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Research Article

ASSESSMENT OF DIFFERENT QUALITY CONTROL PARAMETERS OF MARKET VARIANTS OF PUNARNAVADI GUGGULU

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ABSTRACT

The study was proposed to assess the variation in quality specifications between the standard formulation of the Punarnavadi guggulu made according to the Ayurvedic pharmacopoeia and market variants of Punarnavadi Guggulu so that industry and regulatory agencies can be made aware about the defects if any, and recommendations may be proposed about the quality and consistency status of the products available in the market. Different physico – chemical parameters such as diameter, thickness, weight variation, ash values, extractive values of sample in water and alcohol, Thin Layer Chromatography, HPTLC, heavy metals and aflalatoxins presence were done to ascertain the quality of this drug.

Keywords: Ayurvedic formulation, Standardization, Quality control, Guggulu.

INTRODUCTION

Gum Guggul is the oleoresin of Commiphora mukul, a plant that is native to India, its extracts include compounds known for their hypolipidemic properties—the *Z*- and *E*- isomers of guggulsterone and its related guggulsterols.: guggulsterol I, guggulsterol-II, guggulsterol-III, guggulsterol IV, guggulsterol V, and guggulsterol VI. Guggul is the principal ingredient of several Ayurvedic formulations used for various ailments such as Kaishore Guggul, Navaka Guggul, Yograj Guggul and Punarnavadi Guggul. Punarnavadi formulation contains beta-sitosterol which is helpful for heart conditions, inflammations and reduces all three doshas. [1, 2]. It helps in fluid retention and reduces excess kapha in the system. [3]

MATERIAL AND METHODS

The three sample of Punarnavadi Guggul as Sample A, B and C was taken (in purified form). The authenticity of the Punarnavadi Guggul of the procured herbs was checked and confirmed. Samples of the raw material were then examined for probable adulterants, which were found to be absent. Samples of the purified raw material were then considered for quality analysis in accordance with WHO guidelines for acceptance.

Procurement of drugs and chemicals

The ingredients used for the preparation of standard punarnavadi guggul formulation and the marketed samples for the comparative studies were provided by Ayurvedic Store house, lucknow. Solvents of reagent grade and double distilled water were used in all experiments.

Shuddhi (purification) of guggulu

The physically cleaned guggulu (taken in a quantity equal to that of the triphala mixture) in raw form was mixed with the standardized decoction of guduchi and triphala for purification. The mixture was heated to 60 – 70 ^{0}c with continuous stirring so that guggulu mass got dissolved. During the process a small quantity of ghrita was added to prevent charring of the material. The resultant mixture was filtered through a thin cotton cloth. The material still remaining in the cloth was repeatedly treated with hot water and filtered, for completion of the filtration process. The filtrate obtained was decanted to get rid of any finer impurities. The resultant liquid was heated to remove the water content and for guggulu to remain in the form of a pasty material. At this stage some amount of ghrita was added and heating continued till a semi solid consistency was attained.4, 5

Preparation of Punarnavadi Guggulu

All ingredients were taken according to pharmacopoeial quality. The coarse powder ingredients were soaked in water in the specified ratio for 1 hr and then it was boiled till the volume was reduced to half of its original. The kasaya was cooled and filtered through a muslin cloth; boiled suddha guggulu was added in the above kasaya in an iron vessel and concentrated, followed by addition of fine powders of remaining drugs with continuous stirring. Finally ghrita was added to the above mixture to form a semisolid mass for preparation of vati. The vati of desired weight were obtained by passing them through vati machine fitted with suitable die. The vati's were dried in a tray dryer at a temperature not exceeding 60ºC and then packed in a closed glass container to protect from light and moisture.

Thickness determination Thickness

The crown tablet thickness of an individual tablet was measured with a vernier caliper, which permits accurate measurements and provides information on the variation between the tablets. The data for tablet thickness is shown in Table 1.

Table 1: Tablet thickness in different samples

Samples Code	Thickness (mm)			Mean	Standard deviation
Sample A	0.126	0.131	0.130	0.129	0.002
Sample B	0.179	0.169	0.170	0.172	0.005
Sample C	0.149	0.160	0.141	0.15	0.002

Tablet diameter

The diameter of the tablet was measured by digital caliper**.** The data is shown in Table no. 2.

Table 2: Tablet diameter in different samples

Samples Code	Diameter (mm)			Mean	Standard deviation
Sample A	0.384	0.382	0.380	0.382	0.002
Sample B	0.410	0.404	0.406	0.406	0.003
Sample C	0.448	0.446	0.440	0.444	0.004

Tablet Hardness

To perform this test, a tablet was placed between two anvils, force was applied to the anvils, and the crushing strength that just causes the tablet to break was recorded. Hardness is thus sometimes termed the tablet crushing strength. The data for calculation of tablet weight variation is shown in Table 3.

