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Quantitative Estimation of Gallic Acid in *Triphala Churnam* Tablet by RP-HPLC

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Abstract

A RP-HPLC assay method has been developed and validated for the estimation of gallic acid. Chromatography was carried on C18 column (250 mm x 4.6 mm I.D., 5 µm) by isocratic elution utilizing a mobile phase of acetonitrile and water containing 0.01 % v/v ortho phosphoric acid (in the ratio of 80: 20% v/v) with UV detection at wavelength 270 nm at the flow rate 1mL/min. The proposed method was validated for sensitivity, specificity, linearity, accuracy, precision, ruggedness, robustness and solution stability. The response of the drug was linear in the concentration range of 20-120 µg/mL. Limit of detection and limit of quantification was found to be 6.13 µg/mL and 18.57 µg/mL respectively. The % recovery ranged within 98-102%. Method, system, interday and intraday precision were also found to be within the limits of acceptance criteria. Method was found to be rugged when analysis was carried out by different analyst. The proposed method is rapid, simple and also it can be applied for the routine analysis of herbal formulations.

Keywords*:* Gallic acid, *Triphala churnam* tablets, RP-HPLC.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the Ayurvedic or Siddha formulations is the lack of standard quality control profiles **[1]**. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and hence modern analytical techniques are expected to help in circumvention this problem **[2]**.

Standardization of Ayurvedic or Siddha formulations is the need of the day. Hence, modern methods can be used to set up certain standards for the herbal formulations. *Triphala churnam* tablet formulation consists of one part each of Katukkay tol (*Terminalia chebula*), Nellikay (*Embelica officinalis*) and Thanrikay (*Terminalia belerica*) and also contain the other binding agents. *Triphala churnam tablet* is an herbal formulation used extensively in Siddha system of Indian medicine, treating wounds and local ulcers. Since it contains enormous amount of tannins such as Gallic acid and Ellagic acid, it is extensively used as an astringent **[3-4]**. No work has been carried out in the estimation of marker compounds in the Siddha formulation of *Triphala churnam* tablet formulation. Hence an attempt has been made in the present work to develop simple, precise and accurate reverse phase high performance liquid chromatographic method to estimate gallic acid in *Triphala churnam* tablet formulation.

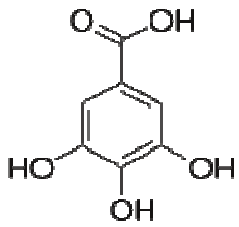

Figure 1 Structure of gallic acid

MATERIALS AND METHODS

Chemicals

Gallic acid marker compound was purchased from Indofine, Inc, USA. All other reagents were of HPLC grade and purchased from SD fine chemicals. *Triphala churnam* tablets were procured from local Ayurvedic Pharmacy.

Instrument

The chromatographic system consisted of Shimadzu, Prominence, and a manual rheodyne injector with a 20 µL fixed loop. The separation was performed on a Phenomenex C18 ODS column (250 mm x 4.6 mm I.D., 5 µm) at room temperature with a UV Visible detector.

Chromatographic conditions

Chromatographic determination was performed using a C18 reverse phase column at ambient temperature with the injection volume of 20 µL at a flow rate of 1 mL/min. The mobile phase composition optimized was acetonitrile and water containing 0.01% v/v ortho phosphoric acid in the ratio of 80: 20 respectively at detection wavelength of 270 nm.

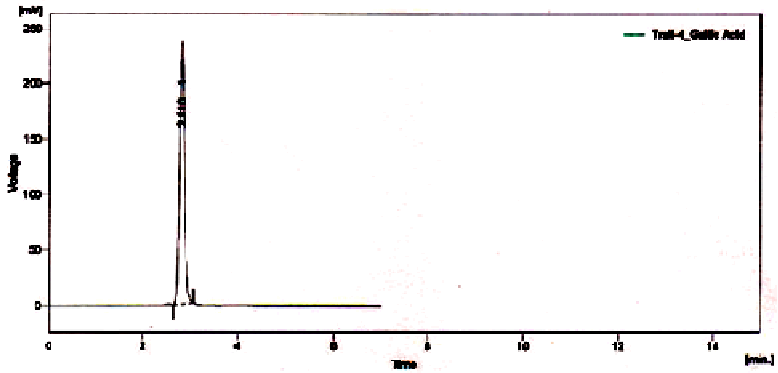

Figure 2 Chromatogram of gallic acid

Preparation of standard solutions

50 mg of gallic acid was taken in a 50 mL standard flask and dissolved in 15 mL of milli-Q-water. The volume was then made up to 50 mL with milli-Q-water to get a concentration of 1 mg/mL.

