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Research Article

ESTIMATION AND VALIDATION OF GALLIC ACID IN POLYHERBAL FORMULATION BY HPTLC

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ABSTRACT

Gallic acid is a phenyl propanoid, chemically it is 3, 4, 5 –Trihydroxy benzoic acid which is widely present in the plant kingdom and possesses activities like antioxidant, antimutagenic anticarcinogenic etc., In our current study we have carried out estimation and validation of gallic acid content in polyherbal formulation by HPTLC analysis which is very cheap and cost effective method. The samples were separated on pre-coated TLC plates with silica gel $60F_{254}$. The mobile phase used was Toluene: Methanol: Ethylacetate: Formic acid 30:5:55:10 (v/v/v/v). The plates were scanned at 280nm using CAMAG densitometer with WINCAT software. The LOD and LOQ for gallic acid were found to be 18ng and 55ng respectively. The developed method was validated as per ICH guide lines.

Keywords: Gallic acid, Polyherbal formulation, HPTLC.

INTRODUCTION

Gallic acidis chemically 3, 4, 5 – Trihydroxy benzoic acid and possess astringent, antioxidant, anticarcinogenic activity[1-5]. A large number of plants are rich sources of gallic acid either in the form of tannin or free base. It is present in tea, red wine, fruits, beverages and various medicinal plants[6,7] . Terminalia species are one of the richest sources of gallic acid and has been extensively used in Ayurveda, Unani and Homeopathic medicine and become cynosure of modern medicine.

In the present polyherbal formation consists of *Terminalia chebula* and *Terminalia arjuna* used for the management of obesity, peptic ulcer[8] associated with various clinical conditions[9]. Literature survey reveals that several methods were reported for the estimation of gallic acid. The determinations of gallic acid on biological samples using hplc[10], mass and thin layer chromatography[11] are also reported. But there is always a difficulty in estimation of active constituents in ayurvedic polyherbal formulations with different combinations. Hence we thought it is worthwhile to develop and validate an accurate and economical HPTLC method for quantification of gallic acid in polyherbal formulations. The proposed method can be applicable for routine analysis and content uniformity test of gallic acid in herbal formulations well with the validation requirements in the pharmaceutical industry. The developed method is validated according to the ICH guidelines

MATERIALS AND METHODS

The capsule formulation was obtained from M/s Varanasi Bio-Research Pvt. Ltd, Varanasi, India and standard Gallic acid was obtained from M/s Sigma Aldrich Ltd. All chemicals used were of analytical grade. All dilutions were performed in standard volumetric flasks.

Instrumentation

HPTLC system – Camag HPTLC system with densitometer and auto samplerLinomat V (Camag,Switzerland), Twin trough developing chamber (Camag) 200 x 100mm, Shimadzu AV 220analytical balance (Shimadzu, Japan) and Lab India Ultrasonication bath were used.

Stock and working standard solution

Gallic acid (4mg) was accurately weighed into a 100mL volumetric flask, dissolved in 50mL methanol. It was then sonicated for 10min and then final volume was made up to 100mL with methanol to get stock solution containing 40µg/mL. Calibration standards were prepared over the concentration range of 2 to 10µg/mL for gallic acid by appropriated dilutions from the above standard stock solution in 10mL volumetric flask with methanol.

Preparation of sample solution

20 capsules were weighed and the average weight was calculated. The powdered drug equivalent to 100mg of the *T.arjuna* in formulation was weighed accurately.Weighed formulation was extracted with 8ml of methanol. After sonicaton it was filtered through Whatmann filter paper, the volume was made upto 10ml with methanol and these solutions were used for analysis(10µg/mL).

