Research Article

Accelerated Stability Study of Khamirae Gaozaban Sada

Sadiya Noor ul Basar*[1], Seema Rani*, Roohi Zaman*, Shariq Shamsi ****
*PG Scholar, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.
**Lecturer, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.
***Reader and HOD, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.
***Corresponding author's E-mail:** snbasar136@gmail.com

Accepted on: 28-06-2013; **Finalized on:** 31-08-2013.

ABSTRACT

Evaluation of Stability Studies of various Unani formulations is need of the time. The keen observations of Unani classical authorities need to be substantiated with empirical evidence using scientific methodology. This study has furnished the feasible methods for evaluating shelf life of Unani Drugs. Accelerated Stability Study for 6 months of Unani formulation Khamirae Gauzaban Sada (KGS) was done. The drug was prepared, packed and kept in stability chamber at 40°C and 75% RH and then analyzed for organoleptic, physical, chemical and microbiological parameters. Samples were analyzed at 0, 3 and 6 months. The formulation was assessed for organoleptic characters, physical, chemical and microbiological changes. High Performance Thin Layer Chromatography (HPTLC) with multiple wavelength densitometric scanning was done. The findings showed degradation changes in drug formulation. The present study showed that the shelf life of Khamirae Gauzaban Sada was from 10 months to 1 year. The data collected at Accelerated Stability Study can be extrapolated for intermediate, long term, bracketing and matrixing so that better storage conditions for KGS can be recommended.

Keywords: Accelerated Stability Study, Chemical, HPTLC, Khamirae Gauzaban Sada, Microbiological, Organoleptic, Physical, Unani Formulation.

INTRODUCTION

As we observe in our routine life that all the objects get spoiled after a specific period. All the living beings go through a cycle of birth, growth, reproduction and death. All the non living substances that are grown or manufactured go through a life span in which they are influenced by environment. Everything made by human hands from the sublime Parthenon to the trivial milkshake is subject to decay. There is no existence of such a substance in world which is imperishable. If there is functionally relevant quality attribute of a drug product that changes with time, evaluation of this change falls within the purview of the pharmaceutical scientists and regulatory authorities who quantify the stability and shelf life of drug product.[1] Stability testing of herbal products is a challenging task, because the entire herb or herbal product is regarded as the active substance, regardless of whether constituents with defined therapeutic activity are known. The objective of a stability testing is to provide evidence on how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature, light, oxygen, moisture, other ingredient or excipient in the dosage form, particle size of drug, microbial contamination, trace metal contamination, leaching from the container, etc. and to establish a recommended storage condition, retest period and shelf-life. Therefore evaluation of the parameters based upon chemical, physical, microbiological, therapeutic and toxicological studies can serve as an important tool in stability studies.[2, 3]

MATERIALS AND METHODS

Identification

The ingredients of the Compound formulation KGS (Table 1) were purchased from the market by purchasing committee and identified by Botanist Dr. Sumathi, Research officer, RMR, FRLHT-IAIM, Bangalore. Specimens were preserved in the Repository of Medical Resources (RMR) and the Accession numbers are Gaozaban *(Onosma bracteatum)*-2729, Gule Gaozaban *(Onosma bracteatum)* --2728, Kishneez *(Coriandrum sativum* Linn.*)*-2733, Behmane Surkh *(Salvia haematodes* M.) -2738, Behmane Sufeid *(Centaurea behen* Linn.)-2736, Tukhme Balangu *(Lallemantia royleana* Benth.)-2732, Tukhme Raihan *(Ocimum sanctum* Linn.*)* -2734, Badranjboya *(Melissa officinalis)*-2730. All the samples were found to be of good quality.

Preparation

The Khamira was prepared as described in National Formulary of Unani Medicine under the guidance of Chief Pharmacist of pharmacy unit, NIUM, Bangalore. Ingredients 1 to 10 were soaked in two liters water overnight and 670 ml of decoction was left and filtered, then Qand Safeid was added and boiled till required consistency and reading of 85 was obtained by Saccharometer/Refractometer. Then Khamira was prepared by continuous stirring till it becomes frothy white.[4]

Storage

Container closure system was procured from market. Each drug formulation weighing about 150 gm was packed in 200 gm air tight containers; the containers taken were of plastic material and are transparent in appearance. All precautions were taken while packing these formulations in the containers. Clean and dry containers were used and fitted with air tight lids.

