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Research Article

QUALITY CONTROL STUDIES AND DETERMINATION OF TOTAL FLAVONOID AND PHENOLIC CONTENT OF AN AYURVEDIC SOLID DOSAGE FORM

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ABSTRACT

Objective: Ayurvedic preparations contain a mixture of herbs. Blood glucose regulator (BGR)-34 is an Ayurvedic tablet meant for the treatment of diabetes. The objective of the present work is to carry out the quality control (QC) studies of BGR-34 tablets.

Materials and Methods: QC tests for the tablet were carried out such as determination of average weight, pH value, tablet hardness, friability, and disintegration time as per official method. Determination of natural antioxidants such as total flavonoid and total phenolic content was also carried out by standard methods. Standardization of ayurvedic preparation involves the determination of marker compound. Gallic acid content was determined using high-performance liquid chromatography.

Results: The results indicated that the tablet passes the QC tests. The tablet was found to have 32.31±0.91 mg quercetin equivalents/100 g of total flavonoid content and 2.285±0.12 mg gallic acid equivalents (GAE)/100 g of phenolic content. The amount of gallic acid present in the sample was found to be 2.06%.

Conclusion: The presence of large amounts of flavonoids may be the reason for its hypoglycemic activity. The present study can be used for the regular QC of the BGR-34 tablets.

Keywords: Ayurvedic preparations, Quality control test, Total flavonoid content, Total phenolic content.

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INTRODUCTION

A number of medicinal plants were used traditionally for the treatment of various diseases. However, they are not scientifically verified with documentation. Hence, it is the need of the hour to standardize herbal preparations and provide scientific evidence. Quality control (QC) tests adopted for tablets comprises of physical, chemical, and biological evaluation. Physical testing comprises the determination of shape, size, color, hardness, friability, and disintegration. Ayurveda is the science of life originated from Indian subcontinent. The ancient Vedic literature specifies instructions to fight the illness using exercises, diet, massages, and herbal drug therapies, thus maintaining health [1]. In the present study, QC of blood glucose regulator (BGR)-34 tablet was carried out as per official method. BGR-34 is developed as a BGR by scientist of the Council of Scientific and Industrial Research in association with the National Botanical Research Institute and Central Institute of Medicinal and Aromatic Plants.

MATERIALS AND METHODS

BGR-34 tablet contains *Berberis aristata* (*Daruharidra* – 1150 mg), Pterocarpus marsupium (Vijaysar – 400 mg), *Gymnema sylvestre* (Giloy – 400 mg), *Rubia cordifolia* (Majeeth – 375 mg), *Trigonella foenum-graecum* (Methika – 350 mg), and *Tinospora cordifolia* (Gudmar – 350 mg). *Daruharidra* helps to improve the pancreas functioning, naturally. Vijaysar, rich in flavonoids, helps to maintain normal blood glucose level. Giloy helps to improve immunity and resistance to infections. Majeeth, an antioxidant, helps to protect vital organs from oxidative damage. Methika, a rich source of micronutrients, nourishes the vital organs. Gudmar delays glucose absorption, thus maintains postprandial blood glucose level.

QC tests for tablets

Average weight of tablets

A total of 20 tablets were selected randomly and weighed on an analytical balance. The average weight was calculated and the percentage deviation was determined.

pH value

About 1 g of sample was weighed in an analytical balance. It was then transferred into a beaker containing 100 ml of distilled water. It was mixed well using a glass rod, filtered with a filter paper and the pH of the filtrate was found using digital pH meter.

Tablet hardness test

Tablet machines hardness tester was used to measure the hardness of the tablet. 10 tablets were taken. The surface that comes in contact with plunger was cleaned with cotton. Tablets were positioned one by one on the lower plunger and the reading was recorded. The threaded bolt was turned until the tablet splintered; again, the reading was noted. Difference between the initial and final readings was recorded. Average hardness of tablet was determined.

