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Evaluation of Stability Study of Laxative Ayurvedic Formulation Constac Plus Granulation

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Abstract

The aim 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. The present study was conducted to evaluate accelerated stability (Temperature: 40 °C ± 2, Relative Humidity (RH): 75% ± 5) and real time stability (Temperature: 25 °C ± 2, Relative Humidity (RH): 60% ± 5) of Constac plus granulation. A study was conducted as per ICH guideline Q1A (R2). The variation in the organoleptic, physico-chemical and microbial load constants of the Constac plus granulation (fine granulation) was observed during 0 (initial), 1, 3 and 6 months. Results of different physico-chemical parameters were taken into consideration to evaluate intercept and slope. Study shows that there was no change in color, odour and taste of Constac plus granulation up to storage of 6 months at accelerated condition. Formulation was suitable at accelerated condition up to 6months storage. It can be extrapolated that shelf life of Constac plus granulation is 26.54 months. The real time stability data of Constac plus granulation showed very good stability up to one year.

Keywords

Accelerated stability, Real time stability, Constac Plus granulation

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INTRODUCTION

The Major weakness of herbal product is consistency in the quality of the product over a range of time[1]. It is necessary to conduct stability studies of herbal products to predict, evaluate, and ensure drug product safety. The legal requirements of stability are aimed at ensuring that the product remains within the specifications established to ensure its identity, strength, quality, and purity[2]. Due to natural origin of herbal medicinal product, questions on microbiological quality arise more often than chemically defined medicinal products[3]. Hence stability testing is essential to improve the quality of the herbal products. According to ayurvedic pharmaceutical science, churna preparations remain potent up to two months, after which they start degrading gradually losing their efficacy[4]. There are two types of stability study one is accelerated stability and other is real time stability. Pharmaceutical products are generally studied for stability profile at accelerated temperature and humidity, the experimental findings of which can be very helpful to predict reliable self-life or expiry date at room temperature by adopting certain assumptions and criterions[5]. In the present study accelerated stability study was carried out to determine shelf-life of Constac plus granulation.

Figure 1 Constac plus granulation

Figure 2 Constac plus product

MATERIALS AND METHODS

Test drug- Constac plus granulation:

CONSTAC PLUS (Figure 1, 2) is an Ayurvedic proprietary polyherbal formulation in granulation form which comprises dried granulations of hirada and bal hirada (*Terminalia chebula), Behada (Terminalia Belerica), Amala (Emblica officinalis), Badishep (Foeniculum Vulgare), Elaichi (Elettaria cardamomum) Narikel lavan (processed salt with coconut), Mulethi (Glycyrhiza glabra), Nishottar (Ipomoea turpethum),* Erand tail (Caster oil). All the ingredients of the formulation have been

used for thousands of years and individual therapeutic efficacy of these herbs as laxative has also been reported in an ancient Ayurvedic literature[6,7]. A freshly prepared Constac plus granulation was considered for stability study. Constac plus granulation was packed in airtight food grade plastic container having aluminum foil covering.

Storage Conditions and Evaluation Parameters: Accelerated stability study and real time stability study were conducted as per ICH guideline Q1. A(R2)[8]. Storage condition are mentioned as below,

- Accelerated stability: Temperature: 40 °C ± 2, Relative Humidity (RH): 75% ± 5
- Real time stability: Temperature: 25 °C ± 2, Relative Humidity (RH): 60 % ± 5

The change was observed during 6 months for accelerated stability and 1 year for real time stability study at an interval of 0,1,3 and 6 months. Real time stability was comparatively carried out to evaluate the actual degradation rate of Constac plus granulation with respect to accelerated condition. 10% degradation was set to extrapolate of the accelerated stability data at the acceptable point. Real time aging factor 5 and 3.3 were used for extrapolation of shelf life.

The following parameters were considered for evaluation of stability study.

1. Organoleptic characters like colour, odour and taste
2. Physico-chemical parameters like Loss on drying, pH, Total ash, Water soluble extractive value, bitter residue, total saponin and total tannin.
3. Microbial load

1. Organoleptic analysis:

The colour indicated the nature of the formulation; odor and taste of the formulation are extremely sensitive criteria.

Color examination: Five gram formulation was taken into watch glass and placed against white background in white tube light. They were observed for their color by naked eye.

Odour examination: Two gram formulations were smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste examination: A pinch of formulation was taken and examined for it's taste on taste buds of the tongue. The time interval among each sample was kept about 15 min., so as to make the taste bud available fresh every time.

