ejpmr, 2017,4(03), 452-457.

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP- HPLC METHOD FOR GALLIC ACID

Fulmali SushmaV and Tatke Pratima A.*

Department of Pharmaceutical Chemistry, C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Juhu Campus, Santacruz (West), Mumbai 400049.

***Corresponding Author: Dr. Tatke Pratima A.**
Department of Pharmaceutical Chemistry, C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Juhu Campus, Santacruz (West), Mumbai 400049.

Article Received on 13/01/2017 **Article Revised on 02/02/2017** **Article Accepted on 23/02/2017**

ABSTRACT

An isocratic stability indicating RP-HPLC method was developed and validated for the assay of gallic acid (GA).The marker and other degradation components were separated on aThermo Hypersil BDS-C_{18} (250 x 4.6 mm, 5.0 µ) column. The mobile phase composed of water acidified with phosphoric acid (0.01%): methanol in a ratio 95:5.The mobile phase flow rate was optimized at 1.0 mL/min. The detection wavelength was selected as 271nm.The method was linear over the concentration range of 5–50.0 µg/mL. Forced degradation studies of GA were carried out under conditions of acid, base and oxidative hydrolysis, sun light irradiation and in water to detect the major degradation products of the GA. The GA was found to be stable under acidic, neutral and in sun light irradiation. The alkaline conditions resulted in higher degradation of GA than oxidative conditions.

KEYWORDS: Gallic acid, HPLC, stability indicating, forced degradation.

INTRODUCTION

During the past decade, an increasing acceptance and public interest in herbal medicines has been observed in both the developing and developed countries. However, one of the problems to the acceptance of herbal formulations is the lack of standard quality control profiles. The quality of herbal medicine (i.e. the profile of the constituents in the final product) has implications for efficacy and safety. Because of the complex nature and inherent variability of the chemical constituents of the plant based products, it is difficult to establish their quality control parameters. The modern analytical techniques are expected to help in circumventing this problem. Various Ayurvedic formulations have been found to be useful remedies for a number of disorders. Although herbal formulations are effective but their use is often associated with a number of undesirable side effects that may be due to the degradation products of drug formed during storage.[1]

The International Conference on Harmonization (ICH) drug stability test guideline Q1A requires that analysis of stability of samples is to be done through the use of validated stability-indicating analytical method, but this concept is often used for synthetic drugs and is not commonly used for herbal drugs. The results of stress test often help to guide the pharmaceutical dosage forms and provide information about the instability of drugs, enabling the development of stability –indicating analytical methods that may be used in the subsequent stability studies of the active ingredient. Hence these studies are also important for herbal dosage form containing the active markers. [2]

Gallic acid is chemically 3,4,5- trihydroxy benzoic acid and possesses astringent, anti-inflammatory, antimutagenic, anticancer and antioxidant activities.[3] A large number of plants are rich source of gallic acid . It is present in large amount in tea, red wine, fruits, and various medicinal plants such as *Terminaliachebula*, *Terminaliaarjuna*, *Punicagranatum* etc.[4]]Thishydrolyzable tannin iseasily hydrolyzed by the action ofacid and enzymes.[5]Various high-performance liquid chromatographic methods are reported for determination of gallic acid which showed that the retention time for gallic acid is around 2-3 min.[6,7] For stability indicating method the retention time must be higher, for elution of more polar degraded products formed during stress testing of gallic acid.

Therefore the present work, aims to develop and validate a stability indicating HPLCmethod for determination of gallic acid and qualitative analysis of its components for subsequent use in the quality control of herbal medicine.

EXPERIMENTAL

Reagents

Gallic acid was purchased from Sigma (purity of 95.0% by HPLC), methanol was of HPLC grade and purchased from S.D. Fine chemicals. Water was purified by doubled distillation and filtered by using 0.2µm nylon membrane filter.

Instrumentation
Agilent 1200 system (Shimadzu, Tokyo, Japan), consisting of a quaternary pump and UV-Vis. Detector.

Chromatographic Conditions
Analysis was performed using a columnHypersilRP-C_{18} (250 x 4.6 mm, 5.0 µm column) with an isocratic run, using the mobile phase consisting ofaqueous o-phosphoric acid (0.01%) and methanol in the ratio of 95:5 at a flow rateof 1 mL/min. The detection was carried out at wavelength 271 nm.

