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

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Abstract

With the use of ellagic acid estimate for Eugenia jamboloma's commercialized formulation and raw material, a UV spectroscopic fingerprinting technique was created. For elagic acid, Beer Lambert's rule holds true between 2 and 12 micrograms per milliliter at maximum absorbance of 280 nanometers. The linearity between concentration and absorbance was evaluated using the correlation coefficient (r2), which showed a value of 0.996. Formulated capsules (one commercial formulation) and Eugenia jamboloma each had their ellagic acid content estimated independently. Eugenia jamboloma and MCM, a commercially available formulation, were analyzed to assess their ellagic acid content. Spectrophotometric measurement of ellagic acid using the current approach was found to be straightforward, precise, accurate, and amenable to routine analysis of ellagic acid in chosen formulations based on the validation results.

Key words :Diabetics, Herbal Capsules, and Ellagic Acid

Introduction

Pharmaceutical analysis is performed with quality control of the medicine in mind. While it's true that you can't "test" quality into a product, careful testing using the right approach and gear may go a long way toward ensuring that pharmaceuticals are up to snuff. Quantitative and qualitative examination of medicinal and herbal products is routinely performed using chromatographic techniques. While sample identities may be learned using qualitative methods,analysis parameters and the patient's condition.Many plants have been documented in the literature to possess disease-curing properties. Different herbal medication formulations are gaining popularity. About twenty-five thousand different plant-based preparations are utilized in traditional medicine in India. India has a $ 1 billion market for herbal pharmaceuticals and a $ 80 million industryfor the export of crude drugs derived from plants.1-3A quantitative approach yields quantifiabledata on the percentage composition of one or more of these elements.Herbal formulations and their phyto-constituents need to be standardized, which necessitates the development of analytical methods for plants and their composition. Herbal formulations come in a wide variety of forms and may be utilized by

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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 ChemicalsAll 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 for2 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

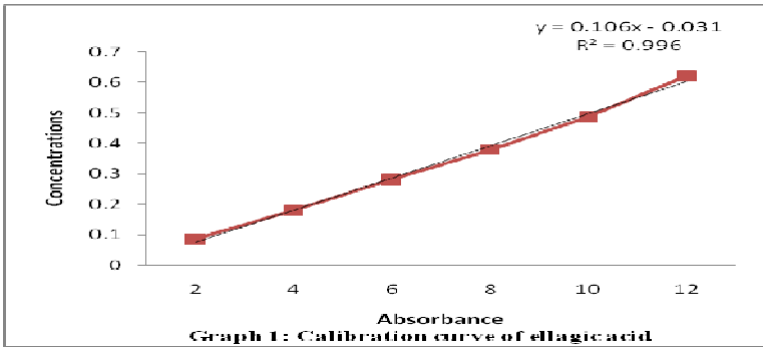
Standard protocols were adopted to determine Precision and accuracy, Limit of quantitation and limit of detection Statistical analysis was carried out using Graph Pad Prism v 5.0. All the results were expressed as Mean±SD and %RSD.

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 similarmanner 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 theseconcentrations (X-axis) and absorbance (Y-axis

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 butnot 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).

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

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

One of the most popular treatments for diabetes mellitus is the polyherbal anti-diabetic pill. For medical purposes, it included Gymnea sylvestre (leaf), Eugenia jamboloma (seeds), Aegle marmelos (leaves), Azadirachta indica (leaves), Cinamomum zeylanicum (leaves), Sphaeranthus indicus (flower), Momordica charantia (fruits), Trivang bhasma, and Shilajeet. The commercially available MCM formulation was acquired from an Indore pharmacy.Using a high-tech UV instrument, a fingerprinting method was developed for each lab batch, its commercialized formulations, and individually for its raw materials Gymnea sylvestre, Eugenia

jamboloma, Aegle marmelos, Azadirachta indica, Cinnamomum zeylanicum, Sphaeranthus indicus, Momordica charantia, Trivang bhasma, and Shilajee.With the use of ellagic acid estimate for Eugenia jamboloma's commercialized formulation and raw material, a UV spectroscopic fingerprinting technique was created. For elagic acid, Beer Lambert's rule holds true between 2 and 12 micrograms per milliliter at maximum absorbance of 280 nanometers. The linearity between concentration and absorbance was evaluated using the correlation coefficient (r2), which showed a value of 0.996. Formulated capsules (one commercial formulation) and Eugenia jamboloma each had their ellagic acid content estimated independently. Eugenia jamboloma and MCM, a commercially available formulation, were analyzed to assess their ellagic acid content. Spectrophotometric measurement of ellagic acid using the current approach was found to be straightforward, precise, accurate, and amenable to routine analysis of ellagic acid in chosen formulations based on the validation results.

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