Table3: Tablet hardness in different samples

Samples Code	Hardness (Kg/cm^2)			Mean	Standard deviation
Sample A	3.0	5.2	6.0	4.7	1.55
Sample B	1.6	1.8	1.6	1.6	0.14
Sample C	2.6	1.3	1.7	1.8	0.66

Weight variation Test

20 tablets were weighed at random and the average weight of the tablets was calculated. The individual weight of the tablet was compared with the average weight. The data for calculation of tablet weight variation is depicted as a graph in Fig .1

Disintegration Test

For the disintegration test one tablet was placed in each tube and the basket rack was positioned in 1 L beaker containing water at 37 ± 2º C. For compliance with USP standards the tablets must disintegrate and all particles must pass through the 10 mesh screen in the specified time.

If any residue remains it must have a soft mass with no palpably firm core. Uncoated USP tablets have disintegration time standards as low as 5 min, but majority of the tablets have a maximum disintegration time up to 30 min. In the case of Ayurvedic tablets the disintegration time should not be less than 60 minutes. The data for calculation of tablet disintegration time is shown in Table 5-

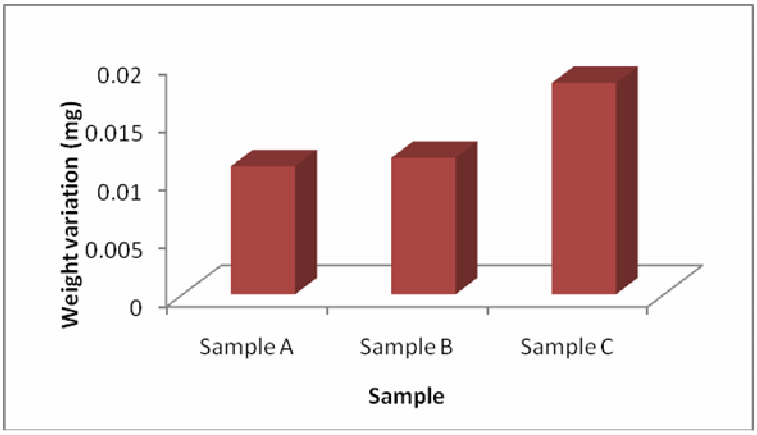


Fig. 1: Weight variation in different samples

Friability Test: 6gms. of the dedusted tablets were subjected to 100 free falls of 6 inches in a rotating drum at 25 rpm. , and then are reweighed. The calculation of tablet friability is shown in Table 4-

Table 4: Percentage (%) friability in different samples

Samples Code	Initial weight(grams)			Final weight(grams)			% friability			Mean	Standard deviation
Sample A	6.16	6.14	6.18	6.11	6.09	6.12	0.8	0.9	0.8	0.83	0.36
Sample B	6.15	6.18	6.07	6.10	6.12	6.00	0.8	0.9	1	0.6	0.05
Sample C	6.07	6.09	6.15	6.00	6.03	6.10	1	0.8	0.8	0.86	0.11

Table 5: Disintegration time of different samples

Samples Code	Disintegration Time(min)			Mean Disintegration Time (min.)	Standard Deviation
Sample A	60	62	65	62.33	4.99
Sample B	20	22	25	22.33	2.51
Sample C	60	61	60	60.33	4.79

pH determination

Standard buffer solution pH 4 -One tablet of pH 4 was dissolved in 100 ml of distilled water. Standard buffer solution pH 9.2- One tablet of pH 9.2 was dissolved in 100 ml of distilled water.10% sample solution was dissolved in distilled water for measuring pH. Then the instrument was switched on for 15 minutes time for initial warming. The standard buffer solution of pH 4 was introduced in the instrument and knob was adjusted followed by standard buffer solution of pH 9.2 .Finally sample solution was introduced and reading was recorded. The readings were taken three times and finally the average was taken. The data for calculation of tablet pH is shown in Table 6-

Percentage loss of drying

4g of the sample was weighed and heated in an oven for 100 -105°c for 5 hours in a previously weighed 100 ml beaker, cooled in a desiccator and reweighed. The procedure was repeated until constant weight was obtained. The percentage of loss in weight of the sample was calculated. The data for calculation of loss on drying of a tablet is shown in Table 7-

Percentage (%) of loss on drying = $\frac{\text{Loss in weight of the sample x 100}}{\text{Weight of the sample by taken}}$

Table 6: pH determination of different samples

Samples Code	pH			Mean	Standard deviation
Sample A	4.3	4.2	4.0	4.16	0.15
Sample B	7.4	7.3	7.2	7.3	0.10
Sample C	5.7	5.5	5.8	5.66	0.21

Table 7: Percentage (%) loss on drying in different samples

Samples Code	ɔn drying	Mean	Standard deviation
Sample A		10.17	4.98
Sample B	6.15	6.12	2.01
Sample C	7.77	7.08	2.16

