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A Meticulous Appraisal of Vyaghri Haritaki Avaleha – An Ayurvedic Medicament WSR to Stability Study

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Abstract

Stability study of the Ayurvedic formulations plays a significant role to provide actual shelf life of the medicament. Hence, it is necessary to perform stability studies of Ayurvedic compound formulations. The stability study was planned to observe whether any physical, chemical, and microbiological change takes place in Vyaghri Haritaki samples by keeping them at three different temperatures (47°C, 37°C, and 25–30°C) for 3 months. The samples kept for accelerated stability study were analyzed after 3 months and the details have been presented in this section.

Keywords: Physicochemical parameters, Stability study, Vyaghri Haritaki Avaleha

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Introduction

The stability of the product is its ability to resist deterioration. It is always expressed in terms of shelf life. Stability information is ubiquitous, but today it is ignored in case of Ayurvedic Pharmaceutical preparations. It is pertinent to emphasize on the stability and quality improvement of these medicines. Therefore, it would be unforeseen and customary to remind the discerning views of Acharya Sharangdhara who judiciously enlightened the concept of shelf life of various Ayurvedic formulations.

Shelf Life and Health-care Products

Shelf life refers to the period from initial preparation and packaging during which the drug dosage forms continuous to remain within its physical, chemical, therapeutic, and toxicological specifications at specified storage conditions.

The product must retain 90% of the labeled claim at the end of shelf life. There are different sets of parameters for evaluating samples of different nature.

- Microbial limits
- There may be a need to specify the total count of aerobic microorganism, the total count of yeast and molds, and the absence of specific objectionable bacteria
- The source of herbal material should be taken into account when considering the inclusion of other possible pathogens (e.g., *Campylobacter* and hysteria species) in addition to those specified in European Pharmacopoeia
- Microbial counts should be determined using pharmacopoeial procedures or other validated procedures. The European Pharmacopoeia gives guidance in acceptance criteria. Following examination should be conducted in laboratory.
  1. Total microbial count
  2. Total fungal count
  3. Coliform count
  4. Pathogens examination
  5. Sterility testing[1,2]

Factors Affecting Stability

- Environmental factors
- Temperature
- Light
- Air
- Oxygen
- Moisture
- Carbon dioxide
- Other ingredients or excipients in the dosage form
- Particle size of drug
- pH of the vehicle
- Microbial contamination
- Trace metal contamination
- Leaching from containers.[3]

How are the Stability Tests Conducted and when do we Need Stability Testing?

Studies are conducted at:

- Accelerated conditions
- Controlled room temperature conditions
- Product samples are analyzed at various intervals by validated analytical methods.

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Microbial Contamination Limits in Medicinal Plant Materials

Different limits are set according to the use of the material and the material itself.

a. Contamination of "Crude" plant material intended for further processing (including additional decontamination by any physical or chemical process). The limits are given for untreated plant material harvested under acceptable hygienic conditions (These could possibly indicate problems occurring during handling practices and would need further investigation)

Per gram – Maximum 10^4 *Escherichia coli*

Maximum 10^5 mold propagules

b. Plant material that has been pretreated (e.g., boiling water as used for herbal teas and infusions) or if the material is used for topical dosage forms:

Per gram – Maximum 10^7 aerobic bacteria

Maximum 10^3 Saccharomycetes and Hyphomycetes

Maximum 10^2 *E. coli*

Maximum 10^4 other enterobacteria

No Salmonellae

c. Other plant material for internal use:

Per gram – Maximum 10^5 aerobic bacterial

Maximum 10^3 Saccharomycetes and Hyphomycetes

Maximum 10^1 *E. coli*

Maximum 10^3 other enterobacteria

No Salmonellae.[3,4]

Materials and Methods

Organoleptic Characters

The organoleptic characters of Vyaghri Haritaki samples such as color, odor, taste, texture, and appearance were noted.

Physicochemical Parameters

The physicochemical parameters of Vyaghri Haritaki samples such as loss on drying[4] –110°C, ash value,[4] acid-insoluble ash,[4] alcohol-soluble extractive values,[4] water-soluble extractive values,[4] and determination of pH[5] were evaluated.