Calibration curve of Gallic acid

All calibration standards were prepared freshly every day using standard stock solution of gallic acid and found to be stable during the analysis time. The plate was developed, dried and scanned as described below. After densitometric scanning the peak area was recorded for each concentration and a calibration plot was obtained by plotting average peak area against concentration of gallic acid (ng/spot). The slope and correlationcoefficient were also calculated and shown in **Fig.2.**

Standard chromatographic HPTLC conditions

Analysis was performed on 10 x 10 cm aluminumplates precoated with silica gel $60F_{254}$ (Merck, Mumbai, India). Standard solution of gallic acid and sample solution were applied to the plates as a bandwidth of 6.0mm, 15.0 mm apart and 10.0mm from the bottom edge of the same chromatographic plate by using of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100µL Hamilton (USA) syringe on HPTLC plate under N_2 gas stream. Ascending development to a distance of 80mm was performed at room temperature (28± 2ºC), with Toluene: Methanol: Ethylacetate: Formic acid : 30 : 5 : 55 : 10(v/v/v/v), as mobile phase, in a Camag glass twin trough chamber previously saturate with mobile phase vapour for 20 min. After development, the plates were dried with a hair dryer and then scanned at 280 nm with Camag TLC Scanner with WINCAT software, using the Deuterium lamp. After densitometric scanning, chromatograms were evaluated via peak area and concentration level. In assay experiment, six samples were weighed separately and each concentration was applied six times to the plate and analyzed. These developed plates are scanned by performingsix replicate for the measurement of peak area of the six bands ofsame concentration. The average peak areas, variations in peak area obtained were expressed as a coefficient of variation (% CV) and concentration of the drug present were automatically calculated by comparing the peak area values of the sample with that of the reference standard. The plot was obtained by plotting average peak area at each concentration against the corresponding band concentrations of gallic acid (ng/spot). From the constructed calibration curve slope, correlation coefficient and %CV of six independent assays values wascalculated.

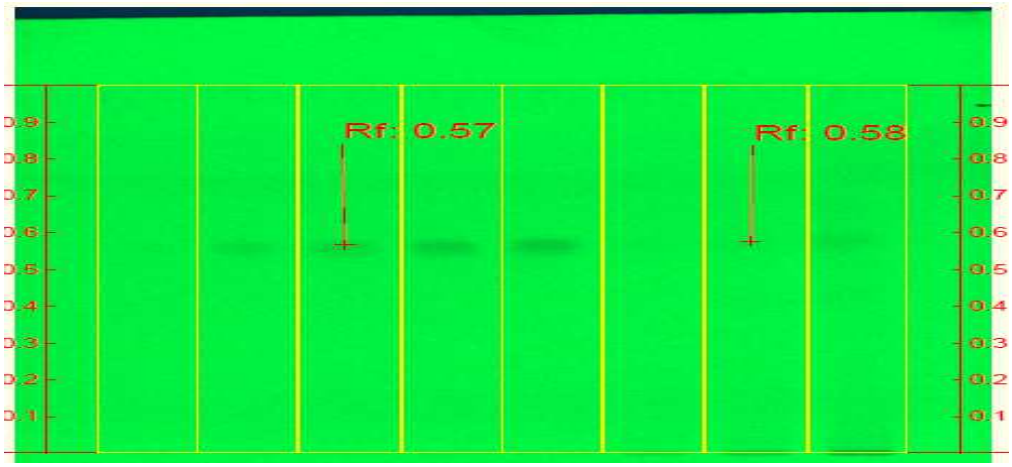

Fig. 2: 1-5 Spots are standard concentrations of Gallic acid and 6-8 spots are different concentrations of Polyherbal formulation.

Method validation

The proposed method was validated according to the ICH guidelines by carryingout, analysis of six replicate samples of the Gallic acid and the method was validated for accuracy, precision, robustness, selectivity, limit of detection,limit of quantification, linearity and range.

Limit of detection and limit of quantification

The limits of detection (LOD) and limit of quantification (LOQ) were estimated experimentally and mathematically using the formula:

LOD = 3: 3 x (standard deviation of intercept/slope of the calibration plot)

LOQ = 10 x (standard deviation of intercept/slope of the calibration plot)

LOD and LOQ values were experimentally verified by diluting known concentrations of a standard solution of Gallic aciduntil the average responses were approximately 3/10 times the standard deviations of the responses for the six replicate determinations. The LOD and LOQ were determined from the graph of the lowest part of the calibration plot.