Preparation of standard stock solution
Gallic acid (5 mg) was dissolved in 50 mL of methanol, sonicated for 30 min (100ug/mL). This solution was diluted appropriately with mobile phase to produce a working solution (20 ug/mL) for assay of the samples. Both solutions were freshly prepared and filtered through a 0.45 µm modified PTFE membrane, prior to injection.

Forced Degradation Studies[8]
A standard stock solution of gallic acid (1mg/mL) was prepared by dissolving 10 mg of the gallic acid in 10 ml of methanol. From the above stock solution, 2 ml was diluted up to 10 ml with 6 M HCl, 0.1 M NaOH, 6% H_2O_2 and HPLC water in separate volumetric flasks to achieve a concentration of 200 µg/ml.The above solutions were exposed to stress tests under acidic condition (10 mL solution in 6 M HCl, 24 h), alkaline condition (10mL solution in 0.1 M NaOH, 1 h), oxidative condition (10 mL in 6% H_2O_2, 1h) and in water for 24 h. All the solutions were refluxed at 80^0C. After the degradation time was achieved, 1 mL of the solution was transferred to another 10 mL volumetric flask, neutralized andvolumewas made up to 10 mL with mobile phase. For photostability studies the gallic acid solution in water was exposed to sunlight for 30 days. After filtration through a 0.45 µm modified PTFE membrane, the degraded samples were injected into the HPLC, and compared with the fresh, non – degraded sample solution.

Method Validation
The developed method was validated as per ICH guidelines. The validation parameters evaluated were as fallows.[9]
1. Linearity
2. Precision
3. LOD & LOQ
3.Robustness
5.Accuracy
6.System suitability

Linearity
The linearity of analytical procedure is its ability (within given range) to obtain the test results which are directly proportional to concentration in the sample. This was studied by analyzing six concentrations in triplicate with the concentration in range of 5-50 µg/mL solution of gallic acid.

Precision
The precision of the method was examined by performing the intra- and inter-day assays of six replicate injections at three concentration levels (10,20 and 30 µg/mL). The intraday assay precision test was performed at the intervals of 4 h in 1 day, while the inter-day assay precision test was performed over 6 days.

LOD &LOQ
The Limit of Detection (LOD) and limit of Quantification (LOQ) were mathematically determined through the calibration curve. The aforementioned factor (3.0 and 10, for LOD and LOQ respectively) were multiplied by the standard deviation of the linear coefficient and divided by the slope, according to the guideline.[7]

Robustness
The robustness of the method was evaluated by changing: (i) the mobile phase flow rate(0.9, 1, 1.1 mL/min), (ii) the mobile phase pH (2, 3 and 4) and (iii) the solvents of different lots. Standard solutions were injected six times for each change. The % RSD was calculated for each component during each change.

Accuracy
Recovery study was performed for determining the accuracy of the method. Sample solution was analyzed in triplicate for each concentration level (80%, 100% and 120%). The results of recovery study are depicted in the Table 3.

System Suitability
A system suitability test was performed to evaluate the chromatographic parameters (capacity factor, separation factor, number of theoretical plate, asymmetry of the peak and resolution between two consecutive peaks. Triplicate injections of the standard solution(20 µg/mL) were analysed.

Analysis of marketed formulations
To determine the content of gallic acid three different herbal formulations (DBEROL Tab., HARTO Tab., TRIPHLA Churna) were procured from local market.

1. DBEROL tablet
Twenty tablets were weighed, their mean weight was determined and they were finely powdered. 0.5 g of tablet triturate was weighed and transferred to 10 mL volumetric flask. 5 mL of methanol was added to it and then sonicated for 30 min. After that volume was made up to 10mL with methanol and again sonicated for 20 min. 1ml of filtrate was taken and diluted to 10 ml with mobile phase.The above solution was filtered through 0.45 micron filterand injected into the HPLC system.

2. HARTO tablets
Twenty tablets were weighed, their mean weight determined and they were finely powdered. 0.5 g of tablet triturate was weighed and transferred to 10 mL

volumetric flask, to it 5 mL of methanol was added and sonicated for 30 min. After that the volume was made up to 10 mL with methanol and again sonicated for 20 min. 3 mL of filtrate was taken and diluted to 10 mL with mobile phase. The above solution was filtered through 0.45 micron filter and injected into the HPLC system.

3. TRIPHALA Churna

0.5 g of powder was weighed and transferred to 50 mL volumetric flask. The volume was made up to 50 mL with methanol and sonicated for 30 min. From the above solution 1 mL was diluted to 10 mL with mobile phase. The above solution was filtered through 0.45 micron filter and injected into the HPLC system.