2. Physico-chemical parameters

Loss of drying:

Loss on drying was determined by weighing about 2gm of the granulated material in previously weighed dried petridish (tarred evaporating dish) and dried in an oven at 105ºC, till two consecutive weights, which do not differ by more than 5mg. 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. [10]

pH:

One gram of Constac plus granulation in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

Water soluble extractive value:

About 5 g of accurately weighed Constac plus granulation was taken in a glass-stopper conical flask. Chloroform (100 ml) and water were added to it and macerated for 6 hrs, shaking frequently and then allowing it to stand for 18 hour. After 24 hours it was filtered rapidly and 20 ml of the filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and evaporated to dryness on a boiling water bath. Then evaporating dish was dried at 105ºC for 6 h and then cooled and weighed. From the weight of the residue the percentage of water soluble extractive was calculated and expressed as % w/w with reference to air dried sample[9].

Total ash:

The ash value was determined by incinerating about 5g of the granulation air-dried material, in a previously weighed crucible at gradually increasing heat up to 450 ºC until it is carbon free. Then cooled in a desiccator and weighed. The percentage of total ash was calculated and expressed as % w/w of air dried material[9].

Total Tannin:

For blank preparation 300 ml of distilled water was taken in a 500 ml conical flask. 25 ml of indigo sulphonic acid solution was added to it and mixed well. It was then titrated against 0.02M $KMnO_4$ solution till stable golden yellow color was developed and the burette reading was noted.

For sample preparation accurately weighed 0.05 g of the sample was transferred to a 500 ml conical flask. To it 50 ml of distilled water was added and mixed well to dissolve completely. To this 250 ml of distilled water was added, mixed well and sonication was done for 10 min. Then to it 25 ml of the indigo sulphonic acid solution was added

and mixed well. Titrated against 0.02M $KMnO_4$ solution till stable golden yellow color was developed. 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[10].

Bitter residue:

One gram of the test material was taken in a 150 ml conical flask. To it 50 ml of methanol was added. It was refluxed for half an hour on a water bath. Then methanol extract was filtered in a 250 ml beaker. The residue was extracted for another two cycles of extraction. Three (or all the extracts if greater than 3) methanol extracts were pooled and evaporated to obtain a thick paste (not free flowing) approximately of 5 ml volume. Then concentrated extract was shaken well with three successive cycles of 25 ml hot water or till all the water soluble matter is extracted or dissolved. Above three (or greater than 3) water washed extracts were pooled and transferred to a separating funnel. This aqueous extract was extracted with minimum 4 cycles of 25 ml of petroleum ether (60-80 0C). Then extracted petroleum ether was washed with 25 ml of ethyl acetate. Ethyl acetate extraction was repeated for another three more cycles. The ethyl acetate extracts were pooled and transferred to a pre-weighed evaporating dish and evaporated to dryness. From the weight of the residue the percentage of bitter residue was calculated and expressed as % w/w with reference to air dried sample[11].

Estimation of Total Saponin:

Five grams of test material were weighed in a conical flask. To this 50 ml 90%v/v methanol was added. Content was mixed well and refluxed for half an hour. Then it was cooled and filtered. After that, residue washed with 90%v/v methanol till it became almost colorless. Methanol extract was combined and evaporated on a water bath to obtain a thick paste like residue. The residue was treated with 25 ml petroleum ether (60-80 0C). Petroleum ether layer was separated and discarded. The residue was treated with 25 ml chloroform. Chloroform layer was separated and discarded. The residue was treated with 25 ml ethyl acetate. Ethyl acetate layer was separated and discarded. Then 5 ml 90%v/v methanol was added to residue. Content was shaken well to dissolve the residue completely. Then solution was poured drop wise with constant stirring into a beaker containing 25 ml acetone to obtain precipitate. The flask was rinsed contain the residue with minimum volume (about 2 ml)

of 90%v/v methanol. Then the organic layer was decanted and the residue was dried to constant weight. The percentage of total saponin was calculated and expressed as % w/w with reference to air dried sample.

3. Microbial load:

Microbial load was conducted as per standard procedure mentioned in Indian Pharmacopoeia[13]. It included a total bacterial count, total Fungal Count, presence of Escherichia coli, Salmonella spices, Pseudomonas aeruginosa and Staphylococcus aureus. (Reference)

All tests were done by Shri Bhaurao Patil College of Bio-informatics and IT Hingoli, Pune.

RESULTS

In the accelerated stability study, Temperature: 40°C ± 2 and Relative Humidity (RH): 75% ± 5 was maintained up to 6 months. The product was analyzed on 0, 1, 3 and 6 months. No change was observed in color, odour and taste of formulation up to storage of 6 months at accelerated conditions (Table 1). Results of microbial load of Constac plus granulation was complying with Ayurvedic Pharmacopeial limits at initial month and up to 6 months (Table 1). Results of different physicochemical parameters were taken into consideration to evaluate intercept and slope (Table 2). The extrapolated shelf life of Constac plus granulation was calculated with 10% degradation rate from physicochemical parameters at accelerated condition 40°C ± 2 and 75% ± 5 RH (Table 3).

