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STABILITY STUDY OF IMPROVED CONSTAC PLUS POWDER A AYURVEDIC POLYHERBAL FORMULATION USED FOR CONSTIPATION

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ABSTRACT: Evidence based medicine is most trusted and accepted methods are now found in around the globe. To provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions. For Ayurveda and siddha real time stability study is recommended. In this stability study parameters successfully evaluated in 24 months was organoleptic, physic-chemical and microbial load in constac plus polyherbal formulation that supports the shelf life of 24 months in storage conditions of temperature $30^0C \pm 2^0C$, relative humidity 60%RH± 5%.

INTRODUCTION

Evidence based medicine is most trusted and accepted methods are now found in around the globe. In India from thousands of years dominant therapies now considered as alternative forms like Ayurveda, which is still effective and safe to use. Stability testing of such medicines using modern technique is the approach which ensures its safety in humans. In Stability testing focused objective as per ICH guidelines is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions.[1] Stability testing done with major aim focusing to give reasonable assurance that the product is fit and quality will remain same for which it is in market place available for supply to the patients. It will be fit for their consumption until the patient uses the last unit of the product.[2] In case of polyherbal formulations difficulty in maintaining consistent quality is a challenge[3] as compared to chemically defined products. In Ayurveda similarity with this mentioned as '*Saviryata avadhi*' which means time period during which *Virya* (potency) of any drug remains unaffected.[4] with consistent quality. For safety and legal concerns it is absolutely necessary that product should remains within the specifications established to ensure its identity, strength, quality, and purity.[5] For modern medicine stability testing is routine procedure for drugs and products which is employed at various stages of product development. For Ayurveda and Siddha accelerated shelf life study in not recommended as per laboratory guidelines for analysis given by central council for research in Ayurveda and siddha.[6] In this study we have performed stability testing of ayurvedic polyherbal formulation indicated for the treatment of chronic constipation.

AIM AND OBJECTIVE

1. To evaluate stability study parameters of Constac Plus Polyherbal formulation using real time.

MATERIALS AND METHODS

1. Test Drug: Constac plus Powder

Constac Plus Powder is polyherbal formulation which includes total 13 ingredients as:

1. Hirada (Fruit)*Terminalia chebula*[7]
2. Balhirada (Fruit)*Terminalia chebula*[7]
3. Behada (Fruit)*Terminalia bellirica*[8]
4. Amala (Fruit)*Emblica officinalis*[09]
5. Ajwain (Seed)*Ptychotis ajowan*[10]
6. Badishep (Fruit)*Foeniculum vulgare*[11]
7. Mulethi (Root)*Glycyrrhiza glabra*[12]
8. Elaichi (Fruit)*Elettaria cardamomum*[13]
9. Erand Tail (Oil)*Ricinus communis*[14]
10. Nishottar (Root*) Ipomoea turpethum*[15]
11. Sonamukhi (Leaves) *Cassia Senna*[16]
12. Narikel lavan (Processed salt with coconut)[17]
13. Permitted Preservatives and excipients.

2. Storage condition and evaluation parameters

As per CCRAS guidelines real-time stability study successfully conducted[6] over a period of 26 months in stability chamber. Carefully, changes in formulation

were noted for 24 months at an interval of 1st, 6th, 12th, 18th and 24th months in maintained temperature of $30^0C \pm 2^0C$ with relative humidity: 60% RH $\pm 5^0C$ in stability chamber. Observations were analyzed, interpreted and prepared a report in next one month. 10% degradation was set as acceptable point.

Using following evaluation parameters for stability study were considered and successfully completed.

- Color, odor and taste known as organoleptic characters
- Loss on drying, pH, Total ash, Water soluble extractive value, bitter residue, total saponin and total tannin known as Physico-chemical parameters
- Microbial load.

➢ **Examination of color, odour and taste**

Color: 5 gm Constac plus granules taken and placed in bright background and light. It was carefully observed by naked eyes as brown color.

Odor: Constac plus granules taken in between thumb and 1st finger and smelled it and found it as Characteristic.

Taste: Constac plus granules taken in a spoon and tasted it on the tongue and examined for the type of taste and found it as salty.