A 20 µL volume of sample solution was injected into HPLC, three times under the condition above. The peak areas were measured and the concentrations in the sample were determined using calibration developed on the same HPLC system under the same condition using the linear regression equation.

RESULT AND DISCUSSION

A simple stability- indicating RP-HPLC method was developed for determination of GA and its degradation products. To optimize the proposed HPLC methods, different mobile phases, were tried for the chromatographic separation of the components and retaining GA. The best resolutionwas achieved using a mobile phase consisting of o-phosphoric acid (0.01%): methanol (95:5). (Fig.no.1, a).

Forced Degradation Studies

Conditions usedfor forced degradation were to achieve degradation in the range of 20-80% of the drug. The following degradation behaviour of the drug was observed during the stress degradation studies.

Acidic condition

The GA was refluxed in a specified concentration of 6 M HCl for 24 h. GA was found to be stable to acid degradation.

Alkaline condition

GA was found to undergo alkaline degradation faster as compared with acid degradation. In 0.1 M NaOH (1 h), the drug decomposed by 20%. A major degradation peak was found at 2.7 min. (Fig.1b).

Oxidative condition

GA was found to be highly labile in terms of oxidation in 6% H_2O_2 at room temperature for 1 h. The major degradation peak was observed at 2.7 min. (Fig.1c).

Neutral (water) condition

GA was found to be stable on refluxing the drug in water at 80^0C for 24 h.

Photolytic condition

GA was found to be stable to photolytic degradation.

HPLC–UV method validation

The method was validated for linearity, accuracy, precision, robustness, system suitability, LOD, LOQ and by the following procedure.

Linearity

The calibration curve was linear over the concentration range 5-50ug/mL for GA. The correlation coefficient (r^2) was found to be 0.999. Linearity data and calibration curve for GA are reported in the Table 1and Fig. 2.

Precision

The precision of the method was examined by performing the intra- and inter-day assays of six replicate injection of the mixture of the standard solution at three concentration levels 10, 20 and 30µg/mL). The % RSD of the assay was less than 2.(Table 2).

LOD and LOQ

LOD and LOQ of the method was determined by Ksd/s, where K is the constant (3 for LOD and 10 for LOQ), sd is the standard deviation of the analytical signal and s is the slope of the concentration /response graph. The LOD and LOQvaluewasfoundto be 0.31µg/mL and0.91µg/mL, respectively.

Accuracy

The accuracy of the method was determined by calculating the recoveries of GA by the method of standard addition. Known amounts of the standard (80, 100, 120%) were added to the pre- analyzed sample solution and the amounts of these standards were estimated by measuring the peak areas. The results from recovery study for accuracy determination are given in the Table 3. The percentage recovery of GA was found to be with in the limit (101.83-102.68%).

Robustness

The robustness of the method was evaluated by changing: (i) the mobile phase flow (0.9, 1, 1.1 ml/min), (ii) the mobile phase pH(2, 3 and 4) and (iii) the solvents of different lots. Standard solutions were injected six times for each change. The % RSD was found to be less than 2.The results are summarized in the Table 4.

System Suitability

All the values of parameter i.e. capacity factor, separation factor, number of theoretical plate, asymmetry of the peak and resolution between two consecutive peak of system suitability were found to be within in the acceptable limits. It is concluded that the method and system are adequate for the analysis to be performed. The results are summarized in the Table 5.

Analysis of marketed formulations

The developed and validated method was applied to determine the content of gallic acid in three marketed herbal formulations (DBEROL Tab., HARTO Tab., TRIPHLA Churna). (Table 6). The gallic acid content was highest in TRIPHALA Churna as compared to the

other two formulations. TRIPHALA Churna contains powder of three herbs, Amla, Harda and Beherda. All the three herbs contain gallic acid and hence, this could be the probable reason for the highest amount of gallic acid in TRIPHALA Churna.