Calibration Curve

From the standard solution, appropriate dilutions were made by taking 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL and 1.2 mL of the standard solution of gallic acid and then making up the volume up to 10 mL with mobile phase resulting in concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL and 120 µg/mL of gallic acid respectively. A calibration curve (peak area Vs concentration) was plotted from the peak areas obtained. The correlation coefficient was found to be 0.999.

Application to Herbal formulation (*Triphala churnam* tablet)

Sample preparation

20 tablets were weighed and powdered. From that 1.6403 g of powder was weighed into a 10 mL standard flask and extracted with 10 mL of milli-Q-water by sonication for 20 min. Later it was kept aside for 12 h to extract gallic acid by cold maceration **[5-7]**, and then filtered through Whatmann No.1 filter paper to obtain a clear solution. From that 0.8 mL was diluted to 10 mL with mobile phase.

Assay procedure

Gallic acid content was calculated in the *Triphala churnam* tablet by comparison with the appropriate gallic acid standard solution. No interferences due to other ingredients and excipients was detected in the spectra or chromatograms produced.

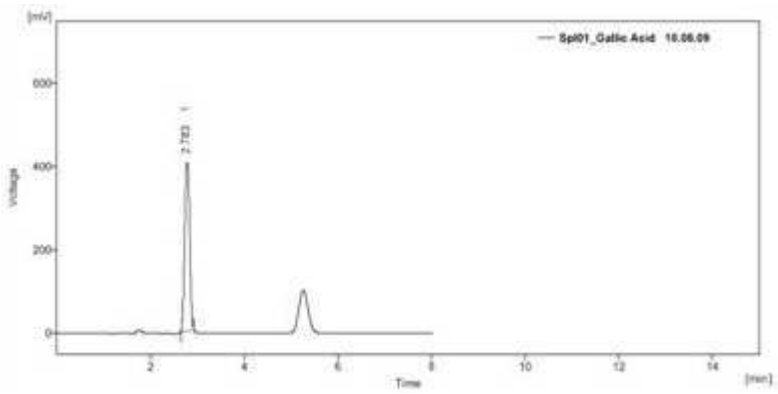

Figure 3 Chromatogram of *Triphala churnam* tablet

RESULTS AND DISCUSSION

In the present study, a simple, precise, accurate and rapid reverse phase HPLC method has been developed and validated for the determination of gallic acid in herbal formulation. The developed analytical method was validated as per ICH method validation guidelines. The validation parameters addressed were LOD, LOQ, linearity, accuracy, precision (inter-day and intra-day), robustness, ruggedness and specificity.

Accuracy

The accuracy of the method was determined by calculating the recovery of gallic acid by the method of standard addition **[8-12]**. The accuracy of the method was checked by intercepting calibration curve (which is plotted between the area under curve on y- axis and concentration of standard solutions on x-axis) with the sample area under curve which is obtained when injecting the 80 µg/mL, 100 µg/mL and 120 µg/mL standard solutions. The % recovery for gallic acid was found to be 98.5 %, 99.5 % and 99.15 % respectively which was shown in the Table 1.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, etc. To perform the specificity parameter, stressed samples (sample heated to 60 $^{\circ}$C for 2 h, sample treated with 1N hydrochloric acid for 2 h, and sample treated with 1N sodium hydroxide for 2 h) and working standard were injected separately [**8-12**]. The results are given in Table 2.

Linearity

The standard stock solution was diluted further to get concentrations in the range of 20 µg/mL to 120 µg/mL of gallic acid. Each concentration was injected in triplicate and the average area was calculated **[8-12]**. From the average area, calibration curve was plotted using peak area vs concentration. The correlation coefficient was found to be 0.999.

Precision

The intraday and inter day precisions of the proposed method were determined by estimating the corresponding response 3 times on the same day and on 6 different days over a period of 1 week for three different concentrations of gallic acid **[8-12]**. The results are reported in terms of relative standard deviation (RSD) in Table 2.

Sensitivity

The LOD was calculated from the slope and was found to be 6.13 µg/mL **[8-12]**. The LOQ was calculated from the slope and was found to be 18.57 µg/mL.

Robustness

Robustness of the proposed method was evaluated by changing the column to a Phenomenex ODS, C18 (250 mm x 4.6 mm I.D., 5 µm) column **[8-12]**. The effect of change in temperature was studied and reported in Table 2.

System suitability

Standard solution was injected 6 times for each change. System suitability parameters and RSD were calculated for each peak **[8-12]**. Recoveries and % RSDs were calculated for each component during each change and is reported in Table 2.

