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RESEARCH ARTICLE

METHOD DEVLOPMENT AND VALIDATION OF GALLIC ACID AND ELLAGIC ACID IN ARGWADHARISTAM

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ABSTRACT

A novel RP-HPLC method has been developed for the estimation of Gallic acid and Ellagic acid in Argwadharistam, which is popular herbal formulation. It is an ayurvedic formulation made out by Terminalia Chebula along with other ingredients. Studies have shown that it has used in all types of dermatitis. The use of gradient elution of Acetonitrile & buffer solution enabled the efficient separation of Gallic acid and ellagic acid in 4.8 min and 8.46 min (Rt), five calibration standards in range of 2.0-10µg/ml for Gallic acid and 10-50µg/ml for Ellagic acid at 254nm quantification was effected at 254nm. Validation was performed according to ICH guidelines. Proposed method showed good linear correlation coefficient (r2=0.999). The LOD and LOQ of Gallic acid and ellagic acid were 0.8µg/ml & 2.5µg/ml and 1.5µg/ml & 5µg/ml respectively. The recoveries are between 98 and 102%. The developed and validated method was simple and accurate can be used for the estimation of Gallic acid and ellagic acid in herbal formulation.

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INTRODUCTION

Plant based medicines has been popular throughout the world. In India, the herbal formulation market is nearly $ 107 billion and the export of herbal medicine is around 2.5% to global markets. Many plant parts contain phenolic flavanoids and widely used as a model system in plant research (Ong, 2004). Most flavanoids exhibit as antioxidant, neuroprotectiv effect with free radical scavenging effects (Zhongbing *et al*., 2006; Kebo *et al*., 2010) and immune-modulator properties (Quality standard of Indian medicinal plants, 2006; Mukherjee, 2005; Quality standard of Indian medicinal plants, 2006). These activity may be due to the presence of Gallic acid, Ellagic acid, Catechin, Quercetin etc. Separation, identification and quantification of polyherbal formulation are very difficult. The gradient elution reverse phase column is commonly applied for analysis of chemical constituents present in herbal formulation. Argwadharistam is well known ayurvedic preparation consists of *Terminalia Chebula* and *Cassia Fistula*. It detoxifies blood and reduces toxins in the body, which also helps in healing the skin lesions due to dermatitis, eczema and psoriasis. Gallic acid and Ellagic acid is main active constituent of *Terminalia Chebula.* Gallic acid (3, 4, 5 trihydroxy benzoic acid) and Ellagic acid (2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde] chromene-5,10-dione) is polyphenolic compound and found in different fruits, berries and nuts. It prevents cancer (Deighton *et al*., 2000; Liu *et al*., 2005; Huang and Nia, 2007) and contributing to strength the body defense system (Suram and Hebar, 2005). In present work, we developed a simple method for quantitation of Gallic acid and Ellagic acid in Argwadharistam.

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MATERIALS AND METHODS

1.Chemicals and solvents: Argwadharistam was procured from local market. The chemical markers Gallic acid and Ellagic acid were obtained from Natural Remedies, Bangalore. HPLC grade acetonitrile, water, methanol were obtained from Merck Specialist Pvt, Mumbai.

2.Instrumentation and chromatographic condition: RP-HPLC equipped with two LC-10AD pumps for high pressure gradient elution, fitted with an auto sampler, a Rheodyne injector fitted with 20µl sample loop, PDA detector and class VP software. Separation was carried out using Hibar, prepacked column Lichrosher 100, RP-18e(5µm) Phenomenx-Luna5µ (250*4.6mm). The typical chromatogram for standard

(Fig 01, 03) and sample (Fig 02, 04) for both the drugs are shown.