Evaluation

The formulations were filled in three stated packs. They were labeled properly. One pack was tested for various parameters at the time of manufacture; other two packs were kept in stability chamber for accelerated stability study. Temperature was regulated at 40° C and relative humidity at 75%. The second pack was opened after 3rd month and studied for various parameters. The third pack was opened after 6th month and studied for various parameters .The procedures were strictly followed according to ICH Tripartite Guidelines.

Appearance

Appearance was recorded according to the consistency whether semisolid, semiliquid etc.[1, 5]

Determination of Color

The color of the drug formulation was noted by using the Munsell color charts . If any changes occurred were noted.[5-10]

Determination of Odor

The description of this feature sometimes may not be accurate because it depends on individual perception. If the material is expected to be innocuous, a small portion of the sample can be examined by slow and repeated inhalation of air over the material. The strength of the odor like weak, distinct, strong is first determined and then the odor sensations like musty, mouldy, rancid, fruity, aromatic etc were determined.[5, 8-10]

Determination of Taste

First of all the depth of organoleptic capacity should be tested. This can be done by asking the volunteer to taste serial dilutions of drugs. It should be noted that the volunteers do not taste in ordinary sense. In so doing they would have to score the degree of flavouring, e.g., was it less than present originally, i.e., was the flavour being lost? They would also have to be able to describe the flavour well originally[5, 8,9,10].

Physical Parameters

Determination of pH

pH of 1% solution- 1 gm of drug was accurately weighed and dissolved in accurately measured 100 ml of water, then filtered and pH was checked with a standardized glass electrode.

pH of 10% solution-10 gm of drug was accurately weighed and dissolved in accurately measured 100 ml of water, then filtered and pH was checked with a standardized glass electrode.[5,11]

Determination of Moisture Content

Azeotropic method (Toluene distillation)

The apparatus consists of a glass flask connected by a tube to a cylindrical tube fitted with a graduated receiving tube and a reflux condenser. The receiving tube is graduated in 0.1-ml divisions so that the error of readings does not exceed 0.05 ml. The preferred source of heat is an electric heater with a rheostat control was taken. The upper portion of the flask and the connecting tube was insulated. Receiving tube and the condenser of the apparatus, was thoroughly cleaned, rinsed with water and dried. 75ml of toluene R and about 10 gm of drug was introduced into a dry flask. Flask was heated to distil the liquid over a period of 6 hours, and then allowed for cooling for about 30 minutes and reading of the volume of water to an accuracy of 0.05 ml (first distillation). Accurately a 10 gm drug material was weighed A few pieces of porous porcelain was added and the flask was heated gently for 15 minutes. When boiling began, 2 drops per second was distilled until most of the water has distilled over, then rate of distillation was increased up to about 4 drops per second. As soon as the water was completely distilled, inside of the condenser tube was rinsed with toluene R. the distillation was continued for 5 more minutes, removed from the heat, the receiving tube was allowed to cool at room temperature and droplets of water adhering to the walls of the receiving tube were dislodged by tapping the tube. The water and toluene layers were allowed to separate; the volume of water (second distillation) was noted. Content of water was calculated as percentage using the formula: $\mathbf{100(n_1-n) / w}$

Where, w = the weight in g of the material being examined

n = the number of ml of water obtained in the first distillation

n_1 = the total number of ml of water obtained in both distillations.[8,11]

Determination of Ash value

Total ash

4gm of the drug was accurately weighed, in a previously ignited and tarred crucible (Silica). Material was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until it turned white, indicating the absence of carbon. Then it was cooled in a desiccator and weighed. Carbon free ash was not obtained in this manner, so crucible was cooled and residue was moistened with about 2 ml of water. It was dried on a water-bath, then on a hot-plate and ignited to constant weight. Residue was allowed to cool in a desiccator for 30 minutes and then weighed without delay. Content of total ash in mg / gm of drug was calculated.[2, 8, 11]

Acid-insoluble ash

To the crucible containing the total ash, a 25 ml of dilute hydrochloric acid, was added and covered with a watch-glass and boiled gently for 5 minutes. Watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. Insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, then dried on a hot-plate and ignited to constant weight. Residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash was calculated in mg/ gm of material.[2,8,11]

Water-soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water-soluble ash in mg/ gm of material was calculated. [2,8,11]

Chemical Parameters

Determination of Aqueous Extractive Value

4gm of drug material was accurately weighed, in a glass-stoppered conical flask. Macerated with 100 ml of the water for 6 hours, shaking frequently, was then allowed to stand for 18 hours. Then rapidly filtered taking care not to lose any solvent and then 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath, then dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was weighed without delay. The content of extractable matter was calculated in mg/ gm of material.[5,8,11]

