GTRP Nature of Pharmaceutical Technology

Quantification and Validation of Gallic Acid in Ayurvedic Herbs and Its Related Formulation by HPLC Method

Deepti Nigam[1], Vivek Kumar Gaur[2], Prachi Srivastava[2], Jitendra Kumar Pandey[3] and Savariraj Dominic Rajkumar[4]

[1]Dr. K.N. Modi Institute of Pharmaceutical Education & Research, Modi Nagar, Ghaziabad, U.P., India.
[2]Standard Analytical Lab Pvt. Ltd., New Delhi, India
[3]Department of Chemistry, St. Andrew College (Pg), Gorakhpur, U.P., India.
[4]Department of Botany, St. Andrew College (Pg), Gorakhpur, U.P., India.

Published: 15 April, 2013; Vol. No. 3(1):15-18; www.gbtrp.com;

Abstract

A new, simple, sensitive, selective and precise high-performance liquid chromatography (HPLC) method for analysis of Gallic acid in raw material & related formulations was developed and validated. Raw materials (*Terminalia chebula, Terminalia bellerica* and *Emblica officinalis*) & finished product (Triphala churna) were taken for the study. The stationary phase was inertsil C_{18} column. The mobile phase consisting of acetonitrile (HPLC grade) and potassium dihydrogen phosphate buffer in a gradient flow were used. The column was equilibrated with the mobile phase (flow rate 1.0 ml/min) & elution was monitored at 270 nm.

Keywords: Gallic acid, HPTLC, method validation, *Terminalia chebula, Emblica officinalis*

Introduction

A large number of plants are rich source of Gallic acid in free form or as a part of tannin molecule (Borde *et al*., 2011). Gallic acid has a molecular formula $C_7H_6O_5$ and its IUPAC name is 3, 4, 5-trihydroxybenzoate. Gallic acid is found in gallnuts, sumac, witch hazel, tea leaves, oak bark, amla, triphala, bahera and other plants. Salts and esters of Gallic acid are termed "gallates". Gallic acid is known to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity. It also seems to have antifungal, antiviral and antibacterial property. Gallic acid was found to show cytotoxicity against cancer cells without harming healthy cells (Borde *et al*.,2011). Gallic acid a common phytoconstituent present in three herbs i.e; *Emblica officinalis* Gaertn (Amla), *Terminalia chebula* Retz (Haritaki) and *Terminalia bellerica* Linn (Bahera) which are used in the formulation of triphala churna (Pawar *et al*.,2009).

In present study we have demonstrated this approach of validation and HPLC method using fruits of *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis* which are known to be rich in polyphenols using Gallic acid as a marker. Triphala churna is one of the well-known powdered preparations in Indian system of Medicine. It has extensive usage both by way of prescription by Ayurvedic doctors and as an OTC preparation. Triphala is categorized as a rejuvenator and antioxidant rich ayurvedic herbal formulation and has traditionally been used in various gastric problems including intestinal inflammation. Triphala is prepared by mixing three myobalans amla, haritaki and bahera in equal proportion based on the observation of Ayurvedic Formulary of India (AFI). The formulation is prescribed in the first line treatment of many aliments and is used as laxative, detoxifying agent (Pawar *et al.*,2009). It is used to treat diseases like anaemia, jaundice, constipation, asthma, fever and chronic ulcers. Research studies showed that Triphala supplementation can be regarded as a protective drug against stress. Currently compressed tablets of Triphala preparation are also available for the consumers. These tablets are prepared by mixing aqueous extracts of triphala herbs to reduce the variability of dose whish may not be possible to achieve in powder preparation. *Triphala churna* is also used to promote immunity. This constituents act as cardio-tonic, control blood pressure, improve blood circulation and reduces cholesterol levels (Mane *et al.,* 2010). The aim of this work was to quantify and develop an accurate, specific, repeatable and robust method for the determination of Gallic acid in three myrobalans and its marketed formulation of *Triphala churna* by HPLC method. The method

was validated in compliance with International Conference on Harmonization guidelines (ICH).

