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Research Article

Qualitative And Quantitative Estimation Of Gallic Acid In Rohitakarishta Formulation

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Abstract

Rohitakarishta is one of the Ayurvedic polyherbal formulation mentions in Ayurvedic Pharmacopoeias, which protect against anemia, spleen and liver disorders. The qualitative estimation of Gallic acid from Rohitakariasta formulation was done by using IR, HPTLC techniques and result stated the significant presence of Gallic acid in Rohitakarishta formulation. The suitability of UV method for quantitative determination was proved by validation as per ICH Q2B guidelines.The quality assessment of Rohitakarishta for total microbial content was also analyzed as per WHO guidelines and shows greater therapeutic efficacy.

Keywords: Rohitakarishta, FT-IR, HPTLC, UV method development.

Introduction

Ayurveda as a traditional Indian System of medicine strongly believe in polyherbal formulation due to its safety and efficacy [1]. Ayurveda is a widely accepted plant based system of medicine consisting of various Ayurvedic formulations such as liquid dosage forms (asava, arishtas), solid dosage forms (pills, powders) and semisolid dosage forms (Ghritas, avlehas) [2]. Rohitakariasta is an Ayurvedicpolyherbal formulation was prepared with decoction of herbs in boiling water [3]. It contains the 5-10% of self-generated alcohol [4]. Rohitakariasta contains the *Tocomella undulata* as a main ingredient along with other herbs as shown in Table 1. It is widely used in the treatment of spleen disease, abdominal disease, localized abdominal swelling, Jaundice, Hapatitis, Loss of appetite, liver disease and many skin disorders. It also reduces the toxins developed due to viral, bacterial or parasitic infections and improved the Lymphocytes production [5].

This research consists of the use of various medicinal plants to achieve greater therapeutic efficacy. The *Tecomella undulate* and *Cinnamom umtamala* are used in liver and spleen diseases. *Piper longum*, *Plumbagozeylanica* and *Elettaria cardamomum* are useful in worm infestation, constipations, throat, lungs, respiratory, skin, menstrual disorders and also helps in maintaining Vata, Pitta, Kaphadosha. *Terminalia chebula, Terminalia belerica* and *Emblica officinalis* are use as powerful antioxidant, rejuvenating, and astringent action. *Piper longum*, *Zingiber officinalis* helps to regulate the secretion of digestive system and correct indigestion. *Plumbago zeylanica*, *Zingiber officinalis* having carminative, laxative, rubefacient, stimulant property. The combination of these different herbs may increase the medicinal potential of formulation and provide higher activity against various diseases. It will help in proper absorption, transportation and to nullify the toxicity of Rohitakarishta[6 and 7].

Rohitakariasta contain Gallic acid as a one of the active ingredient. The present investigation is concerned with the qualitative estimation of Gallic acid from Rohitakariasta formulation by using FT-IR, HPTLC techniques. The WHO has recommended technical guidelines for the assessment of microbial quality of herbal formulation [8]. To ensure that the preparation is free from risk it is mandatory to perform microbial limit test. The UV method was developed and validated in terms of linearity, range, precision, robustness, ruggedness, recovery, limit of detection and limit of quantification. The

preparation and procedures are carried out in accordance with GMP conditions [9].

Table 1: Composition of Rohitakirishta formulation

Sr. No.	Ingredients	Part of Plant Used	Quantity Taken
1	Rohitaka (*Tecomellaundulata*)	Bark	1.2Kg
2	Dhataki (*Woodfordiafruticosa*)	Flowers	192g
3	Pippali (*Piper longum*)	Fruits	12g
4	Pippalimula (*Piper longum*)	Steam	12g
5	Cavya (*Piper cubeba*)	Roots	12g
6	Citraka (*Plumbagozeylanica*)	Roots	12g
7	Shunthi (*Zingiberofficinalis*)	Rhizomes	12g
8	Tvak (*Cinnamomumzeylanicum*)	Stem bark	12g
9	Ela (*Elettariacardamomum*)	Seed	12g
10	Patra (*Cinnamomumtamala*)	Leaf	12g
11	Haritaki (*Terminaliachebula*)	Powder	12g
12	Bibhitaka (*Terminaliabelerica*)	Powder	12g
13	Amalaki (*Emblicaofficinalis*)	Powder	12g
14	Jala for decoction of water	-	q.s. to 300ml
15	Guda (Jaggery)	-	1.5 Kg

MATERIALS & METHODS

Instrumentation and Authentication of Raw material

UV-visible spectrophotometer (Double beam) Shimadzu 1650 PC, Fourier Transform Infrared spectrophotometer (model no.84000S SHIMADZU), HPTLC –CAMAG Linomet. The raw materials were collected from Mahatma PhuleKrushiVidyapeth, Rahuri. Dist-Ahmadnagar, India. Its authentification was carried out at department of botany, S. S. G. M. College Kopargaon, Dist-Ahmadnagar, India.

