



## High-performance Liquid Chromatographic Method for the Quantification of Gallic Acid in Simhanada Guggulu

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### Abstract

Marker compounds quantification with new analytical tools and methods is necessary for establishing the authenticity and usage of Ayurvedic or herbal formulations. Simhanada guggulu or guggul is one of the supportive Ayurvedic medicines for treatment of rheumatoid arthritis and used in various disorders in Ayurveda such as limping, anemia, gout, disease of skin, cough, abdominal lump, pain digestive impairment. Simhanada guggulu is an Ayurvedic herbal formulation made by some selected herbs. The rejuvenating and tonic properties of 'Simhanada guggulu' are considered majorly due to their antioxidant principles, which in turn is due to the presence of phenolic compounds. A high-performance liquid chromatography (HPLC) method has been developed for quantitative determination of the gallic acid in 'Simhanada guggulu'. The acidic mobile phase used in RP18 column which enabled efficient separation of gallic acid. A binary gradient with mobile phase containing solvent A (Acetonitrile) and solvent B (water: 0.3% O-Phosphoric Acid) was used for analysis. Elution was carried out at flow rate of 0.8 mL/min. Pure gallic acid Rt was found to be 5.29 min and peak with same Rt was also observed in prepared formulation. Gallic acid content of prepared formulation was found to be 2.28 %. The developed HPLC-UV method is simple, rapid and help as tool for the standardization of Simhanda guggulu.

### 1 Introduction

Ayurveda, the ancient system of Indian medicine has gained worldwide attention due to its safety and efficacy. An attempt for the standardization of polyherbal formulation has been carried out with respect to the active principles of the medicinal plants used<sup>1, 2</sup>. Herbs are creature having as source of medicinal value since ancient time. A number of medicinal plants are act nature's gift to human beings to maintain disease free healthy life<sup>3</sup>. Even though several other factors such as maturity and nativity of the plant matters its efficacy due to the variations in its percentage of active principle present in the plant material, it can also be well quantified using modern chemical tools. Standardizations of polyherbal formulations are needed for the easy marketing of Ayurvedic medicine. These studies can promote the export of the valuable Indian traditional medicine<sup>4, 5</sup>.

Ayurvedic medicinal plants of Indian origin and formulations have been proved to be safer during their traditional practices since ancient times<sup>6</sup>. Standardization of herbal products is a composite evolutionary process due to their diverse composition, which is in the form of whole plant, plant parts or extracts obtained from plant. To make certain reproducible quality of herbal products, proper control of starting material and standardization of finished product is extreme essential<sup>7</sup>. Standardization by the means of marker profile development has vast significance for quality control and scientific validation of the polyherbal formulations.

Safe clinical practice of traditional medicines required the safety issues of the polyherbal formulations by the mean of chemical consistency<sup>8</sup>. The research work dealing with standardization involves a very important aspect of separation and isolation of

the marker or active constituent. A marker compound is a chemical component present in herb, herbal preparation/formulation that is used for identification and/or quality control purposes of that herb and formulation, especially when the active constituents are not known or identified. It is termed as a chemical marker<sup>9,10</sup>. If the compound with specific biological activity is not known, marker compounds have been used frequently as surrogates for quality determination<sup>11,12</sup>.

Simhanada guggulu or guggul is one of the supportive Ayurvedic medicines for treatment of rheumatoid arthritis and used in various disorders such as anemia, cough, gout limping, skin diseases, abdominal lump, pain, and digestive impairment<sup>13</sup>. It is an Ayurvedic herbal remedy formulated with selected ayurvedic herbs.

The rejuvenating and tonic properties of 'Simhanada guggulu' are considered majorly due to their antioxidant principles, which in turn is due to the presence of phenolic compounds. The preparation of Simhanda guggulu is based on traditional method. Due to lack of modern Pharmacopieal standards laid down and followed for processing of Simhanda guggulu, the formulation prepared by traditional method may not have preferred quality and batch to batch uniformity. Here there is a need for standardization of Simhanda guggulu<sup>14</sup>. In the present study, an attempt was made to quantify gallic acid in in-house prepared formulation of 'Simhanada guggulu' by using HPLC.

