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EVALUATION OF THE SHELF LIFE PERIOD OF A SIDDHA FORMULATION, NILAPPANAI CHOORANAM

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ABSTRACT

Stability or shelf life period is an important matter regarding ASU - Ayurveda, Siddha, Unani - formulations. Stability is the capability of a specific formulation in a particular container/ closure system to remain within its physical, chemical, microbiological, toxicological and therapeutic specifications. The objective of the present study was to evaluate stability study of a Siddha compound formulation, *Nilappanai chooranam*. Accelerated stability study was carried out at a temperature 40°C ± 2 and Relative Humidity (RH) 75% ± 5 and real time stability study at temperature 30°C ± 2 and RH 60% ± 5. The change in organoleptic parameters, physico-chemical parameters and microbial load was observed 6 month 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 *Nilappanai chooranam* with respect to accelerated condition. No change was observed in color, odour and taste of the *chooranam* up to storage of 6 months at accelerated condition. Results of different physico-chemical parameters were taken in consideration to evaluate intercept and slope. Extrapolated shelf life of the *chooranam* was calculated with 10% degradation rate from physico-chemical parameters at accelerated condition, 40°C ± 2 and 75% ± 5 RH. The present study supports that the *chooranam* was suitable at accelerated condition up to 6 month storage. It was observed that the shelf life of *chooranam* is observed

as 1.99 yrs for countries which comes under climatic zone I & II and 1.31 years for countries which comes under climatic zone III & IV. Real time stability data showed very good stability up to 1 year.

KEYWORDS: *Nilappanai chooranam*, physico-chemical parameters, shelf life period, microbial contamination, stability study.

INTRODUCTION

Traditional medicines are widely used in the prevention, diagnosis and treatment of an extensive range of ailments. Nowadays there is an increasing concern for shelf life and stability of Ayurveda, Siddha and Unani systems. Siddha system of medicine is practiced in Tamil speaking parts of India and abroad.[1] The medicinal products of ASU system of medicine include single herb or polyherbal formulations with or without mineral drugs and/or drug of animal origin. Stability or shelf life period is an important parameter for defining the safety and efficacy of the formulation. The Ayurvedic Pharmacopoeia of India[2] prescribes guidelines for stability testing and shelf life determination for new and existing Ayurveda, Siddha and Unani medicines. The guideline covers scope and objective, general information, selection of batches, container and closure system specification, testing frequency, storage condition and evaluation.[3]

Two approaches can be followed to monitor the stability of the product. The first approach is to store the samples of same batch material at standard storage and accelerated storage conditions and test them periodically. Based on the evaluation of the results, the expiry date or shelf life may be determined. The second approach called “cross sectional approach” is applicable for existing products which do not have yet a declared shelf life. The approach is to select samples from batches manufactured over a period of last five years spanning six months and evaluate them simultaneously. Based on the result obtained the expiry date or shelf life may be determined.[3]

Generally, there are five types of testing methods for the determination of stability or shelf life period. They are (1) Accelerated testing, (2) Real time (long-term) testing, (3) Intermediate testing, (4) Stress testing and (5) Forced degradation testing.[4] In accelerated stability testing, the product is subjected to a several high temperatures, humidity, light intensity etc., that accelerates degradation, and shelf life period is calculated.[5] Real time (long-term) testing is normally performed for longer duration to allow significant degradation

of the product under specified storage conditions. Intermediate testing is mainly conducted when the accelerated studies for general case failed to meet the acceptance criteria and are designed to moderately increase the rate of degradation for a drug intended to be stored long-term. Stress testing includes the effect of temperature, humidity, oxidation, photolysis and hydrolysis. Forced degradation testing is performed with objective to provide intrinsic stability assessment of the drug, to elucidate the possible degradation pathways by identifying the likely degradation products and to have an idea of the stability of the analytical process applied for the drug.[4]

A product can be considered to be stable if there is no significant change on keeping. Siddha medicines are widely used in the prevention, diagnosis and treatment of an extensive range medical system. Earlier the practicing physicians used to prepare medicine by themselves for his patients using simple instruments similar to mortar and pestle. Today drug products are usually manufactured on commercial scale. Typically a drug product is transported from manufacturer to distributor, from distributor to wholesaler, from wholesaler to hospital or pharmacy and finally to user. During transfer of product from manufacturer to user, the variations in external factors may create changes in stability. In order to obtain full therapeutic efficacy it is mandatory that the product should be stable at user end.[3]

The shelf life period of different types of Siddha formulations are given in Rule 161-B[5]. The objective of the present study was to evaluate the shelf life period of *Nilappanai chooranam*, a Siddha polyherbal formulation.

