Original Article

Development and Validation of UV-spectrophotometric Method for Estimation of Gallic Acid in *Acalypha indica* Leaf Extract and its Cellulose Nanoparticle Formulation

Kumar Sneha, Muniyappa Geetha, Birendra Swapna, Ramachandra Setty Siddam Setty*

Department of Pharmacology, Government College of Pharmacy, Bengaluru, Karnataka, INDIA.

ABSTRACT

Aim/Background: Quantification of Gallic acid in the hydro-alcoholic extract of *Acalypha indica* and extract loaded cellulose nanoparticles is a characteristic parameter to assess the entrapment efficiency of nanoparticles. Gallic acid is one of the major phenolic acids identified in leaf extract of *Acalypha indica*; hence, it was chosen to be estimated. **Materials and Methods:** A UV spectrophotometric method was developed using distilled water as a suitable solvent system to estimate Gallic acid. The method was validated for parameters such as linearity, precision, robustness and accuracy according to the ICH guidelines. The λ_{max} of 256nm was observed for both Gallic acid and *Acalypha indica* extract, which indicated specificity of the method for further analysis. **Results:** Linearity was established between the concentration range of 5-30 μg/ml with a regression coefficient of 0.999 signifying good correlation between the concentration and absorbance. Average percent recovery of 97.6% demonstrated the accuracy of the method. The recorded RSD of $<2\%$ in precision validation indicated the correctness of the method. Ruggedness and robustness of the method were established with RSD of $<2\%$ suggesting that the analysis is unaffected with change in analyst and wavelength. The detectable and quantifiable limits of Gallic acid were found be 0.045 μg/ml and 0.119 μg/ml respectively, which demonstrated the sensitivity of the method as well. **Conclusion:** From the above results, it was concluded that the UV-spectrophotometric method can be employed to estimate Gallic acid in both leaf extract and extract loaded pharmaceutical formulations.

Keywords: Gallic acid, UV spectrophotometer, *Acalypha indica*, Cellulose nanoparticles, Method validation, Leaf extract.

INTRODUCTION

Gallic acid (GA) is a polyphenolic acid with molecular structure comprising of three hydroxyl groups attached to a benzoic acid. It is a secondary metabolite synthesized in plants from shikimic acid pathway and possesses wide range of therapeutic potential.[1,2] Phenolic compounds such as GA, ellagic acid and syringic acid are ubiquitous across plant kingdom, which are responsible for plants' antioxidant property that can mitigate oxidative stress induced organ damage.[3] It is colourless or slightly yellow crystalline substance with characteristic odour. GA has achieved high altitudes with respect to its utilization in different areas including pharmaceutical industry.[4]

Numerous techniques such as chromatography, spectroscopy, electrophoresis, thermal analysis and chemiluminescent have been recognized to isolate and estimate GA.[2] GA has been quantified using robust instruments like UV spectrophotometer and high-performance liquid chromatography by employing suitable solvents and conditions. Sophisticated instruments such as capillary electrophoresis,[5] and gas chromatography,[6] have also been employed to determine GA in extracts as well as plasma of rats.[7] Estimation of total phenolic content in a plant

Submission Date: 19-07-2021;
Revision Date: 06-12-2021;
Accepted Date: 14-04-2022.

DOI: 10.5530/ijper.56.3.141

Correspondence:
S. Ramachandra Setty
Department of Pharmacology, Government College of Pharmacy, No.2, P. Kalingarao Road, Mission Road, Bengaluru-560027, Karnataka, INDIA.
E-mail: rssiddamsetty@rediff.com

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extract is measured in terms of GA (mg)equivalent/g of extract which is an indication of its antioxidant capacity.[8] *Acalypha indica* (AI) is a plant belongs to the family Euphorbiaceae and grows in wet, temperate and tropical region.[9] A study about AI revealed the presence of GA as an essential flavonoid apart from other constituents such as syringic acid and ellagic acid,[10] and hence, the plant is rendered as an effective antioxidant medicine,[11] for many diseases. The plant has been testified for other pharmacological activities such as cardioprotective,[12] anti-urolithiasis and diuretic,[13] using pre-clinical screening models in rodents. Therefore, in the present study hydro-alcoholic extract of leaf of AI (HAELAI) was chosen to evaluate the nephroprotective property in rats and to compare it with its nano-particulate formulation.