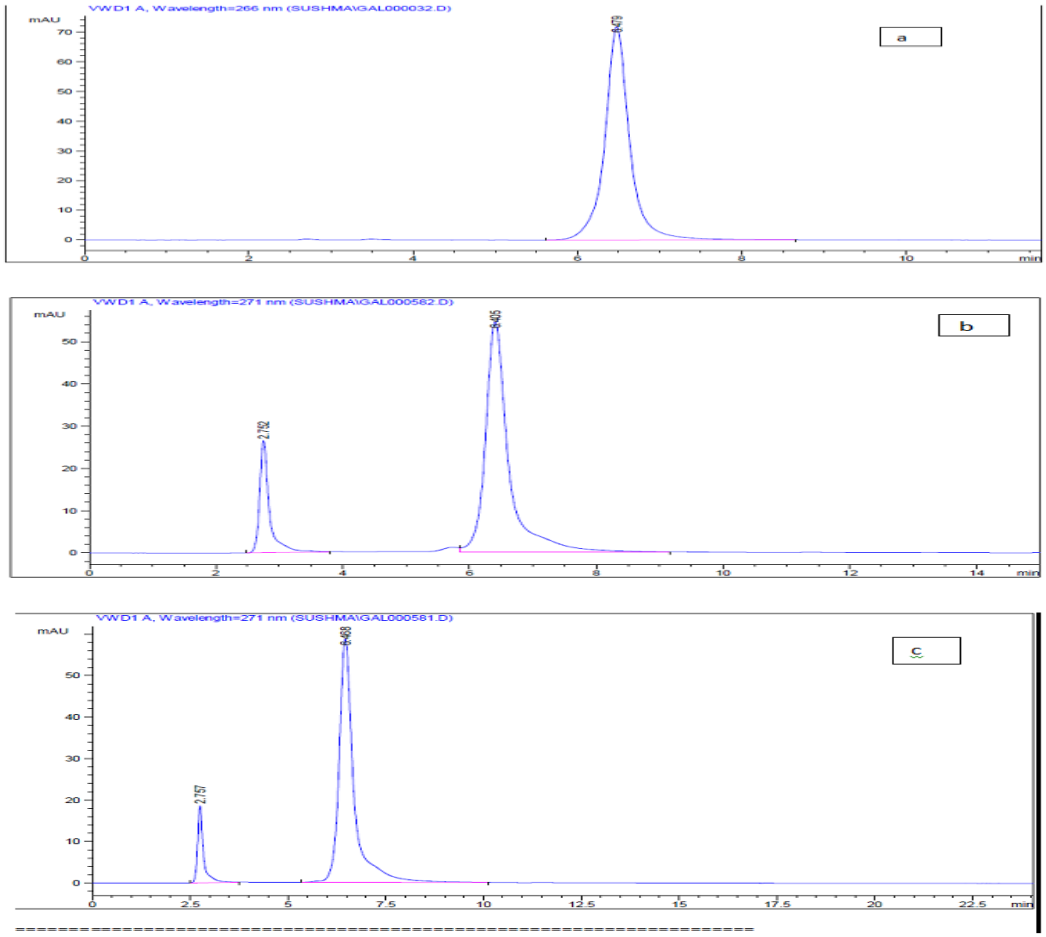

Figure1. HPLC Chromatograms at 271 nm (a) Gallic acid (GA) at 20ug/mL (b) GA after stress with 0.1 M NaOH 1 hr (c) GA after stress with 6% H_2O_2 1 hr.

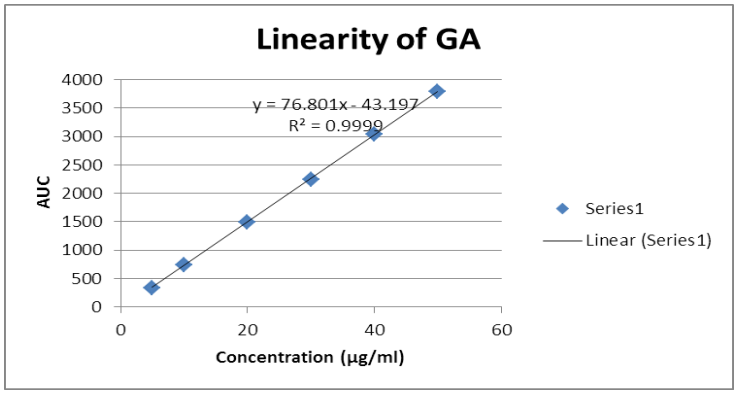

Figure 2: Calibration curve of gallic acid

Table1. Linear regression data for calibration curves (n= 3)

Parameters	GA
Detection wavelength, nm	271nm
LOD	0.31 µg/mL
LOQ	0.91 µg/mL
Linearity range,	5-50 µg/mL
Correlation coefficient	0.9990
Regression equation	y = 76.80x - 43.19

Table: 2. Intra- and inter-day precision of the developed method (n=6)

