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Validation of Spectroscopic method for estimation of Ellagic acid and Gallic acid in Herbal capsule

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Abstract

Analytical method development for herbs and their formulation need to develop in order to standardize the herbal formulations and their phyto-constittuents. There are various available herbal formulations in the market to be used by the patient and their analytical parameters need to be developed. In the present investigation ellagic acid and gallic acid in herbal capsule using UV spectroscopy in raw material and marketed capsule.

Keywords: Herbal Capsule, Spectroscopy, Ellagic acid, Gallic acid

Introduction

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. 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. [1-2]

The present study is an approach to develop spectroscopic and chromatographic method for estimation for herbal formulations (capsule).

Material and Methods

Madhuneel: Anti-diabetic herbal Capsule

Composition of Madhuneel: manufactured by Dhanwantari Pharmaceuticals, India
Antidiabetic herbal Capsule (50 mg)

Development of fingerprinting method

The fingerprinting method was developed for raw materials *Gymnea sylvestre* (leaves), *Eugenia jamboloma* (seeds), *Aegle marmelos* (leaves), *Azadirachta indica* (leaves), *Cinamomum zeylanicum* (leaves), *Sphaeranthus indicus* (flower), *Momordica charantia* (fruits), marketed formulation (MCM) by using UV-visible spectrophotometer. [3-4]

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Table 1: Composition of anti-diabetic herbal capsule

S/No.	Ingredients	Quantity
1.	*Gymnea sylvestre*	10
2.	*Eugenia jamboloma*	5
3.	*Aegle marmelos*	5
4.	*Azadirachta indica*	5
5.	*Cinamomum zeylanicum*	5
6.	*Sphaeranthus indicus*	5
7.	*Momordica charantia*	5
8.	Trivang bhasma	5
9.	Shilajeet	5
10.	Excipients	qs

Development of UV spectroscopy fingerprinting method for ellagic acid

The UV spectroscopy fingerprinting method was developed for herbal capsule (Madhuneel) *Eugenia jamboloma* (seeds) via estimation of ellagic acid which is an important content in formulation.

Experimental Techniques

Chemicals

All the chemicals and solvents were used of A.R. Grade.

Instrument

UV-Visible Spectrophotometer (Shimadzu, UV-1800) was used for estimation of ellagic acid content against standard ellagic acid solution in formulations and raw materials.

Preparation of standard solution of ellagic acid

Accurately weighed ellagic acid (10 mg) was transferred in 100 ml volumetric flask and dissolved in and diluted to 100 ml with methanol. The final solution contained 100 µg of the ellagic acid per ml of the solution.

Calibration curve of ellagic acid

Standard solutions of ellagic acid were pipetted into concentration range 5-30 µg/ml in a series of five 25 ml volumetric flask. The absorbance of the ellagic acid was measured at 280 nm against methanol.

Preparation of ellagic acid extract of formulated capsule

Extract the powdered formulated capsule (1 gm) with 6 volume of denatured spirit on a shaker for 2 hours. Filter the extract and re-extract the marc left with 4 volumes of denatured spirit for another 1hours. Filter and combine the filtrate. Concentrate the denatured spirit extract under vacuum till the semisolid mass is obtained. The same procedure was performed for marketed formulation (MCM) and raw materials *Eugenia jamboloma* (seeds).

Method validation

Standard protocols were adopted to determine Precision and accuracy, Limit of quantitation and limit of detection

Development of UV spectroscopy fingerprinting method for gallic acid

The UV-visible spectroscopy fingerprinting method was developed for raw materials *Gymnea sylvestre* (leaves), *Azadirachta indica* (leaves), *Sphaeranthus indicus* (flower), marketed formulation (MCM) via estimation of gallic acid which is an important content in raw materials and capsule. [5-8]

Experimental Techniques

Chemicals

All the chemicals and solvents were used of A.R. grade was procured from Hi-Media India.

Instrument

UV-Visible Spectrophotometer (Shimadzu, UV-1800) was used for estimation of gallic acid content against standard marker component in formulations and raw materials.

Preparation of standard solution of gallic acid

Accurately weighed gallic acid (10 mg) was transferred in 100 ml volumetric flask and dissolved in and diluted to 100 ml with methanol. The final solution contained 100 µg of the gallic acid per ml of the solution.

Calibration curve for 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.

