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Deepika Sharma*
Department of Pharmacology, K.N Modi University, Newai, Tonk, Rajasthan 304021, India

Yash Paul Singla
Lord Shiva College of Pharmacy, Sirsa, Haryana 125055, India

Correspondence:
Deepika Sharma
Department of Pharmacology, K.N Modi University, Newai, Tonk, Rajasthan 304021, India
Tel: +91 9953244865
E-mail: deepika26jul@yahoo.com

Analysis of Gallic acid and 4-Hydroxy benzoic acid in *Prosopsis cineraria* leaf extract using High Performance Liquid Chromatography

Deepika Sharma, Yash Paul Singla

Abstract

A simple and fast method to determine gallic acid and 4-Hydroxy benzoic acid in hydroalcoholic extract of *Prosopsis cineraria* was established by liquid chromatographic (HPLC). On Agela XBP-C18 (5 µm, 4.6 mm ×150 mm) column, a multistep binary gradient elution program and a simplified sample pretreatment approach were used in the experiment. The method has been validated with respect to various parameters of performance quality including computation regression analysis based on calibration curves, peak resolution factor, asymmetry factor, tailing factor, RSD (%) of retention time and peak area response, limit of quantitation, limit of detection, precision and recovery. The developed method has been applied to the analysis of leaf of *Prosposis cineraria* for gallic acid and 4-hydroxy benzoic acid content.

Keywords: *Prosopis cineraria*, Gallic acid, 4-Hydroxy benzoic acid, High performance liquid chromatography (HPLC).

Introduction

Prosopis cineraria (Mimosaceae) is a small to moderate sized tree found in the regions of Arabia and various parts of India such as Rajasthan, Gujarat, Haryana, Uttar Pradesh and Tamil nadu. This plant is used in pregnancy as a safeguard against miscarriage. The smoke of the leaves is good for eye troubles. The bark is used as a remedy for rheumatism, cough, common cold asthma and scorpion stings.[1, 2] A new piperidine alkaloid spicigerin, prosogerin E along with gallic acid, pautelin, luteolin and rutin.[3] Prosogerin A and B were isolated from flowers.[4] The antimicrobial activity of ethyl acetate and hydroalcohoic extracts of stem bark was proved.[5]

So far, limited efforts have been made to develop an analytical HPLC profile of the major constituents of *P. cineraria*. The HPLC methods reported earlier[6-8] are limited to a few markers. Also, the methods lack baseline resolution and/or their reported analyses times are quite high. Therefore, we have developed a reliable and efficient HPLC method for the analysis of the gallic acid and 4- hydroxy benzoic acid.

The aim of the present work was to identify the content of gallic acid and 4- hydroxy benzoic acid in hydroalcoholic extract of *P. cineraria*.

Material and Methods

Reagent and Materials

For carrying out HPLC, the standards were purchased from Qualikems Fine Chem Pvt. Ltd., Vadodara (India).

Sample Preparation

Fresh leaves of *Prosopsis cineraria* was collected weight, washed with tap water. Leaves were then dried in shade. After drying leaves were powdered, powdered leaves were mixed with distilled water and ethanol in the ratio of 50:50. Hydroalcoholic extracts of the plant were prepared using Soxhlet apparatus for 2 hrs. The extracts were then fed into rotary evaporator to remove the solvent (ethanol) and the dried extract was stored at -5°C.[9]

Preparation of Standard Solutions and Method Validation

Standard Solutions and Calibration Curves

For quantitation, an external standard method was utilized. Peak areas from the HPLC chromatogram were plotted against the known concentrations of stock solutions at varying concentrations. Equations generated by linear regression were used to establish concentrations for herbal medicines and standard solutions.

About 10 mg of a standard of each kind of phenolic acid weighed accurately was dissolved into a 10 mL volumetric flask in 1:1 MeOH/ water to obtain stock solutions. For calibration curves, the stock solution was diluted with 1:4 MeOH/ water to obtain the concentration sequence. The linear range and the equations of linear regression were obtained through such a sequence of 50, 20, 10, 5, 2 and 1µg/L. Mean areas (n) 6 generated from the standard solutions were plotted against concentration to establish calibration equations.

Detection Limits

For the evaluation of detection limits, both the phenolic acids were dissolved into a 10 mL volumetric flask in 1:1 MeOH/ water to obtain stock solutions and a concentration sequence of 50, 20, 10, 5, 2 and 1µg /L of the standards was obtained by diluting these stock solutions. The mean value of the signal-to-noise ratio [(n) 4] generated from the solution that just caused more than 3 times S/N ratio was used to calculate the detection limit [(S/N) 3] of the corresponding phenolic acid.