## 2 Materials and Methods

### 2.1. Materials

Gallic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, water and phosphoric acid were obtained from JT Baker and Fischer scientific Ltd.

### 2.2. Preparation of Simhanda guggulu formulation<sup>15, 16, 17</sup>

All crude drugs (amla, haritiki, bibhtaki, guggulu & sulphur) were purchased from the market, Sagar (M. P.) and authenticated by comparison with herbarium specimens.

Following method as per AFI was adopted for preparation of Simhanda guggulu. The composition of Simhand guggulu shown in Table 1 and Fig. 1.

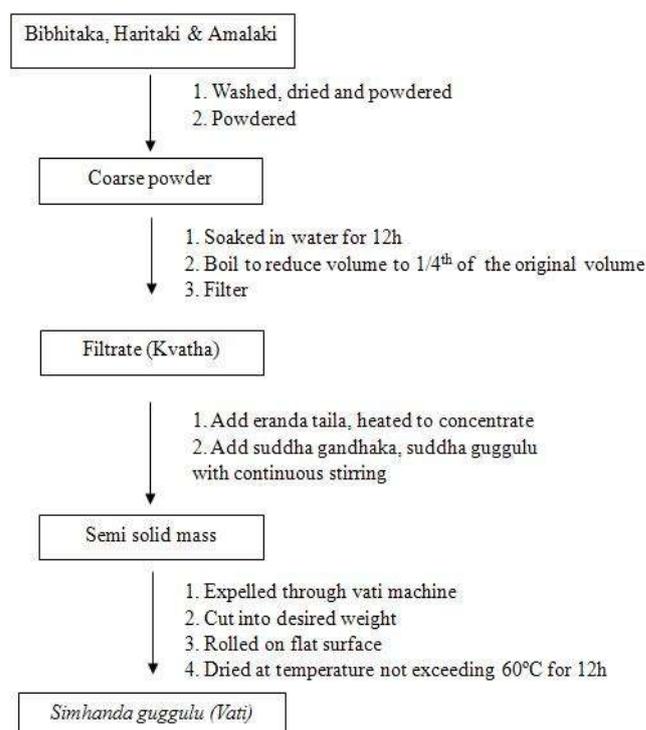
#### 2.2.1. Gandhaka sodhana

Small pieces of *Gandhak* were melted in an iron pan smeared with ghrta and it was poured in to a pot containing godhuga. Mixture was collected after cooling. Process was repeated for the seven times, at the end of the seventh process, the material was washed and dried.

#### 2.2.2. Guggulu sodhana

Big pieces of sandstone, Glass, Wood etc. were manually removed from the *guggulu*. *Guggulu* was cut in to small pieces, bundle in a cloth and immerse in *Dola yantra* containing

*Godugha*. Content was boiled till the whole amount of *guggulu* passes in to the liquid through the cloth. Residue was discard present in the bundle. Liquid was filtered through muslin cloth and the mixture was heated till a semisolid mass was obtained. Mass was dried in sun and stored.



**Fig 1: Preparation scheme of Simhanda guggulu**

2.2.3. All the ingredients of the Pharmacopieal quality were taken:

- Haritiki, Bibhitaka, Amalaki were washed, dried and powdered separately and passed through sieve no. 40
- Coarse powder of Haritiki, Bibhitaka and Amalaki was soaked in potable water for 12 h. The mixture was gently heated to boil and boiling continued to reduce the volume of the mixture to one fourth of its original volume.
- Boiling was stopped and mixture was filtered while still warm through a muslin cloth (*kvatha*). *Suddha gandhaka* was powdered and pass through sieve no 120.
- *Eranda taila* was added to the filtrate (*kvatha*) and gently heated to concentrate. *Suddha gandhaka* and *Suddha guggulu* was added with continuous stirring to obtain a semi solid mass of suitable plasticity.
- Mass was expelled the through vati machine fitted with a suitable die and vatis were cut to a desired weight.