Percentage (%) total ash

2g of the sample was accurately weighed in a previously ignited and tarred Silica dish. The material was evenly spread and ignited in a muffle furnace by gradually increasing the temperature to 600^0 C until it is white, indicating the absence of carbon. The dish was cooled in a desiccator and weighed. If carbon free ash is not obtained in this manner, the dish is cooled and moistened the residue with about 2 ml of water or a saturated solution of ammonium nitrate followed by drying on a water-bath, and then ignited in the muffle furnace to obtain a constant weight. The dish is cooled in a desiccator for 30 min. and then weighed. The percentage of total ash of air-dried material was calculated. The data for calculation of total ash is shown in Table 8-

Percentage (%) of total ash = Weight of ash x 100 / Weight of the sample taken

Table 8: Percentage (%) total ash in different samples %Acid insoluble ash

Samples Code	ash	Mean	Standard deviation
Sample A	6	7.33	1.24
Sample B	12	9.83	1.84
Sample C		14.66	2.05

To the dish containing the total ash, 25 ml of dilute Hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minute. The watch glass was rinsed with 5 ml of hot water .The insoluble matter on an ash less filter paper (Whatman No. 41) was collected and washed with hot water until the residue is free from acid. The filter paper containing the insoluble matter was transferred to the original dish, dried and ignited to obtain constant weight. The dish was cooled in a desiccator for 30 minutes, and then weighed. The percentage of acid insoluble - ash of the air-dried material was calculated. The data for calculation of acid insoluble ash is shown in Table 9-

Percentage (%) of acid insoluble ash = Weight of the acid insoluble residue x 100 / Weight of the sample

Table 9: Percentage (%) acid insoluble ash in different samples

Samples Code	nsoluble ash	Mean	Standard deviation
Sample A	1.5	1.7	0.16
Sample B		3.76	0.20
Sample C	5	5.3	0.29

Percentage (%) Water Soluble Extractive

4 g of the sample was weighed in a glass stoppered flask. 100 ml of distilled water was added and shaken occasionally for 6 hours and then allowed to stand for 18 hours. It was filtered rapidly, taking care not to lose any solvent and 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath, kept in an air oven at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed. The experiment was repeated thrice and then the average was taken. The data for calculation of water soluble extractive is shown in Table 10-

Percentage (%) of Water Soluble Extractive = Weight of the extract x100x100 / 25 x weight of the sample taken

Table 10: Percentage (%) water soluble extractive in samples

Samples Code	r soluble extractive	Average value	Standard deviation
Sample A		12.62	2.19
Sample B	16.4	17.76	3.21
sample C	20.75	18.5	2.30

Percentage (%) of Alcohol Soluble Extractive

4 g of the sample was accurately weighed in a glass stoppered flask. 100 ml of distilled alcohol was added to it and shaken occasionally for 6 hours and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent and 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath, kept it in air oven at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed. The percentage of alcohol extractable matter of the sample was calculated. The experiment was repeated thrice and then the average was taken. The data for calculation of alcohol soluble extractive is shown in Table 11-

Percentage (%) of Alcohol Soluble Extractive = Weight of the extract x 100 x 100 / *25* x weight of the sample taken

Table 11: Percentage (%) alcohol soluble extractive in samples

Samples Code	(% Alcohol soluble extractive)	Mean	Standard deviation
Sample A		11	2.64
Sample B	4	4.66	0.54
Sample C	16	13.6	2.89

Thin layer chromatography - Preparation of Vanillin-Sulphuric acid reagent

5 ml of concentrated sulphuric acid and 95 ml of ethyl alcohol was cooled in refrigerator separately. 1g of Vanillin was weighed and dissolved in 95 ml of cool Ethyl alcohol followed by addition of 5ml cool concentrated sulphuric acid .4g of sample was weighed and 5ml of distilled ethyl alcohol was added to it and kept overnight with occasional shaking followed by boiling for 10 minutes on a water bath, cooling and filtration. The filtrate was concentrated and made up to 10 ml in a volumetric flask with ethyl alcohol. This solution was applied on a pre-coated Merck Silica gel 60F254 and the plate and was developed in a suitable solvent system in a twin trough chamber up to a length of approximately 8cm. The plate was dried, kept inside the photo documentation unit and the photograph was taken in UV 254 & 366 nm in fig 2.

Photograph at 254 nm

UV 254 nm light was switched on. The focus was adjusted and the field of view was kept maximum and an aperture size to 5.6. The knob of the TLC photo documentation unit was adjusted to yellow light and the photograph was taken in fig. 2.