Repeatability

The standard solutions of 1 mg/L, which were near the concentration of those constituents in the herbal drug, were used to achieve repeatability testing for intraday and interday [(n) 5]. The data used to calculate relative standard deviation (RSD) percent of interday repeatability was the mean value of three injections in succession. And the repeatability of peak area of drug extract was also validated in the experiment.

Recovery

For the recovery of the main constituent, gallic acid and 4-hydroxyl benzoic acid, in *P. cineraria* sample, stock solution containing 200 mg of chlorogenic acid was added into 0.5 g of desiccated and triturated *P. cineraria* sample and, after evaporation of solvent, was extracted with 100 mL of solvent.

HPLC analysis

HPLC analysis was performed on an Agilent 1200 series and separations were achieved using a C18 (250*4.6mm 5µ) Shodex column subjected to gradient elution. The two solvents used for the analysis namely Mobile Phase A consisting of 800 ml of water + 200 ml of buffer i.e 11.5 gm H3PO4 + 1000 ml water (pH2.5 with ammonia) and Mobile Phase B consisting of 250 ml Buffer+ 750 ml methanol. Gradient programming of the solvent system was carried out at 27°C and was: initially at 100% A, changed to 0% A at 30.0 min, maintained for the next 5.0 min, changed to 100% A at 35 min, and then maintained to 100% A at 40.0 min at a flow-rate of 1 mL/ min and then at a flow rate of 1.0 mL/min and this solvent composition was maintained until the run time reached 40 min. The wavelength was set at 280 nm.

Results

Gallic acid and 4-hydroxy acid are the most abundant phenolic acids present in medicinal plants. These two are of interest because of their pharmacological actions. Analysis of gallic acid and 4-hydroxy benzoic acid from the hydroalcoholic *Prosopis cineraria* leaf extract was carried out based on chromatographic separation. In the present study phenolic acids were analysed at 280 nm using peak area comparison to a calibration curve derived from standard plots of 4-hydroxy benzoic acid and gallic acid shown in Figures1 and 2 respectively.

