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Determination of Gallic acid from their Methanolic Extract of Punica granatum By HPLC Method

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Abstract: A simple and rapid analytical method for high performance liquid chromatographic (HPLC) procedure based on isocratic elution with UV detection has been developed for the determination of gallic acid from their methanolic extract of punica granatum. Their quantitative estimation from the solvent extracts is a great challenge. Analysis was carried out using Waters' symmetry C-18 column with methanol: ethyl acetate: water (25:5:70) as isocratic elution mode with UV detection (λ=270 nm). The method is pretty linear for Gallic acid in the range of 0.01-0.1 mg/ml (R2 = 0.996). The method was validated by mixing these acids standards in methanol and found that it is accurate, sensitive and has a good reproducibility.
Key-words: Punica granatum, Gallic acid, HPLC.

Introduction

Pomegranate (Punica Granatum L.), a species of punicaceae, has recently become of great interest to the scientists who engage themselves in pharmaceutical, neutrological and pharmacological research, and new drug development [1].The extract from pomegranate peel is the most important in the field of Pharma industries. The flavonoids and tannins such as gallic acid isolated from extract of punica granatum peel. Gallic acid is an interesting natural compound because of its antioxidant, anti-inflammatory, antifungal and antitumor properties [2]. Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid. The physical properties for gallic acid are: chemical formula $C_6H_2(OH)_3COOH$, molecular weight 170 and boiling point 250 °C. The structure of gallic acid is shown in Figure 1 [3]. The determination of this compound in fruit juices using LC with gradient elution has been reported [4,5]. Development and validation of RP-HPLC method for identification of Gallic acid in Triphala churna has been reported [6].This paper reports determination of methanolic extract of Gallic acid from pomegranate (punica granatum) peel by isocratic elution high performance liquid chromatographic method with UV detection.

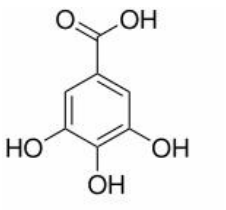

Figure 1: Structure of Gallic Acid

Materials and Method

Materials:

The Peel of pomegranate (Punica granatum) were collected locally (Lonere, Raigad district of Maharashtra, India). Peels were washed with water and shade dried (at room temperature for 4 or 5 days) and powdered. The powdered mass was then sorted using screening technique and 0.106-0.420 mm size was selected for further experiments. Standards of Gallic Acid (99%), obtained from suppliers of nearby place were used for calibration. Solvents like Methanol, Ethyl Acetate and Water of HPLC grade used for High Performance Liquid Chromatography (HPLC) analysis were obtained from S.D. Fine Chemicals, India.

Instrumentation:

Standards of Gallic acid dissolved in methanol and all unknown samples of methanolic extract were analyzed by HPLC system of Waters' (USA). The system consists of Waters HPLC 600 pump, a Rheodyne 7725i injector with 5 µl sample loop, on-line degasser AF (Waters, USA), Waters 2487 Dual λ absorbance UV detector (at λ=270 nm, for Gallic acid) with 0.01 aufs sensitivity. Gallic acid were analyzed using a Symmetry C-18 (4.6 × 250 mm, 5 µm) column equipped with automatic temperature (± 0.1°C) controller module. An isocratic mobile phase of Methanol: Ethyl acetate: Water (25:5:70 v/v) with an elution volume of 0.7 ml/min was selected for identification of acids. The column temperature was maintained at 37°C (± 0.1°C).

Gallic acid calibration:

Standard solutions of Gallic acid (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/ml) were prepared in methanol. The solutions were filtered through 0.2 µm (PALL Ultipor N66) membrane filter and analyzed in HPLC. Evaluation of each point was repeated thrice. The area under peak of Gallic acid vs. concentration plot shows a linear fit with correlation coefficient of 0.996 as shown in Figure 2. The same calibration chart was subsequently used for quantification of unknown samples.

