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VALIDATION OF NOVEL SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF PROPYL GALLIC ACID

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ABSTRACT

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 Folin-ciocaltaeu reagent in alkaline conditions in the presence of Hydrogen peroxide and enzyme horseradish peroxidase to produce purple colored chromogen (λ_{max} at 680 nm). Results of analysis were validated statistically and by recovery studies. This method could be successfully employed for the determination of Propyl gallic acid in oils.

Keywords: Propyl gallic acid, Reduction, Visible Spectrophotometric determination, Validation, Beer's Law.

INTRODUCTION

Gallic acid[1-5] is propyl ester of Propyl gallic acid, n- propyl ester trihydroxy benzoic acid propyl 3, 4, 5-trihydroxy benzoate" Propyl gallate[1-5] is the most effective naturally derived antioxidant for the food industry. Propyl gallate is made from natural Propyl gallic acid, which is obtained by the hydrolysis of tannins from Tara pods. It exhibits excellent antioxidant activity in food and vegetable oils, especially in combination with ascorbyl palmitate. Propyl gallate is mainly used as antioxidant additive in fats, oleaginous foods and medicinal preparations and to stabilize cosmetics, adhesives, and lubricants, food packaging materials. It is used to prevent rancidity in oily substances. A careful review of the literature suggested the availability of very few GC- MS[6-8], HPLC[9, 10] and spectrophotometric [11- 16] methods. Thus by exploiting the various functional groups present in the structure of propyl gallic acid, 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 680 nm). The chemical structure of porpyl gallic acid is as shown below.

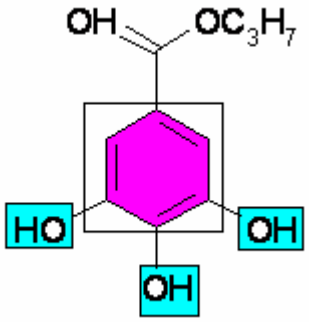

Fig-1: Structure of Propyl Gallic acid with reactive functional groups.

EXPERIMENTAL

Instrumentation

After the calibration of instrument, spectral and absorbance measurements are made using ELICO UV-Visible spectrophotometer model number FGSL159. The wavelength was scanned in the region of 440 to 750 nm and the wave length for maximum absorbance is noted.

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 solutions of Propyl gallic acid (0.1% w/v), FC reagent (1:2 diluted) hydrogen peroxide (0.01M), phosphate buffer (0.1 M, p^H 7.0), sodium carbonate (10% w/v) and extracted enzyme Horseradish Peroxidase were used.

Standard and Sample solution of Propyl gallic acid

About 100 mg of Propyl 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 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, 1.5 ml of 1 :2 diluted folin-ciocaltaeu reagent, 2 ml of 10 % Sodium carbonate solution, 1 ml of hydrogen peroxide (0.01M) and 0.5 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 680 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 of Propyl gallic acid in edible oils and fats.

RESULTS AND DISCUSSION

The results obtained in this method were due to reduction of the functional groups present in propyl gallic acid by phosphomolybdic, phosphotungstic acid present in F/C reagent followed by complex formation in the presence of dilute sodium carbonate solution.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. Recovery experiment was performed and percent recovery values obtained are listed in Table-2. Recovery experiment indicated the absence of interferences from the commonly encountered additives and excipients.

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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Table-1: Optical characteristics, precision and accuracy of the Proposed method for Propyl gallic acid estimation

Parameters	Method
λ max (nm)	680
Beer's law limit (μg/ml)	4-20
Sandell's Sensitivity (μg/cm^2/0.001 abs. unit)	0.01743
Molar absorptivity (litre.mole^{-1}.cm^{-1})	1.217x 10^4
Correlation coefficient I	9998.805
Regression Equation (Y)*	
Slope (a)	0.3313
Intercept (b)	0.00136
% RSD**	1.19
% Range of errors (95% confidence limits	
0.05 level of significance	± 0.99887
0.01 level of significance	± 0.1.4778

Table- 2: Recovery of Propyl gallic acid in various oils.

OIL	Quantity of Propyl gallic acid (μg)	% Recovery by Proposed method
Coconut	10	98.81
Groundnut	10	98.71
Sunflower	10	99.0

REFERENCES

1. R.J.Lewis,Sr. and R.L.Tatken, Eds. Registry of Toxic effects of Chemical substances. *Online Ed.National Institute.*
2. M.Hirose, H.Yada, S.Takahashi and N. Ito, *Carcinogenesis,* **14**, 2355 (1993)
3. B.Halliwell. The biomarker concept. *Nutr Rev*, **57**, 104 (1999).
4. M.F.Muldoon, S.B.Kritchevsky, *Brit Med J*, **312**, 458 (1996).
5. N.G.Lay-Keow, Pieue Lafotaine and Jean Hamns., *Journal of Chromatography A*, **29**,870, 1 (2000).
6. V.Viplava Prasad, K.Ekambareswara Rao and C.S.P. Sastry, *Food Chem.,* **17**, 209 (1985)
7. A. Soucy , *Health Canada, PSL, Project Report* on 2001-02, 2000-0568.

8. O.Martin.Coinceanainn and Michel.J.Hynes, *Journal of Organic Biochemistry*, **85**:2-3,131(2001); **84**, 192,1 (2001)
9. I.S.Bhatia, T.R. Madan and M.L.Nagpal, *Sci., Cult.,* **37**, 490 (1971).
10. C.S.P. Sastry, K. Ekambareswara Raoand, U.V.Viplava Prasad, *Talanta*, **99**, 917 (1982).
11. M.S. Abu-Bark, H.M. Rageh, E.Y. Hashem, M.H. Moustafa, Monatsh, *Chem.,* **125**, 11, 1197(1994)
12. H.J.Vos, H. Wesseles and Six, C.W.T. *Analyst.,* **82**, 362 (1957).
13. Sivilia E. Castillo- Blum and Norah Barba- Behrens, *Co-ordination Chemistry Review*, **3**, 1961, (2000).
14. Innocenzo C.Casella and Maria Gatta *Journal of Chromatography* A,**1**,1 827, (1998).
15. Gustavo.A.S.Pinto, Souni Couri, Elisabeth.B.Goncalves, , *EJEAF Chem..,* **5 (5)**, 1560(2006).
16. P.D. Jadhav, K.S. Laddha, *Indian Drugs,* **41(4)**, 200(2004).

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