MATERIALS AND METHODS

1. Preparation of *Nilappanai chooranam*

Nilappanai chooranam is a compound formulation comprising of seven ingredients - (1) Nilappanai kizhangu (*Curculigo orchiodes* Gaertn.) (2) Poonaikali vithai (*Mucuna pruriens* Bak.) - purified (3) Nerinjil mul (*Tribulus terrestris* Linn.) (4) Nelli vatral (*Emblica officinalis* Gaertn.) (5) Mulillavam pisin (*Bombax malabaricum* DC.) (6) Seenthil sarkarai (*Tinospora cordifolia* Willd., Miers) and (7) Panang karkandu (*Borasses flabellifer* Linn.) in equal proportion. - was prepared by the Clinical Research Section, Siddha Regional Research Institute, Poojappura, Thiruvananthapuram by the method described in Kannusamy parambarai vaidhyam.[7]

2. Storage condition

Accelerated stability study and real time stability study was carried out conducted as per ICH guideline Q1. A (R2).[8] For Accelerated stability study, the storage condition[2] was (1) Temperature: 40°C ± 2 and (2) Relative Humidity (RH): 75% ± 5 and for Real time stability (1) Temperature: 30°C ± 2 and (2) Relative Humidity (RH): 60% ± 5. The changes were observed upto 6 months for accelerated stability and upto 1 year for real time stability study at an interval of 0,1,3,6 and 12 months.

3. Parameters evaluated for the study

a. Organoleptic characters

Colour of the *chooranam* was observed by taking it into watch glasses and placed against white background in white tube light by naked eye. Odour was observed by carefull smelling and taste by taking a pinch of the *chooranam* and examining its taste on taste buds of the tongue.[9]

b. Loss on drying at 105ºC

Loss on drying was determined by weighing about 2 gm of the powdered 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 5 mg. 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.[9]

c. Determination of total ash

The ash value was determined by incinerating about 1 g of the powdered air-dried material, in a previously weighed crucible at gradually increasing heat up to 500-600 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]

d. Acid Insoluble Ash

To the total ash as obtained above, added 25 ml 6N HCl and boil for 5 minutes. Filtered with an ashless filter paper. Washed with hot water until the filtrate was free from acid. Transfer the filter paper containing the insoluble matter into the same crucible and ignite to constant weight. The percentage of acid insoluble ash was calculated and expressed as % w/w of air dried material.[9]

e. Water/ alcohol soluble extractive value

About 4 g accurately weighed *Nilappanai chooranam* was macerated in a glass-stopper conical flask. 100 mL water/ ethyl alcohol water was added and macerated for 6 h, shaking frequently and then allowed to stand for 18 h then after 24 h 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/ alcohol soluble extractive was calculated and expressed as % w/w with reference to air dried sample.[9]

f. Volatile oil

Take 20 g coarsely powdered plant material in a 1 litre R.B. flask. Add 300 ml of water and a few pieces of porous pieces. The flask is connected to a volatile oil apparatus. The contents of the flask are now heated and boiled for 2 hours or until the distillation is completed. The flask is rotated occasionally to wash down any material that adheres to the sides. The apparatus is allowed to cool for 10 minutes and the volume is read. From this, the percentage volatile oil is calculated.[9]

g. Microbial Contamination

The microbial contaminations Total viable count[10] and Total fungal count count[11], were determined at CEPC (Cashew Export Promotion Council of India) Laboratory and Technical Division, Kollam, Kerala.