Precision

Repeatability studies were performed by the analysis of three different concentrations (80, 160 and 320ng/band). Repeatability was determined by running minimum of six analyses per sample and evaluating the coefficient of variation (%CV) for each. Each concentration was applied six times to the TLC plate on the same day. The precision of the method as repeatability intra-day assay precision [%CV] was assessed by performing six independent analyses of sample and qualified reference standards together at 100% of the test concentration. Inter-day precision was determined by repeating the analysis of the same concentration by repeatingthe study by three different analysts on three different days over a period of 1 week also expressed in terms of %CV.

Repeatability and reproducibility

Repeatability was further confirmed by determination of %CV for standard addition at three concentrations levels. In assay experiment six samples were weighed separately, analyzed and the results of independent assay of the 200, 300 and 500ng/band were depicted in **Table 2.**Reproducibility means the precision of the procedure when it is carried out under different conditions.

Accuracy by recovery

Accuracy of the method was ascertained by performing recovery studies by standard addition method at different levels, i.e., 0%, 80%, 100% and 120% by adding standard drug of Gallic acid to previously analyzed capsule powder sample and mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate and the amount of drug found, % recovery was calculated.

Selectivity and specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix in impurities, degradation products and matrix components. UV spectrum of gallic acidwas initially obtained by scanning a developed plate in screening mode to select the detection wavelength. For each sample, UV spectra taken at the edges and maxima of the gallic acidpeaks were compared automatically to verify peak purity. Specificity of the method was ascertained by analyzing standard drug and samples of equivalent concentration (25mg). The specificity of the method was established by analyzing formulated capsule as an experimental sample together with the reference standard using proposed method. The spot for gallic acid in sample was confirmed by comparing the R_f and spectra of the spot in samples with those of standard. The peak purity of sample was judged by comparing the spectra at peak start, peak apex and peak end positions of the spot.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage by introducing small changes in the mobile phase composition; the effects on the results were examined. Mobile phases having diverse composition of Toulene: methanol: Ethylacetate: Formic acidwas also tried at two different concentration levels of 100 and 200 ng/spotand % CV of peak area was calculated.

Analysis of the Gallic acid in polyherbal formulation

In assay the standard solution of gallic acid of 40µg/mL and the experimental solution (extracted from methanol) of strength (50mg/mL) were prepared bythe above described procedure. The standard solution of gallic acid of strength 40µg/mLwasapplied on HPTLC plate together with reference standard, developed and scanned under the optimized conditions by the proposed method. The developed plate, spectrum of gallic acid and polyherbalformualtion was depicted in **Fig. 3-4.**

RESULTS AND DISCUSSION

HPTLC analysis

A mixture of Toluene:Methanol:Ethyl acetate: Formic acid (3:0.5:5.5:1 v/v/v/v) mobile phase gave a good resolution of Gallic acid and reproducible peak at R_f value of 0.57. The calibration curve for the gallic acid was linear over the range of 80 to 400ng/mL/spot. The LOD

of gallic acid by proposed method was determined by repeated scanning of the lowest detectable standard solution, multiplying the standard deviation of the peak are by three and converting area into concentration. The recovery rates were determined at three different concentrations of the marker to the extracts and analyzed quantitavelyin triplicate. Mean recoveries for Gallic acid from formulation was found to be 99.08% which indicates the accuracy of the method. The precision of the HPTLC instrumentation was checked by repeated scanning of the same spots of the markers three times on a same day (intra-day precision) and on the three consecutive days (inter-day precision)and the relative standard deviation data shown in **Table 1&2.** The UV-Vis absorption spectra recorded on the CAMAG TLC scanner at the start, middle and end positions of the gallic acid peak. Gallic acid was well separated in the polyherbal formulations by the HPTLC method and detected in all the concentration at R_f values 0.57. CV[%] for all the validation experiments and sample analyses; all values were within 5%. The summary of the all the validation parameters are given in **Table 4.**