Photograph at 366 nm

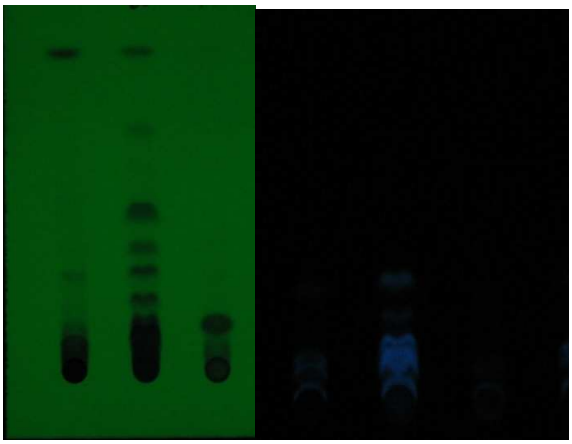
UV 366 nm light was switched on. The aperture size was kept to 3.5 and the knob was adjusted to position B and photograph was taken. The plate was developed in vanillin-sulphuric acid reagent, dried and heated in an air oven at 105 ^{0}C till coloured spots appeared. The photograph was taken in white light using TLC photo documentation unit. The TLC of prepared formulation and different marketed samples are shown in fig. 2.

Rf of each spot = Distance to which the spot moves / Distance to which the solvent moves

4g of sample was weighed and 40 ml of distilled ethyl alcohol was added to it and kept overnight with occasional shaking followed by boiling for 10 minutes on water bath, cooling and filtering. The volume of the filtrate was made up to 10 ml in a volumetric flask. The HPTLC applicator was switched on. The pressure of the nitrogen gas was regulated to 3.5 Pascal in the regulator.

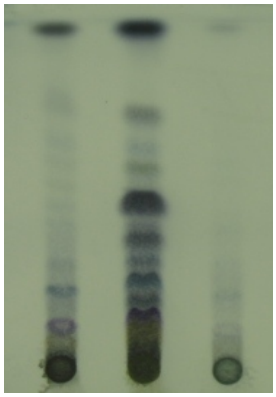
The syringe was filled with extract. Gas flow was checked. 1. Plate width (according to the plate used) 2. Start position (generally 10 mm) 3. Band width (generally 6 mm) 4. Space (generally 6 mm) 5. Speed (10 micro litre per second) 6. Calculation (indication of number of tracks as per the plate width) The gas flow button was pressed and the gas flow was checked by pressing + and downward arrow keys of the applicator. For different extracts volume was fixed and number was tracked each time. The sample solution was applied on a pre-coated Silica gel 60F254 plate (Merck). The plate was developed in suitable solvent system in the twin trough chamber previously saturated with the solvent for 30 minutes. The syringe was washed twice with methanol.

The gas cylinder was closed. The plate was dried and then placed in the scanner. A file was opened, and all parameters of scanning were entered. For the absorption of *I* reflection mode the plate was scanned in 254 nm using Deuterium lamp, for fluorescence *I* reflection mode, the plate was scanned in UV 366 nm using Mercury lamp. The scanner was switched off immediately after UV spectrum scanning stopped. UV spectra of each spots were then compared. The file was closed and the computer was shut down. The graphs of different marketed samples and formulation prepared are shown in Fig. 3,4 and 5- Sample A Wavelength (λ) – 254 nm



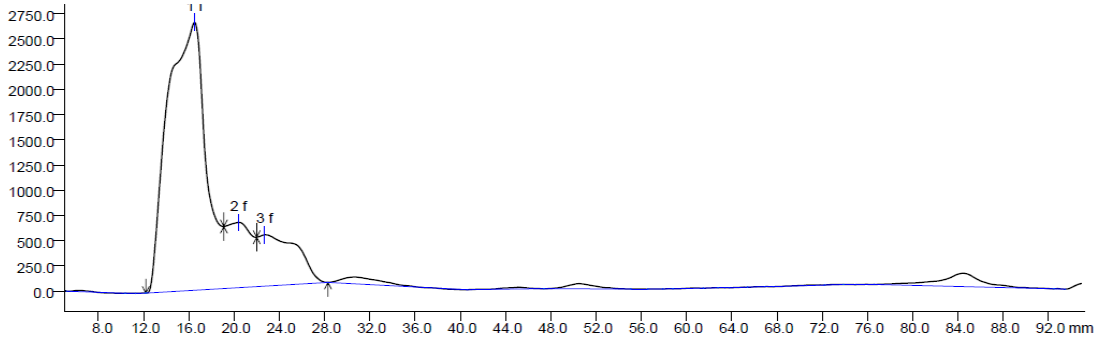
A B C A B C

TLC chromatogram at 254 nm TLC chromatogram at 366 nm



A B C

Fig. 2: TLC chromatogram with visualizing agent High performance thin layer chromatography