➢ **Determination of loss on drying**

2gm weighed of constac plus granules was taken in dried petridish and placed it in oven at $105\text{-}110^0C$. This was repeated in two consecutive times using two different petridish. After losing moisture content in powder were weighed in digital balanced weighing machine. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % w/w with reference to air dried. Sample loss on drying was 3.27%, loss on drying in 1st month was 3.56%, loss on drying after 6 month 4.09%, loss on drying after 12 month 4.15%, loss on drying after 18 months 4.80%, loss on drying after 24 months 5.20%. In this long term study increase in loss on drying were observed.

➢ **Determination of Ph**

1gm constac plus granules were weighed in digital balanced weighing machine and mixed in 100ml of distilled water in volumetric flask. This 100ml solution was sonicated for about 10min. pH of this solution was calculated using digital pH meter. It was measured at initial point and after 1, 6, 12, 18 and 24 months which was 4.4, 4.7, 4.8, 4.9 and 5.0.

➢ **Determination of total ash**

1gm air dried constac plus granules were weighed in digital balanced weighing machine. This powder was incinerated by gradual heating up to $500\text{-}600^oC$ till found it carbon free. After cooling it in desiccators same is been placed in balanced weighing machine. Total ash content was calculated and expressed as % w/w of air dried material.[18] It was successfully calculated as from initial day 0, 1st month, 6th month, 12th month, 18th month, and 24th month and found as 6.12%, 6.95%, 6.96%, 7.12%, and 7.16%.

➢ **Acid insoluble ash value**

Ash found while determining total ash were poured in 250ml beaker without loss of ash and 100 ml of dil. Hydrochloric acid were added. Using water bath this solution were boiled for 5 minutes followed by filtration of the solution and collect the insoluble matter on a ashless filter paper (whatmann no.41). This filtrate has been washed with hot water till get the neutral filtrate. Transferred this filter paper containing insoluble matter to the original crucible and dried on a hot plate at 60^0C using ignition in a muffle furnace (until it becomes white ash). Allowed this residue to cool in suitable desiccators for about 30 minutes and weigh without delay. Repeated the process until constant weight obtained. Calculated this acid insoluble ash with reference to the air dried drug and found as 0.98% on 1st day, 0.97% after 6 months, 0.98% after 12 months, 0.99% after 18 months and 0.99% after 24 months.

➢ **Water soluble ash value**

Prepared as has given under total ash determination procedure and boiled it for 5 minutes with 25ml of water. Collected insoluble matter in a gooch crucible on an ash less filter paper. Washed it with hot water and ignited for 15 minutes at a temperature not exceeding 600^0C. Subtracted the weight of the insoluble matter from the weight of the ash, the difference in weight represent the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug found as 2.67% on 1st day, 2.63% at 6th months, 2.65% at 12months, 2.66% at 18 months, 2.71% at 24th months.

➢ **Determination of water soluble extractive value**

Air dried constac granules were weighed 5 gm in digital balanced weighing machine. These granule (Churna) macerated in glass stopered conical flask in which 100ml chloroform water were added and again macerate it for 6hrs. It was shaked frequently and once stopped allow it to stand for 18hrs. Rapidly filtered it after 24hrs and 20ml of filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and placed above boiling water to evaporate it to dryness. Again evaporating dish dried at 105^oC for 6hrs in oven. After cooling weight of this residue were noted and percentage of water soluble extractive was calculated and expressed as % w/w with reference to dried air sample. It was found that on 1st day of last month it was 48% and same was found at end of 6th month, from 12 and 18 month it was found as 49%. At the end of 24 month it was reduced and found 48%.[18]

➢ **Determination of alcohol soluble extractive value**

Macerated 5g of the constac plus granules with 100 ml of alcohol of specified strength in a closed flask for 24 hours, it has been shaked frequently during 6 hours than

allowed to stand for 18 hours. This solution were filtered rapidly taking precautions against loss of solvent. Evaporated 25ml of the filtrate to dryness in tared flat bottom shallow dish and dried at 105^0C to constant weight. Calculated the percentage of alcohol- soluble extractives with reference to the air-dried drug found as 30% on 1st day, 29% at 6th months, 29.15% at 12th months, 28% at 18th months, and 27% at 24 months.

