International Journal of Applied Ayurved Research ISSN: 2347- 6362
Published online in: https://ijaar.in

EFFECT OF CLASSICAL AND LYOPHILIZED DRYING METHODS ON STABILITY OF JUSTICIA ADHATODA L. POWDER

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https://doi.org/10.70057/ijaar.2024.61102

ABSTRACT

Background: Recent advances in various drying techniques play a significant role in the stability and efficacy of the drug. Powder formulation is the most widely used dosage form.

Aim: To evaluate the stability of classically and lyophilized prepared leaves powder of *Justicia Adhatoda* L. through accelerated stability study as per ICH guideline Q1A .

Materials and Methods: The raw material was collected from the botanical garden of Government Ayurveda Pharmacy, Rajpipla and was certified by Pharmacognosy Department, Food and Drug Laboratory, Vadodara. The drying process of *Vasa* leaves was done by two different methods, one naturally in shade and the other by lyophilization. Both the samples were subjected to accelerated stability study following standard ICH guideline Q1A. For that organoleptic, Physico‑chemical parameters, quantitative HPTLC analysis, heavy metal and microbial contamination were evaluated at specific intervals.

Results: The study showed that no significant changes were observed in the organoleptic and physicochemical parameters up to 6 months of controlled storage under accelerated conditions. Microbial load and heavy metals were found within permissible limits in both the samples. The results of various physicochemical parameters of the initial month were considered to evaluate the intercept and slope.

Conclusion: It can be inferred that the shelf life of shade dried and lyophilized powder of Vasika leaves is 2.22 and 4.43 years respectively.

Key word: *Vasica* (*Justicia adhatoda* L.), Accelerated Stability study, Shelf life, Lyophilization

INTRODUCTION: *Justicia Adhatoda* L. belonging to Acanthaceae family is an important and easily available Ayurvedic medicinal herb [i]. The major alkaloid of the plant, Vasicine has been found to be biologically active and is the subject of many chemical and pharmacological studies [ii].

To date, many researches have been carried out to evaluate the influence of drying methods on particular plants. Classical shade drying process emphasizes by ancestors but it takes more time and inability to achieve consistent quality standards in large scale production. In recent era, more attention is paid to increase the shelf life of drugs in drying methods. Lyophilization has been effectively practiced by many disciplines to overcome the limitations of the traditional method of simple drying and powdering because it takes very less time as compare to classical method and it has some unanticipated and potentially significant effects on constituent profiles and the medicinal action of plants[iii]. This process has been enhancing the stability of

a dry powder as well as the product stability in a dry state [iv].

All these comments about powder stability are empirical and should be scientific. In this era need of long shelf life of formulations for marketing purpose and stability testing of pharmaceutical products is important for patient's safety[v]. Accelerated stability studies are a common approach for the long-term stability of pharmaceutical formulations [vi]. Currently there are two guidelines, ICH and WHO guidelines, which provide sufficient details regarding the parameters under which stability studies can be conducted [vii].

The objective of this paper is to evaluate the stability of classically and lyophilized prepared leaf powder of Justicia adheota L. It is carried out as a rapid stability study of both samples as a function of temperature and relative humidity under tropical conditions as per ICH guidelines, 2003.

Materials and Methods

Collection of raw material

This raw material was collected from the botanical garden of the Government Ayurveda Pharmacy located in Rajpipla. Sample was authenticated by the Pharmacognosy department, Food and Drug Laboratory, Vadodara.

Preparation of samples

Classical shade drying powder (SP) prepared by reference of Sharangdhara Samhita[viii] and lyophilized powder (LP) prepared as per pharmaceutical engineering [ix].

Procedure

For SP, leaves was collected in fresh form, washed thoroughly with water and dried in shade. For D. LP, leaves, first the paste was prepared and freeze dried. Then both the powders were ground separately by mixer grinder and sieved (mesh no. 85). The prepared powder was stored in suitable containers under controlled temperature. Precautions were taken while preparing the powder and packaging these samples.

Packing

The finished products (SP and LP samples) were packed in required quantities in low-density polyethylene containers (LDPE), which were obtained from the local market and used for storage purposes.

Sample quantity

About 50 grams of drug powder was filled into an LDPE container and stored in an accelerated stability study chamber, tightly closed with a lid.

Storage conditions in the stability chamber

Samples were stored at 40°C ± 2°C, and 75 ± 5% relative humidity.

Frequency of withdrawal

Samples were withdrawn at intervals of 0, 1, 3 and 6 months.

