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Spectrophotometric Determination of Phenolic Antioxidants

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A simple and sensitive spectrophotometric method for the determination of gallic acid was developed. This method is based on oxidative coupling reaction between gallic acid with phenol in the presence of hydrogen peroxide and enzyme horseradish peroxidase to produce coloured product, which is measured spectrophotometrically at 420 nm. The colour was stable for 70 min. Beer's law was valid within a concentration range of 25-250 µg/25 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 gallic acid in oils. This method is successfully employed for the determination of gallic acid present in oils.

Key Words: Gallic acid, Phenol, Visible spectrophotometric determination, Beer's law.

INTRODUCTION

Gallic acid is 3,4,5-trihydroxybenzoic acid, monohydrate, an important natural antioxidant, that is obtained by the hydrolysis of tannins from tarapods. It is a clear crystalline compound found in many plants and can be prepared commercially by the hydrolysis of tannic acid with sulfuric acid. It exhibits excellent antioxidant activity in food and vegetable oils, especially in combination with ascorbyl palmitate. Gallate is mainly used as antioxidant[1] additive in fats, oleaginous foods and medicinal preparations[2] and to stabilize cosmetics, adhesives and lubricants, food packaging materials. The reported methods in the literature for the determination of gallic acid are oscillating chemical reaction using the analyte pulse perturbation technique[3], capillary electrophoresis[4], diffuse reflectance spectrometry on polyurethane foam[5], HPLC[6-8], RP-HPLC[9,10] and GS-MS[11]. A colorimetric[12] method has been reported for determination of gallic acid. Among the various methods available for the determination of gallic acid, 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 gallic acid in oils.

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EXPERIMENTAL

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 × 15 s for homogenization of horseradish root.

All materials and reagents were of analytical grade and double distilled water was used. Pure form of gallic acid from Merck. The phenol solution (0.05 % w/v) was prepared by dissolving 500 mg of phenol in 100 mL reagent grade distilled water. Hydrogen peroxide (0.01 M) was prepared by dissolving 0.10 mL of 30 % H_2O_2 in 200 mL of reagent grade distilled water just prior to experiments. Phosphate buffer (0.1 M, pH 7.0): Potassium dihydrogen phosphate-di sodium hydrogen phosphate buffer was prepared as follows.

Stock Solutions for buffer: 0.5 M KH_2PO_4 solution: 68.04 g of KH_2PO_4 is dissolved in 1 L of reagent grade distilled water. 0.5 M Na_2HPO_4 solution: 71 g of Na_2HPO_4 is dissolved in 1 L 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 ºC.

Standard and sample solution of gallic acid: About 100 mg of gallic acid was accurately weighed and dissolved in 100 mL of water 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 min. 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 one week at 4 °C. The extract was suitably diluted for further experimental analysis.

Assay procedure: Into a series of 25 mL calibrated test tubes, 15 mL buffer (pH 7.0) solution, 2 mL of reagent (phenol), 1 mL of hydrogen peroxide (0.01 M) and 2 mL horse radish root solution (1:1 diluted) and aliquots of standard antioxidant (gallic acid) solution, were added and made up to the mark with distilled water. The absorbance was measured after complete colour 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 gallic acid in edible oils and fats.

RESULTS AND DISCUSSION

The proposed method for the determination of gallic acid was based on the formation of the coloured complex from oxidized phenol and phenolic antioxidants (gallic acid) with hydrogen peroxide and peroxidase. The formed coloured complex shows maximum absorbance at 420 nm.

TABLE-1
OPTICAL CHARACTERISTICS AND STATISTICAL DATA OF THE REGRESSION EQUATIONS FOR DETERMINATION OF GALLIC ACID USING THE PROPOSED METHODS

Parameters	Method
λ_{max} (nm)	420
Beer's law limit (µg/25 mL)	25-250
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.111
Molar absorptivity (L mol^{-1} cm^{-1})	1.693 x 10^4
Optimum photometric range (µg/25 mL)	22-244
Time taken for colour development (min)	2
Stability of colour (min)	70
Regression equation	
Intercept (a)	-0.012
Slope (b)	0.4533
Corellation coefficient (R)	0.9995

Investigation of assay parameters

Order of addition of reactants: The suitable order or addition of reactants in the determination of gallic acid for attaining maximum colour and stability was buffer-phenol-hydrogen peroxide-peroxidase enzyme-gallic acid.

Effect of variation of temperature: All experiments and absorbance measurements were carried out at laboratory temperature (28 ± 3 ºC). At low temperatures (20 ºC) the time required for attaining maximum colour is more. At high temperatures (35 ºC) the stability of the coloured species is less. So laboratory temperature is preferred.

Effect of reagent concentration: 2 mL of 0.5 % w/v phenol 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.

Accuracy and precision of the proposed methods: 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 were incorporated in Table-1. The accuracy and precision of the proposed methods was established by measuring the content of gallic acid in pure form. Thus the proposed method was sensitive and accurate.

The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of gallic acid was added to the oils and the nominal value of gallic acid 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 GALLIC ACID IN VARIOUS OILS

Oil	Quantity of gallic acid added (μg)	% Recovery by proposed method
Coconut	10	98.7
Sunflower	10	97.7
Groundnut	10	98.4

Conclusion

The proposed method is quite simple and has wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrophotometric method for the determination of gallic acid demonstrate that the proposed method is accurate, precise and linear and thus can be extended for routine determination of gallic acid in oils and quality control analysis.

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