Original Article

Development and Validation of a RP-UFLC Method for Estimation of Polyphenol (Gallic Acid) in Anti Diabetic Poly Herbal Formulation

Rashmi N.G.* and Gurupadayya B.M.

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Mysore, Karnataka, India

Address for Correspondence

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Mysore, Karnataka, India.
E-mail: ng.rashmi85@gmail.com

ABSTRACT

Objective: The principle destination of our work was to develop a simple, rapid and sensitive reverse phase ultra-fast liquid chromatographic (RP-UFLC) method for the estimation of Gallic acid in anti-diabetic poly herbal formulation (Mehagni).
Methodology: Chromatography was carried on a reverse phase C_{18} column (250 x 4.6 mm) with the mixture of methanol and 2% acetic acid as a mobile phase at the proportion of 70:30 v/v with the flow rate of 1.2 ml/min. The absorbance measured at 272 nm by PDA detector.
Result: Optimized chromatographic conditions were achieved and results showed good peak resolution. The retention time was found at 2.0 min. The parameters, such as specificity, sensitivity, linearity, precision, accuracy, ruggedness, robustness and system suitability were performed. The framework was linear with a correlation co-efficient of 0.995. %RSD of system and method precision were found to be 1.14 and 1.13. The LOD & LOQ for Gallic acid was found to be 10ng/ml and 20ng/ml.
Conclusion: This method is specific, sensitive, accurate, precise, robust and reproducible. The developed method may be used for the quantitative and qualitative estimation of Gallic acid in commercially available herbal formulations.

Keywords: Gallic acid, Mehagni, RP-UFLC, Anti- diabetic herbal formulation.

INTRODUCTION

Standard pharmaceutical "Ayurveda" since extraordinary oldness it has been giving the human kind to act against diseases. Mehagni is a polyherbal formulation[1] holding curcumin, amalaki, madhunasini and ekanayakam, used as anti-

diabetic. Gallic acid is a polyphenol derived from the herbal remedy. Chemically it is 3, 4, 5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark. Gallic acid having antioxidant remedial properties, Oxidative stress results in oxidative alteration of biological macromolecules. It is considered to play a pivotal role in the pathogenesis of aging and degenerative diseases[2-4]. In order to cope with an excess of free radicals produced upon oxidative stress, human bodies have developed sophisticated mechanisms for maintaining redox homeostasis. These protective mechanisms include scavenging or detoxification of reactive oxygen species (ROS), blocking ROS production, sequestration of transition metals, as well as enzymatic and nonenzymatic antioxidant defenses produced in the body, that is, endogenous[5,6], and others supplied with the diet, namely, exogenous ones. Among them, dietary polyphenols have been widely studied for their strong antioxidant capacities and other properties by which cell functions are regulated[7,8]. Polyphenols represent a group of secondary metabolites which widely occur in plant products. They are mostly derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, and phenolic acids. Polyphenols exhibit many biologically significant functions, such as protection against oxidative stress, and degenerative diseases. Experimental data indicate that most of these biological actions can be attributed to their intrinsic antioxidant capabilities. Polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signaling processes such as NF-κB activation, AP-1 DNA binding, glutathione biosynthesis, PI3-kinase/Akt pathway, MAPK proteins (ERK, JNK and P38) activation, and the translocation into the nucleus of Nrf2[9-11]. It might be utilized for the medication of chronic disorder like diabetes, cancer, arthritis, HIV infections. It possess diverse anti-diabetic[12], anti-microbial[13], anti-cancer[14]. The numerous spectrophotometric[14], Thin layer chromato-graphy[15,16], High performance thin layer chromatography[17-20], were also developed. The principle target of this study was to develop a simple, economic, rapid, precise, and validated technique for quantitative estimation active ingredients in commercially available poly herbal formulation.

MATERIALS AND METHODS

Chemicals and reagents

Standard Gallic acid (purity>99%) was purchased from Spectrom research laboratory, Hyderabad. HPLC grade Methanol, acetic acid was obtained from Merck, Germany. Triple distilled water was obtained from the Milli Q unit. Mehagni poly herbal formulation from SNA Oushadhasala Pvt. Ltd., India.