In herbal formulation research, developing nano sized dosage forms viz., polymeric nanoparticles, liposomes, proliposomes, solid lipid nanoparticles, nano-emulsion is beneficial owing to subsequent motives viz., enhancement of solubility, bioavailability, pharmacological activity and stability; protection from toxicity; improving tissue macrophages distribution; sustained delivery; protection from physical and chemical degradation.[14] Further, nanoparticles are extensively used as a novel drug therapeutic strategies to treat chronic ailments like cancers, AIDS, Nephrotoxicity, diabetes and also for wound healing property.[15] Several scholars have utilized AI plant to synthesize metal nanoparticles as a novel therapy for many ailments.[16,17] Hence, research work was further advanced by loading HAELAI into cellulose nanoparticles. Quantification of GA in both HAELAI and HAELAI loaded nanoparticle formulation is an important aspect as it is necessary for determining the drug entrapment efficiency and loading capacity of cellulose nanoparticles.

There is no literature reported till date regarding the estimation of GA in extract loaded cellulose nanoparticles and hence, there is a need for the development of a modest and quick method to quantify GA in extract and as well as from nano-particulate formulation. Thus, the current research aimed at developing and validating UV spectrophotometric method for the estimation of GA in HAELAI and HAELAICNP.

MATERIALS AND METHODS

Materials

Gallic acid (Sigma Aldrich (purity >97%), *Acalypha indica* leaf powder (Prabhu's Herbal Company, Madurai, Tamil Nadu), absorbent cotton (Prabhat surgical cotton, Pvt.Ltd.) and all other chemicals and reagents used were of analytical grade.

Methods

Method development

Preliminary studies were conducted to screen a suitable solvent for developing a UV spectrophotometric method for the estimation of Gallic acid, taking into account the solubility of Gallic acid, extract and the nanoparticle formulation. Water was chosen as a suitable solvent to develop the analytical method.

Preparation of standard stock solution and determination of maximum wavelength of absorption

The standard stock solution was prepared by dissolving 10mg of GA in 100ml of distilled water in a volumetric flask (Stock -I 100 µg/ml). An aliquot of 30 µg/ml concentration was selected and scanned for λ_{max} between 400-200 nm by UV-Visible spectrophotometer (Shimadzu-1800, Japan) against water as blank. The UV absorption spectrum of GA is depicted in Figure 1.

Preparation of calibration curve of Gallic Acid

Aliquots of 6 corresponding concentrations from 5 to 30 µg/ml were prepared from Stock-I and volume was made up by distilled water. Absorbance of these dilutions was measured using UV-visible spectrophotometer at selected wavelength against water as blank. The results of calibration curve are showed in Table 1 and Figure 1.

Validation of UV spectrophotometric Method

The developed UV-Spectrophotometric method was validated as per ICH guidelines in terms of linearity, precision, accuracy and robustness.[18]

Linearity

The linearity was established by measuring the absorbance of 6 concentrations (5-30 µg/ml) and the data was subjected to regression analysis to attain the regression equation and correlation coefficient.

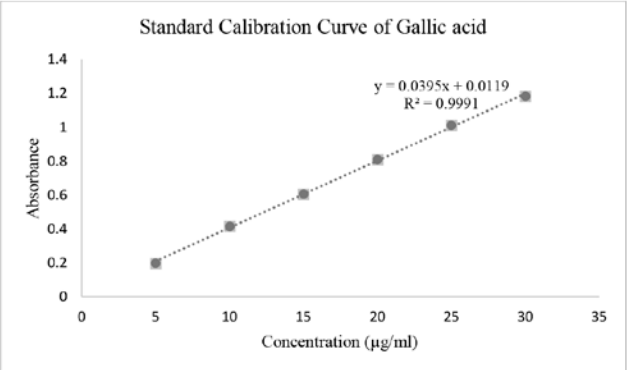

Figure 1: Standard calibration curve of Gallic acid.

Table 1: Linearity data of Gallic acid.

