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The Accelerated Stability Study of *Herbal Tea Bag*- An Ayurvedic Formulation

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Abstract

Herbal Tea Bags was prepared with the incorporation of Adhatoda vasica and Tinospora cordifolia along with other ingredients cardamom, basil and ginger having characteristic odor and bitter salty taste and was evaluated for its stability, as per ICH guidelines Q1A (R2) at accelerated conditions (Temperature: 40 °C ± 2, Relative Humidity : 75% ± 5). The real time stability is estimated at temperature: 25 °C ± 2 &relative humidity 60% ± 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 Tea Bags* 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 °C ± 2 and 75% ± 5 RH. The present evaluation supports that the *Herbal Tea Bags* was appropriate at accelerated condition up to 6 month storage. From this accelerated stability study it could be extrapolated that shelf life is 26.04 months (2.17years) for climatic zone I & II countries.

KEYWORDS: Herbal Tea, Accelerated stability, Real time Stability, Shelf life, Ayurvedic formulations.

Introduction

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

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properties among others **(Pandey *et al.*, 2016)**, which makes it similar to the leaves of *Camellia sinensis* and useful for tea manufacturing. Using these leaves as herbal tea forms the basis for name "Herbal Tea" often given to teas other than from the leaves of *Camellia sinensis*, because they are related with management of different ailments. Ayurvedic drugs from medicinal plants are having different constituents with varied concentrations which make the formulation intricate in nature. To ensure quality, purity and stability of the finished product evaluation of constituents is necessary. Stability study is necessary to ensure that how the quality of a drug substance or product changes with time under influence of variety of environmental factors such as, temperature, humidity and light and also to ascertain a retest period for the drug substance or product and recommended storage conditions **(Bankoti *et al.*, 2012).**

Two type of stability is there: one is accelerated stability and second is real time stability. Every product has definite shelf-life which depends on various physical, chemical, environmental and biological factors.

Methodology

Tea bags were prepared by incorporation of *Adhatoda vasica* (Adusa) and *Tinospora cordifolia* (Giloy) along with other ingredients like basil, ginger and cardamom in definite ratio. For evaluation of stability, a freshly prepared formulation *Tea Bags* was considered. *Tea bag* 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°C ± 2, Relative Humidity (RH): 75 % ± 5.
For 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 0,1,3,6 and 12 months. Real time stability was comparatively carried out to evaluate the actual degradation rate of *Tea Bags* 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

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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 Herbal Tea 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 1050C, 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 0C for 6 hrs and then cooled and weighed.

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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 0C 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 0C). 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.

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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.

Result

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 show that there is no change noticed in color, odour and taste of *Tea bag* up to storage of 6 months at accelerated condition.

Loss on drying (LOD) in initial month is 5.2, 1^{st} month is 5.6, 3rd month it shows 6 and in 6^{th} months it increase to 6.4; total ash in the initial month is 10 followed by 10.18 in 1^{st} month, 10.45 in 3^{rd} month and in the last 6^{th} months it shows to 10.95; pH (acidity) of the product in initial month is 5.1,which in later 1^{st} month increase by 5.3, in 3^{rd} month it was increased by 5.5 and in 6^{th} month pH level up by 5.9; water soluble extractive value of the tea bag in the initial month is 41.15 slightly decrease by 40.75 followed by 40.2 in 3^{rd} months and lastly it declines to the value 39.28 in 6^{th} months; Bitter residue of the tea bag in initial month is 3.45, decreased in 1^{st} month to 3.30 followed by 3.00 in the 3^{rd} month and 2.7 in the last 6^{th} months; Total tannin of Tea Bag in the initial month is 0.004 which slightly decrease by 0.003 followed by 0.002 in the 3^{rd} month and lastly in the 6^{th} month it decrease to 0.001. (Values are in w/w %).

Microbial load including Total plate count have the values 1.77×10^4, 2.01×10^4, 2.30×10^5 and 2.47×10^6 in the initial, 1^{st}, 3^{rd} and 6^{th} months respectively; Yeast and Mould count in the initial, 1^{st}, 3^{rd} and 6^{th} months are 7.27×10^2, 8×10^2, 8.90×10^3 and 9.12×10^4 respectively.