Formulation of Rohitakarishta

Rohitakariasta is prepared by using therapeutically active ingredients as shown in Table1. In this water is used to make the decoction of herbs. The flowers of woodfordiafruticosa are used as a fermenter whereas the addition of jaggery in formulation will increase the rate of fermentation [10]. The fermentation process was carried out in clean and dry room; flow chart for making Rohitakariasta is given in Fig 1.

FT-IR analysis

A drop of sample formulation was squeezed in between two sodium chloride plates (0.1-0.3mm thin), it's absorption of infrared radiations were recorded at 4000-400cm^{-1} using Fourier transform infra-red spectrophotometer model no. 8400S Shimadzu [11].

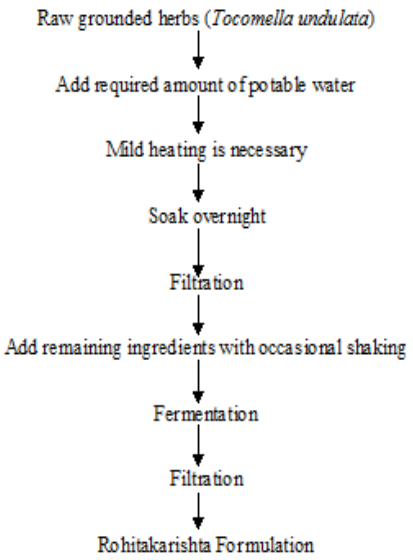

Fig 1: Flow chart for making Rohitakarishta Formulation

HPTLC Analysis

HPTLC study of marketed formulation was carried out along with the marker compound by using High Performance Thin Layer Chromatography (HPTLC) to ensure the presence of active ingredient in the formulation using ethyl acetate: Tolune: Acetone (5:4.2:1) as mobile phase [12].

UV method development

Method validation was performed by using Ultraviolet-vis-spectrophotometer (model no.1650 PC), Shimadzu. It is also use to estimate the quality, reliability and consistency of analytical results. Stock solution was prepared by dissolving 10mg of concentrated powder sample in to 100ml methanol. Method was developed and validated as per ICH Q2B guidelines includes linearity, range, precision, recovery, robustness, ruggedness, LOD and LOQ [13].

Microbial Load Estimation

Microbial Load Determination was done as per WHO guidelines. For herbal formulation, the microbial load should not exceed the stated permissible limits. Total viable aerobic count and test for specific organism such as *Pseudomonas aerugenosa*, *Escherichia coli*, *Salmonella species*, *Shingella Species* was perfrmed[14].

RESULTS AND DISCUSSION

IR spectrum of wave number (cm^{-1}) Vs %T gives sufficient information about the structure of compounds or number of functional groups present in sample. The most important infrared regions are near-infrared (13,000-4000/cm), mid-infrared (4000-400/cm) and far-infrared (<400/cm) [15]**.** The IR spectrum of formulation, Gallic acid was recorded & shown in Fig 2.IR spectra of Rohitakariasta formulation showed the absorption peak of O-H (3215cm^{-1},3308cm^{-1}, 3350cm^{-1}), C-H (2972cm^{-1}), COOH (1720cm^{-1}), C=C (1658cm^{-1}), C_6H_6 (863cm^{-1}), RCOOR'(Out of plane bend for benzene (1730cm^{-1}) indicating the presence of Gallic acid in Rohitakariasta.

Using HPTLC, Good resolution sharp peak with minimum tailing was obtained with mobile phase ethyl acetate: Tolune: Acetone (5:4.2:1v/v/v). The Gallic acid and Rohitakarishta formulation was statistically resolved with R_f value 0.42, 0.43 respectively as shown in Fig 3 and 4. Both R_f value very close to each other, hence prove the significant presence of Gallic acid in the formulation.

For proper selection of wavelength diluted sample was scanned at 200-400nm on spectrum mode and method was developed in photometric mode [16]. The wavelength of maximum absorption (λ_{max}) of Gallic acid and Rohitakarishta formulation were observed at 254nm and 257nm respectively as shown in Fig 5. The calibration curve of formulation was plotted using concentration (ppm) Vs absorbance; curve obtained was linear within the concentration range from 0.2-1.2 ppm. Precision was determined by studying the repeatability and intermediate precision. The method was found to be precise as the RSD values for repeatability and intermediate precision were found to be 0.12% and 0.52% for formulation. LOD and LOQ were confirmed to be 0.078, 0.065 respectively. Accuracy of the method was performed by recovery studies. The percentage recovery of formulation was calculated as 92%, 94.2%, and 96.6% of test concentration as shown in Table 2. Robustness study of the developed method shows that, by varying the condition such as changing in scanning speed of instrument the results of test solutions was not affected the analytical method were concluded as robust. WHO guidelines had stated their maximum tolerance limit for the presence of some pathogenic microorganism and results were within these limits as shown in Table 3.