Concentration (µg/ml)	Absorbance			Average	SD	%RSD
	Trial 1	Trial 2	Trial 3			
5	0.198	0.197	0.198	0.197	0.000471	0.238
10	0.416	0.418	0.415	0.416	0.001247	0.299
15	0.605	0.605	0.605	0.605	0.000471	0.077
20	0.809	0.810	0.808	0.809	0.000816	0.100
25	1.012	1.011	1.012	1.011	0.000471	0.046
30	1.181	1.012	1.182	1.181	0.000471	0.039

Table 2: Results of Recovery studies at three different levels.

Concentration of HAELAICNP before spiking (µg/ml)	Amount of standard (GA) added (µg/ml)	Total concentration of HAELAICNP after spiking (µg/ml)	Concentration of GA in %	Absorbance at 256 nm	Amount of GA recovered (µg/ml)	% Recovery
30	24	54	80	0.264	52.7	97.7
30	30	60	100	0.365	58.8	98.0
30	36	66	120	0.473	64.1	97.1

Accuracy

Accuracy was determined by spiking 30 µg/ml of HAELAICNP with standard GA at three different concentrations viz., low, medium and high respectively indicated as 80, 100 and 120% and the absorbance was measured at 256nm. The % recovery of the drug was calculated and the results are compiled in Table 2.

Precision

Repeatability, intermediate precision, reproducibility are different stages of precision that determines the closeness of the measurements. All the measurements were recorded in triplicates for the 15 µg/ml concentration. Repeatability is also termed as intra-assay or intra-day precision. The measurements were taken 3 times on the same day. The inter-day assessment was carried out for 3 consecutive days of the calendar.

Ruggedness and Robustness

Two different analysts performed the analysis of the 15 µg/ml sample using the same method to determine the ruggedness of the method. While, robustness was estimated by recording the absorbance of the 15 µg/ml sample at ±2 nm of the wavelength (254nm, 256nm and 258nm) selected in the above procedure.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The standard deviation of lowest concentration 5 µg/ml was used to determine LOQ and LOD along with the slope obtained from the calibration curve. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

$$LOD = \frac{3.3 \times \sigma}{S} \quad (1)$$

$$LOQ = \frac{10 \times \sigma}{S} \quad (2)$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve

Quantification of GA in AI leaf extract

100mg of AI leaf extract was transferred to 100 ml volumetric flask and dissolved in distilled water. The final volume was made up with the same solvent to get final concentration of 1 mg/ml (Stock-I) and the solution was filtered through Whatman filter paper to obtain clear solution. Further dilution was made by diluting 1 ml of Stock-I to 10 ml with distilled water to obtain a concentration of 100µg/ml (Stock-II). An aliquot of 15 µg/ml prepared from Stock-II was set as sample solution and scanned for λ_{max} between 400-200 nm. The absorbance of sample solution was recorded at 256nm against water as blank. The analytical result of HEALI is reported in Table 3.

Table 3: Results of quantification of GA in HAELAI and HAELAICNP.

Formulation	Parameters	Drug	Absorbance at 256nm	Concentration
HAELAI	Drug content	Gallic acid	0.178	0.0418 mg/g
HAELAICNP	Drug entrapment efficiency		-	99.2%
	Drug loading capacity			27.28%

Quantification of GA in AI loaded CNP

HAELAICNP equivalent to 10 mg of HAELAI (36mg of HAELAICNP) was extracted with 10ml of dimethyl sulphoxide (DMSO) by subjecting it to centrifugation process (3000 rpm for 5 min). 1.5 ml of the supernatant was diluted to 100 ml with distilled water in a volumetric flask to attain a concentration of 15µg/ml. The absorbance of the sample solution was measured against water as blank. The free drug content was estimated by using standard calibration curve of Gallic acid. Further the drug entrapment efficiency and drug loading capacity were calculated using the following formulae.

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100 \quad (3)$$

$$\% \text{ Drug Loading Capacity} = \frac{\text{Total drug} - \text{free drug}}{\text{Weight of nanoparticles recovered}} \times 100 \quad (4)$$

RESULTS AND DISCUSSION

Method development

Selection of solvent

The hydrophilicity of GA was found to be less i.e., 1.5 g in 100 ml.[19] However, GA quantity in terms of milli grams is soluble in 100 ml of distilled water. On the other hand, cellulose is insoluble in distilled water. The leaf extract of AI was also found to be soluble in water though needed to be filtered. After considering the solubility profile of standard GA, extract and formulation, water was selected as the common solvent for the developing UV spectrophotometric method for the estimation of GA.