Friability test

A total of 20 tablets were taken and weighed in an analytical balance. These pre-weighed tablets were placed in plastic chamber of friability testing apparatus. It was then switched on so that it revolves at a speed of 25 revolutions per minute. The friabilator was switched off after 5 min. Tablets were taken and reweighed. The percentage difference from original weight is used to express friability [2,3].

Disintegration time

Disintegration is defined as the state in which any residue of the tablet except fragment of insoluble portion remaining on screen of apparatus. Six tablets were placed in the disintegration apparatus immersed in water. The apparatus was switched on and the time required by the tablet to disintegrate was noted.

Estimation of total flavonoid and phenolic content

Phenols, flavonoids, tannins, etc., are considered as natural antioxidants as they prevent disease, promote the health, and are used as anti-aging compounds. Phenolics, the secondary plant metabolites possess a range of pharmacological activity. Flavonoids are the polyphenolic compounds with a variety of properties such as nitric oxide scavenging, free radical scavenging, and oxidative and hydrolytic enzymes inhibition [4-7].

Folin–Ciocalteu assay

Folin–Ciocalteu assay was used to determine the total phenolic content. In a 25 ml volumetric flask, 1 ml of extract was mixed with 9 ml of distilled water. To this, 1 ml of Folin–Ciocalteu phenol reagent was added, shaken well, and allowed to stand for 5 min. To this, 10 ml of 7 % sodium carbonate solution was added and the volume was made up to the mark using distilled water. Standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/ml) were prepared. All the solutions were incubated for 90 min at room temperature. The absorbance of the solutions was measured at 550 nm against the reagent blank. Calibration curve for gallic acid was plotted and total phenolic content was expressed as mg of gallic acid equivalents (GAEs).

Aluminum chloride colorimetric assay

Aluminum chloride colorimetric assay is used to measure the total flavonoid content. In a 10 ml volumetric flask, 1 ml of the extract was taken and 4 ml distilled water was added. To this, 0.3 ml of sodium nitrite (5%) was added, allowed to stand for 5 min. Then, 0.3 ml of aluminum chloride (10%) was added followed by 2 ml of 1 M sodium hydroxide. Further, dilution was done using distilled water. Standard solutions containing different concentrations of quercetin were also prepared. Absorbance of the resulting solutions was determined at 510 nm. The total flavonoid content was estimated from the calibration plot for quercetin and the outcomes were given as mg of quercetin equivalents (QE).

Estimation of gallic acid content

High-performance liquid chromatography (HPLC) is an enhanced version of column liquid chromatography. In column chromatography, the solvent is permitted to ooze through a column under gravity; wherein, in HPLC, high pressure allows the solvent to move through the column very fast. The difference in affinity of the substance between the stationary and mobile phase allows the sample to be separated into its constituent part [8,9].

HPLC technique helps in the identification and quantification of compounds. It also helps in chemical separation and purification.

Chromatographic conditions

The mobile phase consisted of acetonitrile and water in the ratio of 20:80, and pH adjusted to 3.2 with O-phosphoric acid. Phenomenex Luna C_{18} (4.6×250 mm, 5 µ particle size) column was used as a stationary phase. Isocratic mode of elution with a flow rate of 1 ml/min and detection wavelength of 266 nm was used for the run. Mixture of water and methanol (9:1) was used as a diluent and the run time was set as 5 min.

Preparation of standard stock solution

About 25 mg of gallic acid was weighed accurately and dissolved in little quantity of the diluent in a 25 ml standard flask. The final volume was made up with diluent to produce a concentration of 1000 µg/ml.

Preparation of sample solution

About 250 mg of powdered drug was taken in a 25 ml standard flask, diluted to 25 ml by the addition of diluent, sonicated for 20 min, and filtered. From the above solution, 1 ml was taken and made up to 10 ml with diluent. The prepared solution was analyzed by HPLC.