Material and Methods

Reagents and chemicals

Gallic acid was purchased from sigma chemicals lot no. 76H0901. Peak purity of this marker was checked before analysis (97.3%). HPLC grade acetonitrile was obtained from RFCL New Delhi. Potassium dihydrogenphosphate and orthophosphoric acid (HPLC grade) was obtained from Fisher scientific (Qualigens fine chemicals). HPLC column was procured from Phenomenex. Ultrapure water was obtained by means of millipore (MiiiQ apparatus). HPLC grade methanol was obtained from Merck specialties Private Ltd, Mumbai.

Formulation

Two formulation of *Triphala churna* A & B were purchased from market.

Collection and Identification of raw material

Dry fruit of individual herbs were received from the Taxonomist, Dabur Research and Development Centre, Sahibabad, Ghaziabad (UP). The raw materials were collected from different places, namely Uttarakhand (UK), Himachal Pradesh (HP) & from Sahibabad, U.P (SBD). The plant material was identified by Dr G. P. Kimothi, Taxonomist, Dabur Research and Development Center, Sahibabad, Ghaziabad (UP). A voucher specimen has been retained in the department for future reference. All the herbs (amla, bahera, haritaki) were powdered and then passed through sieve (85 mesh). The powdered samples were taken for the extraction.

Sample preparation

250 mg of each herb were taken in 100ml of volumetric flask with 50ml of milliQ water and sonicate for 30min at 27±3°C. After shaking the volume was made up with milliQ water.

Standard preparation

10mg of gallic acid was accurately weighed in 100ml of volumetric flask with 50ml of milliQ water and sonicate for 10 min at 27±3°C. After sonication the volume was made up with milliQ water.

Mobile phase

Buffer solution was prepared by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 500 ml of water then 0.5 ml of orthophosphoric acid was added and made upto 1000ml with milliQ water. Mix 975 ml of buffer solution & 25 ml of HPLC grade acetonitrile then filtered through milliQ assembly through 0.45 micron membrane filter paper and degassed.

Column: Inertsil ODS 250mm [023]; Flow rate: 1.0 ml/min; Wave length: 270 nm.

Method Validation

Specificity & Peak Purity

The test was carried out on standard compound, individual herbs and *Triphla churna*. The peak for gallic acid in the sample was confirmed by comparing the retention times of the sample peak with that of the standard. The peak purity of the gallic acid was assessed by comparing the spectra at two levels, viz; peak start(S) and peak end (E) positions.

Precision: Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same sample and was expressed in terms of percent relative standard deviation (% RSD) including Method precision & Intermediate precision.

Linearity & Range: The linearity of an analytical procedure is its ability to obtained test results which are directly proportional to the concentration of analyte in the sample.

Accuracy as Recovery: The accuracy of an analytical procedure is the closeness of test results obtained by that procedure of the true value. The pre-analysed samples were spiked with extra 80%, 100% and 120% of the actual content of gallic acid found in the crude herb by addition of the stock solution of gallic acid and reanalysed. The experiment was conducted six times. This was done to check for the percent recovery of the gallic acid at different levels in the herbs.

Stability: Stability in analytical solution of an analytical procedure is the period upto which the analyte remains unaffected in the prescribed diluents of the analytical procedure. It can be demonstrated by injecting the sample solution at regular time interval of 24hrs till 48hrs at room temperature and under refrigeration (5-10℃).
Robustness: The robustness of an analytical procedure 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 routine usage.

Results and Discussion

In order to develop and validate an efficient method for the analysis of Gallic acid in crude raw material, different detection wavelengths (UV-range), and different compositions of the mobile phase were explored. According to the preliminary results, we finalized the detection wavelength of 270 nm and the mobile phase of acetonitrile/KH_2PO_4 buffer in a gradient flow. Before fully implementing in the quantitative determination of Gallic acid, this method was thoroughly validated for its linearity, specificity, accuracy, precision, intermediate precision, and robustness under various modified conditions.