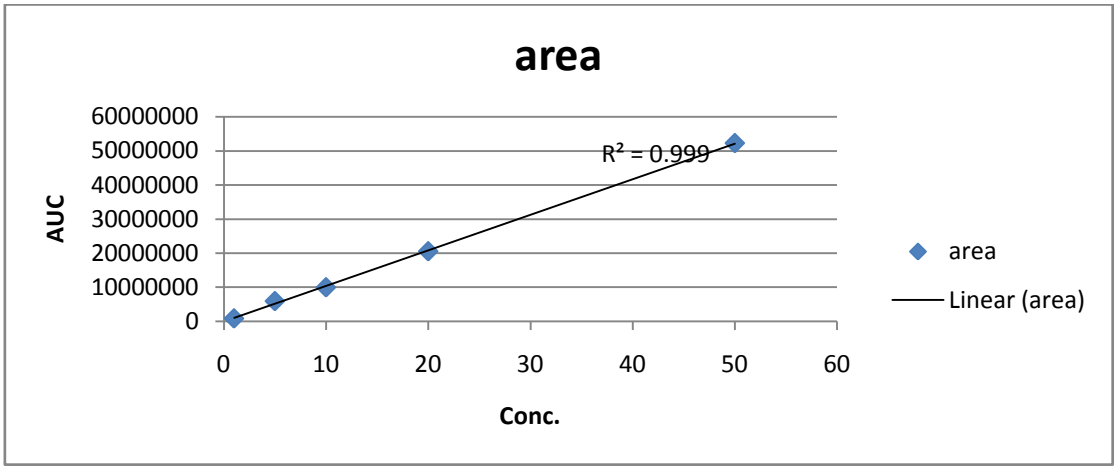

Figure 1: Standard curve plot of 4-Hydroxy Benzoic acid

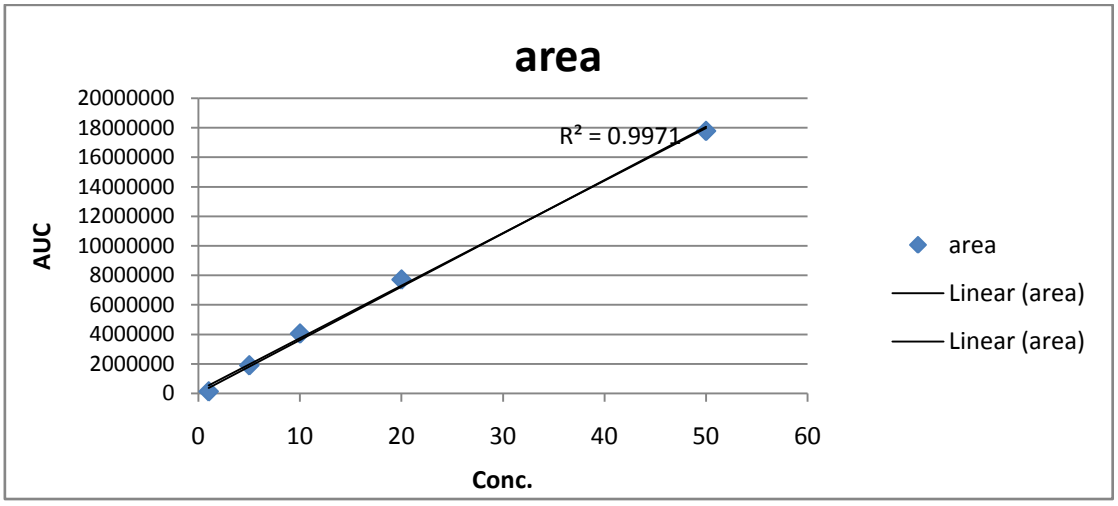

Figure 2: Standard curve plot of Gallic acid

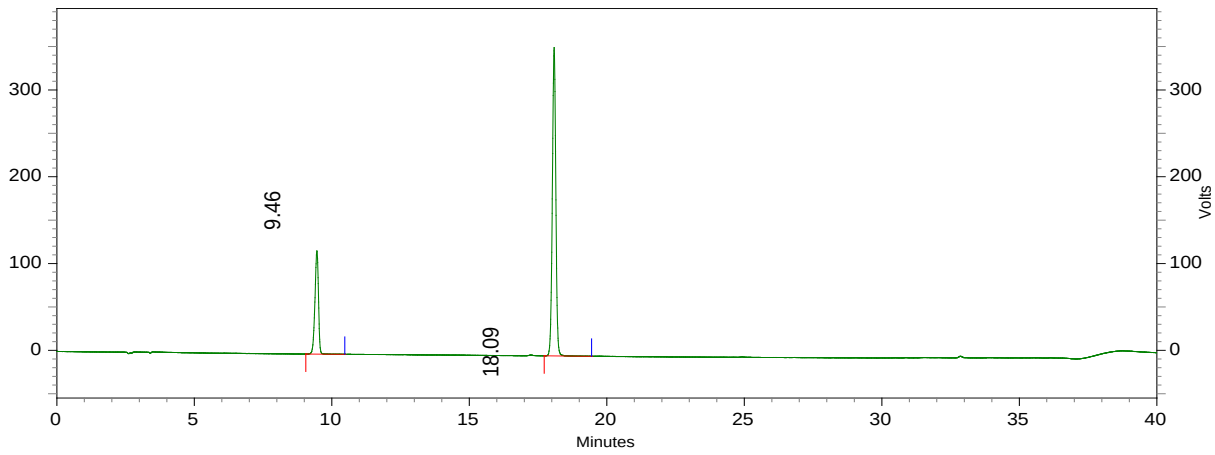

Figure 3: Standard gallic acid and 4-hydroxy benzoic acid

Table 1: Shows the peak area and retention time of Standard drugs

S. No.	Standard Chemical Constituents	Retention Time	Peak Area
1.	Gallic acid	9.46	17784835
2.	4-hydroxy Benzoic acid	18.09	52228838

