Research Article

Quantification of Phenolic Compound Gallic Acid in Polyherbal Ranger Capsule by High Performance Chromatographic Method

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ABSTRACT

A rapid, simple, sensitive, robust, economic and improved RP-HPLC method was developed for the estimation of Gallic Acid in Ranger Capsule, polyherbal formulation. The chromatographic conditions used for the separation was Phenomenex Luna C18 (2) (4.6 x 250mm, 5µ), rheodyne manual injector with capacity of 20µL and mobile phase comprised of water and acetonitrile (80: 20%v/v pH was maintained to 3 with Ortho Phosphoric acid). The flow rate was 1.0mL/min with detection at 272nm. The linearity was found to be in the range of 0.5-50µg/mL for Gallic acid with correlation coefficient of 0.9994. The proposed method is accurate with 100.36% - 100.97 % recovery and The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 0.0178µg/mL and 0.0539µg/mL respectively. The amount of Gallic acid in Polyherbal Capsule was found to be 0.50%.

Keywords: Gallic acid, Method Development, Quantification, Polyherbal Formulation, Polyphenol, Validation.

INTRODUCTION

Phenolic Compounds are a well-documented antioxidant and immuno modulatory agent in Indian System of Medicine (Unani System of Medicine). Gallic acid is extensively used to treat rheumatoid arthritis, Osteoporosis and as a good antimicrobial agent .Structurally Gallic acid has phenolic groups that serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the Phenolic structure.[1,2] The interest in these compounds is due to its pharmacological activity as radical scavengers.[3,4] It has been proved to have potential preventive and therapeutic effects in many diseases Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), a naturally occurring plant phenol is present in nutgalls, amla, tea leaves, grapes, hops, oak bark and other plants, both in its free state and as part of the tannin molecule.[5] Gallic acid and its derivatives have been in use in various industries as antioxidant, photographic developer, in tanning and in the testing of free mineral acids, di-hydroxy acetone and alkaloids.[6] Gallic acid possesses cytotoxicity against cancer cells, anti-inflammatory, antimutagenic, hepatprotective, neuroprotective effect, anti-tumor potential and analgesic activity.[7-13] It is also used in the pharmaceutical industry as a styptic agent and as a remote astringent in cases of internal hemorrhage. Some ointments to treat psoriasis and external hemorrhoids contain gallic acid.

Several chromatographic methods have been documented for determination of gallic acid in plant extracts but 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.[14,15] Objective of the present investigation was to establish and validate the fast and sensitive high performance liquid chromatography (HPLC) method for determination of gallic acid from any plan extract or from Polyherbal Formulation.

Ranger capsule is polyherbal dosage form that contains multiple Ayurvedic herbs including *Emblica officinalis* (Amalaki) as one of the major ingredient. This capsule contains other key ingredients such as *Asparagus racemosus* (Shatavari), *Mucuna pruriens* (Kauncha), *Withania somnifera* (Ashwagandha), *Centella asiatica* (Mandukparni), *Vitis vinifera* (Draksha), *Nardostachys jatamansi* (Jatamansi), *Tribulus terrestris* (Gokshur), *Zingiber officinale* (Shunthi), *Tinospora cordifolia* (Guduchi), *Terminalia arjuna* (Arjun).

MATERIALS AND METHODS

Reagents and standards

Ranger Capsule (An Ayurvedic Proprietary Formulation) Manufactured and Provided by Vasu Healthcare Pvt. Ltd. The reagents utilized were HPLC-grade acetonitrile, Water, Methanol, Ortho phosphoric acid (OPA), made of Merck specialties Pvt. Ltd., Mumbai were used in study. The Gallic acid was purchased from Hi-media Laboratories Pvt. Ltd. Mumbai.

Materials and equipment

Determination of Poly Phenolic compound Gallic acid was made in a HPLC system Shimadzu LC 20 AT, SPD-20A system, consisting of a Series-type double plunger solvent delivery, a Model UV-20A UV–Vis detector with wavelength Range 190-700nm, and a rheodyne injector, with a loop of 20µL capacity. Separation was attained through an Octadecyl Silane (ODS) Luna C18 (2)

Phenomenex column 4.6 × 250 mm, 5 µm and data was processed by a software Spinchrom LC Solution. The following items were also used: UV-Vis Spectrophotometer (Shimadzu UV-1800), precision balances by Shimadzu Unibloc AUX220 with Capacity of 10mg -220g, Toshcon pH meter; vacuum pump; Toshcon SW-2 ultrasound bath; and membrane filters of pore size 0.44µm by milipore.