Instrumentation

The Ultra-Fast Liquid Chromatography consists of Shimadzu LC-20AD solvent delivery system (pump), Photodiode Array Detector (PDA) with a 7725i rheodyne injector with 20µL loop volume (Kyoto, Japan). 20µl of injection volume was used for injection. The LC Solution software was used for integration.

Chromatography conditions

Chromatographic separation was done using Phenomenex C_{18} column (250 X 4.6 mm, 5µ ID). The mobile phase consists of methanol and 2% acetic acid (70:30, v/v). The flow rate was adjusted to 1.2 ml/min and run time was adjusted to 10 min. Gallic acid was detected at a wavelength of 272 NM using a PDA detector with retention time 2.00 min. 20µl of injection volume was used for injection.

Preparation of gallic acid standard solution

100 mg standard Gallic acid was weighed accurately and transferred to a 100 ml volumetric flask and 70 ml of methanol was added and dissolved and the above solution was again made up to volume with methanol to produce 1000µg/ml solution. From the stock solution final concentration (10µg/ml) of the individual working standards was prepared with methanol.

Preparation of sample solution

Twenty tablets (Mehagni) were weighed accurately and the average weight was determined and then grounded into a fine powder. Quantity equivalents to 0.1 g were transferred to 100 ml of volumetric flask and volume was adjusted with100ml with methanol. The solution was centrifuged for 15minutes at 3000 rpm. Centrifugation was found to be faster and more effective than filtration. Centrifugation forms a cake of excipients at the bottom of the test tube, which is not disturbed while drawing out the supernatant solution. This supernatant solution was a pipette out and diluted appropriately with methanol to obtain the concentration of 10µg/mL concentration of Gallic acid.

Mobile phase preparation

HPLC grade methanol (solution A) and acetic acid (solution B). 2% of acetic acid were prepared by dissolving 2ml of acetic acid in 100ml HPLC grade water.

Development of calibration curve of gallic acid standards

Working solutions for the calibration study were prepared from the stock solution by an adequate dilution using Methanol. Calibration standards of concentrations 2-10 µg/ml were prepared for Gallic acid.

Method validation

The validation parameters such as accuracy, precision, linearity, limit of detection and limit of quantification, Robustness, specificity and system suitability has been evaluated as per ICH guidelines[21].

Accuracy

Accuracy is expressed as the closeness of agreement of trueness [21]. It was carried out by recovery studies by adding the known concentration of the standard solution of Gallic acid to the samples of known percentage recovery. The results were shown in the **(Table 1)**.

Precision

Precision studies were carried out in different ways such as system precision, method precision and intraday precision, interday precision[21]. In system precision same concentration was injected six times and method precision same concentration were injected in different six vials of Gallic acid (6 µg/ml). In Intraday precision (4, 6, 10 µg/ml) of Gallic acid was carried out to check the repeatability, interday variation for intermediate precision (4, 6, 10 µg/ml) and at same prescribed conditions.

Linearity

Linearity for Gallic acid was plotted from the standard solution from the concentration of 2µg/ml to 10µg/ml which was analyzed to check the linearity response.

Ruggedness and robustness

The ruggedness of the proposed method was carried out by changing the different instrument, different operators and different column of similar model of C_{18}. Robustness of the method were carried out by small change in flow rate ± 0.1, column temperature ± 5, and % organic strength ±

2%. Peak area was not affected by small variation in parameters.

Limit of detection (LOD)

Limit of detection of the method was determined by measuring the signal to noise ratio. LOD for Gallic acid was found to be 10 ng/ml. Whereas LOD was obtained by the formula.

LOD = 3.3σ/m

Where, 'σ' is the standard deviation of the intercept of regression line and 'm' is the slope of the calibration curve.