Preparation and analysis of extract of punica granatum peels:

8 gm of shed dry and crashed peels (particle size 0.106 to 0.420 mm) was subjected to fully baffled 300 ml stirred borosilicate glass vessel (8 cm ID, and 6.5 cm height) along with methanol (200 ml) as solvent. A four blade turbine type agitator (2 cm diameter) was used for stirring at 700 rpm to ensure all particles remain in suspended condition. Extraction of Gallic acid was carried out at 45°C (± 1°C) for 2 hrs. The schematic experimental setup is as shown in Figure 3. The samples were collected at fixed interval of time. The collected samples were filtered through 0.2 µm membrane filter and analyzed in HPLC system for determination of recoverable Gallic acid from their mixtures.

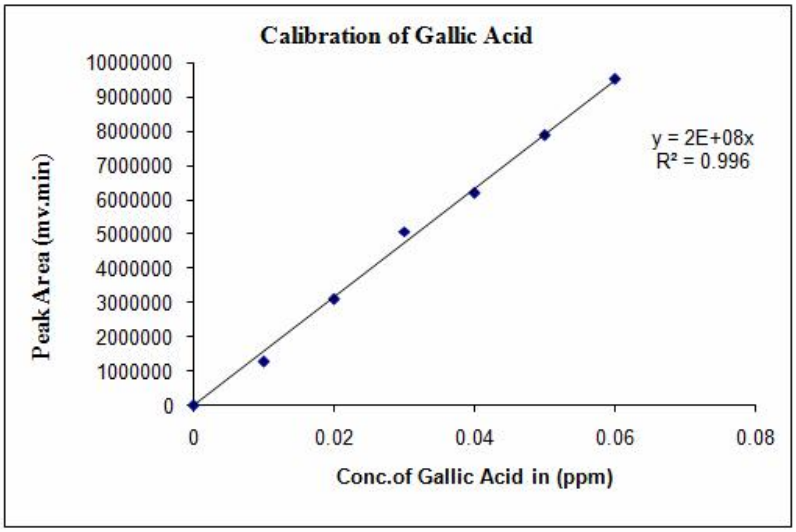

Figure 2: Calibration curve for Gallic Acid by HPLC

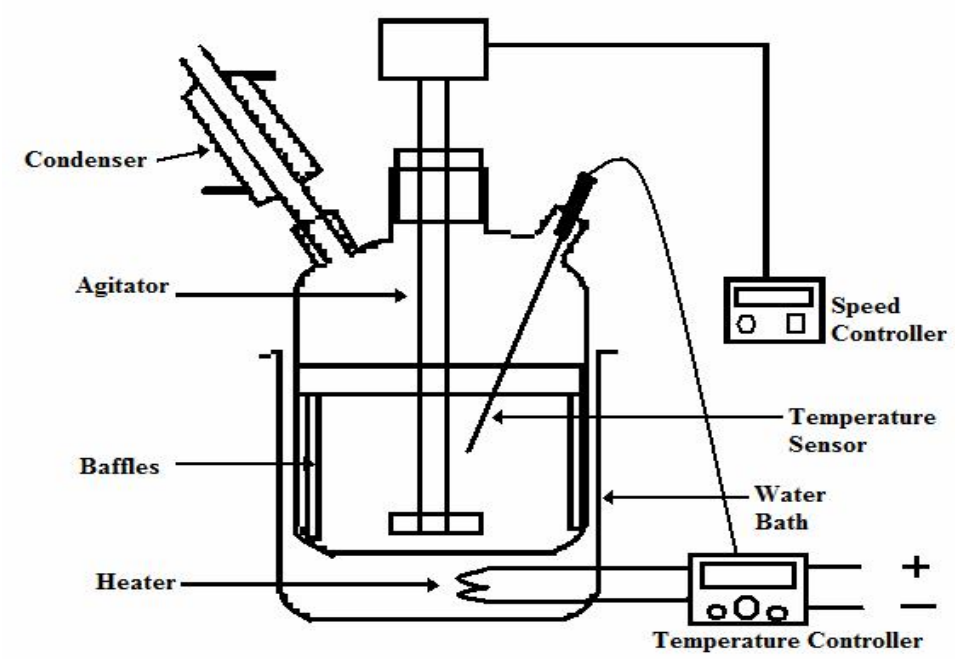

Figure 3: The schematic experimental setup for batch extraction

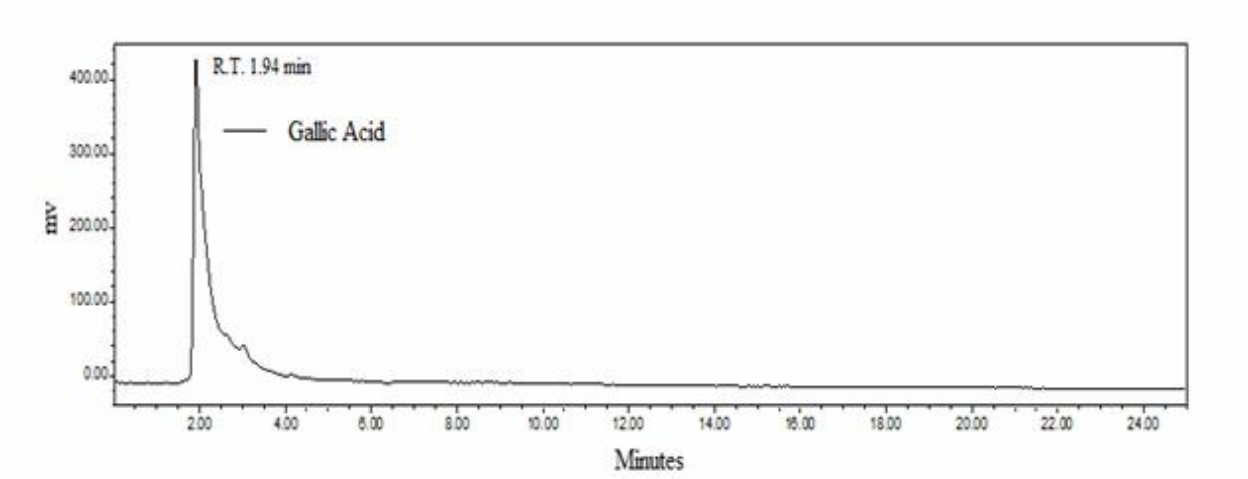

Figure 4: The Chromatogram of Standard of Gallic Acid at Methanol : Ethylacetate : Water = 25:5:70 composition with 0.7 ml/min flowrate.

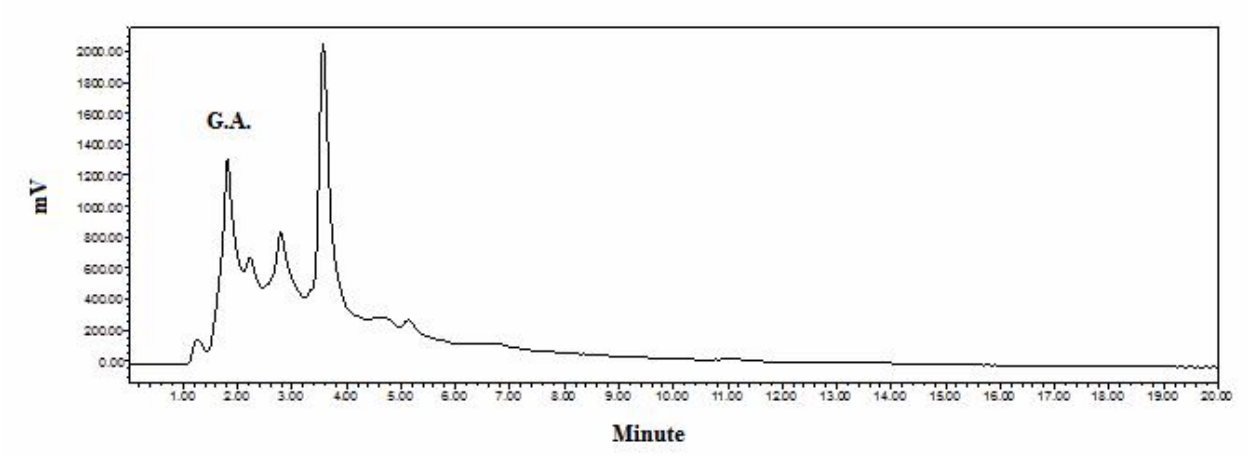

Figure 5: The Chromatogram of methanolic extract of punica granatum at Methanol : Ethylacetate : Water = 25:5:70 composition with 0.7 ml/min flowrate.