Determination of Alcohol Extractive Value

4gm of drug material, accurately weighed, was placed in a glass-stoppered conical flask. Macerated with 100ml of the Ethanol for 6 hours, by shaking frequently, and then was allowed to stand for 18 hours. Then rapidly filtered taking care not to lose any solvent then 25 ml of filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter in mg / gm of material was calculated.[5,8,11]

Determination of tannins

5 gm of drug was accurately weighed and placed, into a conical flask. 150 ml of water was added and heated over water-bath for 30 minutes. The mixture was cooled and transferred to a 250-ml volumetric flask and diluted to 200ml volume with water. The solid material was allowed to settle and filtered through a filter-paper, discarding the first 50ml of the filtrate. The total amount of material that is extractable into water was determined by evaporating 50ml of the extract to dryness; the residue was dried in an oven at 105°C for 4 hours and weighed (T_1).

The amount of drug not bound to hide powder that is extractable into water was determined for which 80 ml of the extract was taken, 6g of hide powder was added and then shaken well for 60 minutes then filtered and 50 ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105°C and weighed (T_2).

To determine the solubility of hide powder, 6g of hide powder R was taken, 80 ml of water was added and shaken well for 60 minutes than filtered and 50ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105°C and weighed (T_0). The quantity of tannins as a percentage was calculated using the following formula:

$T_1-(T_2-T_0) \times 500/ w$

w- Is the weight of the drug[8, 11]

Estimation of Total Alkaloids

5 gm of the drug was taken in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to the sample. The mixture was covered and allowed to stand for 4 hrs. The mixture was then filtered and the extract was allowed to become concentrated in a water bath until it reached 1/4th of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete.

The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, so obtained was alkaloid, which was dried and weighed.[12]

Isolation of Glycosides

The glycosides content of the extracts was determined by dissolving 10g of the extracts in 100ml of 50% H_2SO_4 in test tubes. The mixture was heated in boiling water for 15minutes and 10ml of Fehling solution added, and the mixture boiled. A red precipitate in each extract tested, indicated the presence of glycosides. The percentage glycoside was calculated.[13]

HPTLC Fingerprinting of Unani formulation KGS

Preparation of sample solution

10g given formulation was dissolved in 100ml of HPLC water. Solution was then extracted with Dichloromethane (3x100ml) using separating funnel. DCM fraction was concentrated to dryness and dissolved in 5ml of DCM and used for TLC application.

Chromatographic conditions

Analysis was performed on 2.5x 10cm silica gel 60 F_{254} plates. sample solutions was applied using Linomat 5 (Camag Switzerland) automated spray on band applicator equipped with a 100µl Hamilton syringe and operated with the following settings: Band length 8mm, distance

from the plate edge 12.5mm, and distance from the bottom of the plate 10mm.

Development of the plate was carried out allowing 20min for saturation of the twin trough chamber (Camag Switzerland) at room temperature. Solvent system used was Toluene: ethyl acetate: formic acid (7:2.5:0.5) and migration was 8cm. After development the plate was evaluated under UV 254 and 366 nm, the plate was derivatised with Vanillin sulphuric acid and kept in oven at 110°C and evaluated under visible light using CAMAG TLC Visualiser and scanned using CAMAG TLC SCANNER 3.[14]

Microbiological Assay

Enumeration of micro organisms (bacteria/fungi) by serial dilution agar plate method

Requirements

Sample, 1%Nacl, 9ml dilution blanks, sterile 1ml pipettes, sterile petri plates, nutrient agar, potato dextrose agar medium.

Procedure

50ml each of nutrient agar medium and potato dextrose agar medium was prepared. 1%Nacl was prepared and 10ml was suspended to first test tube and 9ml saline was suspended to the remaining tubes labeled as (10^{-1} to 10^{-6}) each. The above media and tubes containing saline were autoclaved at 121°C for 15mins. After autoclaving contents were brought to laminar air flow and all the tubes containing saline were cooled. 1 gm of sample was weighed and transferred to the first tube containing 1%Nacl and mixed gently for uniform suspension. It was allowed to stand for some time. 1ml of suspension was transferred from the first tube to the next tube labeled as 10^{-1}.

Further the dilutions were made till the tube labeled as 10^{-6}. Once the dilutions were done 0.1ml of suspension was taken from tube labeled as10^{-1} and plated on sterile petri plate. In the same way 0.1ml of suspension was taken from tubes labeled as 10^{-3} and 10^{-5} each and plated on 2 sterile petri plates. Once the plating was done a thin layer of media cooled to 45°C was poured into the plates containing sample and plates were gently rotated for uniform distribution of cells.

