ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

STANDARDIZATION AND QUANTITATIVE ANALYSIS OF AVIPATTIKAR CHURNAM BY UV SPECTROSCOPY

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ABSTRACT

A rapid and sensitive U.V. visible spectroscopic method was developed for the estimation of Gallic acid in Avipattikar churnam and its ingredients were monitored at 271 nm with U.V. detection and there was no interference of diluents found at 271 nm. The method was found to be linear in the range of 2 -7 mcg/ml. The accuracy and precision were determined and validated statistically. The method was validated as per International conference on harmonization [ICH] guidelines. The results showed that proposed method is suitable for the accurate, precise and rapid for the determination of Gallic acid content.

Keywords: Gallic acid, UV spectrophotometer, Avipattikar Churnam

1. INTRODUCTION

Aviptattikar churnam was first time prepared by great Indian saint "Agastya" [1]. It is used for hyperacidity, indigestion, anorexia, heart burn, gastric ulcer, constipation and other abdominal diseases. The churnam is magical remedy for treating gastro esophagus reflex disorders (GERD) which is induced due to backward flow of acid from stomach to esophagus [2-4]. The acid destroys esophagus epithelium mucosa and causes ulcer in esophagus. Avipattikar churnam used for standardization in this work has been prepared as per Bhaisajya Ratnawali, Sastriya Siddha Proyagank and Ayurvedic formulary of India [5]. The churnam is prepared using fourteen different raw ingredients which are: Adrak, Pippali, Kalimirach, Harad, Bahera, Amla, Nagarmotha, Vidang, Ela, Tejpatra, Vida lavana, Clove, Nishodha and Sarkara. The main ingredient, harad, bahera and amla are well known tridosa (Vata, Pitta, Kapha) nasak materials [6]. There are many marketed Avipattikar churnam which show different amount of raw material and lack in establishment of quality parameters [7]. Thus, in this work, a UV standardization of Avipattikar churnam is developed using Gallic acid as marker compound [8]. Analysis of marker compounds is necessary to ensure the quality and identity of formulation [9]. The current work aims to the study of methanolic extract of Avipattikar churnam for estimation of Gallic acid.

2. MATERIALS AND METHODS

Raw materials, i.e., Harad, Bahera and Amla were collected from farm house of Bundelkhand region. The remaining materials were purchased from local market. Authentication of raw materials was done by Dr. Amita Arjariya, Professor of Botany Department of Government Maharaja PG College, Chhatarpur, M. P., India. All chemicals used in analysis and extraction were of analytical reagent grade (AR). Gallic acid marker was purchased from Sigma Aldrich.

2.1. Preparation of formulation

Raw materials had been processed as per composition described in Ayurvedic formulary of India and Dhanvantri Sastriya Siddha Proyagank. The ingredients used for preparation are described in table 1.

2.2. UV spectroscopy Standardization of Avipattikar churnam using Gallic acid marker

UV spectroscopy analysis method was developed for determination of marker compound, Gallic acid, which is main constituent of Avipattikar churna & its ingredients [10, 11]. The AVI-I, AVI-II, AVI-III, MAVI and its ingredients (Amla, Harde, Bahera) containing Gallic acid were estimated for marker Gallic acid solution on Shimadzu UV-1700 spectrophotometer.

Table 1: The ingredients used for preparation of Avipattikar

Ayurvedic Name	Botanical Name	Quantity (gm)
Amla	*Embica officinalis*	10
Bahera	*Termanalia bellerica*	10
Harad	*Termanalia chebula*	10
Adrak	*Zingiber officinale*	10
Kali Mirach	*Piper nigrum*	10
Vidanga	*Embelia ribes*	10
Ela	*Elettaria cardomomum*	10
Pippli	*Piper longum*	10
Lavanga	*Syzygium aromaticum*	110
Trivitra (Nishodh)	*Operculina turpethum*	440
Musta	*Cyperus rotundus*	10
Tej Patra	*Cinnamoum tamala*	10
Sakkara	*Saccharum officinarum*	660
Vida Namak	*Vida lavana*	10

2.3. Preparation of Gallic Acid extract of Avipattikar Churna:

One gm of Avipattikar churnam was taken in round bottomed flask in 50ml of methanol and refluxed for 60 minutes, cooled, extracted and filtered. The marc of filtration was further refluxed for 30 min with 50ml of methanol, cooled, extracted and filtered. Both filtrates were combined and concentrated under vacuum (rota vapour) to obtain semisolid mass and the residue obtained was dissolved in 100 ml methanol and filtered to obtain clear solution. All samples of each batch, marketed formulation, Amla, Harde and Bahera were prepared in the similar manner.

2.4. Experimental

2.4.1. Preparation of Standard Stock Solution of Gallic acid

For preparation of stock solution, 10mg of Gallic acid (marker) was transferred into 100ml volumetric flask, and prepared 1000 mcg/ml solution of Gallic acid by methanol. Using stock solution, standard solutions of different concentrations *i.e.*1,2,3,4,5,6,7,8,9 and 10µg per ml were prepared.

2.4.2. Preparation of Calibration Curve of Gallic Acid

Standard calibration curve of marker Gallic acid was established by plotting concentration on X axis and absorbance on Y axis according to Beer Lambert's law within the range of 2 to 7 µg per ml at absorbance maxima 271 nm against the blank sample. Calibration data of Gallic acid is represented in table 3 and fig.1.

2.4.3. Precision and Accuracy Analysis

Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard of a series of measurements. Accuracy of method is shown by recovery study and spiking working standard in the placebo at levels 80, 100 and 120 % of the working standard.