Limit of quantification (LOQ)

The smallest concentration of the analyte which can be quantified based on the signal to noise ratio. Limit of quantification for Gallic acid was found to be 20 ng/ml. Whereas LOQ was obtained by the formula.

LOQ = 10σ/m

Where, 'σ' is the standard deviation of the intercept of regression line and 'm' is the slope of the calibration curve.

Specificity

Specificity is the method's ability to show good separation of analyte in the presence of compounds which may not be affected by the matrix[21], degrades, impurities or any other plant matrix. The specificity of the method was carried out by comparing the standard retention time spectra and the sample retention time spectra. No endogenous substance were interfered with the of the drug response. Delegate run of the chromatograph was shown in **(Figure 3 and 4)**.

RESULTS AND DISCUSSION

Method development

Optimization of the chromatographies condition was carried out by changing the methanol concentration, strength and acetic acid concentration. Improvement of the chromatographic conditions revealed good separation of Gallic acid with the mobile phase of methanol: 2%acetic acid (70:30) v/v. Retention time for Gallic acid was found to be 2.0min **(Figure 3)**. Increase in acetic acid concentration shifts the peak to the void volume side. Decrease in the acetic acid leads to the loss of resolution. Increase or decrease in methanol concentration leads to the peak splitting. The calibration curve for the standard Gallic acid was plotted from 2-10 µg/ml **(Figure 1)** and it was found to be linear in range. The regression equation for Gallic acid was found to be 0.995respectively.

Accuracy

The results indicate that the recovery of Gallic acid **(Table 1)** was found to be accurate, the % nominal mean was found to be 99-100%, percentage of Standard Deviation (SD) and Relative Standard Deviation (RSD) were found to be within the limits. Which proves the method was accurate.

Precision

Precision studies were carried out by methods such as system precision, method precision and intraday precision, interday precision. In system precision injecting same concentration six times and method precision injecting same concentration in different six vials of Gallic acid (6µg/ml). In Intraday precision (4, 6, 10 µg/ml) of Gallic acid was carried out to check the repeatability, interday variation for intermediate precision (4, 6, 10 µg/ml) and at same prescribed conditions. The percentage Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated and found to be within the limits **(Table 2 & 3)**.

Ruggedness and robustness

The method was discovered to be rugged and robust, since there were no changes in the chromatogram by changing the optimized chromatographic conditions, instruments, operator and column.

LOD and LOQ

The LOD & LOQ are calculated as given in the above formula and found to be 10 ng/ml &20ng/ml respectively.

Specificity

The Specificity of the method showed good separation of the standard Gallic acid and it is not affected by the other plant constituents and matrix.

CONCLUSION

A simple, sensitive and rapid method for estimation of Gallic acid, which is present in Mehagni anti-diabetic formulation has been developed and validated as per the ICH guidelines. This method is specific because the Gallic acid not affected by other plant constituents which present in formulation. It shows very sensitive even in nano gram level also detectable and quantifiable. Robust and reproducible even after changing the optimized chromatographic parameters like temperature, flow rate, mobile ratio in the range of ± 0.2. The developed method can be used for the quantitative and qualitative estimation of Gallic acid in herbal formulations.

Conflict of interest

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Table 1. Accuracy (% recovery data)

S. No.	% Of Drug Added	Amount of Drug Taken (µg/ml) (std)	Amount of Drug Added (µg/ml) (sample)	Total Amount of Drug	Total amount of drug found	% Recovery	Mean	% SD	%RSD
1	50%	4	2	6	5.9	99	100	0.8	0.8
					6	100			
					6	100			
2	100%	4	4	8	8	100	100	1	1
					7.8	98			
					8	100			
3	150%	4	6	10	10	100	100	0.6	0.6
					9.9	99			
					10	100			