4. Evaluation of Shelf life period

Real time stability was carried out to evaluate actual degradation rate of the *chooranam* with respect to the accelerated condition. 10% degradation was set to extrapolate of the accelerated stability data as the acceptable point. Real time aging factor 5 and 3.3 was used for extrapolation of shelf life for climatic Zone I & II countries and climatic Zone III & IV countries respectively. Ambient temperature and humidity for Zone I & II countries are 21°C/ 45% RH and 25°C/ 60% RH respectively. For Zone III & IV countries 30°C/ 35% RH and 30°C/ 70% RH respectively. India comes under climatic Zone III & IV.[8] Number of months when 10% degradation was occurred was calculated using following formula

$$\text{Months when 10\% degradation occurs} = \frac{[0 \text{ month assay value} - \{(0 \text{ month assay value} \times 10)/100\}] - \text{intercept}}{\text{slope}}$$

The samples were analysed by determining the physico-chemical parameters in the 0th, 1st, 3rd and 6th months. *Chooranam* was kept in airtight glass containers. Five containers of the *chooranam* of 50 g each were packed and stored well. Samples were withdrawn at the intervals of 0th, 1st, 3rd and 6th months. Basic analytical parameters including loss on drying at 105°C, ash values, volatile oil, water soluble extractives and alcohol soluble extractives were evaluated at regular intervals.

Based on the values obtained at different stages; intercept, slope, expected time (in months) for 10% of degradation were calculated for individual parameters of the formulation. As India falls in Zone III; the mean obtained of these months was multiplied with 3.3 to extrapolate shelf-life period.

RESULTS AND DISCUSSION

In the accelerated stability study, Temperature: 40°C ± 2, Relative Humidity (RH): 75% ± 5 was maintained up to 6 months. The organoleptic parameters of the chooranam observed during 0th, 1st, 3rd and 6th months are given in Table 1. No change was noticed in color, odour and taste of the *chooranam* up to storage of 6 months at accelerated condition.

Table 1: Organoleptic characters of *Nilappanai chooranam* at 40 °C ± 2 and 75% ± 5 RH at different intervals.

Sl. No.	Parameters	Initial month	1st month	3rd month	6th month
1.	Colour	pale brown	Complies	Complies	Complies
2.	Odour	characteristic smell	Complies	Complies	Complies
3.	Taste	astringent	Complies	Complies	Complies

The results of microbial load of the *chooranam* falls within Pharmacopeial limits at initial month and up to 6 months (Table 2).

Table 2: Microbial load of *Nilappanai chooranam* at 40°C ± 2 and 75% ± 5 RH at different intervals.

Sl. No.	Parameters	RESULTS			
		Initial month	1st month	3rd month	6th month
1.	Total viable count (cfu/g)	$62x10^4$	$61x10^4$	$78x10^4$	$82x10^4$
2.	Total fungal count (cfu/g)	300	1000	$29x10^2$	$34x10^2$

Results of different physico-chemical parameters during 0th, 1st, 3rd and 6th months were taken in consideration to evaluate intercept and slope and are given in Table 3.

Table 3: Intercept and slope of different physico-chemical parameters of *Nilappanai chooranam* kept in accelerated condition.

Sl. No.	Parameters	Initial month	1st month	3rd month	6th month	Intercept	Slope
1.	Loss on Drying at 105°C %	11.78	12.96	13.34	19.74	8.062	1.908
2.	Total Ash Content %	5.84	6.00	6.29	6.4	5.741	0.10
3.	Acid Insoluble Ash %	1.58	2.06	2.11	2.76	1.352	0.209
4.	Water Soluble Extractive %	24.48	22.76	21.89	15.66	27.27	1.743
5.	Alcohol Soluble Extractive %	15.34	15.04	14.23	9.39	16.54	0.746

Number of months when 10% degradation was occurred was calculated using following formula given in Materials and methods and is given in Table 4. Extrapolated shelf life of the *chooranam* from different physico-chemical parameters, kept in accelerated condition, was also calculated. No change was noticed in the volatile oil content which was found to be 0.5% in the initial month and upto storage of 6 months in accelerated condition.

Table 4: Phyico-chemical parameters obtained in Initial month and Months when 10% degradation occurs.