Component GA(µg/mL)	Intraday				Interday			
	Retention time		Peak area		Retention time		Peak area	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
10	6.09	0.42	744.75	0.65	6.09	1.07	729.63	1.23
20	6.08	0.69	1489.91	0.77	6.08	0.25	1504.14	0.90
30	6.01	0.64	2246.16	0.68	6.06	0.59	2266.98	0.74

Table3. Recovery study of GA added to the pre- analyzed samples using the proposed method(n=3)

Components	% Quantity added(µg/mL)	Total quantity present(µg/mL)	Amount quantity found(µg/mL)	%Recovery	%RSD
GA	80	20	10.268	102.68	1.80
	100	30	20.367	101.83	1.75
	120	40	30.727	102.42	1.09

Table: 4. Robustness evaluation of proposed HPLC method(n=3)

Factor	Level	Retention time	Asymmetry
A:Flow rate(mL/min)			
0.9	-1	6.6	0.95
1.0	0	6.5	0.95
1.1	+1	5.5	0.78
Mean±%RSD(n=3)		6.2± 0.60	0.89 ±0.09
B: Ph range			
2	-1	5.9	0.66
3	0	6.5	0.95
4	+1	6.1	0.65
Mean±%RSD(n=3)		6.1± 0.30	0.75± 0.17
C: Solvent of different lot			
First lots		6.5	0.94
Second lots		6.3	0.85
Mean± %RSD(n=3)		6.4± 0.14	0.89± 0.06

Table5: Results of system suitability parameters of the proposed HPLC method (n=3)

Sr.No	Parameter	GA
1	Retention time (min)	6.4
2	Capacity factor (k')	1.37
3	Separation factor	2.37
4	No. of theoretical plate	2935
5	Resolution (Rs)	2.07
6	Asymmetry (As)	0.98

Table 6: Assay results of marketed herbal formulations using the developed HPLC method (n=3)

Marketed formulation	Content of gallic acid (%w/w)
DBEROL tablet	0.37
HARTO tablets	0.12
TRIPHALA Churna	0.76

CONCLUSION

A stability indicating HPLC method was successfully developed and validated for estimation of gallic acid as per ICH guidelines. The method was applied for quantitative estimation of gallic acid in three marketed herbal formulations.

ACKNOWLEDGEMENT

The authors gratefully acknowledge UGC-BSR for financial support and C. U. Shah College of Pharmacy S.N.D.T. Women's University, Mumbai for providing the necessary facilities for carrying out this research work.

REFERENCES

1. P. Raj, A. Pathak. Stability-Indicating reversed phase liquid chromatographic method for determination of Aconitine and piperine in a polyherbal formulation. JAOAC, 2009; l4: 1047-1053.
2. Giovana F, LucianaC, Block. Development and validation of a stability indicative HPLC–PDA method for kaurenoic acid in spray dried extracts of *Sphagneticolatrilobata* (L.) Pruski, Asteraceae, Talanta, 2012; 101: 530–536.
3. Mohan K, Estimation and validation of gallic acid in polyherbal formulation by HPTLC., IJPPS, 2013; 5: 204-206.
4. Wang H, HelliwellK, Xiaoqing. Isocratic elution system for the determination of catechins, caffine and gallic acid in green tea using HPLC. J. Food Chemistry, 2000; 68: 115-121.
5. Yean-Yean Soong , Philip J. Barlow. Quantification of gallic acid and ellagic acid from longan (*Dimocarpuslongan Lour*.) seed and mango (*Mangiferaindica* L.) kernel and their effects on antioxidant activity. Food Chemistry, 2006; 97: 524–530.
6. Sampath M., Isolation and identification of gallic acid from *PolyalthiaLongifolia* (Sonn) thawaites, Int J Pharm Bio Sci., 2013; 42: 966–972.
7. Son R, ChengY, Tian Y,. Zun-Jian1, A validated solid-phase extraction HPLC method for the simultaneous determination of gallic acid, catechin and epicatechin in rhubarb decoction. Chinese Journal of Natural Medicines., 2012; 10(4): 275-278.
8. Saranjit S, Mahendra j, Gajanan M, Tiwari H,Kurmi M, Parashar N,. Forced degradation studies to assess the stability of drugs and products. Trends in Analytical chemistry, 2013; 49: 71–88.
9. ICH, Validation of Analytical Procedures: Textand Methodology—Harmonized Tripartite Guideline, Geneva, 2005.