Variation in Parameters taken for the analysis was found linear according to the Pharmacopeia of India. Results of microbial load of *Tea Bag* was complies with Ayurvedic Pharmacopeial limits at initial month and up to 6 months.

Table 1. Results of different parameters of Tea Bag

Sr. No	Parameter	Initial Month	1^{st} Month	3^{rd} Months	6^{th} Months
1.	Colour	Green	Dark Green	Dark Green	Dark Green

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		Brownish	Brown	Brown	Brown
2.	Flavour	Characteristic	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter	Bitter
4.	Loss on drying (%w/w)	5.2	5.6	6.0	6.4
5.	pH value (1% w/v solution)	5.1	5.3	5.5	5.9
6.	Total ash (%w/w)	10.00	10.18	10.45	10.95
7.	Water soluble extractive value (%w/w)	41.15	40.75	40.20	39.28
8.	Bitter residue (%w/w)	3.45	3.30	3.00	2.7
9.	Total tannin (%w/w)	0.004	0.003	0.002	0.001
10.	Total plate count (CFU/g)	1.77×10^4	2.01×10^4	2.30×10^5	2.47×10^6
11.	Total yeast and mould (CFU/g)	7.27×10^2	8×10^2	8.90×10^3	9.12×10^4
12.	*E. coli*	Absent	Absent	Absent	Absent
13.	*S.aureus*	Absent	Absent	Absent	Absent

Table 2. Intercept and slope of different physico-chemical parameters of Tea Bags

Parameter	Initial Month	1st Month	3rd Month	6th Months	Intercept	Slope
Loss on Drying	5.2	5.6	6.0	6.4	5.32	0.19
pH value	5.1	5.3	5.5	5.9	5.12	0.12
Total ash	10.00	10.18	10.45	10.95	10.00	0.15
Water soluble extractive value	41.15	40.75	40.20	39.28	41.10	0.305
Bitter residue	3.45	3.30	3.00	2.7	3.42	0.125
Total tannin	0.004	0.003	0.002	0.001	0.003	0.0005

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).**

$$\text{Months when 10\% degradation occurs} = \frac{[0 \text{ Month Assay Value} - \{(0 \text{ Month Assay Value} \times 10/100\}] - \text{Intercept}}{\text{Slope}}$$

Table 3. Extrapolated shelf life of Tea Bags

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Parameter	Initial Month	Result at 10% Degradation	Months when 10% degradation occurs
Loss on Drying	5.2	4.68	3.38
pH value	5.1	4.59	4.18
Total ash	10.00	9	6.43
Water soluble extractive value	41.15	37.03	13.32
Bitter residue	3.45	3.10	2.56
Total tannin	0.004	0.003	1.45
Mean months at accelerated condition			**5.22**
Climatic Zone 1 & 2			**26.1 (2.17 years)**
Climatic zone 3 & 4			**17.16 (1.43 years)**

Table 3 reveal the expected shelf life of tea bag that was calculated with 10% degradation rate from physicochemical parameters at accelerated condition 40°C ± 2 and 75% ± 5 RH (Table 3).

Discussion

Tea Bag is an herbal formulation which is having green brownish colour, characteristic flavour and aroma. The Tea Bag did not show any marked change in its Organoleptic parameters in accelerated thermal condition which simply fulfils the condition of storage 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 a 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**

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The percentage change of total ash at different time interval is 10.18%, 10.45% and 10.95% 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 fulfills the optimum requirement. As one of the research study 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 41.15%, 40.75% , 40.20% and 39.28% from initial month, 1, 3 and 6 months respectively. Change in bitter residue from 3.45% to 2.7%. 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, tea bag confirms to the standards set by WHO and (AyurvedicPharmaoepia of India) 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 labeled potency is commonly considered as the minimum acceptable potency level.

For evaluation of stability of *Tea bag*, 5% variation limit was fixed as per the ICH guidelines to assess the physico-chemical parameters. It has been proposed that 3 months at 40°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 × 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

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accelerated study period. It means if the product is stable for 6 months at 40°C/75%RH, its shelf life will correspond to 20 months at 30°CC/70% RH (climatic zone IV).

Thus, in the view of above interpretations, it can be safely affirmed that *Tea Bag* has the shelf life of 26 months at room temperature.

Conclusion

The present investigations supports that the *Herbal tea Bag* 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 26 months (2.17 years).

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