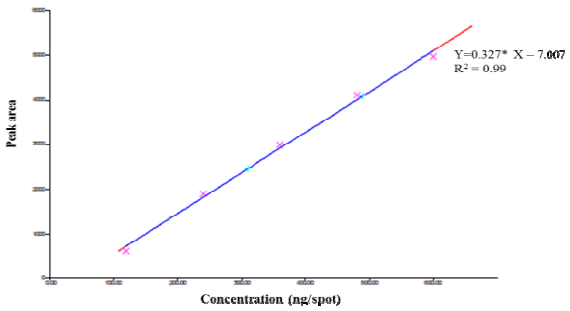

Fig. 3: Linearity of Gallic acid

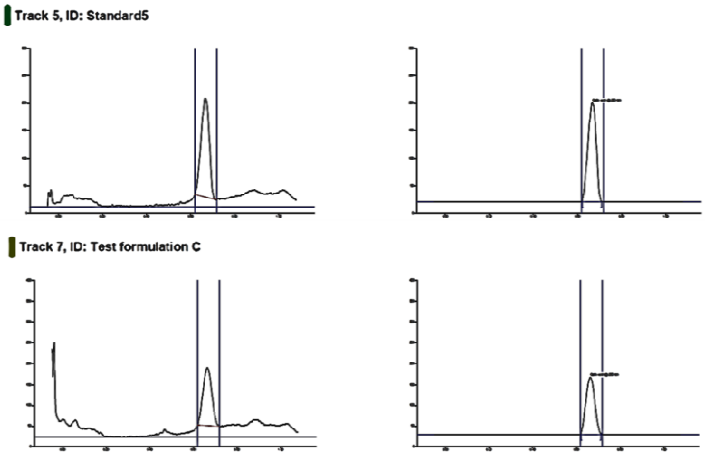

Fig. 4:Chromatogram of gallic acid and polyherbal formulation

Table 1: Summary of intra-day and inter-day method precision

Amount [ng spot^{-1}]	Intra - day precision by peak area			Inter-day precision by peak area		
	Average peak area	SD of peak area	CV [%]	Average peak area	SD of peak area	CV [%]
160	1177.2	4.5	0.38	1186.8	8.1	0.55
240	1483.6	26.1	1.76	1473.5	22.2	1.51
360	2938.1	38.6	1.31	2974.8	58.6	1.97
Precision			1.51			1.38

Each value is a mean of three observations

Table 2: Results from the study of the recovery of Gallic acid

S. No.	Amount of Gallic acid present in formulation	Amount of Gallic acid added	Amount found	%Recovery	%R.S.D	S.E.M	CV
1	1482	0	1479	99.86	0.20	1.76	0.21
2	1482	80	1550	99.23	0.25	2.31	0.26
3	1482	160	1611	98.12	0.18	1.76	0.19
4	1482	240	1689	98.95	0.26	2.6	0.26
Average recovery				99.04	0.22	2.1	0.23
S.E.M				0.50			
%R.S.D				0.72			

Table 3: System precision studies of the developed method

	Repeatability of the measurement of peak area of Gallic acid 160ng spot^{-1}	Repeatability of the sample application of peakarea of Gallic acid 160 ng spot^{-1}
	1172.4	876.3
	1181.6	864.2
	1173.6	870.2
	1177.2	874.6
	1183.2	869.4
	1177.0	871.5
Mean area	1178	871.0
S.E.M	1.74	1.73
%R.S.D	0.35	0.48
CV%	0.36	0.49

Table 4: Summary of method validation parameters

Parameter	Gallic acid
Linearity range (ng)	80-400
Correlation coefficient	0.9986
Limit of detection(µg)	18
Limit of quantification(µg)	55
Recovery (mean ± S.E.M.)	99.08±0.5
Precision (CV)	
Repeatability of application (n=6)	0.49
Repeatability of measurement (n=6)	0.36
Intra-day (n=6)	1.51
Inter-day (n=3)	1.38
Robustness	Robust

Estimation of the Gallic acid in polyherbal formulation

A single spot at R_f = 0.57 was observed in the chromatogram of the polyherbal formulations. There was no interference of the other active components and excipients present in the formulations. The gallic acid present in the polyherbal capsule formulation was found to be 1.7116%w/w.

CONCLUSION

The low %CV value obtained indicates that the suitability of this method for routine analysis and quantitative determination of the gallic acid in the polyherbal formulations by HPTLC. The proposed method for the estimation of gallic acid is accurate, linear, rugged, simple and rapid than the reported methods. The method can, therefore, be used for routine quality-control analysis and quantitative determination of gallic acid in formulations.

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