- **Estimation of bitter residue**

1 gm air dried constac plus granules were weighed in digital balanced weighing machine, poured it to 150ml conical flask and mixed it with 50ml of methanol. In water bath it was refluxed for half an hour. Methanol extract was collected after filtration in 250ml of beaker. This residue again extracted similarly 2 times and total 3 methanol extracts were separated and evaporate it to obtained 5ml volume thick paste. Using 25 ml hot water in three cycles shake it till water soluble matter is extracted or dissolved. Separate this water washed extracts and pour it to separating funnel. Using 25ml of petroleum ether (60-80^{0}C) with minimum 4 cycles aqueous extract was extracted. This extract of petroleum ether was washed using 25 ml ethyl acetate and repeated 3 more cycles of ethyl acetate extraction. This extract then separated and transferred to pre-weighed evaporating dish and after evaporation dry residue were obtained. From the weight of the residue, the percentage of bitter residue was calculated and expressed as % w/w with reference to air dried sample[19] which was found as 3.72% on day 1st, 3.64% at 6 months, 3.29% at 12 months, 3.20% at 18 months and 3.15% at 24 months .

- **Estimation of Total Saponin**

5gm air dried constac plus granules were weighed in digital balanced weighing machine taken into the conical flask. 90%v/v methanol in volume of 50ml added and mixed, and refluxed it for half an hour. Once the cooling done it was filtered and residue was washed with 90%v/v methanol till get a colorless extract. On water bath combined methanol extract evaporated and thick paste like residue obtained. This residue treated by using petroleum ether (60-80^{o}C) than separated the layer of petroleum ether. Residue than treated with 25ml chloroforms and separated it also. Using ethyl acetate 25 residues treated and then layer of ethyl acetate separated. 90%v/v methanol in volume of 5 ml added to this residue and mixed well till residue dissolved completely. This solution poured into beaker containing 25 ml acetone drop by drop with constant stirring to obtain precipitate. Flask rinsed using 90%v/v methanol (about 2ml). Decant the organic layer and residue to constant weight. The percentage of total saponin was calculated and expressed as % w/w with reference to air dried sample[19] which was found as 3.1% on day 1st, 2.8% at 6th months, 2.8% 12th months, 2.7% at 18th months, 3.4% at 24th months.

- **Estimation of Total Tannin: For blank, For sample**

For blank: In 500 ml conical flask 300ml distilled water and 25 ml indigo sulphonic acid solution added and mixed well. This solution than titrated against 0.02M $KMnO_4$ solution till stable golden yellow colored developed and burrette reading was noted.

For sample: 0.05gm air dried constac plus granules were weighed in digital balanced weighing machine. This sample poured in 500ml conical flask and 50ml distilled water added to it till the sample dissolves completely. In this solution 250ml distilled water carefully added and again mixed well. This solution sonicated for 10min and 25ml indigo sulphonic acid solution was added and mixed well. Carefully titrated this solution against 0.02M $KMnO_4$ solution till get stable golden yellow color. The burette reading was noted. The percentage of total tannin was calculated using following factor. 1 mL of 0.02M KMnO4 is equivalent to 0.00415g of tannin substance[20] found as 26% on 1st day, 25% at 6th months, 25.55% at 12th months, 25.60% at 18th months, 24% at 24th months.

Microbial load

As per Indian pharmacopeia[18] standards microbial load calculated in which bacterial count, total fungal count, presence of *Escherichia coli, Salmonella spices, Pseudomonas aeruginosa* and *Staphylococcus aureus* were calculated which was found as absent during the study period. Pure culture was obtained from NCIM pune and media used for microbial limit test were HiMedia Pvt. Ltd.

RESULTS

This study was conducted as real time stability study in temperature 30^0C±2^0C, relative humidity 60%RH±5% was maintained till 24th month during the study. Product was analyzed from 1st day of 1st month, 6, 12, 18, till 24th month. No change was observed over a period of 24 months as mentioned in Table 1.

Table 1: Real time stability study parameter of constac Plus granules (Churna).