Standard solutions

An accurately weighed quantity of Gallic acid (10mg) was transferred to a 10mL volumetric flask, dissolved and diluted up to the mark with Water: Methanol (9:1 %v/v) to obtain standard stock solution of 1000µg/mL Aliquots of 0.1, 0.3, 0.5mL standard stock solution (1000µg/mL) was transferred to 10mL of volumetric flasks and made up to the mark with Water : Methanol (9: 1 %v/v) to get concentration of 10, 30, 50µg/mL aliquots of 0.1, 0.2, 0.5, 1mL from 50µg/mL was transferred to 10mL of volumetric flasks and made up to the mark with Water: Methanol (9: 1 %v/v) to get concentration of 0.5, 1, 2.5, 5µg/mL

Sample preparation

20 capsules were taken from that, accurately weighed 200mg powder transferred to 10mL standard flask. Volume is made up to the mark with Water: Methanol (9: 1 %v/v), sonicated for 10 min. It is filtered with 0.22µ filter to obtain sample stock solution. Aliquot of 1mL from this sample stock solution is transferred to 10mL standard volumetric flask. Volume is made up to the mark with Water: Methanol (9: 1 %v/v). Then it is filtered with 0.22µ filter. Prepared sample solution was analyzed.

Chromatography

Analysis and sample drug quantifications were undertaken by Reversed-Phase HPLC, coupled to UV detection (λ = 272nm). Elution was isocratically made by a flow rate of 1mL/min. The mobile phase consisted of a mixture of acetonitrile (20%) in water and pH -3 with OPA, previously filtered and degassed. Drug identification was performed through retention times and their quantification from the peak area, intersecting the value read in a calibration curve constructed from the injected standards on the same day.

RESULTS AND DISCUSSION

Development and optimization of the analytical methodology

Chromatographic conditions were optimized to improve the performance of the method. Different Mobile phase were initially tried, but was unable to separate Gallic acid from individual ingredient in Polyherbal Formulation, which was eluted together or with very close retention times. It was then observed that the use of Methanol leads to broader peak with less Efficiency. Hence Acetonitrile was preferred over the Methanol. While use of Gallic acid instead of OPA leads to improper Asymmetry. The influence or dissuade of the relative percentages of acetonitrile in the mobile phase was studied, and it was noted that the 20% acetonitrile mixture was the most adequate and gave higher detection of Gallic acid. Higher or Lower percentages of acetonitrile reduced peak Height and Area. Detection wavelength was defined from the absorption spectrum of Gallic acid in the UV–Vis Spectrophotometer. Gallic acid gave λ_{max} at 272nm, which was selected as detection wavelength.