Wavelength (λ) - 366 nm

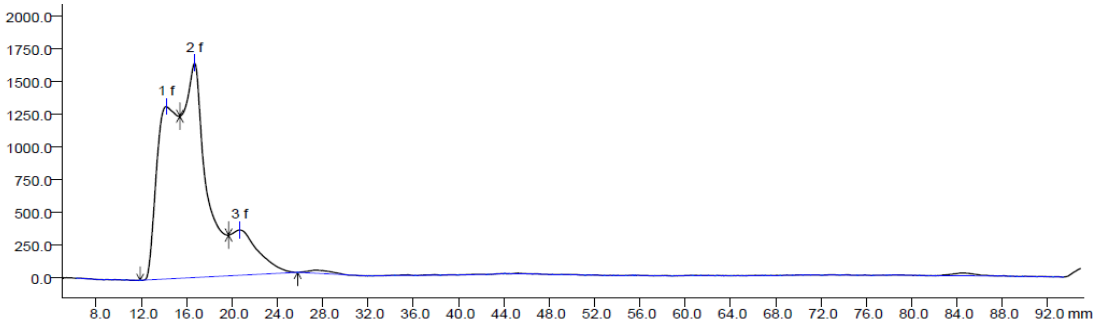
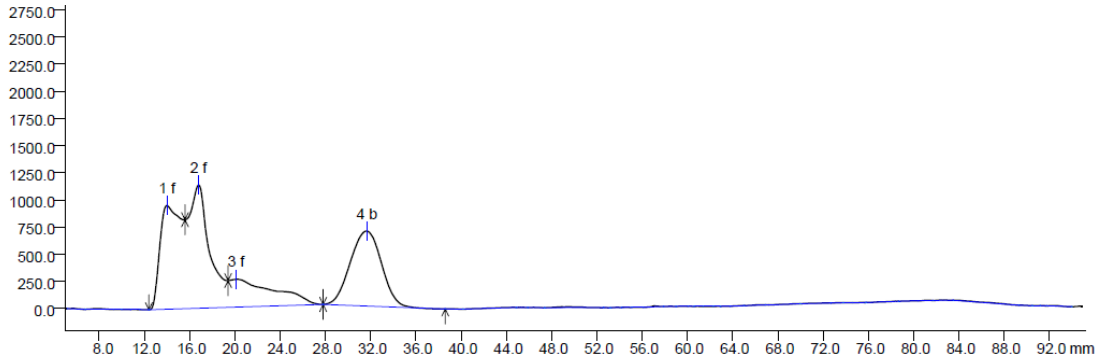
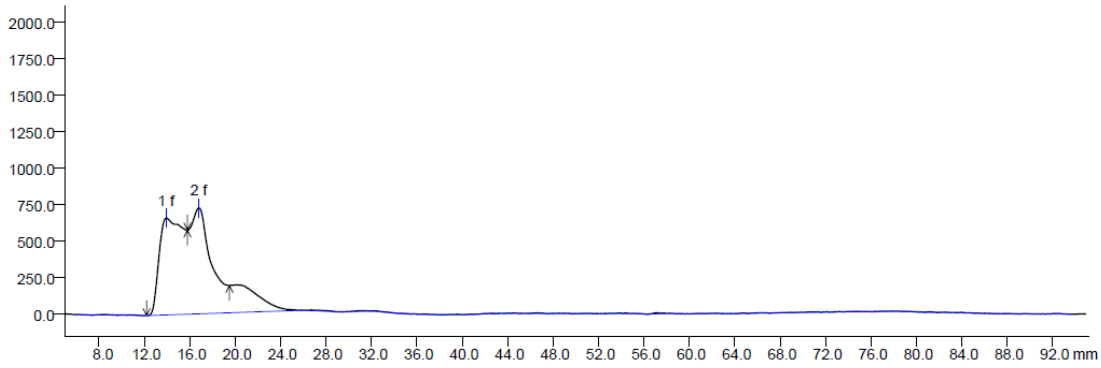


Fig. 3: HPTLC chromatogram of Sample A at wavelength 254 and 366 nm Sample B Wavelength (λ) – 254 nm