Results and Discussion

Determination of Gallic acid from methanolic extract:

After changing different mobile phases with C18 column and UV detection, optimum chromatographic conditions were obtained. The reported HPLC method was found suitable for Gallic acid identification and quantification simultaneously. In this method, Methanol: Ethyl acetate: Water (25:5:70 v/v) was used as mobile phase with 0.7 ml/min elution flow rate, λ=270 nm UV wavelength with 0.01 aufs sensitivity. Symmetry C-18 (4.6 × 250 mm, 5 µm) column was used for quantification and identification of Gallic acid. The HPLC chromatograms for standard Gallic acid and methanolic extract of punica granatum peels using the developed method are shown in Figure 4 and Figure 5.

The retention time for Gallic acid is 1.94 min (± 0.2 min). The method developed certainly indicates improved resolution of peaks and better selectivity.

Batch extraction of Gallic acid from peels:

The batch extraction experiment was carried out with methanol as solvent and at 45°C to extract the Gallic acid from peels of punica granatum. The collected samples were analyzed with proposed HPLC method to determine the Gallic acid content. Figure 6 shows, how the concentration of Gallic acid increases with increase in extraction time. This increase in concentration is due to diffusion mechanism of solute (Gallic acid) in solvent (methanol).

This experiment was carried out to verify the validity of the proposed method. It is found that the proposed HPLC method is valid for determination of Gallic acid from peels of punica granatum.

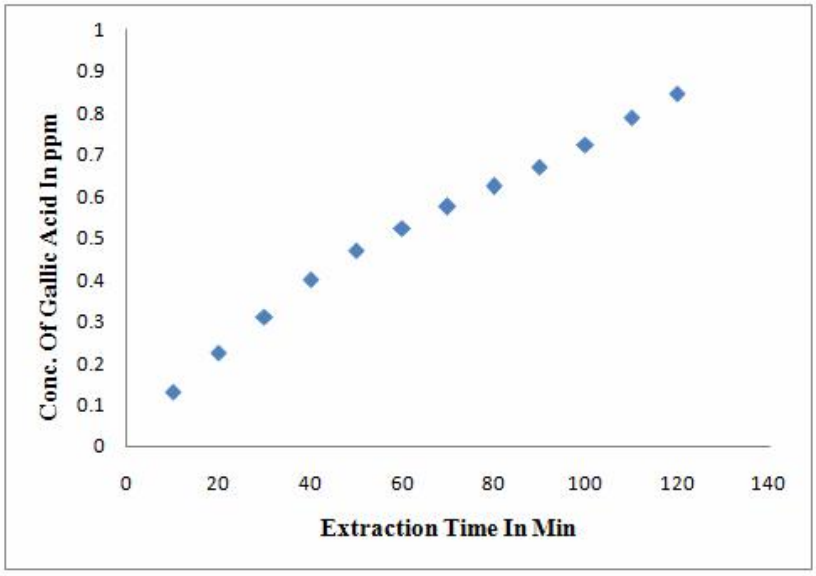

Figure 6: HPLC method validation plot for Gallic Acid

Method Validation For HPLC:

The HPLC method was validated by selectivity, linearity and range. Linear correlation was obtained between peak area and concentration of Gallic acid in the range 0.01-0.06 mg/ml. The value for regression coefficient was found 0.996 and the linearity equation shows the method is valid for the given range of concentrations. The results obtained are shown in Table 1.

Table 1: Regression parameters, Linearity and range of the proposed HPLC method

Sr. No.	Parameters	Gallic Acid
1	Linearity range	0.01-0.06 mg/ml
2	Regression equation Y = mx + c*	08X+2E
3	Correlation coefficient	0.996

Conclusions

The method for determination of Gallic acid from Peels of punica granatum developed on HPLC is suitable one. It also showed linearity for Gallic acid at different concentrations. From the batch extraction experiment results, the method is accurate, sensitive and has a good reproducibility.

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