Table 1 Constac plus granulation results at 40 C +/- 2 C and 75% +/- 5 RH in different interval

S. No.	Parameters	Initial Month	1st month	3rd month	6th month
1	Colour	Brown Colored Powder	Complies	Complies	Complies
2	Odour	Characteristic	Complies	Complies	Complies
3	Taste	Bitter and salty	Complies	Complies	Complies
4	Loss on drying(% w/w)	3.27%	4.09%	4.80%	5.20%
5	pH value (1%w/v solution)	4.9	5.6	5.9	6.1
6	Total Ash (% w/w)	6.95%	7.67%	6.94%	6.59%
7	Solubility (%w/w)	34.72%	32.16%	31.02%	33.264 %
8	Bitter residue (%w/w)	3.89%	3.68%	3.29%	3.15%
9	Total Saponin (%w/w)	Present	Present	Present	Present
10	Total Tannin (%w/w)	Present	Present	Present	Present
11	Total Bacterial Count	$29x10^3$	$48x10^3$	$23x10^4$	$33x10^5$
12	Total yeast and mould	$16x10^1$	$22x10^1$	$38x10^2$	$71x10^3$
13	*E.coli*	Absent	Absent	Absent	Absent

14	*P.areuginosa*	Absent	Absent	Absent	Absent
15	*S.aureus*	Absent	Absent	Absent	Absent
16	*S.spp*	Absent	Absent	Absent	Absent

Table 2 Constc plus granulations -Intercept and slope of different physio chemical parameters

S. No.	**Parameters**	**Initial Month**	**1st month**	**3rd month**	**6th month**	**Slope**	**Intercept**
1	Loss on drying(% w/w)	3.27%	4.09%	4.80%	5.20%	0.30	3.59
2	pH value	4.9	5.6	5.9	6.1	0.17	5.19
3	Total Ash(% w/w)	6.95%	7.67%	6.94%	6.59%	0.11	7.32
4	Solubility (%w/w)	34.72%	32.16%	31.02%	33.264 %	0.15	33.16
5	Bitter residue (%w/w)	3.89%	3.68	3.29%	3.15%	0.12	3.81
6	Total Saponin (%w/w)	Present	Present	Present	Present		
7	Total Tannin (%w/w)	Present	Present	Present	Present		

Table 3 Extrapolated shelf life of Constac plus granulation from different physiochemical parameters

S. No.	**Parameters**	**Initial Month Results**	**Results at 10% degradation**	**Months when 10% degradation occurs**
1	Loss on drying (% w/w)	3.27%	2.943	2.16
2	pH value	4.9	4.41	4.59
3	Total Ash (% w/w)	6.95%	6.255	7.1
4	Water Soluble Extractive Value (%w/w)	34.72%	31.248	10.11
5	Bitter residue (%w/w)	3.89%	3.501	2.58
6	Total Saponin (%w/w)	Present	Present	
7	Total Tannin (%w/w)	Present	Present	
Mean Months at accelerated condition				5.308
Extrapolated shelf life of Constac plus granulation				26.54 months

DISCUSSION

It is important to recognize and be aware of the potential for instability in both manufactured and extemporaneous products. There is a need to specify storage control[12]. In the past it was the practice in many pharmaceutical manufacturing companies to evaluate the stability of the pharmaceutical preparations by observing them for a year or more, corresponding to the normal time that they would remain in stock and in use. Such a method was time consuming and uneconomical[14]. These days accelerated studies at higher temperatures are also used.

Therefore, stability studies were performed. The main objective of stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product, to establish shelf life or expiration period and to support the label claim. Ayurvedic Formulary of India also has given the time period from the date of manufacture within which the formulations should be consumed for best results. For example Churna a period of 6 months has been mentioned in AFI[15]. As per Drug and Cosmetic act, the optimal climatic condition for the storage of medicine is 25 °C/60% RH. If we can maintain same climatic condition for the storage of Constac plus granulation then shelf life of Constac plus granulation is near to 26.54 months. It is matched with the implemented rule, namely 161 B to display the date of expiry of the ASU drugs and propose shelf life of Ayurvedic formulations i.e. shelf life of Churna (fine / course granulation drugs) as 2 years.

CONCLUSION

The present investigation support the formulation was suitable at accelerated condition up to 6 month storage and real time stability data showed very good stability up to one year.

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