Precision:

Method Precision: The % RSD for six replicate injections of the standard drug Gallic acid and measurement of peak areas was found to be 1.01%. Six samples of a single batch of the crude herb powder were prepared and analysed by the proposed method. The % RSD of 1.01% indicates that the method has an acceptable level of precision (Table-1).

Table-1: Showing the Method Precision

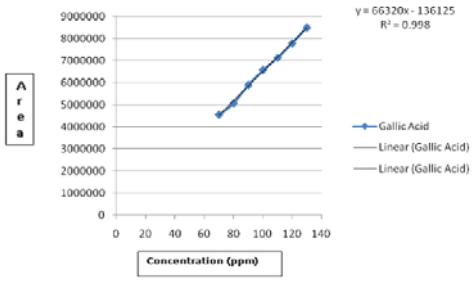
Samples	Gallic acid content (%w/w)
Sample 1	4.64
Sample 2	4.72
Sample 3	4.71
Sample 4	4.62
Sample 5	4.69
Sample 6	4.73
Average	4.69
RSD (%)	1.01

B. Intermediate precision (Ruggedness): The analysis in replicates was performed on different days, using different columns and different system (Table-2).

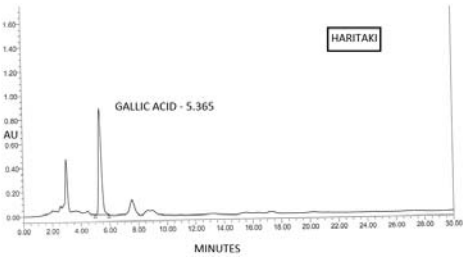
Table-2: Showing the Intermediate precision

Samples	Gallic acid content(%w/w)
Sample 1	4.10
Sample 2	4.00
Sample 3	4.11
Sample 4	4.08
Sample 5	4.00
Sample 6	4.09
Average	4.10
RSD (%)	1.20

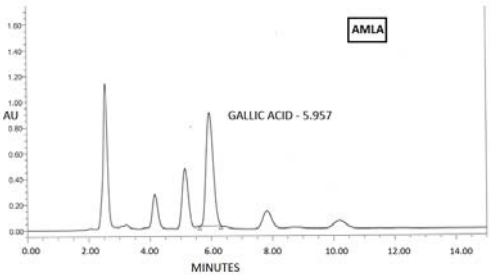
Calibration curve and linearity: The calibration curve was generated from seven concentration levels, i.e., 70, 80, 90, 100, 110, 120, and 130 µg/ml and the corresponding peak areas. It demonstrated an excellent linearity in a range of 70–130 µg/ml of Gallic acid. The correlation coefficient was 0.998 (Graph-1).



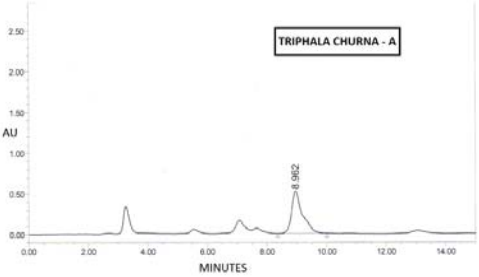
Graph - 1: Correlation coefficient of Gallic acid



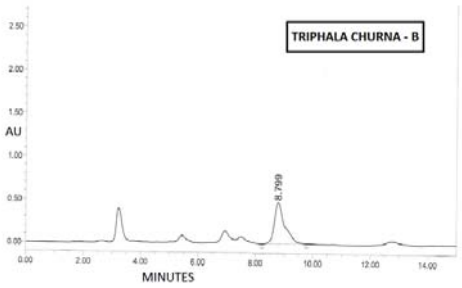
Graph-2: HPLC chromatogram of Haritaki



Graph-3: HPLC chromatogram of Amla



Graph-4: HPLC chromatogram of marketed *Triphala churna* – A



Graph-5: HPLC chromatogram of *Triphala churna* - B

Robustness: Robustness of the method was demonstrated by deliberately changing the chromatographic conditions (flow rates, column dimensions and temperature). It was observed that there was no marked change in the chromatograms.

