M Na_2HPO_4 solution: 71g of Na_2HPO_4 is dissolved in 1 liter of reagent grade distilled water.
39 ml of 0.5 M KH_2PO_4 + 53.6 ml of 0.5 M NaH_2PO_4 were diluted to 1000 ml at 25^0C.

Standard and Sample solution of butylated hydroxyl anisole:
About 100 mg of butylated hydroxyl anisole was accurately weighed and dissolved in 100 ml of alcohol in a volumetric flask to make a solution of 1 mg/ml standard solution and further dilutions are made with the same solvent.

Extraction of the enzyme (Horseradish Peroxidase):
A turnip (Horseradish root) weighing 40 g was Peeled, washed, and cut into 1" cubes. The sliced pieces were homogenized in 200 ml of buffer in a blender at high speed for 15 minutes. The extract is clarified by centrifugation (10-15,000 rpm/ 10 min.) and filtered through Whatman No. 1 filter paper. The extract for stability was stored in toluene for at least a week at 4°C. The extract was suitably diluted for further experimental analysis

Assay Procedure:
Procedure for the determination of butylated hydroxyanisole in pure form:
Into a series of 25 ml calibrated test tubes, 15 ml buffer (pH 7.0) solution, 2 ml of reagent (orcinol), 1 ml of hydrogen peroxide (0.01 M) and 2 ml horse radish root solution (1:1diluted) and aliquots of standard antioxidant (butylated hydroxyanisole) solution, were added and made up to the mark with distilled water. The absorbance was measured after complete color formation at λ_{max} of 420 nm against reagent blank. The amount of antioxidant was computed from the calibration graph and the results were incorporated in Table-1**.** This method could also be extended for the recovery of butylated hydroxyl anisole in edible oils and fats.

Procedure for the determination of butylated hydroxyanisole in edible oils:
10 gm of oil (coconut, groundnut, sunflower) was dissolved in 10 ml of petroleum and successively extracted with 5 x 15 ml aliquots of acetonitrile. The combined acetonitrile phase was diluted with water and reextracted with petroleum. The light petroleum was evaporated just to dryness and residue was dissolved in 50% (approximate volume of 1:1) ethanol in order to obtain the desired concentration of butylated hydroxyanisole. The concentration of butylated hydroxyanisole was determined by the proposed method.

RESULTS AND DISCUSSION

The proposed method for the determination of butylated hydroxyl anisole was based on the formation of the colored complex from oxidized orcinol and phenolic antioxidants (butylated hydroxyanisole) with hydrogen peroxide and peroxidase. The formed colored complex shows maximum absorbance at 420nm.

Investigation of Assay Parameters:
Order of addition of reactants: The suitable order or addition of reactants in the determination of butylated hydroxyl anisole for attaining maximum color and stability was buffer-orcinol-hydrogen peroxide-peroxidase enzyme- butylated hydroxyl anisole.

Effect of variation of temperature: All experiments and absorbance measurements were carried out at laboratory temperature ($28^o \pm 3^o$). At low temperatures (20^o) the time required for attaining maximum color is more. At high temperatures (35^o) the stability of the colored species is less. So laboratory temperature is preferred.

Effect of Reagent Concentration: 2 ml of 0.3% w/v orcinol was the most suitable concentration for the proposed spectrophotometric method.

Effect of pH: Different phosphate buffers with pH range of 5-8 were tried and pH 7 was the pH of choice for getting maximum absorbance.

Volume of buffer: 15 ml of buffer was needed to bring the suitable pH in 25 ml of solution.

Analytical data:
A linear correlation was found between absorbance and concentration. The correlation coefficients, intercepts, slopes, molar extinction coefficient, optimum photometric range and Sandell's sensitivity values of the proposed method were calculated and the results are incorporated in Table-1. The precision and accuracy were found by analyzing five replicate samples containing known amounts of the butylated hydroxyanisole and the results are summarized in Table-1. Thus the proposed method was sensitive and accurate.

Table: 1 Optical characteristics and statistical data of the regression equations for determination of butylated hydroxyl anisole using the proposed method

Parameters	Method
λ_{max} (nm)	420
Beer's law limit (µg/ml)	5-25
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.045
Molar absorptivity(Litre.mole-1.cm-1)	4.0 x 10^4
Optimum photometric range (µg/ml)	4.4-22.4
Time taken for color development (Min)	2
Stability of Color (min)	5
Regression equation	
Intercept (a)	0.056
Slope(b)	0.3256
Correlation coefficient®	0.9894

The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of butylated hydroxyl anisole was added to the oils and the nominal value of butylated hydroxyl anisole was estimated by the proposed method. No interference from the common excipients was observed. The results of the recovery studied are incorporated in Table-2.

Table: 2 Recovery of butylated hydroxyl anisole in various oils

Oil	Quantity of Butylated hydroxyl anisole added (µg)	% Recovery by proposed method
Coconut	10	97.6
Sunflower	10	98.3
Groundnut	10	97.4

CONCLUSION

The proposed method was quite simple. The proposed method has wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrophotometric method for the determination of butylated hydroxyanisole demonstrate that the proposed method

is accurate, precise and linear and thus can be extended for routine determination of butylated hydroxyanisole in oils and quality control analysis.

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