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A Novel Spectrophotometric Method for Determination of Propyl Gallic Acid by Oxidative Coupling with Orcinol

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A simple, sensitive and reproducible spectrophotometric method is developed for the determination of propyl gallic acid. This method is based on oxidative coupling reaction between propyl gallic acid with orcinol in the presence of Hydrogen peroxide and enzyme horseradish peroxidase to produce colored chromogen (λ_{max} at 550 nm). Results of analysis were validated statistically and by recovery studies. This method is successfully employed for the determination of propyl gallic acid in oils.

Key words: Propyl gallic acid, orcinol, Visible Spectrophotomety, Beer's Law.

Propyl gallic acid [1-8] is "Propyl ester of gallic acid, n- propyl ester of 3,4,5-trihydroxy benzoic acid, propyl 3,4,5-trihydroxy benzoate" is an important naturally derived antioxidant. Propyl gallate is made from natural gallic acid, which is obtained by the hydrolysis of tannins from Tara pods. It is a fine, white to nearly white powder. It exhibits excellent antioxidant activity in food and vegetable oils, especially in combination with ascorbyl palmitate[9, 10]. Propyl gallate is mainly used as antioxidant additive in fats, oleaginous foods[11] and medicinal[2] preparations and to stabilize cosmetics, adhesives, and lubricants, food packaging materials. It is used to prevent rancidity in oily substances. Exploiting the various functional groups present in the above compounds, the authors have made attempts in this direction and succeeded in developing a spectrophotometric method for the determination of propyl gallic acid to produce colored chromogen (λ_{max} at 550 nm).

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EXPERIMENTAL

Instrumentation

Spectral and absorbance measurements are made with Systronics UV-Visible Double beam spectrophotometer model 2201.

Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Freshly prepared solutions were always used. Aqueous solution of propyl gallic acid (0.1% w/v), orcinol (0.3 % w/v), hydrogen peroxide (0.01M), phosphate buffer (0.1

M, p^H 7.0) and extracted enzyme Horseradish Peroxidase were used.

Standard and Sample solution of Gallic acid

About 100 mg of Propyl gallic acid 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

Into a series of 25ml calibrated test tubes, 15ml buffer (pH 7.0) solution, 2 ml of reagent (orcinol), 1 ml of hydrogen peroxide (0.01M) and 2 ml horse radish root solution (1:1diluted) and aliquots of standard antioxidant solution, were added and made up to the mark with distilled water. The absorbance was measured after complete color formation at λ_{max} of 550 nm against reagent blank. The amount of antioxidant was computed from the calibration graph and the results were incorporated in Table 1**.** The proposed method is sensitive and accurate with reasonable precision and accuracy. The method could also be extended for the recovery propyl gallic acid in edible oils and fats.

RESULTS AND DISCUSSION

The optimum conditions for the color development was established by varying parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for the purpose and the conditions so obtained were incorporated in Table 1**.** The absorbance's at corresponding series of varying one and fixing the other three parameters (pH, concentration of reagent and enzyme (HRP)/H_2O_2 concentrations) containing in a total volume of 25 ml are measured against corresponding blank in each case. Performed recovery experiment and percent recovery values obtained are listed in Table 2. Recovery experiment indicated the absence of interferences from the commonly encountered additives and excipients.

Table 1. Optical characteristics, precision and accuracy of the Proposed method for propyl gallic acid estimation

Parameters	Method
λ_{max} (nm)	550
Beer's law limit (µg/ 25 ml)	100 - 500
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.083
Molar absorptivity (Litre.mole^{-1}.cm^{-1})	2.0162 ×10^4
Optimum photometric range(µg/ 25 ml)	97 - 447
Time taken for Color development (Min)	5
Stability of Color (Min)	60

Table 2. Recovery of Propyl gallic acid in various oils

Oil	Quantity of propy gallic acid (µg)	% Recovery by Proposed method
Coconut	10	98.4
Groundnut	10	97.2
Sunflower	10	98.5

The 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 proposed method is sensitive and accurate. The method has been extended for the recovery of propyl gallic acid in edible oils and fats. Thus the proposed method is simple and sensitive with reasonable precision and accuracy. This can be used for the routine determination of propyl gallic acid in quality control analysis.

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