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Comparative stability study of formulated ayurvedic health supplement and marketed product

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ABSTRACT

The purpose of the stability testing is to provide proof of how the quality of a finished product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Different parameters like moisture content, pH, total ash, crude fiber, acid in soluble ash, total microbial count were checked at regular intervals [6] . These studies are conducted at 25°C ± 2°C/ 60 % RH ± 5 % RH and 40 °C±2°C /75 % ± 5% RH noted for significant changes occurs at any time during one year. If any significant change occurs at any time during six months, testing at the accelerated storage condition, and additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. In our study no intermediate storage condition should be included because no significant changes occur between three and six months. In this the newly formulated health supplement under gone long term testing over a minimum of 12 months and marketed product was compared for the following parameters at specified conditions. Organoleptic, chemical, biological and microbiological characteristics of a finished product, during and beyond the expected shelf life and storage periods of the sample under the storage conditions expected in the intended market are determined with the help of stability studies[4]. These results helped to establish the re-test period or shelf life, to confirm the projected re-test period and shelf life, and pave a way to recommend a storage condition. Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. . If any considerable changes are noticed in one or more properties of the formulation, with in the time limit, it results inefficacy problem

Key words: Shelf life, poly herbal formulation, total ash, total microbial count.

INTRODUCTION

The most important aspect in the evaluation of the stability study of a product is its storage condition. Based on the climatic conditions only storage conditions can be determined. Before launching the product in to market, the products efficacy, safety, and ethical issue have to be confirmed. Herbal medicinal products have to fulfill the legal requirements with regard to quality including stability testing, but have certain particularities such as a complex nature, an often low concentration of constituents and a natural variability of their raw materials[1]. Due to their natural origin, questions on microbiological quality arise more often for herbal medicinal products than for chemically defined medicinal products[2]. For this reason, particularly in case of new applications, a detailed

microbiological investigation program is often set up for herbal medicinal products. The W.H.O is the directing and coordinating authority in International Health with in the United Nation's system. WH.O plays a major role in health research. Here ICH guidelines are followed during stability studies. These studies are done over the final dosage form with the final packing in which the drugs is prescribed for marketing [7].

MATERIALS AND METHODS

The plant materials for the formulated drug was collected from different parts of Kerala and Tamilnadu, India during month of july-December2009 and got authenticated by Pradeep Kumar, Herbarium curator, Department of Botany, Calicut University, Kerala.

The formulation consists of six medicinal plants based on folklore use. The newly formulated Ayurvedic health supplement undergone physical, chemical, biological and microbiological characteristics study.

The studies are conducted at 25°C ± 2°C/ 60 % RH ± 5 % RH and 40 °C±2°C /75 % ± 5% RH noted for significant changes occurs during one year at an interval of 0,3,6,and 12months [5] .The followed parameters are checked during this interval. Tables 1-8 explain the details.

Organoleptic analysis:
Colour, Odour, taste, was checked. The colour indicated the nature of the formulation; odor and taste of the formulation are extremely sensitive criteria.

Chemical analysis:
1) Determination of pH:
The pH of the poly herbal formulation and marketed formulation in 1% w/v was determined using standard glass electrode in accordance to the prescribed standard method in Indian Pharmacopoeia [8].

2) Extractive values:
The amount of active constituents in a given amount of formulation was determined when extracted with solvents. The water soluble and alcohol soluble extractives was used for evaluating formulation [8].
a) Water soluble extractive:
About 5 grams of the powder is macerated with 100 ml of distilled water in a closed flask for 24 hrs. Shake frequently during 6 hrs and allow the same for standing for 18 hrs. It is filtered rapidly and 25 ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105 °c and weighed. The percentage of water soluble extractive is calculated with reference to the air dried powder[8].