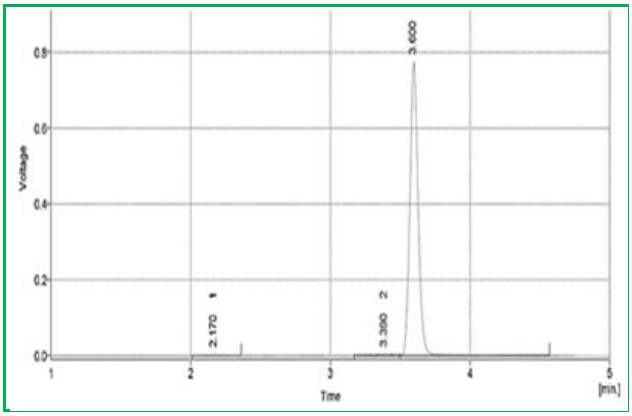

Figure 1: Chromatogram of Gallic acid Standard 50µg/mL

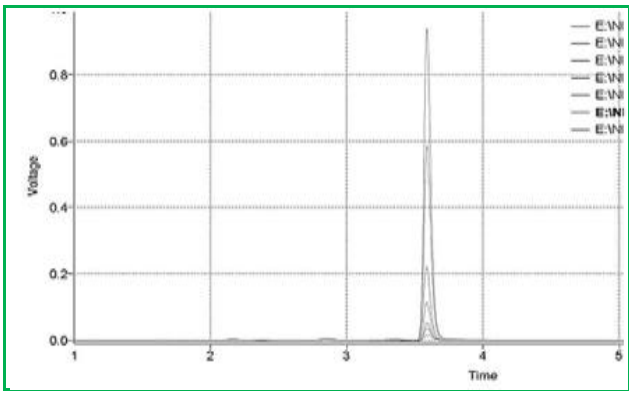

Figure 2: Chromatogram of Calibration of Gallic acid

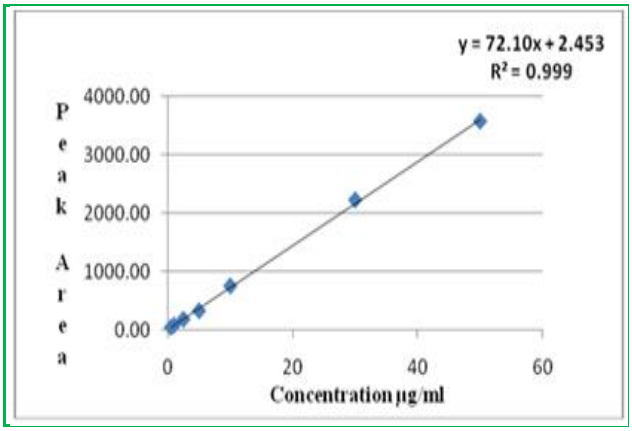

Figure 3: Calibration curve of Gallic acid

Validation of the Method[16]

The optimized Chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005).

System Suitability Test

20µL of Gallic acid standard solution of 50µg/mL was injected under optimized Chromatographic conditions to evaluate the suitability of system [Table 1].

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analyte from other potential components such as impurities, degradants or excipients. A volume of 20µL of individual ingredients and excipients solution were injected and the chromatogram was recorded. Peaks of excipients were not found at retention time of 3.60 min. Hence, the proposed method was specific for Gallic acid.

Table 1: System Suitability of Gallic acid

Conc. (µg/mL)	Peak area	Theoretical Plates (N)	Resolution R_s
50	3154.62	14734	1.24
	3198.56	14653	1.27
	3175.06	14650	1.37
	3242.22	14664	1.23
	3140.39	14655	1.30
Mean	3182.17	14671.20	1.28
% RSD	1.26		

Table 2: Linearity of Gallic acid

Conc. (ppm)	Set-1	Set-2	Set-3	Set-4	Set-5	Mean	SD	RSD
	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area			
0.5	37.75	38.27	37.59	39.09	38.05	38.15	0.59	1.54
1	74.59	75.35	75.72	75.38	74.13	75.03	0.65	0.87
2.5	175.17	175.50	175.52	177.35	176.17	175.94	0.87	0.49
5	318.96	316.30	318.98	330.05	328.02	322.46	6.14	1.90
10	749.29	729.23	740.02	762.91	757.85	747.86	13.57	1.81
30	2228.89	2215.07	2195.96	2242.21	2236.68	2223.76	18.59	0.84
50	3543.19	3582.44	3563.41	3600.69	3571.41	3572.22	21.42	0.60
Slope	71.68	72.27	71.780	72.64	72.15	72.10	0.3899	0.54
Intercept	4.57	-3.27	0.130	5.16	5.67	2.45	3.8824	
R^2	0.9991	0.9995	0.9996	0.9994	0.9992	0.9994	0.0002	0.0218

Table 3: Intraday and Interday precision of Gallic acid

Conc. (ppm)	0 Hour		3 Hour		6 Hour		Mean	SD	% RSD
	Rt (min)	Peak Area	Rt (min)	Peak Area	Rt (min)	Peak Area			
0.5	3.58	38	3.58	38	3.58	38	37.87	0.36	0.95
5	3.59	319	3.58	316	3.58	319	318.08	1.54	0.48
50	3.59	3543	3.59	3582	3.60	3563	3563.01	19.63	0.55
Conc. (ppm)	**Day 1**		**Day 2**		**Day 3**		**Mean**	**SD**	**% RSD**
	Rt (min)	Peak Area	Rt (min)	Peak Area	Rt (min)	Peak Area			
0.5	3.58	38	3.62	39	3.62	38	38.29	0.70	1.83
5	3.59	319	3.62	330	3.62	328	325.68	5.90	1.81
50	3.59	3543	3.65	3601	3.63	3571	3571.76	28.75	0.80