Evaluation of accelerated stability testing

Both powders were filled in containers and labelled properly including the drug name and date of preparation. The accelerated stability study was carried out for the period of 6 months[x]. Temperature was regulated at 40°C ± 2°C with relative humidity (RH) 75% ± 5%[xi]. 10% degradation was set as an acceptable point to extrapolate the accelerated stability data. Real time aging factors of 5 and 3.3 were used for extrapolation of shelf life for Climatic Zone I and II countries and Climatic Zone III and IV countries respectively. India falls under Climatic Zone III and IV. Number of months when

10% degradation was calculated using following formula:

Months when 10% degradation occurs = [0 month assay value - {(0 month assay value×10/100)}-Intercept] / Slope

Analytical parameters

Physico chemical analysis was done by testing pH of 10% solution, loss on drying[xii], total ash[xiii], acid insoluble ash[xiv], water soluble extractive[xv] and alcohol soluble extractive values[xvi]. Qualitative high‑performance liquid chromatography (HPLC) was carried out to evaluate the changes in active ingredient[xvii]. Both the samples were also evaluated for the Heavy metal analysis Atomic absorption spectrophotometer as per the methods described in API.[xviii] Total plate count, Total yeast and mould count and the specific pathogens i.e. Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus were analysed as API[xix].

Observation and Results

SP and LP samples were prepared and tested for organoleptic characteristics and various physicochemical parameters under accelerated stability conditions at 0, 1, 3 and 6 months. Overall, minor differences were observed in organoleptic characteristics (Table 1).

LOD values were found more in SP than LP sample which indicates the presence of certain extent of moisture in SP sample, while freeze drying technique dehydrate the sample by mechanical process applied in it. The results of total ash and acid insoluble ash on both samples indicated that there were no more inorganic impurities in the samples. The values of water soluble extract and alcohol soluble extract were in compliance with the quality parameters as per API limits (Table No. 2). While microbial analysis was in permissible limit[xx].

To confirm the shelf life / stability of product, change in the assay from its initial value should not vary more than 5% and meet the acceptance criteria such as appearance, physical and chemical attributes etc. However, even 90% of labelled potency is commonly considered as the minimum acceptable potency level.[xxi] Based on the values obtained at different stages; intercept, slope (Table No.6) and expected time (in months) for 10% of degradation were calculated for individual parameters of both drugs (Table No.7). India comes under climatic zone IV the mean obtained of these months was multiplied with 3.3 to extrapolate shelf-life (Table No.8).

Table.No.1: Organoleptic Characters of both samples

Sr. No.	Sample	Organoleptic Characters			
		Colour	Odour	Texture	Taste
1	SP*	Green	Characteristic	Slightly rough	Bitter
2	LP*	Dark Green	Characteristic	Slightly rough	Bitter

SP* Shade dried powder, LP* Lyophilized powder

Table.No.2: Physico-chemical parameters of both samples

Parameters	P.L. as per API	'0' month		'1' month		'3' months		'6' months	
		SP*	LP*	SP*	LP*	SP*	LP*	SP*	LP*
pH (%)	-	8.06	8.69	8.06	8.69	8.17	8.70	8.23	8.77
LOD (%)	NMT* 12%(IP)	6.36	0.891	6.60	2.73	6.68	6.03	9.13	7.79
Total Ash (%)	NMT* 21%	12.92	14.06	13.47	14.54	13.76	14.58	13.84	14.83
AIA (%)	NMT* 2%	0.50	0.44	0.61	0.59	0.64	0.68	1.28	1.10
ASE (%)	NLT* 3%	11.99	11.91	12.81	13.26	14.44	14.54	17.09	19.51
WSE (%)	NLT* 22%	22.81	23.67	25.56	27.10	26.76	27.22	26.05	28.61

LOD* Loss on drying, AIA*Acid Insoluble Ash, ASE* Alcohol Soluble Extractive, WSE*Water Soluble Extractive, SP*Shade dried powder, LP* Lyophilized powder, P.L.* Permissible Limit, NMT* Not more then, NLT* Not less then, API* Ayurvedic Pharmacopeia of India, IP* Indian Pharmacopeia

Assay of Vasicine by HPLC

Table No. 3: Result of Assay of Vasicine by HPLC of both samples

Sr. No.	Name	0 Month	6 Months	As per IP* Value[xxii]
1	SP*	1.69 %	0.55 %	NLT* 0.6%
2	LP*	1.73 %	0.56 %	

SP*Shade dried powder, LP* Lyophilized powder, NLT* Not less then, IP* Indian Pharmacopeia