- Vati were rolled on flat surface to round them by circular motion of palm covered with glove and smeared with *eranda taila*.

Vatis were dried in a tray-dryer at a temperature not exceeding 60°C for 12 to 15 h. It was Packed tightly in a closed containers to protect from light and moisture.

**Table 1: Composition of Simhanda Guggulu (as per AFI)<sup>12</sup>**

Vernacular Name	Botanical Name	Part	Wt.
Haritki API	<i>Terminalia chebula</i>	Pericarp	48g
Bibhtaka API	<i>Terminalia bellerica</i>	Pericarp	48g
Amlaki API	<i>Embelica officinalis</i>	Pericarp	48g
Jala for decoction	Water	-	576 mL
reduced to			144mL
Gandhaka API-Suddha	Sulphur	-	48g
Guggulu API-Suddha	<i>Commiphora wightii</i>	Oleo-resin	48g
Citra (Eranda API) Taila	<i>Ricinus communis</i>	Seed Oil	30g

### 2.3. HPLC-PDA system

Analyses were performed on a liquid chromatography Consisting Shimadzu HPLC system LC-10 AVP coupled to a PDA detector and SIL 20AC auto-sampler was used for analysis. All the analytical separations were performed on C<sub>18</sub> column (250 mm X 4.6 mm, 5µ, make: Phenomenex) analysis and detection was performed at 254 nm. A binary gradient with mobile phase containing solvent A (Acetonitrile) and solvent B (water: 0.3% O-Phosphoric Acid) was used for analysis. The gradient elution program was as follow: 0-5 min, 90 – 88%; 5-6 min, 88-86%; 6-9.5 min, 86 -88%; 9.5 -10.5 min, 80-79%; 10.5-12 min, 79-78%; 12-22 min, 78-76%; 22-30 min, 76-90% of solvent B. Each run was followed by a 10 min wash with 10 % Acetonitrile & 90 % O-Phosphoric acid in Water (0.3%) and an equilibration period of 15 min .Elution was carried out at flow rate of 0.8 mL/min. Retention time of gallic acid was 5.29 min.

### 2.4. Sample preparation for quantification of gallic acid by HPLC

Formulation was coarsely powdered; 5g of powder was taken into 250 mL of a round bottom flask. Methanol (75 mL) was added into it, reflux for 3h and filter. This process was repeated 3 times. The extracts were pooled and concentrated at reduced pressure and temperature (40°C) on a rotary evaporator.

10 mg extracts of formulation was dissolved in methanol, and volume was made up to 100 mL with methanol. The samples were used for quantification of gallic acid by HPLC. Samples were filtered through a 0.45 µm nylon membrane filter prior to use.

### 2.5. Calibration

Pure gallic acid (100 mg) was dissolved in 100 mL of methanol to obtain a concentration of 1 mg/mL (stock solution). Different

dilutions from 10 µg/mL to 100 µg/mL were prepared from stock solution. Calibration curve established with six dilutions of standard, at concentrations ranging from 10 to100 µg/ml. Each concentration was measured in triplicate on reversed phase C<sub>18</sub> column and detection wavelength was 254 nm. The corresponding peak areas were plotted against the concentration of the standard gallic acid injected.

### 2.6. Validation parameters for Simhanda guggulu<sup>14</sup>

The linearity of the detector response for the prepared standard was assessed by means of linear regression with respect to the amounts of standard, measured in micrograms, and the area of the corresponding peak on the chromatogram.

To determine the limit of detection (LOD) and quantification (LOQ) standard solution was further diluted in methanol. LOD and LOQ were defined as the amounts for which signal-to-noise ratios (S/N) were 3 and 10, respectively.

