*Biosciences, Biotechnology Research Asia* Vol. **5**(1), 491-492 (2008)

# Spectrophotometric methods for determination of Gallic acid by oxidative coupling with orcinol

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(Received: April 25, 2008; Accepted: June 18, 2008)

**ABSTRACT**

A simple, sensitive and reproducible spectrophotometric method is developed for the determination of gallic acid . This Method is based on oxidative coupling reaction between gallic acid with orcinol in the presence of Hydrogen peroxide and enzyme horseradish peroxidase to produce colored chromogen ($\lambda_{max}$ at 620 nm). Results of analysis were validated statistically and by recovery studies. This method is successfully employed for the determination of gallic acid in oils.

**Key words:** Gallic acid, orcinol, Visible Spectrophotometric determination, Beer's Law.

Gallic acid[1-7] is "3,4,5-Trihydroxybenzoic acid, monohydrate; gallic acid monohydrate" is 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[1]**.** It exhibits excellent antioxidant activity in food and vegetable oils, especially in combination with ascorbyl palmitate[8, 9]. Gallate is mainly used as antioxidant additive in fats, oleaginous foods and medicinal preparations and to stabilize cosmetics, adhesives, and lubricants, food packaging materials. 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 gallic acid to produce colored chromogen ($\lambda_{max}$ at 620 nm).

**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 solutions of 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 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 What man 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 $\lambda_{max}$ of 620 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 gallic acid in edible oils and fats.

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.

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 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 Gallic acid in quality control analysis.

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

| Parameters | Method |
|---|---|
| $\lambda_{max}$ (nm) | 620 |
| Beer's law limit (µg/ 25 ml) | 20 - 100 |
| Sandell's Sensitivity (µg/cm$^2$/0.001 abs. unit) | 0.166 |
| Molar absorptivity (Litre.mole$^{-1}$.cm$^{-1}$) | $1.128 \times 10^4$ |
| Optimum photometric range (µg/ 25 ml) | 16-94 |
| Time taken for Color development (Min) | 5 |
| Stability of Color (Min) | 60 |

**Table 2: Recovery of Gallic acid in various oils.**

| Oil | Quantity of Gallic acid (µg) | % Recovery by Proposed method |
|---|---|---|
| Coconut | 10 | 96.8 |
| Groundnut | 10 | 97.0 |
| Sunflower | 10 | 99.0 |

**ACKNOWLEDGEMENTS**

The authors are grateful to Managements of Siddhartha Academy, Vijayawada and K.L.C.E. Vaddeswaram for their continuous support and encouragement and for providing the necessary facilities.

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