Estimation of Sugar Content[5]

Solution A

Accurately weighed about 1–2 g of sample and 25 ml of distilled water, heated to about 60–70°C on a hot plat for 5 min, filtered through filter paper and the residue was washed with hot distilled water. To this filtrate, 10% w/v lead acetate solution was added till precipitation was completed. This was filtered and 10% w/v solution of sodium oxalated was added to the filtrate till complete precipitation and filtered. Volume of the filtrate was made to 250 ml by washing the precipitate continuously with hot water (solution A).

Procedure for reducing sugar

A 20 ml of solution was taken from solution A and to it 25 ml of each Fehling A and B were added. It was boiled for 3 min and followed by filtration through Whatman filter paper no. 1 and a bed of glass wool. The entire residue was dissolved quantitatively in acidic ferric ammonium sulfate solution. The water was added to the glass wool bed to transfer the complete acidic ferric solution to the flask and then it was titrated with 0.1 N KMnO4 solution using O-phenanthroline as indicator; when reaction was completed, the brownish color was changed to greenish.

1 ml of 0.1 N KMnO4 solution = 0.00636 g of Cu.

From the content of Cu, % of sugar was calculated from the Hammond table.

Procedure for total sugar

For the estimation of total sugar, 20 ml of stock solution was hydrolyzed by addition of 5 ml 6 N HCl. The solution was heated on water bath for 3 min maintaining temperature around 70°C. The solution was cooled and neutralized with 10% w/v of NaOH solution using Phenolphthalein indicator. Volume was made to 100 ml with distilled water (solution B).

From this, 20 ml of solution was pipetted out and processed further same as that of reducing sugar. From the burette reading, amount of copper was calculated and % of total sugar was calculated from the Hammond table, which is equivalent to the amount of copper. By deducting the reducing sugar from total sugar, we can get the percentage of the non-reducing sugar.

Estimation of Total Alkaloid[6]

Accurately weighed, 10 g of sample was taken and soaked with 100 ml ethanol (95%) and was kept overnight. The alcoholic extract was filtered through simple filter paper and the filtrate obtained was taken in an evaporating dish and evaporated. The alcoholic extract was then acidified with about 30 ml of dil. HCl, to convert the alkaloids to their salts. This was then transferred into separating funnel and was extracted with 100 ml of chloroform and the chloroform extract was discarded. To this aqueous acidic solution, strong solution of ammonia was slowly added to make the solution alkaline. This alkaline solution was then transferred into separating funnel and extraction was then carried out by shaking with organic immiscible solvent, that is, chloroform for 5–6 times. All the portions of chloroform extracts were then combined and taken in a conical flask. This chloroform layer was then washed several times with distilled water to make it alkaline free (using Phenolphthalein as indicator). The chloroform extract so obtained was still not free from water. Hence, for removing water, a quantity of anhydrous sodium sulfate (usually 1–2 g for 60–80 ml of chloroform extract) was added and was kept for half an hour. This was then filtered through simple filter paper to remove sodium sulfate. The extract so obtained, was then taken in a previously weighed evaporating dish, and was evaporated to dryness by keeping it on water bath. This leaves a residue containing a mixture of the alkaloids from the original plant material (a total alkaloids extracts) reasonably free from non-alkaloidal inorganic and organic impurities. The alkaloidal residue obtained, was then kept in oven at 105°C till constant weight and from this, the percentage of total alkaloids was calculated.

Estimation of Tannin Content[7]

About 2.5 g of the powdered drug was accurately weighed and mixed with 150 ml of water and was boiled over hot plate

for ½ h with continuous stirring and then cooled. After cooling, it was filtered in 250 ml volumetric flask through simple filter paper. Volume was made up to 250 ml with distilled water (stock solution).

From this stock solution, 5 ml of decoction was taken in 250 ml conical flask. To it, 12.5 ml Indigo carmine solution and 100 ml distilled water were added and titrated against 0.1 N KMnO4. At the end point, the color changes from green to yellow (Reading A).

In an another flask, 50 ml of decoction was taken and 25 ml gelatin solution, 50 ml acidified NaCl solution, and 5 g Kaolin powder were added and shaken for few minute. The mixture was then allowed to settle down and then filtered.

A 12.5 ml of this filtrate is taken in 250 ml conical flask and to it, 12.5 ml Indigo carmine and 100 ml distilled water were added and titrated against 0.1 N KMnO4 with continuous stirring. At the end point, the color changes from green to yellow (Reading B).

