Research Article
CODEN (USA): IJPLCP

ISSN: 0976-7126
Patidar & Jain, 14(2):38-42, 2023

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Development and Validation of UV- Spectrophotometric method for estimation of Gallic acid in Poly-herbal formulation

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Article info

Received: 31/12/2022

Revised: 26/01/2023

Accepted: 18/02/2023

www.ijplsjournal.com

Abstract

Herbal plants have global market worth about us $.62 billion per annum and Indian's share is only 0.2%, which will grow up to about 15% in near future. According to the WHO, approximately 80% of the world's population uses herbal drugs as part of their normal health case routine. In this present work an attempt was made to estimate Gallic acid present in Marketed Herbal formulation of Amla tablet, Neem tablet, Baheda churna, Herbal tea, Mixed fruit Jam and Herbal chyawanprash. The developed method was found to be simple, precise, and economic, can be utilized for routine method for quantification of Gallic acid. The proposed spectrophotometric method is validated in terms of linearity, accuracy, precision and reproducibility. The gallic acid content in Amla tablet, Neem tablet, Baheda churna, Herbal tea, Mixed fruit Jam and Herbal chyawanprash was quantified.

Keywords: Polyherbal Formulatiom, Gallic acid, Estimation

Introduction

Its importance grew, and in the process, absorbed techniques and skills from all other four branches so by the 1950s, analytical chemistry was finally accepted as a branch of chemistry in it own right. There are basically two types of analysis, qualitative analysis and quantitative analysis. The former identifies the nature of substance, and if it is mixture, the nature of the components present, whereas, the latter determines the elemental composition of the substance and/or the quantitative distribution of each component. Most analytical procedures start with some type of separation process, filtration, distillation, extraction, and centrifugation and, what is most likely today, some form of chromatography. Chromatography, in any one of its different forms, is probably the most important technique available to the analyst. Chromatography not only separates a mixture into its constituents, but also provides assistance in their identification and gives a quantitative estimation of the amount of each constituent present in the mixture. Any analytical laboratory devoid of any chromatographic technique would, indeed, be restricted in its scope and performance.

Literature support that many herbs have potential to cure the diseases. Herbal drugs are increasingly used in various formulation forms. In India, there are around 25,000 plant-based formulations available which are used in folk medicine. The herbal drug market is about $ 1 billion and the export of plant-based crude drugs is around $ 80 million in India.

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ISSN: 0976-7126
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In Ayurveda, various plant-based preparations like asava, arista, churna avaleha, kvatha, decoction etc. have been explored for the treatment of diabetes from ancient time. The pharma companies like Himalaya, Zandu, Dabur, Hamdard, Maharishi, shipachem, baidyanath etc. are already involved in herbal drug manufacturing and pharma companies like Ranbaxy, Lupin, Alembic, etc. are planning to start manufacturing of herbal formulations. Although polyherbal formulation have great potential to treat the diseases but the problem of reproducibility of result is there.

The present study is an approach to develop spectroscopic method for estimation for poly herbal formulations.

Material and Methods

Spectrophotometry was carried out by using UV-Visible spectrophotometer. Wavelength 256.20 nm was selected for measurement of the absorbance.

Methods

Preparation of stock solution(1000µg/ml)

Anaccuratelyweighed100mgofpuregallicacidwastransferred to 100 mL volumetric flask and 30 mL water was added to it. Solution was shaked for 5 minutes to solubilize compound and finalvolume was made upto mark with water.

Preparation of working standard solution

Appropriate aliquots were withdrawn from the stock solution and diluted upto 10mL with water to obtain standard solutions of different concentrations (5,7.5,10,15and20µg/mL).

Methods for Marketed Herbal Formulations Analysis

Standard procedure were adopted

Validation of developed method

ICH guidelines were followed for the validation of analytical methods developed for precision, repeatability, accuracy, LOD and LOQ.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism v 5.0. All the results were expressed as Mean±SD and %RSD.