Table 2. Intraday and interday precision

Value		Parameter
Interday	Intraday	
1.2	0.9	Standard deviation
1.3	1	%RSD

Table 3. System and method precision

Value		Parameter
Method	System	
1.2	1.1	Standard deviation
1.2	1.1	%RSD

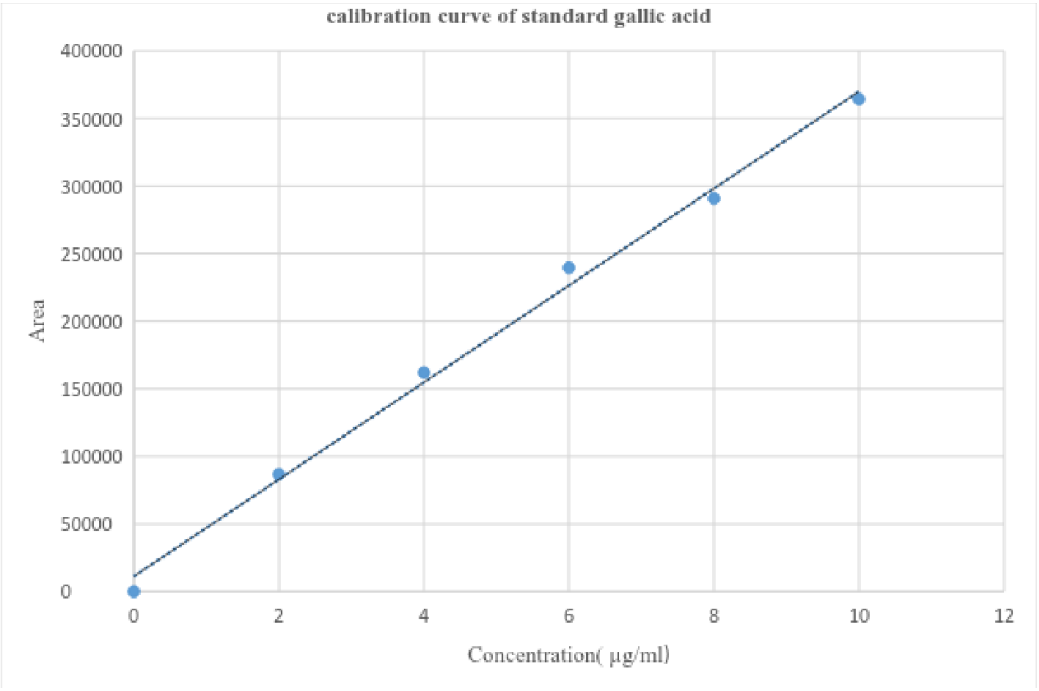

Figure 1. Calibration curve of standard gallic acid