Stability Study of Vyaghri Haritaki Avaleha

The Accelerated Stability Study of laboratory sample of Vyaghri Haritaki was carried out by keeping it in three glass containers at different temperatures for the period of 3 months. Recommended storage conditions and duration of studies are prescribed in Table 1.

Sample Preparation

A 1 g of each stability sample of *Vyaghri Haritaki* was accurately weighed and a suspension (10 mg/ml) was prepared in distilled water in aseptic conditions. The suspension was used for the microbiological study.

Microbial Assessment

Medicinal plant materials normally carry a large number of bacteria and molds, often originating from the soil, with a large range of bacteria and fungi from the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate. The current practices of harvesting, handling, and production may cause additional contamination and microbial growth. The determination of total count, yeast, and molds, *Salmonella* and *Escherichia coli* may indicate the quality of production.[5,6]

Total bacterial count

The total viable count was determined using plate count method. For that Petri dish, 9–10 cm in diameter was used. A 20 ml of liquefied N. agar (nutrient broth jelled by the addition of 1.2% agar) was poured in a plate and kept at 37°C for 24 h to solidify the medium. A 10 µl of the sample was inoculated on the solidified agar and kept for incubation for 24 h.[7] After that, colonies were counted and total viable count was calculated as per equation given below:

N = Y/VX

Where,

N = No. of organism/ml

Y = No. of colonies

V = Volume used for streaking the sample

X = Dilution factor.

Test for E. coli

The medium used for testing the presence of *E. coli* was MacConkey agar and plates were prepared with this media, sample (10 µl) was streaked on the plate and incubated at 37°C for 24 h. After 24 h, results were observed and details noted. Growth of rod, generally known mucoid colonies of Gram-negative rods of red color, sometimes surrounded by reddish zone of the precipitation indicates the presence of *E. coli*.[7]

Test for Salmonella

The medium used for testing the presence of *Salmonella* was MacConkey agar and plates were prepared with this media, sample (10 µl) was streaked on the plate and incubated at 37°C for 24 h. After 24 h, the result was observed and details noted.

Salmonella are Gram-negative rods. Colonies are large, 2–3 mm in diameter, circular, low convex, and smooth. They are more translucent than coliform colonies. On MacConkey media, colonies are colorless due to the production of H_25.[7,8]

Yeast and molds

For testing the presence of yeast and molds, the plates were prepared with Sabouraud agar. Sample (10 µl) was streaked on prepared plates and incubated at 37°C. After 48 h, the result was observed and the details were noted. The microbial examination was carried out at SHREY PATHOLOGY LAB, Jamnagar.

Results and Discussion

Organoleptic Characters

The organoleptic characters of the samples kept for stability study are presented in Table 2.

The organoleptic characters of all the three stability samples were found similar and no significant variation occurs after 3 months accelerated stability study.[8]

Physicochemical Parameters

The analytical data of physicochemical parameters of three stability sample of *Vyaghri Haritaki* kept for 3 months are prescribed in Table 3.

Table 1: Duration and storage conditions for stability study

Name of sample	*Study type*	*Storage conditions*	*Duration of study*
Stability sample-1	Accelerated testing	47°C±2°C	3 months
Stability sample-2	Accelerated testing	37°C±2°C	3 months
Stability sample-3	Accelerated testing	25–30°C	3 months

Table 2: Organoleptic characters of Vyaghri Haritaki samples after 3 months

Organoleptic characters	*Stability sample-I (kept at 47°C)*	*Stability sample-2 (kept at 37°C)*	*Stability sample-3 (kept at 25–30°C)*
Color	Dark brown	Dark brown	Dark brown
Odor	Aromatic	Aromatic	Aromatic
Taste	Sweet and bitter	Sweet and bitter	Sweet and bitter
Appearance	Thick semisolid mass	Thick semisolid mass	Thick semisolid mass

From the above table, it was observed that no significant variations were found in all the three stability samples kept at different temperatures for 3 months.