Table 1: Organoleptic parameters of BGR-34 tablets

S. No.	Parameters	Observation
1	Color	Dark brown
2	Taste	Bitter
3	Odor	Characteristic
4	Form	Solid

BGR: Blood glucose regulator

Table 2: QC parameters of BGR-34 tablets

S. No.	Parameters	Result
1	Weight variation	Average weight: 0.639 g Minimum weight: 0.629 g Maximum weight: 0.651 g
2	Tablet hardness	15.5 kg/cm
3	Disintegration test	25 min
4	Friability test	0.0057%
5	pH	3.14±0.06

BGR: Blood glucose regulator, QC: Quality control

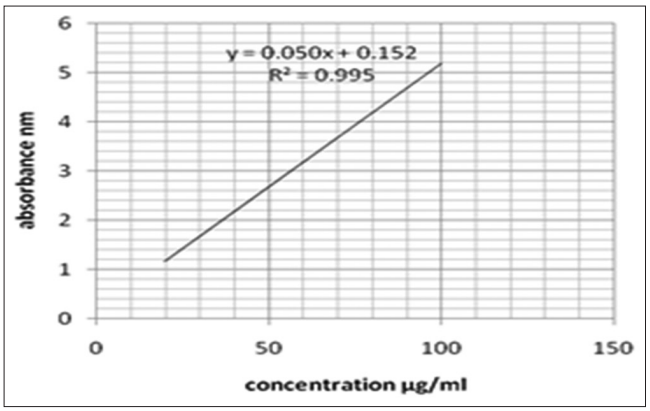

Fig. 1: Calibration curve of gallic acid as a reference for total phenolic content

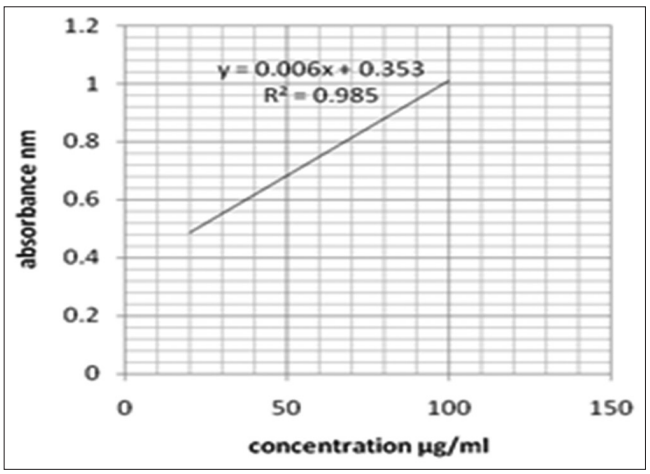

Fig. 2: Calibration curve of quercetin as a reference for total flavonoid content