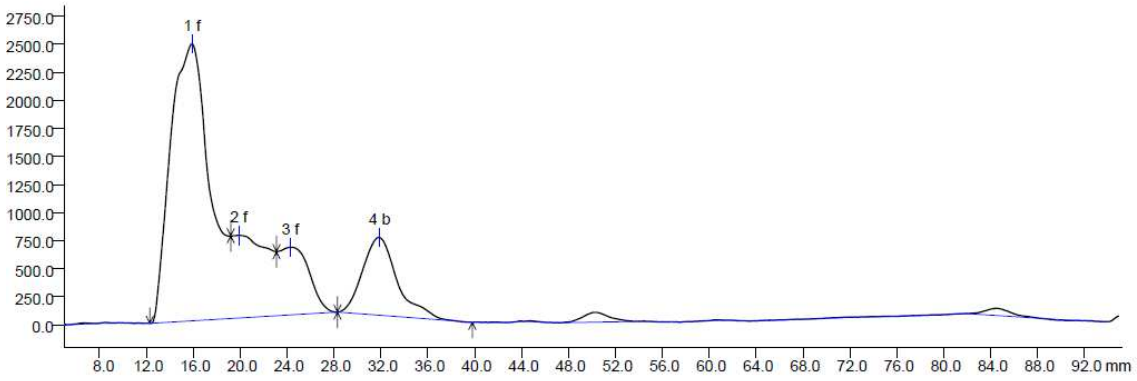


Wavelength (λ) - 366 nm



Wavelength (λ) – 254 nm

Fig. 4: HPTLC chromatogram of Sample B at wavelength 254 and 366 nm Sample C



Wavelength (λ) - 366 nm

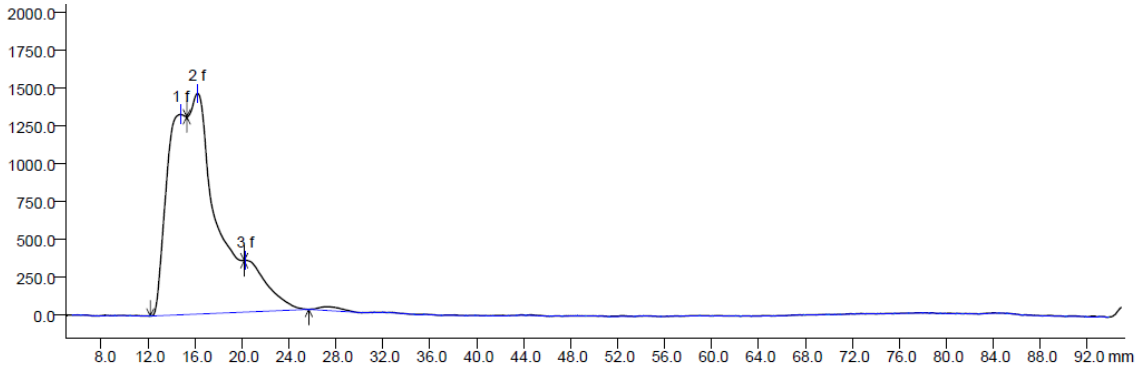


Fig. 5: HPTLC chromatogram of Sample C at wavelength 254 and 366 nm

Determination of heavy metals (lead, arsenic) Method (direct calibration method)

Three reference solutions of the element being examined having different concentrations were prepared covering the range recommended by the instrument manufacturer .Separately the corresponding reagents were added for the test solution and the blank solution was prepared with the corresponding reagents. The absorbances of the blank solution and each reference solution were measured separately, and the readings were recorded. A calibration curve was prepared with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa. A test solution of the substance being examined was prepared as specified in the monograph. The concentration was adjusted such that it falls within the concentration range of the reference solution. The absorbance was measured 3 times, and the readings were recorded and the average value was calculated. The mean value was interpolated on the calibration curve to determine the concentration of the element.

Determination of lead and arsenic Preparation of lead standard stock solution

An accurate quantity of lead single – element was measured to prepare a standard stock solution in 2 % nitric acid solution, containing 1 µg per ml of Pb. It was stored at 0 – 5 ºC.

Preparation of calibration curve

Accurate quantities of lead standard stock solutions were taken and diluted with 2% nitric acid solution to test concentration 5, 20, 40, 60, 80 µg per ml respectively. 1 ml of 1 % ammonium dihydrogen phosphate (ADP, $NH_4H_2PO_4$) and 0.2% magnesium nitrate [Mg $(NO_3)_2$], was added to it and mixed well.20 µl was pipetted out to inject into the atomic generator of graphite oven and their absorbance was recorded. Then a calibration curve was plotted with absorbance as vertical axis and concentration as horizontal ordinate.

Preparation of test solution

0.5 g of the coarse powder of the substance being examined was weighed accurately and transferred into a casparian flask and 5 – 10 ml of the mixture of nitric acid and perchloric acid (4:1) was added to it and macerated over night. Then it was transferred into a 50 ml volumetric flask, followed by washing of the container with 2% nitric acid solution. One ml of the test solution and its corresponding blank solution was measured respectively and 1 ml of solution containing 1 % ammonium dihydrogen phosphate and 0.2% magnesium nitrate were added to it .10 - 20 µl was pipetted out to determine the absorbance. The lead (Pd) content in the test solution was determined from the calibration curve.

Determination of arsenic (As) Preparation of As standard stock solution

An accurate quantity of As single – element was measured to prepare standard stock solution in 2% nitric acid solution, containing 1.0 µg per ml of As. It was stored at 0- 5º C.