Table 4: Accuracy of Gallic acid

Gallic acid amount	Amount added (ppm)	Peak area	Mean	% Recovery	Mean
14.33µg/mL	12	1934.09	26.49	100.62	100.36
		1922.702	26.34	100.03	
		1930.35	26.44	100.43	
14.33µg/mL	15	2162.64	29.63	101.01	101.01
		2156.311	29.54	100.71	
		2169	29.71	101.30	
14.33µg/mL	18	2386.474	32.69	101.12	100.97
		2392.76	32.78	101.38	
		2369.804	32.46	100.41	

Table 5: Robustness of Gallic acid

Conc. (ppm)	Rt	PA	Rt	PA	Rt	PA	Rt	PA	Rt	PA	Rt	PA
	Wavelength						Flow Rate					
	267 nm		272 nm		277 nm		0.98 mL/min		1.00 mL/min		1.02mL/min	
10	3.58	689	3.58	740	3.57	706	3.65	746	3.58	740	3.51	718
	3.58	695	3.58	729	3.58	707	3.65	748	3.58	729	3.51	717
	3.58	690	3.58	729	3.58	709	3.65	746	3.58	729	3.51	719
Mean	3.58	691	3.58	733	3.58	707	3.65	747	3.58	733	3.51	718
SD	0.002	2.94	0.002	6.49	0.002	1.23	0.002	1.43	0.002	6.49	0.002	0.98
%RSD	0.048	0.43	0.05	0.89	0.06	0.17	0.05	0.19	0.05	0.89	0.05	0.14
Conc. (ppm)	pH						Mobile Phase					
	pH-2.94		pH-3.00		pH-3.06		78.5 : 21.5		80 : 20		81.5 : 18.5	
10	3.74	739	3.68	728	3.65	720	3.67	723	3.68	728	3.72	735
	3.72	737	3.67	726	3.64	717	3.66	721	3.67	726	3.70	731
	3.71	734	3.66	725	3.63	717	3.65	719	3.66	725	3.69	728
Mean	3.72	737	3.67	726	3.64	718	3.66	721	3.67	726	3.70	731
SD	0.014	2.64	0.01	1.65	0.01	1.64	0.01	1.79	0.01	1.65	0.02	3.40
%RSD	0.36	0.36	0.27	0.23	0.28	0.23	0.28	0.23	0.27	0.23	0.45	0.46

Where, **Rt**= Retention time, **PA**= Peak Area

Linearity

Linearity was evaluated in the interval of concentrations of 0.5–50 µg/mL for Gallic acid using seven standard solutions. A standard calibration curve was constructed, and linearity was evaluated by the correlation coefficient obtained through the treatment of the results. Each standard was analyzed five times. Obtained results demonstrated that the method was linear in the concentration range of 0.5-50µg/mL for Gallic acid, with average correlation coefficients (r^2) of 0.9994 [Table 2] [Figure 2, 3].

Precision

The precision of the method was determined by repeatability, interday and intraday precision.

Repeatability

The repeatability of the proposed method was ascertained by injecting five replicates of 50µg/mL concentration, within the Beer's range and finding out the peak area by the proposed method, from this peak area % RSD was calculated.

Intraday precision

Three different concentration (0.5, 5, 50 µg/mL) of Standard Gallic acid was injected three times in a single day and % RSD value was calculated to determine Intraday variation which is within limit (i.e. % RSD >2) for Gallic acid which is found to be 0.55-0.95% [Table-3].

Interday precision

Here, three different concentration (0.5, 5, 50µg/mL) of Standard Gallic acid was injected three times on different days and % RSD value was calculated to determine Interday variation which is within limit (i.e. % RSD >2) for Gallic acid which is found to be 0.83-1.83% [Table 3].

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (80%, 100% and 120% of final concentration). A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory [Table 4].

Limits of detection and quantification

The LOD was calculated by standard formula

$LOD = 3.3 * \sigma/S$

Where σ = the standard deviation of the response, S = the slope of the calibration curve was LOD fro Gallic acid was found to be 0.0178µg/mL for Gallic acid.