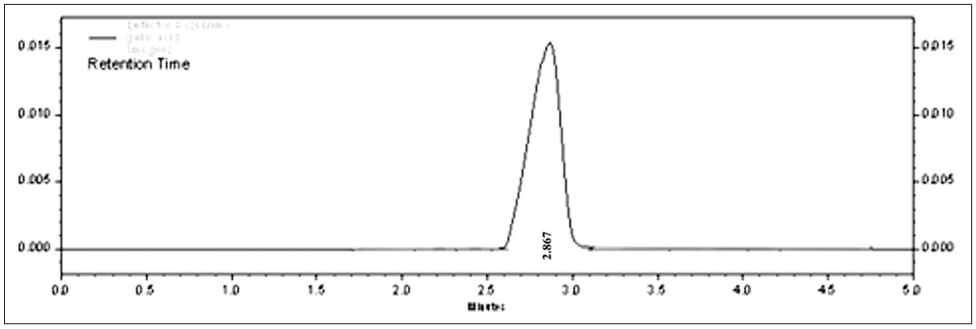

Fig. 3: Chromatogram of gallic acid reference standard

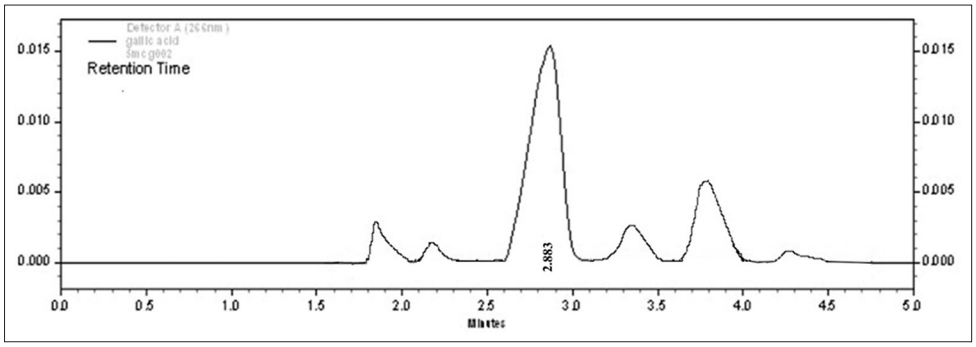

Fig. 4: Chromatogram of gallic acid content in polyherbal tablet

RESULTS AND DISCUSSION

QC tests for tablets

Herbal tablets are generally given orally [10]. The organoleptic parameter forms the basic criteria to verify the finished product. The smooth texture of tablets indicated that the surface is uniform and no cracks found. It is the initial character to judge the quality of the tablet [11]. The tablets were dark brown in color with bitter taste. A characteristic odor was observed due to specific property of various ingredients present in it. The results are depicted in Table 1.

Table 2 shows the QC parameters of BGR-34 tablets. The average weight of tablet was found to be 0.639 g. Weight variation was found to be <5%, which is within the limit. The hardness was found to be 15.5 kg/cm, and the tablet disintegrated within 25 min. Even though the hardness was more, the tablets disintegrated within 25 min. Friability was found to be very less indicating that there is no loss of ingredient during transportation or handling.

pH usually represents the acidity and alkalinity. pH of BGR-34 tablet was found to be 3.14±0.06 which shows weakly acidic nature of the polyherbal tablet.

Estimation of total flavonoid and phenolic content

Folin–Ciocalteu assay is the method adopted for the estimation of phenolic content. Gallic acid was used as a reference standard. Fig. 1 represents the calibration graph plotted with y=0.0504x+0.1528. The correlation factor was found to be 0.9951. The given sample was analyzed in triplicate and found to contain 2.285±0.12 mg GAE/100 g.

The total flavonoid content was measured using aluminum chloride colorimetric assay taking quercetin as a reference standard. The calibration graph was plotted with y=0.0066x+0.3537 and shown in Fig. 2. The correlation factor was found to be 0.9858. The given sample was analyzed in triplicate and found to contain 32.31±0.91 mg QE/100 g.

Plants are the rich sources of natural antioxidants. They are used for the treatment of various ailments. Their protective effects are mainly due to the presence of phenolics and flavonoids. They act as reducing agents and stabilize lipids against peroxidation.

Estimation of gallic acid content

The chromatogram of gallic acid reference standard and polyherbal tablets is given in Figs. 3 and 4, respectively.

A good separation under satisfactory peak was obtained using Phenomenex Luna C_{18} column of particle size 5 µ. The mobile phase had water and acetonitrile in the ratio of 80:20, with a pH of 3.2 attained using orthophosphoric acid. The mobile phase passed through the column at a rate of 1 ml/min. UV detector was set at 266 nm for the detection of eluent. The retention time was found to be 2.8 min. The quantity of gallic acid present in the polyherbal tablet (BGR-34) was determined as 2.06%. Gallic acid can be used as a marker for the standardization of BGR-34 tablets.

CONCLUSION

The present study can be used for the regular QC of the BGR-34 tablets. All the QC tests were carried out as per the WHO guidelines. Gallic acid can be used as a marker for the QC studies of the selected polyherbal tablet. The presence of phenols (2.285±0.12 mg GAE/100 g) and high content of flavonoids (32.31±0.91 mg QE/100 g) support the use of this compound as an antidiabetic drug.

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AUTHORS' CONTRIBUTIONS

First author: Guidance and preparation of manuscript, Second author: Carried out the experiment, Third author: Performed the calculations.

CONFLICTS OF INTEREST

The author's declares no conflicts of interest.

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