Results and Discussion

UV Spectra of Gallic acid used for analysis

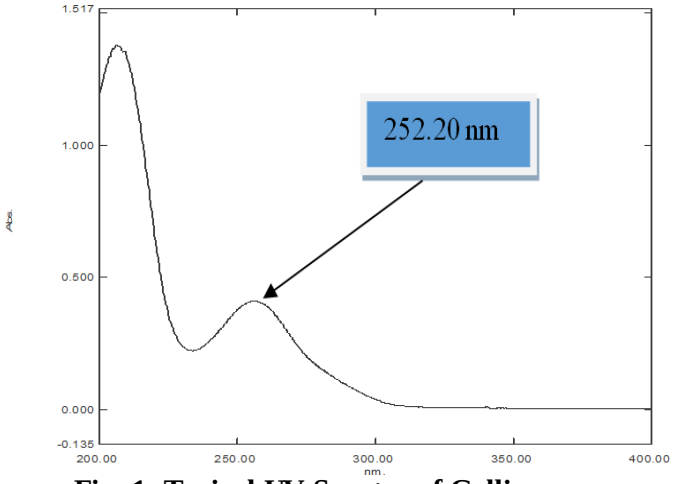

Fig. 1: Typical UV Spectra of Gallic

Linearity of Standard Gallicacid (5-20µg/ml)

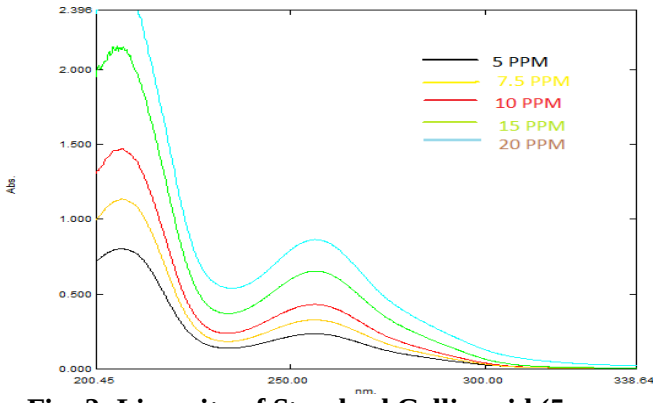

Fig. 2: Linearity of Standard Gallic acid (5-20µg/ml)

Calibration curve of Standard Gallicacid

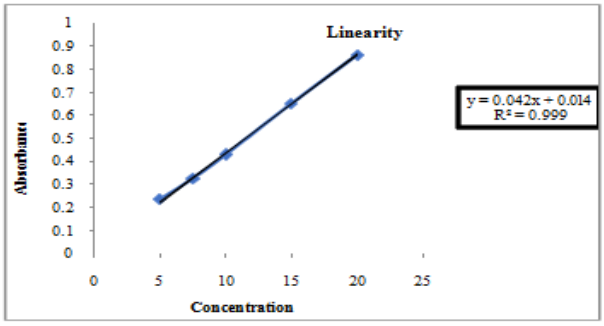

Figure 3 Calibration curve of Standard Gallic acid

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Table 1: Linearity study of Standard Gallic acid (n=5)

S/No.	Conc. µg/ml	Absorbance at 256 nm
1	5	0.234
2	7.5	0.327
3	10	0.430
4	15	0.652
5	20	0.864

Table 2: LOD and LOQ data of Gallic acid at 256.20nm

S/No.	Slope	Intercept
1	0.042	0.014
2	0.042	0.008
3	0.042	0.006
MEAN	0.041667	0.009333
SD	0.000577	0.004163
LOD	0.329	
LOQ	0.999	

Results of Validation parameters of various marketed formulation

Table 3: Assay of Gallic acid

Sample	Weight of sample	% Drug content
Amla Tablet	120 mg	16.24
Neem Tablet	119 mg	12.19
Herbal Tea	1 gm	52.32
Kisan Jam	0.1 gm	14.19
Chyawanprash	0.1 gm	10.18
Bahera Churna	100 mg	34.86

