



THE ACCELERATED STABILITY STUDY OF *HERBAL DIGESTIVE POWDER* - AN AYURVEDIC FORMULATION

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Abstract

Herbal Digestive Powder was prepared with the incorporation of *Adhatoda vasica* and *Tinospora cordifolia* along with other ingredients Dry Mango Powder, Asafetida (Heeng), Black Salt, Fennel, Ammonium Chloride, Pomegranate seeds, Bishop's weed (Ajwain) having characteristic odor and bitter salty taste and was evaluated for its stability, as per ICH guidelines Q1A (R2) at accelerated conditions (Temperature: $40^{\circ}\text{C} \pm 2$, Relative Humidity: $75\% \pm 5$). The real time stability is estimated at temperature: $25^{\circ}\text{C} \pm 2$ & Relative Humidity: $60\% \pm 5$. The study sample was observed for changes in different parameters like physico-chemical, organoleptic and microbial load for 6 month under accelerated conditions and for real time stability study observed for one year. Real time stability was comparatively estimated to evaluate actual degradation rate of *Herbal Digestive Powder* with respect to accelerated conditions. Up to six months of storage at accelerated condition, no change was observed in organoleptic parameters like colour, odour and taste. Changes in of different physico-chemical parameters were taken into account to evaluate intercept and slope. Extrapolated shelf life was calculated with 10% degradation rate from physico-chemical parameters at accelerated condition $40^{\circ}\text{C} \pm 2$ and $75\% \pm 5$ RH. The present evaluation supports that the *Herbal Digestive Powder* was appropriate at accelerated condition up to 6 month storage. From this accelerated stability study it could be extrapolated that shelf life is 22.4 months (1.87 years) for climatic zone 1 & 2 countries and 14.85 months (1.23 years) for climatic zone 3 & 4 countries.

Key words: Herbal Digestive Powder, Accelerated stability, Real time Stability, Shelf life, Ayurvedic formulations.

Introduction

In health practices the tradition of Ayurveda has been well acknowledge and Ayurvedic formulations are used immensely by the people since long time. In this regard powdered form (Churna) formulations are widely used for treatment. It is seen that sometimes many patient find it difficulty in swallowing the tablets, especially the elderly ones, thus do not abide by prescription, which results in high incidence of non-compliance and ineffective therapy convince (Tatiya *et al.*, 2015). Thus, newer drug delivery system in forms of powder or churna is helpful in fulfilling the need of patients. *Adhatoda vasica* (*Adusa*) and *Tinospora cordifolia* (*Giloy*) have so many attributes such as the green nature of the leaves, the antioxidant properties, polyphenol content, phytochemicals properties and so on (Pandey *et al.*, 2016). These herbs are present in combination with other herbs that are available in market. Numerous formulations using in combination or alone are prescribed in various forms such as choorna, vati, arka, quath etc. to treat various ailments (Sonal *et al.*, 2018). In the management of disease churna or herbal powder is very helpful due to the presence of Kapha Dosha which is among of the Tridasha which is very well described in Ayurveda. Mangal *et al.* (2015) stated that Churna or laxative powder improves digestion and ensures timely evacuation, improves functioning of liver, cures hyperacidity, heart burn and acidic belching.

Methodology

Digestive Powder were prepared by incorporation of *Adhatoda vasica* (*Adusa*) and *Tinospora cordifolia* (*Giloy*) along with other ingredients like Dry Mango Powder, Asafetida (Heeng), Black Salt, Fennel, Ammonium Chloride, Pomegranate seeds, Bishop's weed (*Ajwain*) in definite ratio. For evaluation of stability, a freshly prepared formulation *Digestive Powder* was considered. *Digestive Powder* was packed in airtight food grade plastic container having aluminum foil covering.

Parameters of evaluation

As per ICH guideline Q1. A (R2) evaluation of accelerated stability and real time stability study was conducted. The study sample was stored under specific conditions as mentioned below,

For Accelerated stability: Temperature: $40^{\circ}\text{C} \pm 2$, Relative Humidity (RH): $75\% \pm 5$.

For Real time stability: Temperature: $25^{\circ}\text{C} \pm 2$, Relative Humidity (RH): $60\% \pm 5$.