Preparation of gallic acid extract of formulated capsule

The marketed formulation (MCM) and raw materials were extracted with 10 ml methanol: water (4:1 v/v) for 2 hr. The mixture was filtered through buchner funnel and the methanol was evaporated in a rotatory evaporator. The residual aqueous phase was acidified to pH 2 by addition of some drops of HCl (3N), and the volume was adjusted to 10 ml with distilled water. In order to isolate the gallic acid, another extraction by

ethanol using a decanting bulb was carried out. The ethanol phase was dried by using anhydrous sodium sulphate then evaporated and residue was dissolved in methanol. The same procedure was performed for *Gymnea sylvestre* (leaves), *Azadirachta indica* (leaves), *Sphaeranthus indicus* (flower), and marketed formulation (MCM).

Method validation

Standard protocols were adopted to determine Precision and accuracy, Limit of quantitation and limit of detection. The method was validated according to ICH Q2(R1) guidelines for validation of analytical procedures. Typical validation characteristics such as specificity, linearity, range, precision, accuracy and robustness were considered for evaluation. These measurements regarded as the most important for the validation of assay type analytical procedure. [5-8]

Specificity

Specificity was confirmed by UV-spectrophotometric scanning of each curcumin standard solution (1-5 µg/mL) in the range of 400-600 nm against ethyl acetate as blank.

Linearity

The linearity was determined by analyzing absorbance of the standard concentrations (1-5 µg/mL) at specific nm against methanol as blank. The calibration curve was plotted using concentration against absorbance. A regression equation and correlation coefficient were determined for standard concentrations (1-5 µg/mL).

Range

The data obtained from the linearity and accuracy studies was used to assess the range of the method.

Precision

Precision was evaluated by using repeatability and intermediate precision. Repeatability was analyzed using standard (1 µg/mL) for six times in the same day (intra-day). The intermediate precision was analyzed using three standard concentrations (1, 3 and 5 µg/mL) for three times on three consecutive days (inter-day).

Accuracy

Accuracy was established by percentage recovery of known added concentrations of standard (1, 2 and 3 µg/mL) to the pre-analyzed sample solutions (2 µg/mL). The method was repeated for three times for each concentration.

Robustness

Robustness was measured for standard solution (5 µg/mL) by different analysts and different instruments. The percentage relative standard deviation (%RSD) values for different analysts (analyst 1 and 2) and different instruments and were calculated.

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

Estimation of Ellagic acid

The stock solution of ellagic acid was prepared by dissolving 10 mg of ellagic acid in 100 ml of methanol. This solution was diluted as needed to prepare different concentrations of standard solutions. A stock solution of ellagic acid (100 µg/ml) was prepared in methanol. The absorbance was measured at absorption maxima 280 nm, against the reagent blank prepared in similar manner without the ellagic acid. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted (Table 1).

The linear correlation between these concentrations (X-axis) and absorbance (Y-axis) were graphically presented and the slope (b), intercept (a), and correlation coefficient (r^2) were calculated out for linear equation (Y= bx+a) by regression analysis using the method of the least square (Graph 1).

Table 1: Calibration curve data for ellagic acid

S. N0.	Concentration	Absorbance
1.	2	0.085
2.	4	0.179
3.	6	0.282
4.	8	0.379
5.	10	0.487
6.	12	0.623

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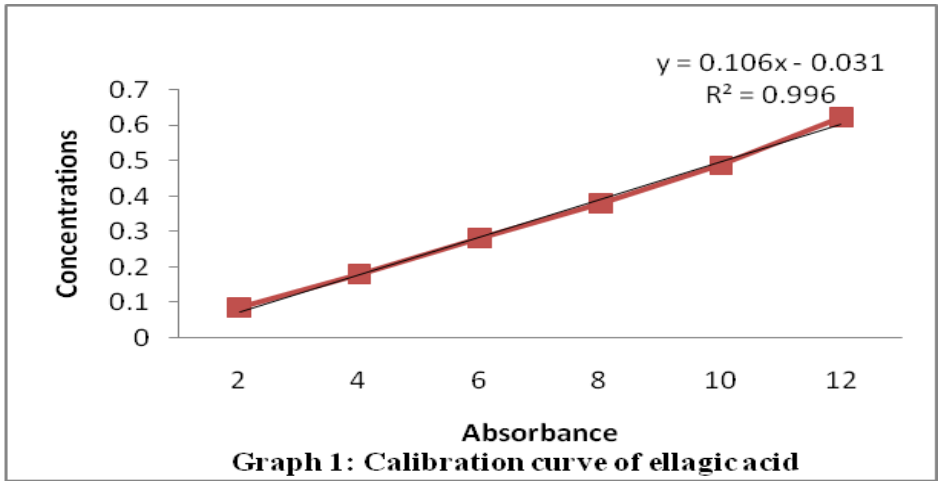

Graph 1: Calibration curve of ellagic acid

Method validation

Precision and accuracy

The method was validated for precision and accuracy, by performing the recovery studies at two levels by adding known amount of ellagic acid extract of formulated capsule, of which the ellagic acid content have been estimated previously. The data were obtained and recovery was calculated (Table).