2.4.4. Intraday and Interday precision

The experiments of UV spectroscopy were repeated 5 times (1st hr, 2nd hr, 3rd hr, 4th hr) for intraday precision and 5 day (1st day, 2nd day, 3rd day, 4th day and 5th day) for interday precision. Precision activity results of intraday and interday studies are expressed in table 4 & 5.

2.4.5. Estimation of Gallic acid in Avipattikar churna

The appropriate aliquots from gallic acid extract of AVI-I, AVI-II, AVI-III, MAVI and its ingredients (Amla, Harde, Bahera) were withdrawn in 10 ml aliquots and absorbance value at 271 nm was determined. Concentration of Gallic acid against various absorbance values are tabulated in table 6.

2.4.6. Recovery Study of Gallic acid in Avipattikar churna

The Recovery study was performed by spiking known amount of Gallic acid to Avipattikar churna at different levels of working standard. The samples were prepared according to the assay procedure. The results are shown in Table 7.

3. RESULTS AND DISCUSSION

The estimation of Gallic acid batches AVI-I, AVI-II, AVI-III and marketed formulation MAVI, Amla, Bahera and Harad were carried out using UV spectroscopy analysis method for determination of marker compound, Gallic acid which is main constituent of Avipattikar churna and its ingredients. The AVI-I, AVI-II, AVI-III, MAVI and its ingredients (Amla, Harde, Bahera) containing Gallic acid were estimated for marker Gallic acid solution on Shimadzu UV-1700 spectrophotometer, the calibration curve of Gallic acid shown in figure 1, table no.3. The parameters for UV spectroscopy are shown in table 2.

Table 2: Parameters of UV spectroscopy Standard using Gallic acid marker

Parameter	Value
Absorbance maxima wavelength	271 nm
Beer's Law limit	2-7 µg/ml
Regression analysis equation	y = 0.164x - 0.23
Accuracy (%)	98.87

Table 3: Calibration of standard of UV spectroscopy absorbance at 271 nm

Concentration (µg/ml)	Absorbance
2	0.0980
3	0.2620
4	0.4260
5	0.5905
6	0.7540

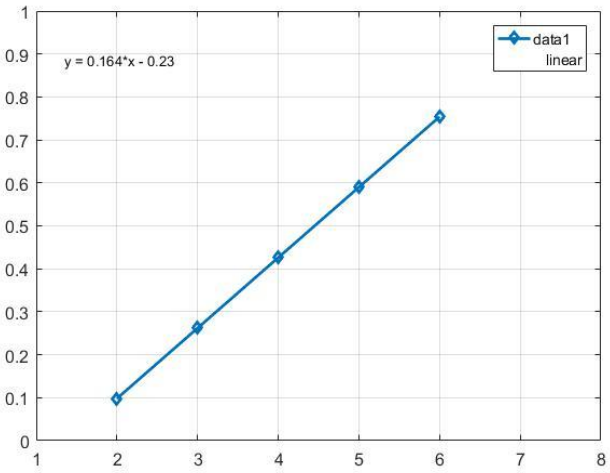

Fig. 1: Calibration curve of Gallic acid Standard

Estimation of Gallic acid content by using spectroscopy in Amla, Harad, Bahera, AVI-I, AVI-II, AVI-III and M-AVI were 3.213±0.011, 3.481±0.015, 7.334±0.021, 0.312±0.001, 0.242±0.001, 0.233±0.002, 0.232±0.001 % w/w (table 6). The intraday and interday study has been shown in table 4 and table 5. The % R. S. D. of intraday and interday was 0.129 and 0.587.The recovery study results are according to limit as expressed in table 7. The mean value of recovery study was 98.87%.

Table 4: Intraday precision activity of Gallic acid

Hr	Concentration (µg/ml)	Absorbance (Au)
1st hr	5	0.5905
2nd hr	5	0.5914
3rd hr	5	0.5906
4th hr	5	0.5912
5th hr	5	0.5924
	Mean	0.59122
	S. D.	0.00062
	% R. S. D.	0.12904

Table 5: Interday precision activity of Gallic acid

Day	Concentration (µg/ml)	Absorbance (Au)
1st day	5	0.5905
2nd day	5	0.5923
3rd day	5	0.5912
4th day	5	0.5990
5th day	5	0.5914
	Mean	0.59288
	S. D.	0.00284
	% R. S. D.	0.58713

Table 6: Estimation of Gallic acid content using UV spectroscopy

Name	Gallic Acid (%w/w±SD)
Amla	3.2134±0.011
Harad	3.4810±0.015
Bahera	7.3341±0.021
AVI-I	0.3122±0.001
AVI-II	0.2423±0.001
AVI-III	0.02327±0.002
M-AVI	0.2321±0.001

Table 7: Recovery study of Gallic acid content using UV standard in Avipattikar churna

Amount of Gallic acid				RSD%	Recovery%
Sample Amount	Added Amount	Estimated Amount	S.D.		
100	20	117.13	0.01	0.008538	97.60833
100	40	139.11	0.02	0.014377	99.36429
100	60	158.01	0.02	0.012657	98.75625
100	80	179.10	0.03	0.016750	99.50000
100	100	198.21	0.05	0.025226	99.10500

4. CONCLUSION

In this work, Avipattikar Churnam and its marketed formulations were evaluated according to standard procedures by UV spectroscopy. The standards established are accurate, precise, sensitive and efficient. The standard established for the formulation can be used as reference by Ayurvedic manufacturers.

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