Preparation of calibration curve

Accurate quantity of arsenic standard stock solutions, diluted with 2% nitric acid to test concentrations of 2, 4, 8, 12 and 16 µg per ml respectively. 10 ml each was transferred into 25 ml volumetric flask followed by addition of 1 ml of 25% potassium iodide solution. Finally it was shaken and diluted with hydrochloric acid solution (20 – 100) to make up the volume. The stopper was closed and the flask was immersed in a water bath at 80^0C for 3 minutes. Proper quantities of each solution was transferred into the hydride generator device, the absorbance was determined , and the calibration curve with peak area (absorbance) at vertical axis and concentration as horizontal ordinate was plotted.

Preparation of test solution

0.5 g of the coarse powder of the substance being examined was weighed accurately, and transferred into a casparian flask, and 5 – 10 ml of the mixture of nitric acid and perchloric acid (4:1) was added to it. It was macerated over night, and then transferred into a 50 ml volumetric flask, followed by washing of the container with 2% nitric acid solution. Ten ml of the test solution and its corresponding reagent blank solution was pipetted out. 1 ml of 25% potassium iodide solution was added to it and the content of As in the test solution was calculated from the calibration curve. The data for concentration of Pd and As (ppm) in prepared and marketed samples is shown in Table12.

Heavy metals (In ppm) = Concentration × Dilution Factor Sample Weight Dilution Factor = 50

Table 12: Concentration of Lead and Arsenic (ppm) in Samples

Samples Code	Sample weight (gm.)	Concentration of lead (ppm)	Concentration of arsenic (ppm)
Sample A	1.1349	1.63009	0.045
Sample B	1.3366	0.2618	Absent
Sample C	1.0843	11.25	3.42

Test for Aflatoxins

This test is provided to detect the possible presence of Aflatoxin B_1, B_2 G_1 and G_2 in any material of plant origin.

Zinc Acetate – Aluminium chloride reagent

20g of zinc acetate and 5 g of aluminum chloride were dissolved in sufficient water to make 100 ml.

Sodium Chloride Solution

5 g of sodium chloride was dissolved in 50 ml of water.

Test Solution 1

200g of plant material was grinded to make fine powder. 50g of the powdered material was transferred to a glass – stoppered flask. 200 ml of a mixture of methanol and water (17: 3) was added to it, shaken vigorously by mechanical means for not less than 30 minutes and filtered. The first 50 ml of the filtrate was discarded and the next 40 ml was collected. The filtrate was then transferred to a separatory funnel. 40 ml of Sodium Chloride Solution and 25 ml of solvent hexane was added to it and was shaken for 1 minute. The layers were allowed to separate and the lower aqueous layer was transferred to a second separatory funnel. Twice the aqueous layer was extracted in the separatory funnel, each time with 25 ml of methylene chloride, by shaking for 1 minute. Then layers were allowed to separate. Lower organic layer was separated and the combined organic layers were collected in a 125 ml conical flask. The organic solvent was evaporated to dryness on a water bath and the residue was cooled. The residue obtained was dissolved in 0.2 ml of a mixture of chloroform and acetonitrile (9.8: 0.2) and was shaken by mechanical means.

Test solution 2

100 ml of the filtrate was collected and transferred to a 250 ml beaker. 20 ml of Zinc Acetate – Aluminum Chloride reagent and 80 ml of water was added to it, followed by stirring. Then it was allowed to stand for 5 minutes. 5 g of suitable filtering aid such as diatomaceous earth was added to it, mixed and filtered. The first 50 ml of the filtrate was discarded and the next 80 ml portion was collected.

Aflatoxin Solution

Accurately weighed quantities of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1 and Aflatoxin G_2 were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of Aflatoxin B_1 and Aflatoxin G_1 and 0.1 µg per ml each of Aflatoxin B_2 and Aflatoxin G_2.

Procedure

Aflatoxin Solution of 2.5 µl, 5 µl, 7.5 µl and 10 µl and three 10 µl applications of either Test Solution 1 or Test Solution 2 were separately applied to a suitable thin layer chromatographic silica

gel mixture. Aflatoxin Solution of 5 µl was superimposed on one of three 10 µl applications of the Test Solution. The spots were allowed to dry and the chromatogram was developed in an unsaturated chamber containing a solvent system consisting of a chloroform, acetone and isopropyl alcohol (85: 10:5) until the solvent front has moved not less than 15 cm from the origin. The plate was removed from the developing chamber and was air dried. The spots were located on the plate by examination under UV light at 365 nm, the four applications of the Aflatoxin solution appeared as four clearly separated blue fluorescent spots. The intensity of the Aflatoxin spot, if present in the test solution, when compared with that of the corresponding aflatoxin in the aflatoxin solution will give an approximate concentration of Aflatoxin in the test solution. The observations for Aflatoxins are shown in the Table 13.