The loss of drying of the sample-1 which was kept at 47°C for 3 months was slightly decreased (15.35%) while the pH of the sample-2 and sample-3 which were kept at 37°C and 25–30°C for 3 months was slightly increased (5.06 and 5.02, respectively).[7-9]

Estimation of Sugar Content

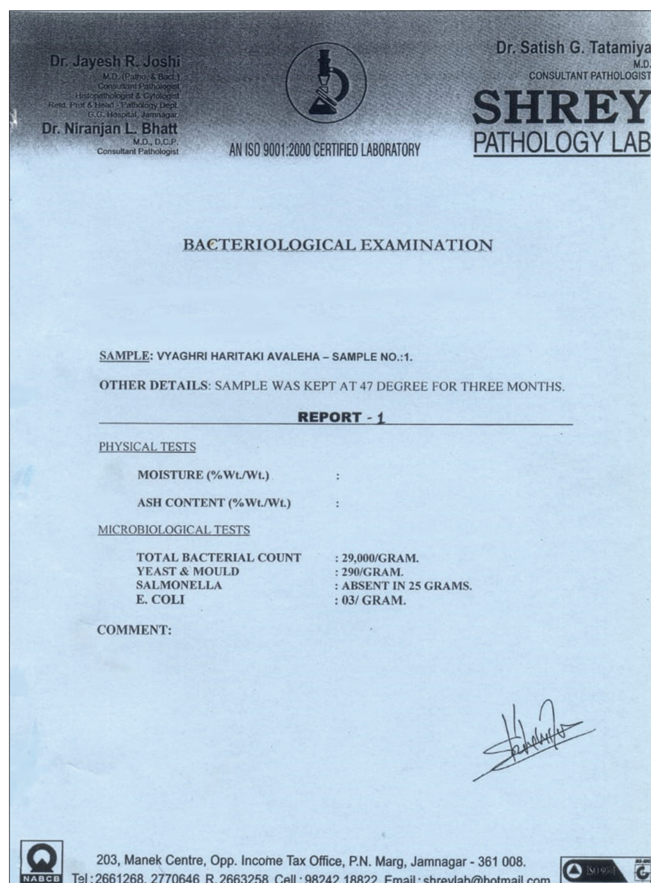
The sugar content of all the three stability samples was determined after 3 month's accelerated stability study and the data obtained are presented in Table 4.

From the above table, it was observed that no significant variation occurred in sugar content of *Vyaghri Haritaki* after 3 months accelerated stability study by keeping it at different temperatures (i.e., 47°C, 37°C and 25–30°C).

Estimation of Alkaloidal Content

The total alkaloids of all the three stability samples were determined after 3 month's accelerated stability study and the data obtained are presented in Table 5.

From the above table, it was observed that no significant variations were occurred in alkaloidal content of *Vyaghri Haritaki* after 3 month's accelerated stability study by keeping it at different temperatures (47°C, 37°C, and 25–30°C).[9]



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BACTERIOLOGICAL EXAMINATION

SAMPLE: VYAGHRI HARITAKI AVALEHA – SAMPLE NO.:1.

OTHER DETAILS: SAMPLE WAS KEPT AT 47 DEGREE FOR THREE MONTHS.

REPORT - 1

PHYSICAL TESTS

MOISTURE (%Wt./Wt.) :

ASH CONTENT (%Wt./Wt.) :

MICROBIOLOGICAL TESTS

TOTAL BACTERIAL COUNT : 29,000/GRAM.
YEAST & MOULD : 290/GRAM.
SALMONELLA : ABSENT IN 25 GRAMS.
E. COLI : 03/ GRAM.

COMMENT:

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Report 1: Stability sample-1 which was kept at 47°C for 3 months

Estimation of Tannin Content

The tannin content of all the three stability samples was determined after 3 month's accelerated stability study and the data obtained are presented in Table 6.

From the above table, it was observed that no significant variation was found in all the three stability samples kept at different temperatures for 3 months. The tannin content of the sample 1 which was kept at 47°C for 3 months was slightly decreases (1.8%), while the samples 2 and 3 kept at 37°C and 25–30°C showed similar results.[9]

Table 3: Data of physicochemical parameters of stability samples of Vyaghri Haritaki after 3 months

S. No.	*Parameters*	*Stability sample-I (kept at 47°C)*	*Stability sample-2 (kept at 37°C)*	*Stability sample-3 (kept at 25–30°C)*
1.	Loss of drying – 110°C (%w/w)	15.35	17.04	17.28
2.	Ash value (%w/w)	4.5	4.38	4.28
3.	Acid-insoluble ash (%w/w)	0.16	0.18	0.16
4.	Alcohol-soluble extractive (%w/w)	23.6	24.1	24.59
5.	Water-soluble extractive (%w/w)	78.19	79.23	79.26
6.	pH value of 5% aqueous solution	4.79	5.06	5.02