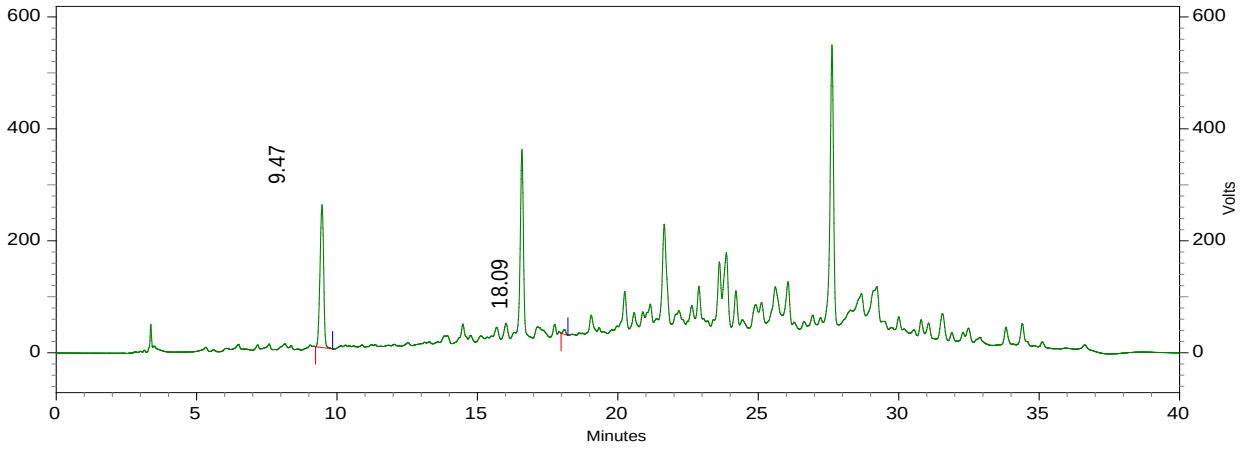

Figure 4: Hydroalcoholic extract of *Prosopsis cineraria* containing gallic extract and 4-hydroxy benzoic acid

Table 2: Shows the peak area and retention time of Hydroalcoholic extract of *Prosopsis cineraria* containing gallic extract and 4-hydroxy benzoic acid

S. No.	Extracts	Retention Time	Peak Area
1.	Gallic acid	9.47	38476525
2.	4-Hydroxy Benzoic acid	18.09	925251

HPLC chromatograms of standard 4-hydroxy benzoic acid and gallic acid were shown in Figure 3. Retention time recorded for 4-hydroxy benzoic acid and gallic acid was 18.09 and 9.46 respectively (Table 1). From the calibration curve results, the amount of gallic acid and 4-hydroxy benzoic acid, in the sample injected was calculated. In the present investigation, gallic acid and 4-hydroxy benzoic acid was identified based on the absorbance at 280 nm for both samples and standard. Figure 4 shows the HPLC peaks of hydroalcoholic extracts of *Prosopis cineraria* containing gallic acid and 4-hydroxy benzoic acid whereas the peak area and retention time of the extract containing gallic acid and 4-Hydroxy benzoic acid were shown in Table 2. Hence the present method was applicable in the analysis of gallic acid and 4-hydroxy benzoic acid in any plant material using HPLC technique.

Discussion

The HPLC parameters for the analysis were selected after screening the previously reported solvent systems, gradient profiles and adsorbents. The isocratic methods reported for gallic acid and 4-hydroxy benzoic acid were not considered suitable for the analysis, as the sample matrix was too complex and could not be reliably analysed by isocratic systems. Hence the binary gradient method was applied to enable discrete resolution and unambiguous quantification that could preclude overlapping of peak of the above two. After trials of different compositions of methanol– water as mobile phase and use of different gradient shapes of mobile phase solvents to resolve the compounds in the standard mixture of the leaf extract, the complete resolution could be achieved using methanol–water as the solvent system under time-programmed gradient conditions starting (0 min) with solvent A (800 ml water containing 200 ml buffer viz.,11.5 gm H3PO4 in 1000 ml water) at 100% and solvent B (750 ml methanol containing 250 ml buffer) at 0%, changing to 0% A and 100% B at 30.0 min, holding the mobile phase composition for the next 3.0 min followed by changing to 0% A at 33min, and then to 100% A at 35.0 min with a flow rate of 1.0 mL/min, and finally maintaining this composition until the run-time reached 40 min. Standard calibration curves were obtained for 4-hydroxy benzoic acid (Figure 1) and gallic acid (Figure 2). Under these HPLC conditions, the mean retention times (Rt) for gallic acid and 4-Hydroxy benzoic acid respectively, were 9.47 and 18.09 min at the detection wavelength of 280 nm for gallic acid and 4–Hydroxy benzoic acids (Figure 3).[10] The validity of the method with reference to these compounds was confirmed by comparing their on-line UV spectra with those of the reference compounds using library matching. Also, the full identity of the HPLC-PAD chromatograms was taken as evidence for the compositional homogeneity of the peaks resolved by the HPLC method.

Conclusions

The developed gradient HPLC method allows rapid and simultaneous determination of gallic acid and 4-Hydroxy benzoic acid in *Prosopsis cineraria* leaf extract. Thus the method described offers better resolution and is simple, and can be easily and reliably applied to the quantitative analysis of gallic acid and 4-Hydroxy benzoic acid.

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