Selection of wavelength

Among the 6 concentrations from 5-30 µg/ml, the concentration 30 µg/ml aliquot was chosen for determining the maximum absorption of wavelength using UV spectrophotometer within a scanning range of 400-200nm. The wavelength of maximum absorption of UV light by GA was observed to be 256nm (Figure 2).

Validation

The validation was carried out for the developed method according to the ICH guidelines Q2 R (1) with respect to linearity, precision, robustness, ruggedness, LOD, LOQ and accuracy.

Linearity

The linearity was established between the concentration of 5 -30 µg/ml with a regression coefficient of 0.999 (Figure 1 and Table 1), indicating good correlation between concentration and the absorbance.

Accuracy

Accuracy (bias) expresses the closeness of the agreement between true value and the test value. The % recovery of GA in HAELICNP was found to be 97.1 - 98% (Table 2). The consistency in % recovery at different concentrations demonstrated the recovery efficiency of developed method with reasonable accuracy.

Precision

The method was validated for its precision in terms of intra-day and inter-day precision. The intra-day precision involved measurement of absorbance in morning, afternoon and evening of the same day. While

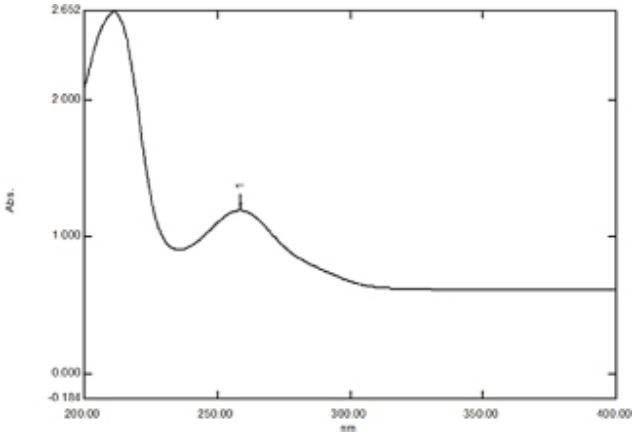

Figure 2: UV-Absorption Spectrum of Gallic acid showing lambda max at 256 nm.

Table 4: Results of Intra and Inter Day precision method for Gallic acid.

Concentration (µg/ml)	Absorbance					
	Intra-Day Precision			Inter-Day Precision		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
15	0.609	0.608	0.591	0.609	0.605	0.605
15	0.611	0.607	0.597	0.611	0.617	0.61
15	0.608	0.602	0.594	0.608	0.606	0.595
Average	0.609	0.606	0.594	0.609	0.609	0.603
SD	0.00125	0.00262	0.00245	0.00125	0.00544	0.00624
SEM	0.00072	0.00152	0.00142	0.352	0.352	0.349
RSD (%)	0.205	0.433	0.412	0.205	0.892	1.034

Table 5: Results of Ruggedness and Robustness.

Concentration (µg/ml)	Absorbance				
	Ruggedness		Robustness		
	Analyst 1	Analyst 2	254 nm	256 nm	258 nm
15	0.609	0.611	0.59	0.609	0.619
15	0.611	0.613	0.576	0.611	0.618
15	0.608	0.61	0.571	0.608	0.621
AVG	0.609	0.611	0.579	0.609	0.619
SD	0.00125	0.00125	0.00804	0.00125	0.00125
SEM	0.00072	0.00072	0.00464	0.00072	0.00072
RSD %	0.205	0.204	1.389	0.205	0.201

Table 6: Results of LOD and LOQ.

Concentration Range	Equation for LOD	LOD	Equation for LOQ	LOQ
5-30 µg/ml	(3.3*SD)/ slope	0.0405 µg/ml	(10*SD)/ slope	0.119 µg/ml

inter-day absorbance was recorded on three consecutive days. The % RSD values were observed to be less than 2% which indicated a good precision of the developed method (Table 4).

Ruggedness and Robustness

Ruggedness of the method was determined by performing the analysis by two different analysts and the absorbance for 15 µg/ml concentration was noted. Robustness was evaluated by measuring the absorbance at 256 ± 2. The calculated % RSD values of <2% represented the repeatability, reproducibility and robustness of the method. (Table 5).