Table 4: Data of sugar content of stability samples of Vyaghri Haritaki

Sample	*Sugar content (%w/w)*	
	Total sugar	*Reducing sugar*
Stability sample-1	33.82	11.65
Stability sample-2	33.28	12.13
Stability sample-3	33.21	12.01

Table 5: Data of total alkaloids in stability samples of Vyaghri Haritaki

S. No.	*Sample*	*Total alkaloids (%w/w)*
1	Stability sample 1	0.12
2	Stability sample 2	0.119
3	Stability sample 3	0.12

Table 6: Detail of tannin contents in stability samples of Vyaghri Haritaki

S. No.	*Sample*	*Tannin content (%w/w)*
1	Stability sample 1	1.8
2.	Stability sample 2	2.1
3.	Stability sample 3	2.36

Table 7: Microbial examination

S. No.	*Microbiological Tests*	*Sample 1 (kept at 47°C for 3 months)*	*Sample 2 (kept at 37°C for 3 months)*	*Sample 3 (kept at 25–30°C for 3 months)*
1	Total bacterial count	29,000/g	34,000/g	56,000/g
2	Yeast and mold	290/g	320/g	490/g
3	*Salmonella*	Absent in 25 g	Absent in 25 g	Absent in 25 g
4	*Escherichia coli*	03/g	05/g	12/g

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BACTERIOLOGICAL EXAMINATION

SAMPLE: VYAGHRI HARITAKI AVALEHA – SAMPLE NO.:2.

OTHER DETAILS: SAMPLE WAS KEPT AT 37 DEGREE FOR THREE MONTHS.

REPORT - 2

PHYSICAL TESTS

MOISTURE (%Wt./Wt.) :

ASH CONTENT (%Wt./Wt.) :

MICROBIOLOGICAL TESTS

TOTAL BACTERIAL COUNT	: 34,000/GRAM.
YEAST & MOULD	: 320/GRAM.
SALMONELLA	: ABSENT IN 25 GRAMS.
E. COLI	: 05/ GRAM.

COMMENT:

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Report 2: Stability sample-2 which was kept at 37°C for 3 months

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BACTERIOLOGICAL EXAMINATION

SAMPLE: VYAGHRI HARITAKI AVALEHA – SAMPLE NO.:3

OTHER DETAILS: SAMPLE WAS KEPT AT 25-30 DEGREE FOR THREE MONTHS.

REPORT - 3

PHYSICAL TESTS

MOISTURE (%Wt./Wt.) :

ASH CONTENT (%Wt./Wt.) :

MICROBIOLOGICAL TESTS

TOTAL BACTERIAL COUNT	: 56,000/GRAM.
YEAST & MOULD	: 490/GRAM.
SALMONELLA	: ABSENT IN 25 GRAMS.
E. COLI	: 12/ GRAM.

COMMENT:

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Report 3: Stability sample-3 which was kept at 25–30°C for 3 months

Microbial Examination

The bacteriological examination was carried out after 3 months accelerated stability study of laboratory sample of *Vyaghri Haritaki*. The result of the bacteriological examination has been represented in three different reports which are as follows:

Report-1: Stability sample-1 which was kept at 47°C for 3 months

Report-2: Stability sample-2 which was kept at 37°C for 3 months

Report-3: Stability sample-3 which was kept at 25–30°C for 3 months.

From the Reports 1, 2, and 3, it was observed the total bact. count, yeast, and mold and *E. coli* and the data obtained are presented in Table 7 and shown in Reports 1, 2, and 3.

E. coli found in the stability samples may be due to contamination during opening and transferring or it might have come from atmosphere into samples during opening and transferring of sample into other containers. The pathological bacteria, that is, *Salmonella* were found absent in all the three stability samples.[9,10]

Conclusion

On the basis of this stability study, it can be said that this formulation is stable for the period of more than 3 months. The data evolved in the present study will be very useful for routine quality control of *Vyaghri Haritaki* and also to control the batch-to-batch variation. The paper provides a brief explanation on the necessity of stability testing, types of stability tests, the measures, and guiding principle of stability study and the extrapolation of the generated data.

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