Table 13: Presence of Aflatoxins in different samples

Samples Code	Presence of Aflatoxins (B_1, B_2, G_1, G_2)
Sample A	Absent
Sample B	Absent
Sample C	Absent

Stability data for different parameters after 3 months at 25ºC

Stability testing was carried out on the basis of non ICH guidelines mentioned in USP NF 2007 for 3 months for the marketed and standard formulation prepared according to Ayurvedic Pharmacopoeia and the following changes in different parametric values were seen at ambient temperature condition (25 ºC). The stability study data for marketed samples (A, B, and C) are shown in Table 14.

Table 14: Stability study data of samples (A, B, and C)

Parameters	Sample A Mean ± S.D	Sample B Mean ± S.D	Sample C Mean ± S.D
Weight variation (mg)	0.39± 0.01	0.62± 0.011	0.416± 0.018
Hardness (kg/cm^2)	4.5± 1.50	1.60± 0.09	1.60± 0.096
Disintegration Time (min)	35.40±4.04	22.30±2.5	62.30±4.98
Friability (%)	0.5±0.32	0.80±0.47	0.860±0.11
pH	3.34±0.19	7.2±0.09	5.60± 0.149
Loss on drying (%w/w)	10.170±4.89	6.120±2.0	7.080±2.15
Total ash(%w/w)	86±4.30	88±2.54	64.6±5.19
Acid-insoluble ash(%w/w)	12.19±.824	12.19±2.8	9.7±2.298
Alcohol-soluble extractive(%w/w)	10.9± 2.63	4.59±0.53	13.3±2.8
Water-soluble extractive(%w/w)	12.59±2.194	17.70±3.2	18.3±2.21

RESULT AND DISCUSSION

The average thickness and diameter of Sample B and C deviated from Sample A which is the standard formulation. The tablet hardness (Kg/cm^2) of Sample B and C were found to be 1.66 which again showed deviation from standard formulation (Sample A). This may be due to variation in the compression force employed during the formulation of the tablets. The average weight of all the samples (A, B and C) was 250mg and above. Hence they followed the deviation scheme of 5% according to the 'Quality Control Manual for Ayurvedic, Siddha and Unani Medicine'. The average values of all the Samples A, B and C was found to be 0.011031, 0.011758 and 0.01820 respectively. Tablets of all the samples were found to be in range and passed the weight variation test. While in case of friability Sample A and C were found to be within range i.e. 0.8 – 1% while Sample B was beyond range i.e. 0.6. This may be due to the presence of moisture content at the time of granulation or during the formulation of finished tablets. In case of disintegration time sample A was found to be in range according to Ayurvedic Pharmacopoeia i.e. not less than 60 minutes while Sample B and C deviated from the result, this may be due to less concentration of Guggul in these samples which resulted in faster disintegration of the tablets. The pH value Sample A was found to be in range according to Ayurvedic Pharmacopoeia i.e. 4.0 to 4.5 while Sample B and C deviated from the range , this may be due to change in the nature of any one of the ingredients which may have imparted more basic character to the tablets. The Results of loss in drying showed that values of all the Samples were in prescribed range i.e. (N.M.T 13%). Sample A was found to be in prescribed range for total ash (i.e. N.M.T 9%) while Sample B and C deviated from the limits. For % acid insoluble ash Sample A was found to be within range (i.e. N.M.T 2%) while Sample C and D deviated from the range. The observations for water soluble extractive showed that values of all the samples were out of mentioned range (i.e. N.L.T 34%).. The values for alcohol soluble extractive showed that all the samples were out of the mentioned range (i.e. not less than 40%). The extract for TLC was prepared in n- hexane. A mixture of n- hexane and ethyl acetate in the ratio 8.5: 1.5 was taken as a mobile phase and vanillin- sulphuric agent was used as a visualizing agent. HPTLC was performed both at 254 and 366 nm. The Rf and % area covered of different samples were calculated. The Rf of 1st peak of sample A and C were found to be same i.e. 0.13, so this could be concluded from this data that the constituent present in these samples are same. The results for heavy metals showed that market variants contained heavy metals in small proportion but are within limits. Aflatoxins B_1, B_2 G_1 and G_2 were found to be absent in all the samples (A, B and C) which was a good indication. The Stability studies were performed for 3 months at room conditions i.e. at 25 ºC (according to the non ICH guidelines mentioned in USP NF) and no significant changes was seen in the data obtained after stability testing

CONCLUSION

Different quality control tests were performed on both the prepared formulation and marketed samples .The data revealed that there was variation in many samples for different parameters. There are limits mentioned in the Ayurvedic Pharmacopoeia and the marketed samples do not wholly comply with those limits. The manufacturers of Ayurvedic formulation should try to comply with pharmacopoeial specifications so that quality of the products could be maintained and variations could be kept within prescribed limit.

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