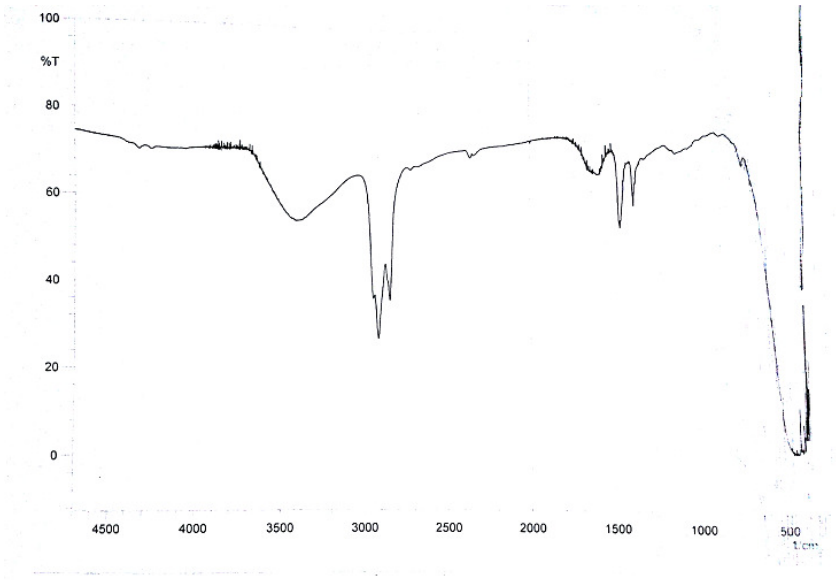

Fig 2. IR spectrum of Rohitakarishta formulation

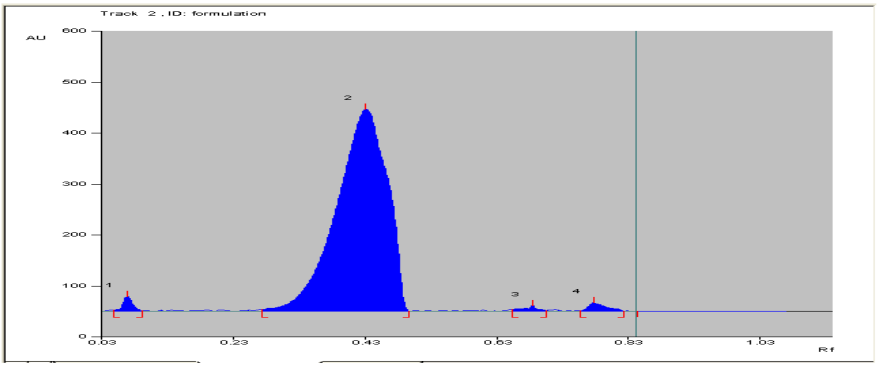

Fig 3: HPTLC chromatogram of Rohitakarishta formulation

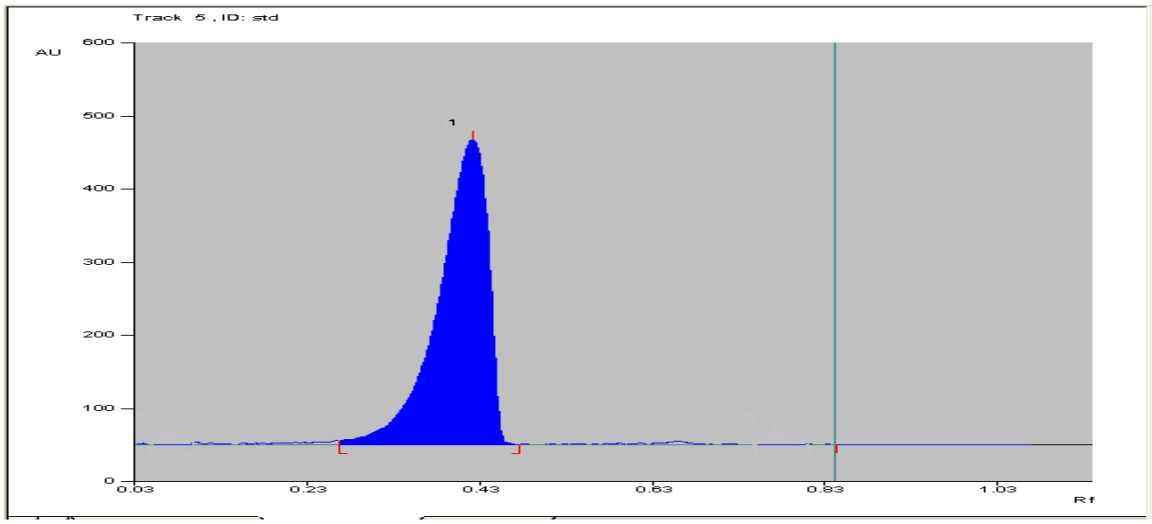

Fig 4: HPTLC chromatogram of standard Gallic acid

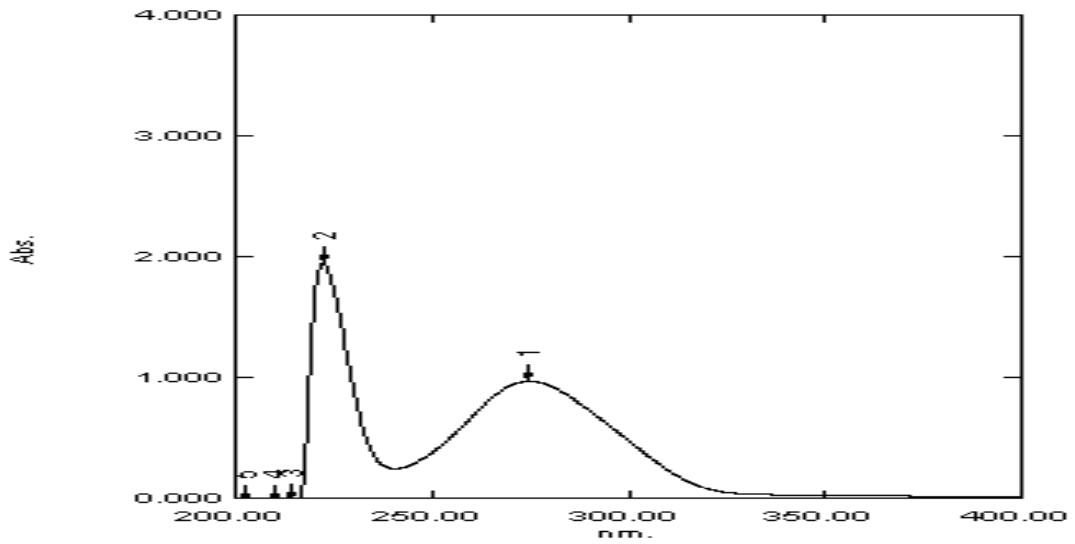

Fig 5: UV spectrum of Rohitakarishta Formulation

Table 2: UV Method validation parameters

Sr. no.	Parameters	Rohitakarishta formulation	
1.	λ_{max}	257nm	
2.	Linearity a)Correlation coefficient b)Slope c)Intercept d)Range	 0.99 0.59 0.004 0.2-1.2µg/ml	
		SD	%RSD
3.	Precision a)Intraday precision b)Interday precision c)Intermediate precision	 0.0010 0.00056 0.0051	 0.12 0.036 0.52
4.	Ruggedness Analyst I Analyst II	 0.0011 0.00021	 0.34 0.064
5.	Robustness (Scanning speed-fast)	0.0094	0.74
6.	LOD	0.078	
7.	LOQ	0.065	
8.	% Recovery	50% - 92% 100% - 94.2% 150% - 96.6%	

Table 3: Observation table along with permissible limits for microbial load

Sr. No.	Parameters	Observed value	Permissible Limits
1	Aerobic viable bacteria	150 cfu/ml	10^7/g
2	*Escherichia coli*	Absent	100/g
3	*Shingella Species*	Absent	absent/g
4	*Pseudomonas aerugenosa*	Absent	absent/g
5	*Salmonellae species*	Absent	absent/g

CONCLUSION

After analysis it can be concluded that, HPTLC chromatogram confirmed qualitative presence of Gallic acid in Rohitakarishta formulation. Chemical structure of formulation was observed by IR spectrum; it confirmed the presence of Gallic acid and enhances the resistance of the body against disease and infection. The proposed analytical method for Rohitakarishta were developed, validated and was found to be simple, linear, accurate, precise and robust. The formulation also passes the test for total microbial load these gives rise to quality of Rohitakarishta with wider therapeutic efficacy.

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