Precision was determined as the intra-day and inter-day variation of results from analysis of six different standard solutions. Intra-day precision was determined by triplicate analysis of each solution on a single day. Inter-day precision was determined by triplicate analysis of the solutions on three successive days.

The accuracy of the method was determined by application of the standard addition method. Accurately known amounts of the standard was added to formulation and then extracted and analyzed in duplicate as described above. The total amount of each compound was calculated from the corresponding calibration plot, and the recovery of each compound was calculated by use of the equation:

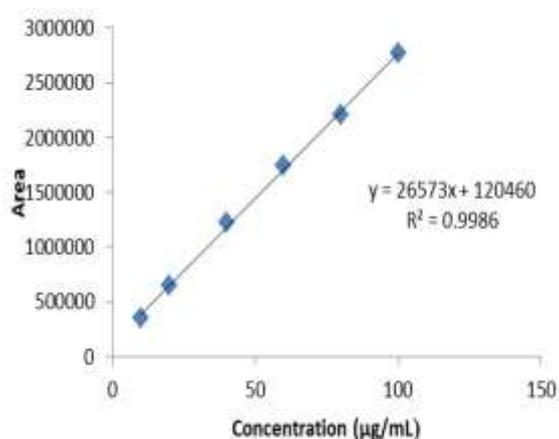
Recovery (%) = (amount found – amount contained)/amount addedx100

### 3 Results and Discussions

Standard curve of gallic acid is linear at the concentrations range of 10–100 µg/mL. The linear equation of calibration curve was found as  $y = 26573x + 12046$  and correlation coefficient ( $r^2$ ) 0.998 was obtained. Linearity data and curve are given in Table 2 and Fig. 2 respectively.

**Table 2: Linear Regression data for Calibration Curve of gallic acid**

Concentration (µg/mL)	Area
10	350340
20	649070
40	1229700
60	1748900
80	2212098
100	2770390



**Fig 2: HPLC calibration curve of Gallic acid (254 nm)**

LOD and LOQ for the analytical method were determined. LOD was obtained as signal to noise ratio equal to 3 and LOQ was obtained as signal to noise ratio equal to 10. LOD and LOQ were found to be 2 µg/ml and 5 µg/ml respectively (Table 3). The LOD and LOQ values show that the developed analytical method showed very good sensitivity.

The intra-day and inter-day precisions of the above method were determined by estimating the corresponding response 3 times on the same day and on 3 different days for three different concentrations of gallic acid. The RSD (%) for Intraday precision (n=3) and Inter day precision (n=3) was found 0.124% and 0.0307 respectively.

The accuracy of analytical method was performed by spiked Simhanda guggulu sample solution with Gallic acid in concentrations of 60, 80, 100 µg/ml to evaluate recoveries of gallic acid for this method. Good recoveries exhibited for gallic

acid, ranging from 98.2 to 98.3% which demonstrates that the developed analytical method has good accuracy (Table 4).

**Table 3: Parameters of quantitation for gallic acid (254 nm)**

Parameter	Result
Rt of standard gallic acid	5.29 min
Rt of simhanda guggulu extract	5.29 min
Linear range (µg/ml)	10–100µg/ml
Regression equation <sup>a</sup>	$y = 26573x + 12046$
Linearity ( $r^2$ )	0.998
LOD (µg/ml) <sup>b</sup>	2 µg/ml
LOQ (µg/ml) <sup>c</sup>	5 µg/ml