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, 6 and 12 months. Real time stability was comparatively carried out to evaluate the actual degradation

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rate of *Digestive Powder* 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 parameters considered for evaluation of stability were: (1) Organoleptic characters like colour, flavour and taste, (2) Physico-chemical parameters like Loss on drying, pH, Total Ash, Water soluble extractive value, Bitter Residue and Total Tannin, (3) Microbial Load.

1) Assessment of Organoleptic Parameters

The nature of the Ayurvedic formulation indicated by its colour while taste and flavour of the formulation are very susceptible indicators.

- a) **Color:** Sample of Digestive Powder in quantity of 5 grams was taken into watch glasses. A perfectly white background was used and the sample was observed for colour with naked eye in white tube light.
- b) **Flavour:** 2 gram sample was smelled for flavour test.
- c) **Taste:** A pinchful of sample was tasted on taste buds of tongue.

2) Physico-Chemical Parameters

- a) **Loss of drying:** Loss on drying was determined by weighing about 2gm of sample in previously weighed dried petridish (tarred evaporating dish) and dried in an oven at 105°C, 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 (API, 2008).
- b) **pH:** For pH determination 1 g of sample mixed with 100 ml distilled water in a 100 ml volumetric flask. The solution was sonicated for about 10 minutes. The pH was measured with the help of digital pH meter.
- c) **Water soluble extractive value:** About 5 gm accurately weighed sample was macerated in a glass-stopper conical flask. 100 ml chloroform water was added and macerated for 6 hours, shaking frequently and then allowed to stand for 18 hours then after 24 hrs 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 105°C for 6 hrs 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.
- d) **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°C 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.

- e) **Total Tannin For blank preparation:** In a 500 ml conical flask 300 ml of distilled water was taken and 25 ml of indigo sulphonic acid solution was added to it and mixed well. It was titrated against 0.02M KMnO₄ solution till stable golden yellow color was developed. The burette reading was noted.
- f) **For sample preparation:** Accurately weighed about 0.05 g of sample was taken in to a 500 ml conical flask and 250 ml of distilled water was added and mixed well, and then sonicate it for 10 min. 25 ml of the indigo sulphonic acid solution was added and mixed well. Titrated against 0.02M KMnO₄ 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.
- g) **Bitter residue:** One gram of accurately weighed sample was taken in a 150 ml conical flask and 50 ml of methanol was added. It was refluxed for half an hour on a water bath. Then it was filtered and the methanol extract was collected in a 250 ml beaker. The residue was extracted for another two cycles of extraction. Three methanol extracts were pooled and evaporated to obtain approximately 5 ml thick paste. The concentrated extract was shaken with three successive cycles of 25 ml hot water or till all the water soluble matter is extracted or dissolved. This water washed extracts were pooled and transferred it to a separating funnel. This aqueous extract was extracted with minimum 4 cycles of 25 ml of petroleum ether (60-80°C). This extract was washed with 25 ml of ethyl acetate. Ethyl acetate extraction was repeated for 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.
- h) **Microbial load:** Microbial load was carried out as per standard procedure mentioned in Indian Pharmacopoeia. It included total plate count, total yeast and mould count. Presence of *Escherichia coli* and *Staphylococcus aureus* by NCIM: 2065; ATCC: 739 and ATCC 6358 method.

Digestive Powder

In the accelerated stability study, Temperature: 40°C ± 2, Relative Humidity (RH): 75% ± 5 was maintained up to 6 months. The product was analyzed on 0, 1, 3 and 6 months. Table 1 shows that there is no change noticed in color, odour and taste of *Digestive Powder* up to storage of 6 months at accelerated condition.

Loss on drying (LOD) in initial month is 5.3, 1st month is 5.8, 2nd month it shows 6.3 and in 6th month it was 6.6; total ash

in the initial month is 10.18 followed by 10.30 in 1st month, 3rd month it shows to 10.52 and lastly in last 6 months it shows to 10.97; pH (acidity) of the product in initial month is 5.0, which in later month increase by 5.03, in 3rd month pH level up by 5.5 and in 6th month it was 6.2; water soluble extractive value of the Digestive Powder in the initial month is 42.13 slightly decrease by 41.97, in 3rd month it declines to the value 41.05 and lastly in 6th month the value reduced to 39.65; Bitter residue in initial month is 3.50, decreased in 1st month to 3.43 followed by 3.29 in the 3rd month and lastly reduced to 2.95 in the final 6th months, Total tannin of Digestive Powder in the initial month 0.005 which slightly decrease by 0.004 followed by 0.003 and 0.001 in the last month. (Values are in w/w %).