3) Ash determinations:
a) Acid insoluble ash:
Acid insoluble ash is frequently necessary to evaluate the crude drug, which indicates contamination with silicious material.
Procedure:
Acid insoluble ash can be obtained by boiling for five minutes with 25 ml of 2M hydrochloric acid and filtered through an ash less filter paper. The filter paper is ignited in the silica crucible, cooled and then acid insoluble ash is weighed. Weight of acid insoluble ash divided by weight of sample multiply with 100 gives the percentage of acid insoluble ash [8].
b) Total ash:
It is an important parameter for the purpose of evaluation of a formulation. Total ash is designed to measure the total amount of material produced after complete incineration of the formulation at a low temperature to remove all the carbons. The total ash usually consists of physiological ash and non physiological ash.

Procedure:
Sample to be heated in silica crucible to red heat for 30 minutes. Then allowed to cool in a desiccators and weighed.1 gm sample was evenly distributed on the crucible. Dry at 100^{o}c to 105^{o}c for one hour and ignite to constant weight in muffle furnace at 600 ^{o}±25 ^{o}c. Allow the crucible to cool in a dessicator after each ignition. The material should not catch fire. On prolonged ignition a carbon free ash cannot be .Exhausted the charred mass with

hot water, then collected the residue on an ash less filter paper. Incinerate the residue and filter paper until the ash becomes white. Then calculate the % of ash with reference to air dried drug by using following equation. Weight of the sample taken minus weight of the ash divided by weight of the sample taken [8] .

4) Moisture content:
Moisture is an inevitable component of the formulation .The presence of moisture results in the active growth of common moulds and bacteria.

Procedure:
Weigh about 1 gm of the powdered crude drug in to a weighed flat and thin porcelain dish. Dry in the oven at 100-105˚c for half an hour. Cool and weigh the contents. Keep back the contents in oven and repeat the drying at 100˚-105˚c for half an hour. Cool the contents and check its weight. Repeated the process to get the concordant value and same process was done for marketed sample also.

5) Crude fiber determination:
Estimation of crude fiber denotes the measurement of the content of cellulose, lignin and cork cell. Excess of crude fiber in the formulation indicates adulteration and it depends on the degree to which the material has been ground up.

Procedure:
About 2 gm of drug is weighed accurately and transferred to a porcelain dish.50 ml of 10% nitric acid is added and boiled for 30 seconds with constant stirring and filtered through fine mesh cotton cloth. The residue is washed with 5 ml of boiled water. The material from the cloth is collected in a porcelain dish and boiled with 50 ml of 2.5% caustic soda. Then the liquid is filtered by using the fine mesh cotton cloth. The residue is washed with 100 ml of boiling water. Then the fiber is collected in a dried and weighed crucible. The crucible is then placed at 105˚c for 2 hours. It is then placed in desiccators and cooled. The cooled crucible is weighed. From the weight of the residue crude fiber content is calculated. Compare it with the marketed preparation.

6) Microbiological determination:
The microbial count and detection of pathogens is of primary importance because this may cause alteration of physicochemical characteristics of the formulation [8].

a) Total Microbial count:
This was carried out by plate method as per I.P. preliminary testing of the sample was done to determine its inhibitory activity.

One milli liter of diluted 24hr broth culture of specified organism (E.coli, B.Subtilis) was added to the first dilution (in buffer solution, pH 7.2) of the test material. The tubes were inoculated for specified period and observed for growth. Recovery of the viable culture in the test tubes after inoculation period showed that the test specimen did not of themselves inhibit the multiplication under the test condition of micro organism that could be present.

Pre treatment of the sample
For total microbial count 10 gm of the sample was suspendedin100ml of sterile saline.