3.Chromatographic seperation of standard gallic acid and ellagic acid: Stock solution were made by dissolving Gallic acid (10mg) in water and Ellagic acid(10mg) in methanol transferred to a 100 volumetric flask to obtain 100µg/ml. Aliquots of 2-10µg/ml for Gallic acid and10-50µg/ml Ellagic acid standard stock solution. Each concentration was measured in triplicate. The optimized gradient elution was used; solvent A was Acetonitrile and solvent B water. The flow rate was 1.5ml/min and detection wavelength was 254nm.

Sample preparation of Gallic acid: Accurately weight 2ml of Argwadharistam was transferred to 50ml volumetric flask and dissolved in 20ml water and kept for sonicating for 10mins. Volume was made with water to obtain 100µg/ml as standard stock solution.

Sample preparation of Ellagic acid: Accurately weighed 5ml of Argwadharistam was dissolved in methanol and kept for heating for 5mins and sonicated for 10mins. Volume was made with methanol to obtain 100µg/ml as standard stock solution.

4.HPLC method development: Gallic acid and Ellagic acid were analyzed by HPLC technique using following chromatographic condition:

5.HPLC method validation: The analytical method validation was performed according to ICH Q2A guidelines. (Table no 02)

Table 2. Validation Parameter of Gallic acid and Ellagic acid

Parameters	Gallic acid	Ellagic acid
Retention time	4.46min	8.4min
Beer's law limit	2-10 µg/ml	10-50µg/ml
Wavelength	254nm	254nm
Slope	102724	33992
Intercept	15697	86995
Correlation coefficient(r^2)	0.999	0.991
Accuracy (%)	98-102%	98-102%
Limit of detection(LOD)	0.8 µg/ml	1.5 µg/ml
Limit of quantification(LOQ)	2.5 µg/ml	5 µg/ml

Specificity: System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

Linearity: Linearity of the method was performed by linear regression and was linear range 2-10µg/ml and 10-50µg/ml forGallic acid andEllagic acid, three phase's validation done. The graph was plotted the mean peak area versus the concentration of each analyte.

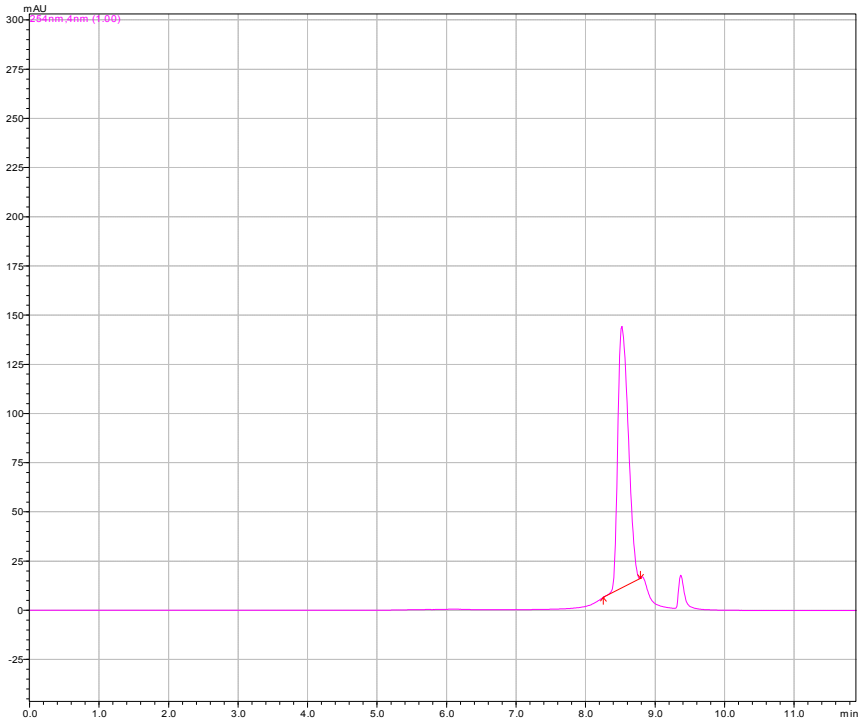

Fig.1. Chromatogram for standard Ellagic acid

Table 1.