Microbial load including total plate count have the values 1.91×10^4 , 2.12×10^4 , 2.25×10^5 and 2.30×10^6 in the initial, 1st, 3rd and 6th month respectively; Yeast and Mould count in the initial, 1st, 3rd and 6th month is 1.09×10^2 , 2.98×10^2 , 4.03×10^3 and 5.0×10^4 respectively. Variation in Parameters taken for the analysis was found linear according to the Pharmacopeia of India. Results of microbial load of *Digestive Powder* was complies with Ayurvedic Pharmacopeial limits at initial month and up to 6 months. Table 2 shows the intercept and slope of different physico-chemical parameters. An extrapolated result at 10 % degradation and months when 10 % degradation occurs was calculated by using the reported formula that is by calculating the slope and intercept values for the above deviations (Biswajyoti *et al.*, 2014).

Months when 10% degradation occurs =

$$\frac{[0 \text{ Month Assay Value} - \{(0 \text{ Month Assay Value} \times 10/100)\} - \text{Intercept}}{\text{Slope}}$$

Table 3 reveal the expected shelf life of *Digestive Powder* that was calculated with 10% degradation rate from physicochemical parameters at accelerated condition $40^\circ\text{C} \pm 2$ and $75\% \pm 5$ RH. Digestive Powder is an herbal formulation which is having green brownish colour, characteristic flavour, taste is bitter and salty, uniform solid powder without any tufts. The digestive powder did not show any marked change in its Organoleptic parameters in accelerated thermal condition which simply fulfils the condition of storage that states the any change in Organoleptic characters affects the loss of its shelf life (Pharmacopeial forum). Only Organoleptic attributes is not enough to see the shelf life of an herbal product. According to as per the guidelines of Ministry of AYUSH, other food and drug regulatory agencies as well as WHO, the physico-chemical and microbial stability data is essential to decide the shelf life of formulations.

Physico-chemical parameters

1. Loss on drying

The percentage of moisture content of the product did not show any significant change perhaps due to the container in which product was kept is air tight and prevents moisture adsorption. Aulton (2009) stated that excess amount of water present in plant drugs causes hydrolysis of constituents, other biochemical reactions and the growth of bacteria and fungi however water content can vary between 8% and 14%.

2. Ash values

The percentage change of total ash at different time interval is 10.30%, 10.52% and 10.97% at 1st, 3rd and 6th months respectively. As these changes were <5%, is acceptable as per to the ICH guideline (Junior *et al.*, 2011).

3. pH values

Changes in pH value are below 5% considering the negligible change as per the ICH guideline proving that the product fulfils the optimum requirement. As one of the researches quoted that a neutral and alkaline pH discriminated the high contamination level of herbal preparation (Abba *et al.*, 2003). Microbial count was also found to be within limit as per WHO guidelines and similar findings were mentioned in previous researches (Abba *et al.*, 2003).

4. Extractive values and presence of acidic substances

The change in percentage of Water soluble extractive value was from 42.13%, 41.97%, 41.05% and 39.65% from initial month at 1st, 3rd and 6th months respectively. Change in bitter residue from 3.50% to 2.95%. Percentage change in total tannin was less than 1%. All the changes were not more than 5% and hence met the criteria given by ICH.

5. Microbial Analysis

Formulation showed absence of bacteria *E. coli* and *S. aureus*. Thus, digestive powder confirms to the standards set by WHO and API. According to a Draft issued by the Government of India to determine the shelf life of a product, changes in parameters from initial value should not be more than 5% and also meet the acceptance criteria such as appearance, physical, and chemical parameters, etc., However, even 90% of labelled potency is commonly considered as the minimum acceptable potency level.