Heavy metal analysis

Table No. 4: Heavy metal analysis in both samples

Parameters	Permissible limit	SP*	LP*
Lead	10 ppm	0.888 ppm	0.173 ppm
Cadmium	0.5 ppm	N.D.	N.D.
Arsenic	3 ppm	2.675 ppm	0.234 ppm
Mercury	1 ppm	N.D.	N.D.
Note: It is compliance with standard Parameters of API			

SP*Shade dried powder, LP* Lyophilized powder

Microbial Test

Table No. 5: Microbial test in both the samples

Parameters	Permissible limit	'0' month		'6' months	
		SP*	LP*	SP*	LP*
Total Plate Count (cfu/g)	10^5 cfu/g*	2115	1701	655	345
Total Yeast & Mould Count (cfu/g)	10^3 cfu/g	538	95	296	Nil
Escherichia Coli	Should be Absent	Absent	Absent	Absent	Absent
Salmonella Spp	Should be Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Should be Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Should be Absent	Absent	Absent	Absent	Absent

cfu/g* Colony forming unite per gram

SP* Shade dried powder, LP* Lyophilized powder

Table No. 6: Intercepts and Slope of both samples

Parameters	Intercepts		Slope	
	SP*	LP*	SP*	LP*
pH (%)	8.05	8.68	0.031	0.013
LOD (%)	6.06	1.50	0.45	1.14
Total Ash (%)	13.16	14.23	0.134	0.10
AIA (%)	0.44	0.44	0.125	0.105
ASE (%)	24.20	24.99	0.43	0.66
WSE (%)	11.95	16.01	0.84	0.51

LOD* Loss on drying, AIA* Acid Insoluble Ash, ASE * Alcohol Soluble Extractive, WSE* Water Soluble Extractive, SP* Shade dried powder, LP* Lyophilized powder

Table No. 7: Approximate period for 10% Degradation

Parameters	Initial month Value		10% Degradation		Months required for 10% degradation	
	SP*	LP*	SP*	LP*	SP*	LP*
pH (%)	8.05	8.69	7.254	7.821	25.67	63.16
LOD (%)	6.36	0.891	5.724	0.801	0.74	0.61
Total Ash (%)	12.92	14.06	11.62	12.65	11.51	15.76
AIA (%)	0.50	0.44	0.45	0.396	0.08	0.42
ASE (%)	22.81	23.67	20.52	21.303	8.56	5.58
WSE (%)	11.99	11.91	10.755	10.719	1.44	10.35
Mean Months					8	15.98

SP*Shade dried powder, LP* Lyophilized powder, LOD* Loss on drying, AIA* Acid Insoluble Ash, ASE* Alcohol Soluble Extractive, WSE* Water Soluble Extractive, P.L.*Permissible Limit

Table No. 8: Extrapolation of Shelf life

Sample	Mean Months for 10% degradation	Multiplication Factor	Shelf life	
			Months	Years
SP*	8	3.33	26.64	2.22

LP*	15.98	3.33	53.21	4.43

SP* Shade dried powder, LP* Lyophilized powder

DISCUSSION: Indeed, in the absence of advanced analytical tools in ancient times, organoleptic properties (like color, odor, taste, and texture) served as key indicators for determining the quality and efficacy of medicinal formulations. These sensory characteristics were essential in ensuring that the products maintained their intended therapeutic effects. With the advancement of modern science, there is an increasing need for more rigorous methods to assess shelf life, especially in the case of herbal medicines. Organoleptic characteristics alone cannot provide conclusive evidence of stability, particularly when chemical degradation occurs on a microscopic level, which may not be visually detectable. That's why additional evaluations such as physico-chemical testing (e.g., pH, moisture content, and active compound stability) and microbial assessments (to detect contamination or spoilage) are crucial for confirming the actual shelf life and safety of the product.

CONCLUSION: Under Rule 161-B of the Drugs and Cosmetics Act, the shelf-life for powder is 2 years. On the basis of the accelerated stability study conducted for SP and LP sample are found to have 2 years and 2 months and 4 years and 4 months respectively. Similar obtained in HPLC analysis of both the samples were initially and after six months showed the minimum deterioration of the product. Heavy metals like arsenic and lead are present within permissible limits, while cadmium and mercury have not been detected. In terms of physico-chemical parameters, lyophilized powder is more stable than shade dry powder.

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Source of support: Nil Conflict of interest: None Declared

Cite this Article as : [Hiral Khunt : Effect of Classical and Lyophilized Drying Methods on Stability of Justicia Adhatoda L. Powder] *www.ijaar.in : IJAAR VOL VI ISSUE XI NOV - DEC 2024Page No: 489-495*

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