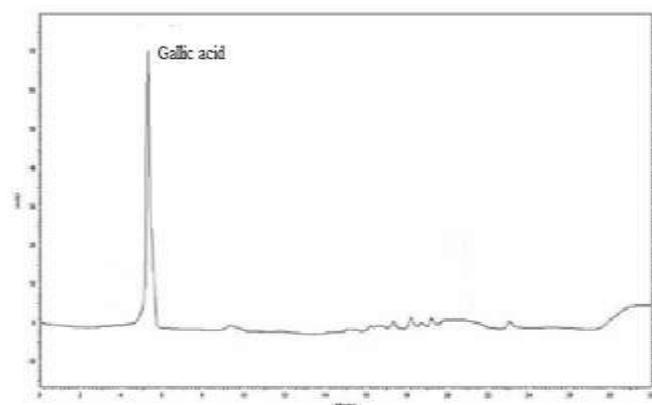
a:  $Y=AX+B$ , where Y is peak area, X is the concentration of the analyzed material.; b: Limit of detection (LOD): signal to noise ratio = 3.;c: Limit of quantitation (LOQ): signal to noise ratio = 10

**Table 4: Validation of accuracy of the analytical method for gallic acid**

Spiked level (µg/ml)	Recovery (%) <sup>a</sup>
60	98.2±0.18
80	99.3±0.21
100	98.98±0.35

a All values are mean±SD, obtained by triplicate analyses in a day

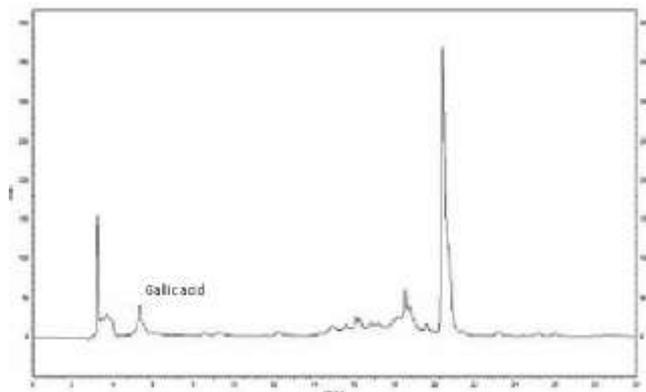
Retention time of pure gallic acid was found 5.29 min. HPLC chromatogram of Simhanda guggulu formulation extract showed peak with same Rt i.e. 5.29 min., having same uv absorbance. HPLC chromatogram of standard gallic acid and Simhanda guggulu extract are shown in Fig 3 and Fig 4, respectively.



**Fig 3: HPLC chromatogram of standard Gallic acid (254 nm)**

A reverse phase C-18 column with the mobile phase containing solvent A (Acetonitrile) and solvent B (water: 0.3% O-Phosphoric Acid) provided good separation of gallic acid from

the formulation extract shown in Fig. 4. The detection wavelength was maintained at 254 nm as gallic acid showed good UV absorption in this region with no interference from the mobile phase or other components of the extract.



**Fig 4: HPLC chromatogram of Simhanda guggulu extract (254 nm)**

The amount of gallic acid present in sample extract was determined by compare the peak area from the standard and calculated by standard linear equation. Gallic acid content of prepared formulation was found to be 2.28 %.

#### 4 Conclusions

In the present work, a simple and rapid RP-HPLC method was developed and validated for estimation of gallic acid in Simhanda guggulu. The gallic acid content in Simhanda guggulu was quantified. The developed HPLC method will help in standardization of Simhanda guggulu using chemical marker (gallic acid). Now a day's traditional and Ayurvedic formulations gained popularity through worldwide as they are belief to be safe, effective, time tested since ancient time and having no added chemicals. So standardization of Ayurvedic formulation is very important to fulfill the requirement of quality products in terms of quality control and batch to batch consistency. A RP-HPLC method Developed for quantification of gallic acid in Simhanda formulation and validation of method was performed in terms of linearity, LOD, LOQ, accuracy and precision.

#### 5 Conflict of interest

The authors declare that there are no conflicts of interest.

#### 6 Author's contributions

NJ carried out literature review and collection of material for experimental process. SJ performed the experimental work. AKJ and MLK carried out draft the manuscript.

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