For evaluation of stability of *Digestive Powder*, 5% variation limit was fixed as per the ICH guidelines to assess the physico-chemical parameters. It has been proposed that 6 months at $40^\circ\text{C}/75\%$ RH is roughly equivalent to 24 months at room temperature (25°C) (Baertschi *et al.*, 2013). As per the deliberation, it can be confirmed that Stability study will be stable for 3.3 years at room temperature. According to the "Shelf life Recommendations for Supplements Guidelines for Manufacturers", if a study was carried out at 10°C temperature above the room temperature, an estimate of shelf life equals to $\times 2$ accelerated storage time (Drugs and Cosmetics (amendment) Rules, 2005). However, the most popular concept in this regard is Grimm's statement. Grimm (1998) mentioned that, predictive factor for zone IV (India) was 3.3 of the accelerated study period. It means if the product is stable for 6 months at $40^\circ\text{C}/75\%$ RH, its shelf life will correspond to 20 months at $30^\circ\text{C}/70\%$ RH (climatic zone IV).

Thus, in the view of above interpretations, it can be safely affirmed that *Digestive Powder* has the shelf life of 22 months at room temperature for zone 1 and 2 and 14 months for zone 3 and 4. Gajanan *et al.* (2017) also found the shelf life of churna prepared by mixing dried powder of herbal plants including

rock salt, fennel, ajwain as other ingredient to enhance the taste was found to be 1 year for zone 3 which is similar to the finding of study. Biswajyoti *et al.* (2014) also prepared a polyherbal formulation by mixing dried leaves of *Tinospora cordifolia* and found the shelf life of Churan and it was 1.10 years which is similar to our study.

Conclusion

The present investigations supports that the *Herbal Digestive Powder* was appropriate and stable under accelerated conditions of storage up to 6 months. From the accelerated study this can be extrapolated that the average shelf life is 14 months (1.23 years).

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Table 1: Results of different parameters of Digestive Powder

S. No.	Parameter	Initial Month	1 st Month	3 rd Month	6 th Months
1.	Colour	Green Brownish	Dark green brown	Dark green brown	Dark green brown
2.	Flavour	Characteristic	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter & Salty	Bitter	Bitter	Bitter
4.	Loss on drying (%w/w)	5.3	5.8	6.3	6.6
5.	pH value (1% w/v solution)	5	5.03	5.5	6.2
6.	Total ash (%w/w)	10.18	10.30	10.52	10.97
7.	Water soluble extractive value (%w/w)	42.13	41.97	41.05	39.65
8.	Bitter residue (%w/w)	3.50	3.43	3.29	2.95
9.	Total tannin (%w/w)	0.005	0.004	0.003	0.001
10.	Total plate count (CFU/g)	1.91×10^4	2.12×10^4	2.25×10^5	2.30×10^6
11.	Total yeast and mould (CFU/g)	1.09×10^2	2.98×10^2	4.03×10^3	5.0×10^4
12.	<i>E. coli</i>	Absent	Absent	Absent	Absent
13.	<i>S. aureus</i>	Absent	Absent	Absent	Absent

Table 2: Intercept and slope of different physico-chemical parameters of Herbal Digestive Powder

Parameter	Initial Month	1 st Month	3 rd Month	6 th Month	Intercept	Slope
Loss on Drying	5.3	5.8	6.3	6.7	5.47	0.22
pH value	5	5.03	5.5	6.2	4.90	0.20
Total ash	10.18	10.30	10.52	10.97	10.19	0.13
Water soluble extractive value	42.13	41.97	41.05	39.65	42.15	0.42
Bitter residue	3.50	3.43	3.29	2.95	3.50	0.09
Total tannin	0.005	0.004	0.003	0.001	0.004	0.0006

Table 3: Extrapolated shelf life of Digestive Powder

Parameter	Initial Month	Result at 10% Degradation	Months when 10% degradation occurs
Loss on Drying	5.3	4.77	3.16
pH value	5	4.5	1.94
Total ash	10.18	9.16	7.87
Water soluble extractive value	42.13	37.91	9.96
Bitter residue	3.50	3.15	3.78
Total tannin	0.005	0.004	0.29
Mean months at accelerated condition			4.5
Climatic Zone 1 & 2			22.5 (1.87 years)
Climatic zone 3 & 4			14.85 (1.23years)