Table 1 Recovery study of gallic acid by HPLC method

Conc (µg/mL)	Peak area	Average	Recovery (µg/mL)	% Recovery
80	2994.344	2995.397	78.8	98.5
	2995.545			
	2996.303			
100	3782.275	3782.854	99.5	99.5
	3781.005			
	3785.282			
120	4522.803	4523.479	118.98	99.15
	4524.971			
	4522.664			

Table 2 Summary of validation parameters of embelin

Parameters	Results
Linearity	
Range	20-120 µg/mL
Linear equation	Y = mx + C
Slope (m)	38.07
Intercept (C)	-5.791
Correlation coefficient (r^2)	0.999
Standard deviation (SD)	0.031
Precision (% RSD)	
Intraday precision (n=3)	% RSD = 0.124
Inter day precision (n=3)	% RSD = 0.0307
Limit of Detection (LOD)	6.13µg/mL
Limit of Quantification (LOQ)	18.57µg/mL
Ruggedness	% RSD = 0.32
Robustness	Robust
Specificity	Specific
System suitability	% RSD = 1.23
Quantification	3.06 mg/tablet

Ruggedness

For demonstrating ruggedness of the method, the standard drug solution was injected in triplicate by two different analysts **[8-12]**. The % RSD value was found to be 0.059 which is in the limit.

A simple HPLC method was adopted for the estimation of gallic acid in herbal formulation. To optimize the proposed HPLC method, all of the experimental conditions were investigated. For the choice of stationary phase, reversed-phase separation was preferred due to the drawbacks of the normal phase. To optimize the mobile phase, different systems were tried for chromatographic separation of the components; the best resolution was achieved using a mobile phase consisting of acetonitrile and water containing 0.01% v/v ortho phosphoric acid in the ratio of 80: 20, which gave good sensitivity.

The calibration curve constructed for the marker was linear over the concentration range of 20-120 µg/mL for gallic acid. Peak areas of the marker was plotted versus the concentration and

linear regression analysis performed on the resultant curve with the correlation coefficient 0.999 for gallic acid. The precision result of the solution at medium concentration (Table 2) indicate that the RSD value of retention time was less than 1%, while the RSD value of peak area was less than 2 % both for intra-day assay and inter-day assay precision (Intra 2 h six injections, inter 3 days). The LOD was found to be 6.13 µg/mL. The LOQ was found to be 18.57 µg/mL. The robustness study indicated that the selected factors remained unaffected by small variations of parameters. The recovery obtained was found to be 99.5% which is under acceptance criteria according to be ICH guidelines. Therefore, it can be concluded that the method is consistent for selected column and solvent brand. A system suitability test was performed to evaluate the chromatographic parameters like capacity factor, separation factor, column efficiency, number of theoretical plates and HETP, asymmetry of the peak (Table 1). The gallic acid content in *Triphala churnam* tablet was found to be 3.06 mg.

CONCLUSION

In the present study, a simple and reproducible method for the estimation of gallic acid in herbal formulation by reverse phase HPLC method is developed. The gallic acid content in *Triphala churnam* tablet was quantified. The advantage of the method lies in the simplicity of the sample preparation and less run time. The validated parameters indicate that the developed method is quick, selective and cheap. Hence the developed method is more suitable for the estimation of gallic acid in multi-component herbal formulation.

REFERENCES

[1] Indian Herbal Pharmacopoeia, Revised New Edition, Indian Drug Manufacturers Association, Mumbai, **2002**, 206-213.
[2] JO Kokwara; Medicinal Plants of East Africa, East Africa Literature Bureau, Nairobi, Kenya, **1976**.
[3] www.phytochemicals.info/reaserch
[4] http://www.sigmaaldrich.com/catalog/lookup
[5] NS Jaganathan; K Kannan; *Res. J. of Phytochem*., **2008**, 2 (1), 1-9.
[6] 6. JX Qu; W Wenquan; Z Yanqing; B Yin; *J. Pharm. Biomed. Anal*., **2007**, 45(3), 450-455.
[7] YS Yean; JB Philip; *Food Chemistry*, **2006**, 97, 534-530.
[8] RS Lloyd, JK Joseph; LG Joseph; Practical HPLC Method Development, John Wiley and Sons, Inc, Noida, **1997**, 2, 235-251.
[9] MB David, Validating Chromatographic Methods – A Practical Guide, John Wiley and Sons, Inc, Noida, **2006**, 6, 1-5.
[10] ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, **1994**, Q2R (1).
[11] Center for Drug Evaluation and Research (CDER), 1994. Reviewer Guidance, Validation of Chromatographic Methods.
[12] AN Robert; HW Alfred; Pharmaceutical Process Validation, Replica Press P. Ltd, New Delhi, **2003**, 3, 507-524.