Limit of quantitation and limit of detection

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution and the lowest concentrations assayed (Table).

Table 2: % recovery of ellagic acid

S. No.	Amount of ellagic acid (µg/ml)			RSD%	SE	Recovery%
	Sample	Added	Estimated			
1.	100	50	148.05±0.70	0.482	0.291	99.17±0.62
2.	100	100	201.11±0.64	0.324	0. 243	100.01±0.10
Mean				**0.403**	**0. 145**	**99.51**

Mean ± SD of six determinations, RSD =Relative Standard Deviation, SE = Standard Error

Table 3: Validation parameter of ellagic acid

S. No.	Parameter	Observations
1.	Absorption Maxima	280 nm
2.	Beer's Law limit	2-12µg/ml
3.	Regression equation (y= bx+a)	y = 0.106x - 0.031
4.	Intercept (a)	-0.031
5.	Slope (b)	0.106
6.	Correlation coefficients (r^2)	R^2 = 0.996
7.	Precision (n=6, % RSD)	0.397

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8.	Accuracy (%)	99.61
9.	LOQ	0.360 µg/ml
10.	LOD	0.127 µg/ml

Estimation of ellagic acid in raw materials and capsule

The appropriate aliquots from ellagic acid extract of *Eugenia jamboloma* (seeds), and marketed formulation (MCM) separately were withdrawn in 10 ml volumetric flask. Absorbance for aliquots of each was noted at 280 nm. The corresponding concentration of ellagic acid against respective absorbance value was determined using the ellagic acid calibration curve. The statistical analysis for checking uniformity in batches is also performed (Table).

Table 4: Estimation of ellagic acid in raw materials and capsule

S. No.	Name	Ellagic acid content % w/w	Confidence level (95%)
1.	*Eugenia jamboloma*	1.45 ± 0.498	±0.361
2.	MCM	0.140± 0.212	± 0.431

Mean ± SD of six determinations

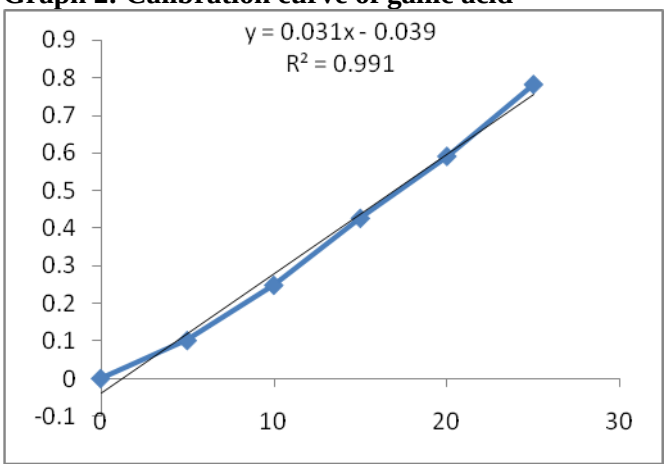
Estimation of Gallic acid

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 5: Calibration curve data for gallic acid

Concentration	Absorbance
0	0
5	0.101
10	0.248
15	0.426
20	0.591
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

Estimation of gallic acid in raw materials and capsule

The marketed formulation (MCM) and raw materials were transferred in 10 ml volumetric flask. Absorbance for aliquots of each was noted at 270 nm. The corresponding concentration of gallic acid against respective absorbance value was determined by using calibration curve (Table 7).

Table 7: Estimation of gallic acid in raw materials and capsule

S. No.	Name	Gallic acid content (% w/w)	Confidence level (95%)
1.	*Gymnea sylvestre*	2.83±0.52	±0.264
2.	*Azadirachta indica*	4.13±0.19	±0.421
3.	*Sphaeranthus indicus*	2.11±0.11	±0.357
4.	MCM	0.50±0.18	±0.285

Mean ± SD of six determinations

Conclusion

The UV spectroscopy fingerprinting method was developed via estimation of gallic acid and ellagic acid for, marketed formulation and raw material. The proposed UV method was found suitable for determination of gallic acid. Statistical data analysis proves that the method is precise and reproducible.

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