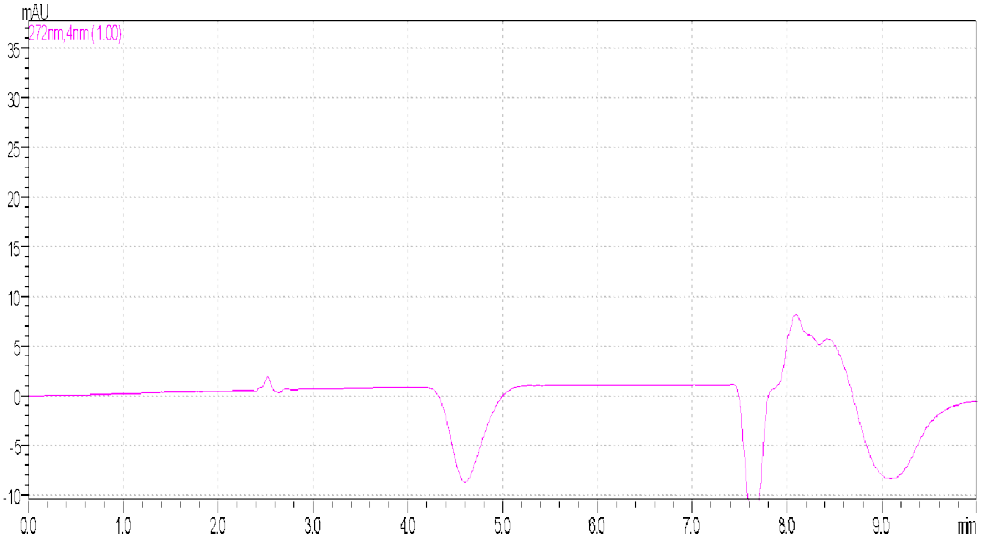

Figure 2. Typical UFLC chromatogram of blank

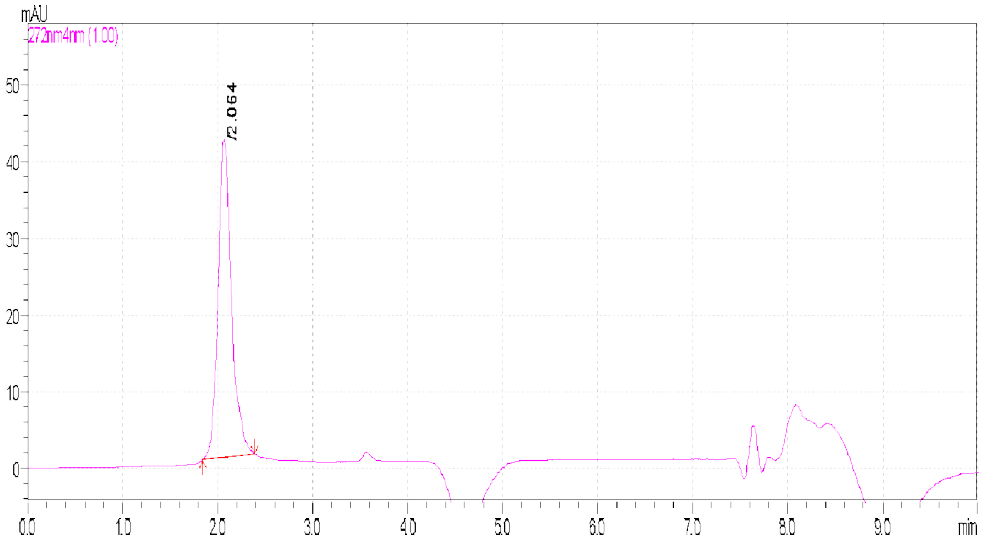

Figure 3. Typical UFLC chromatogram of standard gallic acid

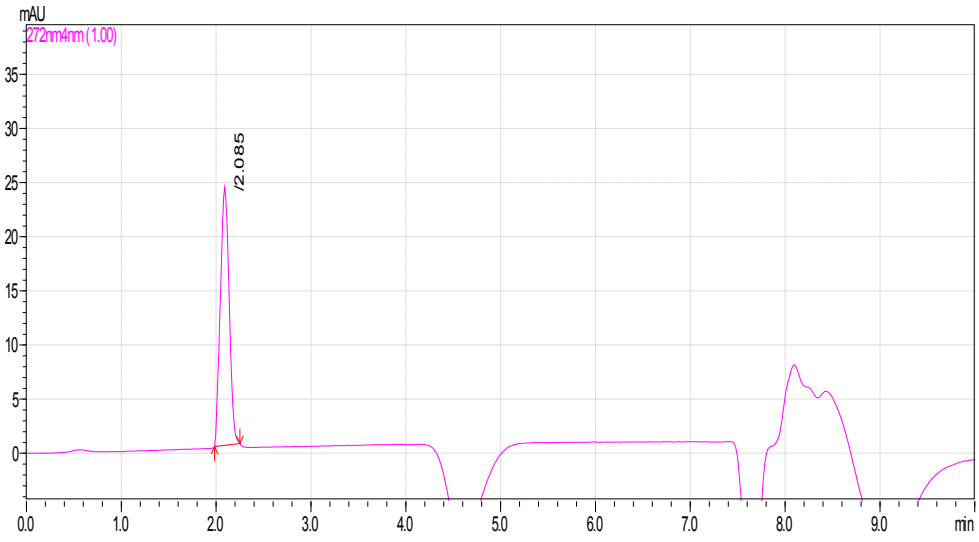

Figure 4. Typical UFLC chromatogram of sample (Mehagni)