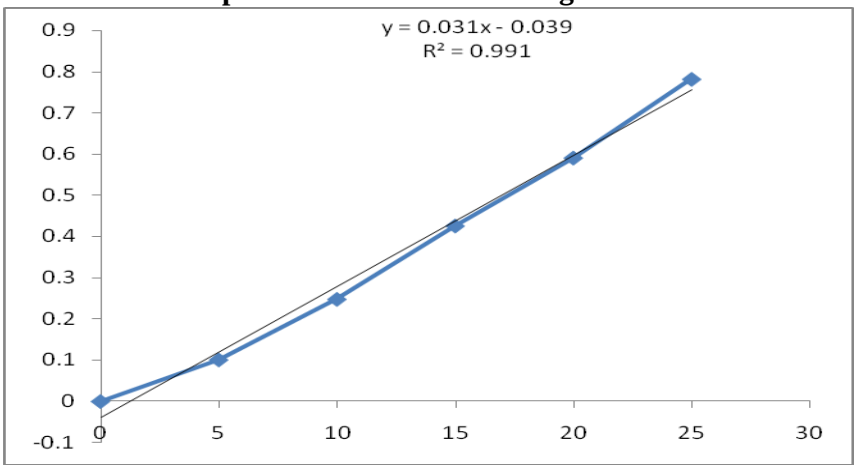
Estimation of Gallic acid

Standard solutions of gallic acid were pipetted into concentration range 5-30 µg/ml in a series of five 25 ml volumetric flask. The absorbance of the gallic acid was measured at 270 nm against methanol. The values are shown in table and graph.

Table 4: Calibration curve data for gallic acid

S. N0.	Concentration	Absorbance
1.	0	0
2.	5	0.101
3.	10	0.248
4.	15	0.426
5.	20	0.591
6.	25	0.782

Graph 2: Calibration curve of gallic acid



Method validation

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of gallic acid was accurately weighed and assayed. The repeatability of sample application and measurement of absorbance for active compound were expressed in terms of relative standard deviation (R.S.D %). Method repeatability was obtained from R.S.D. value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assessed by the assay of two, six-sample sets on different days (inter-day precision).

Limit of detection and limit of quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was run six times. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of gallic acid until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations (Table).

Recovery studies

The pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard gallic acid and the mixtures were analyzed by the proposed method. The experiment was conducted six times. This was done to check the recovery of the drug at different levels in the formulations (Table).

Table 5: % recovery of gallic acid

Excess drug added to the analyte (%)	Conc. found	SD	Recovery (%)	R.S.D. (%)
50	14.98	0.05506	99.68	0.3682
100	19.98	0.1428	99.89	0.7289
150	24.89	0.06070	99.78	0.2442

Table 6: Validation parameter of gallic acid

S. No.	Parameters	Observations
1.	Absorption maxima	270 nm
2.	Beer's law limit (µg/ml)	5-30
3.	Correlation coefficient (r^2)	0.991
4.	LOD	0.34
5.	LOQ	0.121
6.	Recovery (%)	99.79
7.	Regression equation (y^*) Slope (a) Intercept (b)	y = 0.031x - 0.039 0.031 0.039
8.	Precision (% R.S.D.) Repeatability (n = 6) Intraday precision (n = 3) Interday precision (n = 3)	 0.44308 0.21813 1.14941

Conclusion

The gallic acid content in Amla tablet, Neem tablet, Baheda churna, Herbal tea, Mixed fruit Jam and Herbal chyawanprash was quantified. The content of Gallic acid in Herbal marketed formulation was quantified is: Herbal tea (50.86%) > Baheda churna (33.06%) > Mixed fruit jam (18.66%) > Amla tablet (18.24%) > Herbal Chyawanprash (13 .97%)>Neem tablet(13.88%).So from result we conclude that there is higher amount of Gallic acid content present in herbal tea than another herbal formulation.

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Cite this article as:
Patidar V. and Jain S.K. (2023). Development and Validation of UV- Spectrophotometric method for estimation of Gallic acid in Poly-herbal formulation**.** *Int. J. of Pharm. & Life Sci.,* 14(2): 38-42.

Source of Support: Nil
Conflict of Interest: Not declared
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