Parameter	Description	
Column	Hibar, Prepacked Column, Lichrospher 100, RP-8e	
Column size	4.6mm*250mm*5µ	
Detector	Photodiode array detector	
	Gallic acid	Ellagic acid
Mobile phase	Acetonitrile : Water	Acetonitrile : Water
Flow rate	1.5µl	1.5µl
Wavelength	254nm	254nm
Retention time	4.46min	8.46min
Injection volume	20 µl	20µl

Accuracy: Three replicate injection containing known amount of Gallic acid and Ellagic acid at 50%, 100%, and 150% with respect to assay concentration (10µg/ml). The developed method satisfies the acceptance criteria and ensures accuracy of method.

Precision:

Interday: Assay method was analyzed by three independent sample solutions and from the area obtained, concentration was calculated and results were expressed as %RSD.

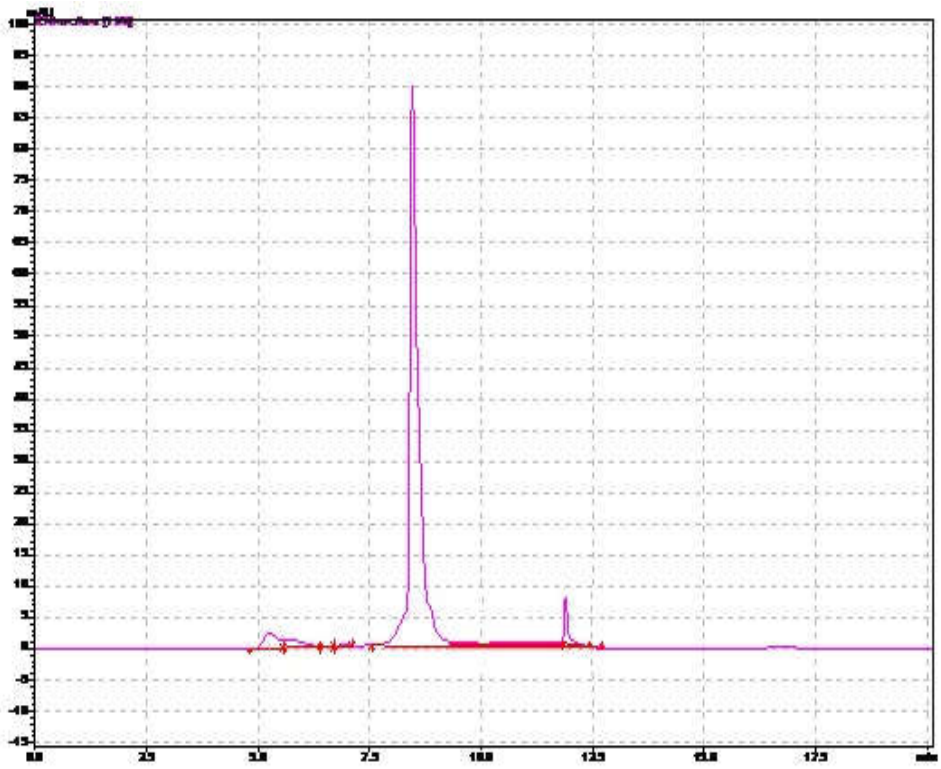

Fig . 02. Chromatogram for sample Ellagic acid

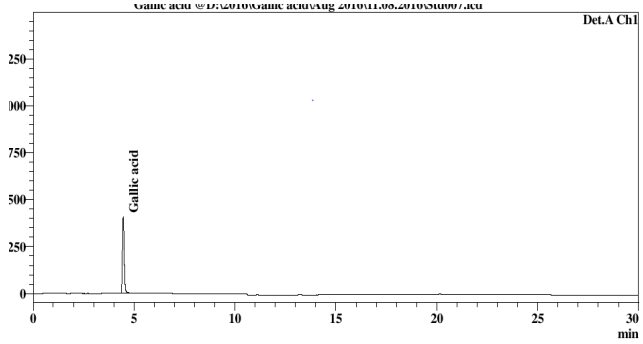

Fig. 3. Chromatogram for standard Gallic acid

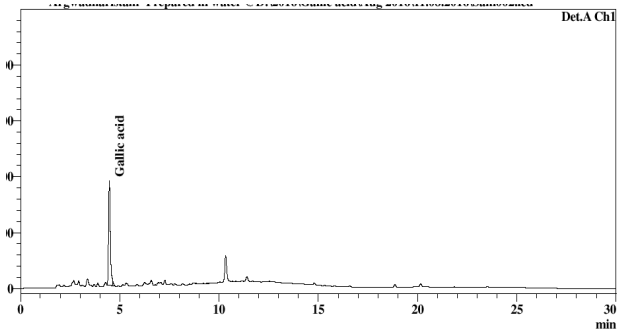

Fig. 4. Chromatogram for sample Gallic acid

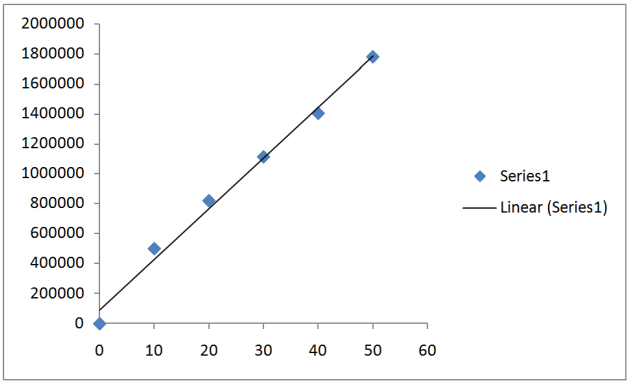

Fig.5. Standard calibration graph of Ellagic acid

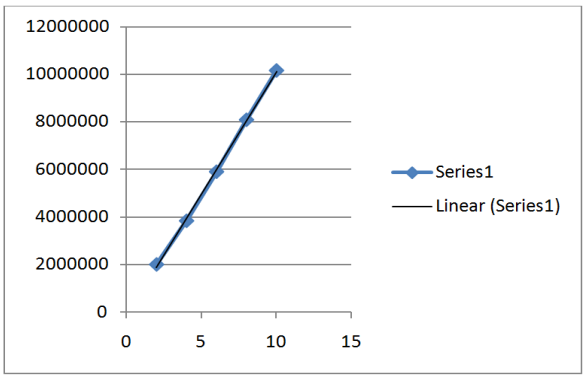

Fig.6. Standard calibration graph of Gallic acid

Intraday: The method was analyzed by six carrying out the experiment on different day, different analyst and different columns.

LOD and LOQ: Limit of detection and limit of quantification were determined by formula 3.3xσ/slope and 10xσ/slope respectively.

Where σ = standard deviation
Slope = slope of calibration curve.

RESULTS AND CONCLUSION

Estimation of Gallic acid and Ellagic acid in Argwadharistam is found to be economical. Use of mobile phase buffer and

acetonitrile at a flow rate 1.5µl showed a single peak for the both standard and sample at 254nm.The standard curve showed a linear response (Fig. 05, 06) in a concentration range 2.0-10µg/ml and 10-50µg/ml with correlation coefficient 0.991. The result of accuracy studies indicated high recovery values 98-102%. The low coefficient of variation values of intraday and interday precision showed the developed method is précised. LOD and LOQ were found to be for Gallic acid 0.8µg/ml & 0.4-5µg/ml and Ellagic acid 1.5µg/ml & 5µg/ml respectively. The developed method can be adopted for the route analysis and quality control of Gallic acid and Ellagic acid in polyherbal formulation.

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