Sl.No.	Parameters	Result for Initial Month	Result for 10% Degradation	Months when 10% degradation occurs
1.	Loss on Drying at 105°C %	11.78	12.360	1.331
2.	Total Ash Content %	5.84	6.424	4.85
3.	Acid Insoluble Ash %	1.58	1.738	0.3316
4.	Water Soluble Extractive %	24.48	22.022	11.635
5.	Alcohol Soluble Extractive %	15.34	13.806	5.667
Mean Months at accelerated condition was obtained as				**4.76292 months**

Stability period for Climatic zone I & II = (4.76292× 5) = 23.846 months = 1.99 yrs

Stability period for Climatic zone III & IV= (4.76292× 3.3) = 15.718 months = 1.31 yrs

In the real time stability study, Temperature: 30°C ± 2, Relative Humidity (RH): 60% ± 5 was maintained up to 1 year. The product was analyzed on 0, 1, 3, 6 months and 1 year. It was carried out to check the properties of the *chooranam* such as organoleptic characters, different physico-chemical parameters and microbial load at optimal condition. Results of different parameters at real time condition are given in Table 5.

Table 5: Results of different parameters of *Nilappanai chooranam* at 30°C ± 2 and 60% ± 5 RH in different intervals.

Sl.No.	PARAMETERS	Result for Initial Month	1st month	3rd month	6th month	1 year
1.	Colour	pale brown	Complies	Complies	Complies	Complies
2.	Odour	characteristic smell	Complies	Complies	Complies	Complies
3.	Taste	astringent	Complies	Complies	Complies	Complies
4.	Volatile oil %	0.5	0.5	0.5	0.5	0.5
5.	Loss on Drying at 105°C %	11.78	12.62	14.75	15.07	16.19
6.	Total Ash Content %	5.84	6.08	6.40	6.58	7.02
7.	Acid Insoluble Ash %	1.58	2.08	2.16	2.27	2.79
8.	Water Soluble Extractive %	24.48	25.89	22.29	21.08	19.67
9.	Alcohol Soluble Extractive %	15.34	15.22	14.34	13.45	10.08

The total viable aerobic count was found to be $86x10^4$ cfu/g and total yeast and mould count $46x10^2$ cfu/g after maintained up to 1 year in real time condition. The permissible limit according to WHO is Total viable count should be $<10^7$cfu/g and Total fungal count should be $<10^5$ cfu/g.

The recommended storage conditions for accelerated study are 40°C ± 2° C / 75% RH ±5% RH for minimum of 6 months and for long term study conditions are 30°C ±2°C / 60% RH ± 5% RH for minimum of 12 months respectively. An ASU drug can be considered to be stable if "no significant change" occurs at any time of testing at accelerated storage condition or real time storage condition.[8] In the case of *Nilappanai chooranam*, no change was observed in organoleptic characters such as colour, odour and taste. Considering physico-chemical parameters, no significant change for the drug means, the parameters shall not vary beyond 25% of the initial value. Hence it may be stated that in the present study, the physico-chemical parameters are stable. After keeping the *chooranam* for 6 months in accelerated condition and 1 year in real time condition, the total bacterial count and total fungal count falls within the permissible limits.

As per ICH guidelines, countries coming under climatic zones I and II are having climatic condition, temperature 21°C/ relative humidity (RH) 45% and temperature 25°C/ relative humidity (RH) 60% respectively. Countries coming under climatic zones III and IV are

having climatic condition, temperature 30°C/ relative humidity (RH) 35% and temperature 30 °C/ relative humidity (RH) 70% respectively. India comes under climatic zone III & IV.[7] Stability period for Climatic zone I & II was obtained as 23.846 months (1.99 yrs) and for Climatic zone III & IV was obtained as 15.718 months (1.31 yrs). According to Siddha Formulary of India[12], *chooranams* retain their potency for 3 months. In the present study, it is observed that real time stability data of *Nilappanai chooranam* showed very good stability for more than 1 year.

CONCLUSION

The potency of herbal preparations will be reduced after a certain period of time. To have efficacy and safety for these formulations, it is mandatory that product should be stable physically, chemically, microbiologically and therapeutically. The formulation should be free from toxicity. In the present study, no change is observed in organoleptic characters such as colour, odour and taste. In the present study, the stability of the drug is confirmed since there is "no significant changes" in physico-chemical parameters at accelerated condition up to 6 month storage. The present investigation supports that the *chooranam* was suitable at accelerated condition up to 6 month storage. It was observed that shelf life of *Nilappanai chooranam* is 23.846 months = 1.99 yrs for countries which comes under climatic zone I & II and 15.718 months = 1.31 yrs for countries which comes under climatic zone III & IV.

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