The LOQ was calculated by standard formula

$LOQ = 10 * \sigma/S$

LOQ for Gallic acid was found to be 0.0539µg/mL

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the pH of the mobile phase (±2%), organic solvent content (±2%), Flow Rate (±2%) and wavelength (±2%). None of these alterations caused change in % RSD of peak area or retention time. Although the change in the retention time was significant, yet quantification was possible [Table 5].

Assay of Formulation

Solution of different individual ingredients and marketed formulations Ranger Capsule was prepared, injected in optimized mobile phase and assay was carried out. Amount of Gallic acid in Ranger capsule was found to be 0.50%.

Statistical analysis

Statistical calculations were carried out with the Microsoft Excel 2007 for Windows software package. Average, Sum, Standard Deviation (STDEV), Regression (RSQ) for Statistical Calculation, and Scattered Chart were used for Linearity; P values > 0.05 were considered to be significant.

CONCLUSION

The developed HPLC method is simple, sensitive, specific, and adequate to the quantification of polyphenolic Gallic acid. The method was validated according to ICH guidelines and proved to be precise and accurate. The developed method can be used in the laboratory to routinely quantify Gallic acid and to evaluate the physico-chemical stability of referred Polyherbal Formulations.

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REFERENCES

1. K Robards, PD Prenzler, G Tucker, P Swatsitang, W Glover, Phenolic compounds and their role in oxidative processes in fruits, Food Chem., 66, 1999, 401-436.
2. KM Nikolic, Theoretical study of phenolic antioxidants properties in reaction with oxygen-centered radicals, J Mol Struc: THEOCHEM, 774, 2006, 95-105.
3. M Karamaæ, A Kosiñska, RB Pegg, Comparison of radical–scavenging activities of selected phenolic acids Pol, J. Food Nutr. Sci., 14, 2005, 165–170.
4. S Kaur, H Michael, S Arora, PL Harkonen, S Kumar, The in vitro cytotoxic and apoptotic activity of Triphala –an Indian herbal drug, J. Ethnopharm., 97, 2005, 15–20.
5. Singleton VL, Naturally occurring food toxicants: phenolic substances of plant origin common in foods, Adv Food Res., 27, 1981, 149-242.
6. Inoue M, Suzuki R, Koide T, Sakaguchi N, Ogihara Y, Yabu Y, Antioxidant, gallic acid, induces apoptosis in HL-60RG cells, Biophys Res Commun., 204, 1994, 898-904.
7. Gali HU, Perchellet EM, Klish DS, Johnson JM, Perchellet JP, Antitumor-promoting activities of hydrolysable tannins in mouse skin, Carcinogenesis., 13, 1992, 715-718.
8. Stich HF, Rosin MP, Brison L, Inhibition of mutagenicity of a model nitrosation reaction by naturally occurring phenolics, coffee and tea, Mutat Res., 95, 1982, 119-128.
9. Ohno Y, Fukuda K, Takemura G, Toyota M, Watanabe M, Yasuda N, et al., Induction of apoptosis by gallic acid in lung cancer cells, Anticancer Drugs, 10, 1999, 845–851.
10. Anjana J, Monika B, Shukla S, Protective effect of Terminalia belerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats, Journal of Ethnopharmacology, 109, 2007, 214–218.
11. Zhongbing L, Guangjun N, Peter S, Belton C, Huiru T, Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives, Neurochemistry International, 48, 2006, 263–274.
12. Chiara D, Giorgia P, Federica M, Gianni Z, Gaetano B, Silvia M, Gianluca V, Anti-tumour potential of a gallic acid-containing phenolic fraction from Oenothera biennis, Cancer Letters, 26, 2005, 17–25.
13. Krogh R, Yunes R, Structure–activity relationships for the analgesic activity of gallic acid derivatives, Farmaco., 55, 2000, 730–735
14. Y Amakura, M Okada, S Tsuji, Y Tonogai, Determination of phenolic acids in fruit juices by isocratic column liquid chromatography, J Chromatogr A., 891, 2000, 183-188.
15. M Rizzo, D Ventrice, MA Varone, R Sidari, A Carini, HPLC determination of phenolics adsorbed on yeasts, J Pharm Biomed Anal., 42, 2006, 46-55.
16. ICH Harmonized Tripartite Guideline, Q2 (R1): Validation of Analytical Procedures: Text and Methodology Current Step 4 versions, 2005.

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