Limit of Detection and Limit of Quantification

The LOD and LOQ results (Table 6) demonstrated that GA can be detected and quantified at lowest concentration of 0.0405 µg/ml and 0.119 µg/ml respectively, demonstrating sensitivity of the method.

Quantification of GA in HAELAI and HAELAICNP

The retention of peak at almost same λ_{max} as that of GA of 256 in the spectrum of extract indicates that the method is specific for the estimation of GA in the extract. The drug content in HAELAI (Figure 1) and free drug content in formulation (HAELAICNP) was estimated. The results are gathered in the Table 3. Further concentration of free drug content was substituted in the equation (3) and (4) to determine the drug entrapment efficiency and drug loading capacity which was found to be 99.2% and 27.28% respectively.

CONCLUSION

The findings clearly suggested that the method developed to estimate GA was found to be precise, robust, highly subtle and accurate. Hence, the proposed UV spectrophotometric method can be adopted for determining GA content in leaf extract and pharmaceutical formulations in research laboratories. The current UV-Spectrophotometric method also

facilitates feasible quantification of drug loaded in the cellulose nanoparticles.

ACKNOWLEDGEMENT

The authors wish to thank Government College of Pharmacy and Drugs Control Department, Government of Karnataka for providing necessary facilities to carry out research.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest involved in the study.

ABBREVIATIONS

GA: gallic acid; **HAELAI:** hydro-alcoholic extract of leaf of *Acalypha indica*; **HAELAICNP:** hydro-alcoholic extract of leaf of *Acalypha indica* loaded in cellulose nanoparticles; **Abs:** absorbance; **UV:** ultra-violet; **ICH:** international conference on harmonization; **ISO:** international organization for standardization; **LOD:** limit of detection; **LOQ:** limit of quantification; **RSD:** relative standard deviation.

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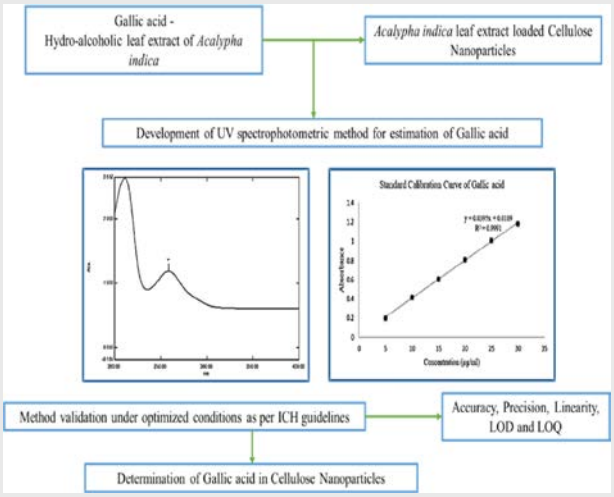
PICTORIAL ABSTRACT



SUMMARY

In Phyto-formulation research, quantification based on phytochemicals is essential to determine the amount of extract loaded in nanoparticles. Therefore, a modest and quick analytical method was developed to quantify Gallic acid in extract and nanoparticulate formulation. The analytical procedure was validated following ICH guidelines, and the technique is found to be suitable for the estimation of Gallic acid in both leaf extract and extract loaded cellulose nanoparticles.

About Authors

Sneha Kumar is a Post-Graduate student, Department of Pharmacology, Government College of Pharmacy, Bengaluru.

Swapna Birendra is Assistant Professor, Department of Pharmacology, Government College of Pharmacy, Bengaluru.

M Geetha is Assistant Professor, Department of Pharmaceutics, Government College of Pharmacy, Bengaluru.

Ramachandra Setty S is Principal, Senior Professor and Head, Department of Pharmacology, Government College of Pharmacy, Bengaluru.

Cite this article: Sneha K, Geetha M, Swapna B, Setty RSS. Development and Validation of UV-Spectrophotometric Method for Estimation of Gallic Acid in *Acalypha indica* Leaf Extract and its Cellulose Nanoparticle Formulation. Indian J of Pharmaceutical Education and Research. 2022;56(3):881-7.