Sr. No	Parameters	Observations				
		1st Year studies		2nd Year studies		
A	**Physiochemical Parameters**	**1st day of 1st Month**	**Last day of 6th Month**	**Last day of 12th Month**	**Last day of 18th Month**	**Last day of 24th Month**
1	Color	Brown colored Granules	Brown colored Granules	Brown colored Granules	Brown colored Granules	Brown colored Granules
2	Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
3	Taste	Bitter & Salty	Bitter & Salty	Bitter & Salty	Bitter & Salty	Bitter & Salty
4	Particle size	Pass Through 12mesh	Pass Through 12mesh	Pass Through 12mesh	Pass Through 12mesh	Pass Through 12mesh
5	Loss on Drying	3.56%	4.09%	4.15%	4.80%	5.20%
6	P^H (10% Aq Solution)	4.4	4.7	4.8	4.9	5.0
7	Total Ash Content	6.12%	6.95%	6.96%	7.12%	7.16%
8	Water Soluble Extractive Values	48%	48%	49%	49%	48%
9	Alcohol Soluble Extractive Value	30%	29%	29.15%	28%	27%
10	Acid Insoluble ash value	0.98%	0.97%	0.98%	0.99%	0.99%
11	Water Soluble ash Value	2.67%	2.63%	2.65%	2.66%	2.71%
B	**Bitter residue**	3.72%	3.64%	3.29%	3.20%	3.15%
C	**Total Tannins**	26%	25%	25.55%	25.60%	24%
D	**Total Saponin**	3.1%	2.8%	2.8%	2.7%	3.4%
E	**Total Bacterial Count**	$48x10^3$	$23x10^4$	$33x10^5$	$25x10^4$	$30x10^5$
F	**Total Yeast & Mould**	$22x10^1$	$38x10^2$	$71x10^3$	$40x10^2$	$41x10^3$
G	**Specific Pathogen**					
	Staphylococcus aureus	Absent	Absent	Absent	Absent	Absent
	Escherichia coli	Absent	Absent	Absent	Absent	Absent
	Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent
	Salmonella Species	Absent	Absent	Absent	Absent	Absent

DISCUSSION

Constipation is the symptoms affecting almost all ages people predominantly in females and elderly aged population.[20,21] Constipation not only affects physical but also disturbs mentally which indirectly reduce quality of life of an individual if not treated timely.[22] In India since ancient times this was effectively treated with various shastrokta churna. In Ayurveda it is found as "saviryata avadhi" mentioned after 13th century in various authentic Ayurvedic texts like Vanga Sen, Shrangdhar Samhita, and Yogaratnakar. This stability studies ensure the efficacy and quality of active compounds in product, to establish shelf life or expiration period and to support the label claim. In this era of evidence based therapies it is very demandable to have stability studies to prove action of such polyherbal preparations which ensures the finished product is having constant quality in its shelf life. Central council of research in Ayurveda (CCRAS) have recommended real time stability studies in India[6] and other two guidelines which include International Conference on Harmonization (ICH) and the World Health Organization provide details on parameters for stability study of pharmaceutical products but ICH guidelines from Q1 to Q11 are generally followed.[1] Past many decades it was ignored due to various reasons its early developmental stages with modern views, utilization of drugs instantly, lack of mass production and unavailability of such instruments. In past decade with polyherbal formulation stability testing giving quality to herbal drugs that established more safety and efficacy with modern views and suggested procedures. Use of recently developed packaging and storage technology developed the confidence for the establishment of the stability period based on some scientific study data. Research in this demanded area already initiated that helps present era is to thoroughly conduct such type of study on every formulation individually. Constac plus a polyherbal formulation were successfully tested for real time stability studies for 24 months, procedures suggested by CCRAS, India and found good to use in humans found in the results (Table 1). Further clinical and long term studies needed which can prove further safety and efficacy.

CONCLUSION

Ingredient in constac plus polyherbal formulation already proved its use in constipation in Ayurveda granth but with help of modern techniques shelf life can be increased. As per CCRAS guidelines Present study conducted on constac plus churn by applying real time stability study. We have found shelf life of 24 months in storage conditions of temperature $30^0C \pm 2^0C$, relative humidity 60%RH± 5%.

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