METHOD
Using petri dishes of 10cm in diameter, 1mlof pre treated sample and 15 ml of liquefied sterile soya bean casein digest agar medium at not more than 45°C was added to each dish. Two such petri dishes were incubated at 37°C for 5 days and the number of colonies that were formed was counted

TABLE. 1: DATA SHOWING THE STABILITY STUDIES OF "0" MONTH AT 25 °C±2°C AND 60 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.23 %w/w	10.65 %w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3% w/w	4% w/w
Crude fiber	5.2% w/w	4.8%w/w
Acid insoluble ash	0.9 %w/w	1.9 %w/w
Total microbial count	15 CFU /gm	18 CFU /gm

TABLE. 2: DATA SHOWING THE STABILITY STUDIES OF "0" MONTH AT 40 °C±2°C AND 75 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.22 %w/w	10.65 %w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3% w/w	4%w/w
Crude fiber	5.2% w/w	4.6%w/w
Acid insoluble ash	0.9 %w/w	1.9 %w/w
Total microbial count	15 CFU /gm	18 CFU /gm

TABLE. 3: DATA SHOWING THE STABILITY STUDIES OF "3" MONTH AT 25 °C±2°C AND 60 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.22 %w/w	10.65 %w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3%w/w	4.1%w/w
Crude fiber	5.2%w/w	4.4%w/w
Acid insoluble ash	0.7%w/w	1.9%w/w
Total microbial count	15 CFU /gm	20 CFU /gm

TABLE. 4: DATA SHOWING THE STABILITY STUDIES OF "3" MONTH AT 40 °C±2°C AND 75 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.21 %w/w	10.65 %w/w
Water soluble extractive	36% w/w	35 %w/w
Moisture content	3.1 %w/w	4.5 %w/w
Crude fiber	5.2% w/w	4.4% w/w
Acid insoluble ash	0.7 %w/w	1.9 %w/w
Total microbial count	15 CFU /gm	20 CFU /gm

TABLE. 5: DATA SHOWING THE STABILITY STUDIES OF "6" MONTH AT 25 °C±2°C AND 60 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.21 %w/w	10.65%w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3.1%w/w	4.8%w/w
Crude fiber	5.2%w/w	4.1%w/w
Acid insoluble ash	0.8 %w/w	2 %w/w
Total microbial count	15 CFU /gm	20 CFU /gm

TABLE. 6: DATA SHOWING THE STABILITY STUDIES OF "6" MONTH AT 40 °C±2°C AND 75 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.21 %w/w	10.65 %w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3.3%w/w	4.8%w/w
Crude fiber	5.2%w/w	3.8%w/w
Acid insoluble ash	0.8 %w/w	2 %w/w
Total microbial count	15 CFU /gm	20 CFU /gm

TABLE.7: DATA SHOWING THE STABILITY STUDIES OF "12" MONTH AT 25 °C±2°C AND 60 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.21 %w/w	10.65 %w/w
Water soluble extractive	36 %w/w	35%w/w
Moisture content	3.1%w/w	4.8%w/w
Crude fiber	5.2%w/w	4.1%w/w
Acid insoluble ash	0.8 %w/w	2 %w/w
Total microbial count	16 CFU /gm	20 CFU /gm

TABLE.8: DATA SHOWING THE STABILITY STUDIES OF "12" MONTH AT 40 °C±2°C AND 75 %±5% RH

Parameter	Fabricated formulation nature	Marketed formulation nature
pH	Neutral	Neutral
Total ash	12.21 %w/w	10.42 %w/w
Water soluble extractive	36 %w/w	35 %w/w
Moisture content	3.1%w/w	4.8%w/w
Crude fiber	5.1%w/w	4.1%w/w
Acid insoluble ash	0.8 %w/w	2 %w/w
Total microbial count	16 CFU /gm	22 CFU /gm

RESULTS AND DISCUSSION

The following parameters are evaluated and compared with the marketed preparation. No significant changes were reported for the formulated product. Marketed preparation shows significant changes but it was nearly on the limit of W.H.O protocol. The present study indicates that the poly herbal formulation was stable for one year

CONCLUSION

The present investigation supports that the formulation was suitable on different climatic conditions. It also suggests that the sample was perfect for one year. The outcome of this study indicates that the formulation could be preserved at low temperature as well as moderate temperature. When compared to the marketed formulation, the poly herbal formulation shows a very good response in stability studies.

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