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Development and Validation of UV Spectroscopic method for estimation of Ellagic acid in Herbal capsule used for the treatment of Diabetes

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Abstract

The UV spectroscopy fingerprinting method was developed via estimation of ellagic acid for, marketed formulation and raw material *Eugenia jamboloma*. Ellagic acid was found to follow Beer Lambert's law in concentration range 2-12µg/ml at λ_{max} 280 nm. The correlation coefficient (r^2) was calculated, where the r^2 value 0.996 indicates the good linearity between the concentration and absorbance. The estimation of ellagic acid content of formulated capsules (one marketed formulation) and *Eugenia jamboloma* was carried out separately. The concentration of ellagic acid present in raw material was determined in *Eugenia jamboloma* and in marketed formulation MCM. From the validation data it was observed that the present method of spectrophotometric determination of ellagic acid is simple, precise, accurate and suitable for routine analysis of ellagic acid in selected formulation.

Keywords: Diabetes, Herbal Capsule, Ellagic acid

Introduction

The primary goal of the pharmaceutical analysis is to assure drug quality. It is well known that quality cannot be tested in to a product; however, well planned testing with suitable methodology and instrumentation can help build quality in to a drug product. Chromatographic methods are commonly used for quantitative and qualitative analysis of pharmaceutical and herbal preparations. A qualitative method provides information about the identity of sample, whereas, a quantitative method provides numerical information as to the relative amount of one or more of these components.

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.

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.[1-3]

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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 herbal formulations (capsule).

Materials and Methods[4-7]

Anti-diabetic herbal Capsule (50 mg)

Table 1: Composition of anti-diabetic herbal capsule

Ingredients	Quantity
Gymnea sylvestre	10
Eugenia jamboloma	5
Aegle marmelos	5
Azadirachta indica	5
Cinamomum zeylanicum	5
Sphaeranthus indicus	5
Momordica charantia	5
Trivang bhasma	5
Shilajeet	5
Excipients	qs

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.

Development of UV spectroscopy fingerprinting method for ellagic acid

The UV spectroscopy fingerprinting method was developed for herbal capsule *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

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

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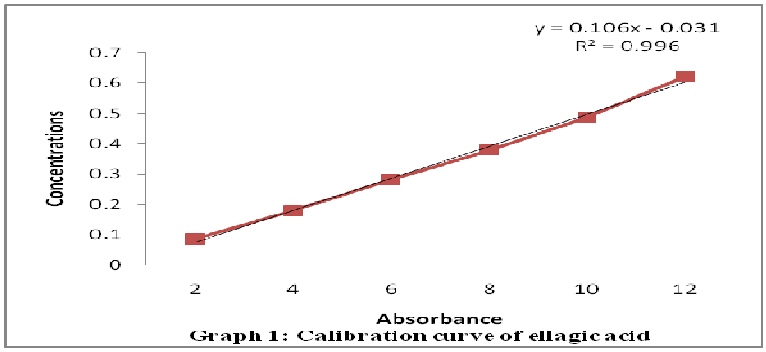
manner without the ellagic acid. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted (Table 2).

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 2: 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



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 3: % 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 4: 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
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 5: 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

Conclusion

Polyherbal anti-diabetic capsule is one of the most common formulations used for diabetes mellitus. It comprised of the some medicinally important plants, *Gymnea sylvestre* (Leaves), *Eugenia jamboloma* (seeds), *Aegle marmelos* (leaves), *Azadirachta indica* (leaves), *Cinamomum zeylanicum* (leaves), *Sphaeranthus indicus* (flower), *Momordica charantia* (fruits), Trivang bhasma and Shilajeet. The marketed formulation designated MCM was purchased from local pharmacy store of Indore.

Fingerprinting method was developed for each laboratory batch, its marketed formulations and separately its raw material *Gymnea sylvestre*, *Eugenia jamboloma*, *Aegle marmelos*, *Azadirachta indica*, *Cinamomum zeylanicum*, *Sphaeranthus indicus*, *Momordica charantia*, Trivang bhasma and Shilajeet by using sophisticated instrument UV.

The UV spectroscopy fingerprinting method was developed via estimation of ellagic acid for, marketed formulation and raw material *Eugenia jamboloma*. Ellagic acid was found to follow Beer Lambert's law in concentration range 2-12µg/ml at λ_{max} 280 nm. The correlation coefficient (r^2) was calculated, where the r^2 value 0.996 indicates the good linearity between the concentration and absorbance. The estimation of ellagic acid content of formulated capsules (one marketed formulation) and *Eugenia jamboloma* was carried out separately. The concentration of ellagic acid present in raw material was determined in *Eugenia jamboloma* and in marketed formulation MCM. From the validation data it was observed that the present method of spectrophotometric determination of ellagic acid is simple, precise, accurate and suitable for routine analysis of ellagic acid in selected formulation.

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