Recovery: The results showed high efficiency for extraction of Gallic acid from crude herb powder. The recovery of Gallic acid ranged from 98.88 – 101.78%. This confirms that the proposed method can be used for determination and quantification of Gallic acid (Table-3).

Table-3: Showing the Method recovery

Samples	Recovery (%)	Mean (%)
Recovery 80% (A)	98.17	98.88
Recovery 80% (B)	99.59	
Recovery 100%(A)	101.30	101.78
Recovery 100%(B)	102.25	
Recovery 120%(A)	98.30	99.30
Recovery 120%(B)	100.29	

Table-4: The total Gallic acid content found in different herbs

Name of Sample	Gallic acid (% w/w)
Raw Herbs	
Amla (UK)	2.02±0.14
Amla (SBD)	2.03±0.28
Amla (HP)	2.95±0.10
Bahera (UK)	4.68±0.12
Bahera (SBD)	4.71±0.08
Bahera (HP)	2.15±0.09
Haritaki (UK)	4.76±0.007
Haritaki (SBD)	1.94±0.035
Haritaki (HP)	2.06±0.28
Marketed Formulations	
Triphala Churna (A)	1.7±0.28
Triphala Churna (B)	1.95±0.35

There are lot of variation had been noticed in the presence of Gallic acid in raw herbs. Pawar *et al.,* (2009) had found Gallic acid in Amla : 3.01 and 0.87±0.04%, in Bahera: 1.79 and 0.93±0.05% and in Haritaki: 1.24 and 1.05±0.02 respectively. But in our studies we had found gallic acid in Amla: 2.33±0.53%, in Bahera: 3.84±1.46% and in Haritaki: 2.29±1.59%.

The RP- HPLC technique we have described is precise, specific, and accurate for the determination of Gallic acid. Statistical analysis proves that the method is reproducible and selective for the analysis of Gallic acid. Its advantages are speed and simplicity of sample treatment, satisfactory precision, and accuracy. The results indicate that all the three herbs Amla, Haritaki and Bahera contains a high amount of Gallic acid (Graph -2, 3, 4), as well as it is also present in Triphala churna (Graph -5). The developed RP-HPLC method will assist in the standardization of all three herbs and *Triphala churna* using biologically active chemical markers. All three herbs also contained a number of other constitute, which are currently the subject of further investigation, apart from those standard studies. With the growing demand for herbal drugs and with increased belief in the usage of herbal medicine, this standardization toll will help in maintaining the quality and batch to batch consistency of these important Ayurvedic preparations.

References

Borde,V.U., Pangrikar,P.P. and Tekale, S.U. 2011. Gallic acid in Ayurvedic Herbs and Formulations. *RRST, Scholar Journals*, 3(7): 51-54.

Pawar, V., Lahorkar, P. and Anantha Narayana, D.B.2009. Development of a RP-HPLC method for analysis of *Triphala churna* and its applicability to test variations in *Triphala churna* preparations. *Indian Journal of Pharmaceutical Sciences*,7 : 382-386.

Mane, R., Sagar., Subbiah, P.I., Jadhav, A. Pradip., Salunkhe, V.R.,Bhise, S.B. and Rakesh, Sachin. U.2010. Formulation, HPTLC method development and validation of Gallic acid in health drinks. *Scholars Research Library*,2(1):363-370.

Manuscript Progress Date
Received : 13.02.2013
Revised : 23.03.2013
Accepted : 28.03.2013