For Bacteria—Nutrient agar media was poured and after solidification the plates were incubated at 37°c for 24 hours in inverted position.

For Fungi Potato dextrose agar media was poured and after solidification the plates were incubated at room temperature for 5days.[15]

RESULTS AND DISCUSSION

The observation from the stability study of **KGS** shows that there was a considerable change in organoleptic characters from 3rd month onwards. Appearance of the drug formulated was semisolid at 0 month, at 3rd month also it was semisolid with little caking and at 6th month caking was seen predominantly. Colour of the drug formulated matched YR colour of Munsell colour charts, YR is yellow red colour. Colour changes were noticed at 3rd month. It changed from 7.5YR5/6 at 0 month to 7.5YR4/6 at 3rd month and 7.5YR3/6 at 6th month.[9,10] The odour of the KGS was distinct at 0 month, and its distinctness changed from 3rd to 6th month. Changes in taste were noticed in from 3rd month onwards of KGS. The results from organoleptic observations indicated that the KGS showed significant changes at 3rd month, when these results were inferred from ICH Tripartite Guidelines.[16]

Increase or decrease in percentage from based data to 3rd month and 6th month was calculated by using percent change formula.

Moisture content increased from 21% at 0 month to 21.5% at 3rd month and 22.5% at 6th month; there were 2% increase in moisture from 0 month to 3rd month and 4.6% increase in moisture from 3rd to 6th month. It showed that there was 7% increase in moisture from 0 month to 6th month and remained equally high in 3rd and 6th month. It implies that packaged material was not of standard quality.[2, 3,9,10]

The pH at 1% solution of the KGS decreased from 5.63 at 25^0 C in 0 month, 5.54 at 25^0 C at 3rd month and 5.1 at 25^0 C at 6th month. It indicated that there was 1.5% difference from 0 month to 3rd month and 7.9% difference from 3rd to 6th month which shows that there was 9.4% decrease in pH from 0 month to 6th month. For pH at 10% solution it was 4.83 at 0 month and 4.76 at 3rd month and 4.3 at 6th month; it showed 1.4% difference from 0 month to 3rd month and from 3rd to 6th month difference was 9%. It indicates that there was 10% decrease in pH from 0 month to 6th month. This implies that there was significant change in pH and moisture content, thus it can be assumed that chemical degradation was occurring in the drug. But Ash values did not have any changes from 0 to 6th month, which implies that there was no trace metal contamination.[2,9,10,17]

Regarding the chemical stability, aqueous extractive value showed 8.20% of extractive value at 0 month, 8.1% at 3rd month and 7.5 % at 6th month. Aqueous extractive value from 0 month to 3rd month showed degradation of 1.2% and from 3rd to 6th month; it showed degradation of 7.4 %, this indicates that degradation from 0 month to 6th month was 8.5%. It implies that there was significant change in aqueous extractive value from 0 month to 6th month.[9,10,16,18]

Alcohol extractive value showed extractive value of 4.2% at 0 month and 4% at 3rd month and 3.8% at 6th month. Alcohol extractive value from 0 month to 3rd month showed degradation of 4% and from 3rd to 6th month it shows degradation of 5%, this indicates that degradation from 0 month to 6th month was 9.5 %. It shows there was significant change in Alcoholic extractive value from 0 month to 6th month. The decrease in extractive values

denotes that there is depletion in concentration of the drug constituents.

Alkaloids content of KGS was 0.46% at 0 month, 0.45% at 3rd month and 0.42 % at 6th month. Alkaloid content from 0 month to 3rd month showed degradation of 2.2% and from 3rd to 6th month it showed degradation of 6% this indicates that degradation from 0 month to 6th month was 8.6%. It implies that there was significant change in Alkaloid content from 0 month to 6th month.[18]

Glycoside content of KGS was 0.9% at 0 month and 0.88% at 3rd month and 0.82 % at 6th month. Glycoside content from 0 month to 3rd month showed degradation of 2.2% and from 3rd to 6th month showed degradation of 6.8% this indicates that degradation from 0 month to 6th month was 8.8%. It shows there was significant change in Glycoside content from 0 month to 6th month.[9,10]

Tannins content of KGS is 0.5% at 0 month and 0.49% at 3rd month and 0.46 % at 6th month. Tannins from 0 month to 3rd month showed degradation of 2.0% and from 3rd to 6th month shows degradation of 8%, this indicates that degradation from 0 month to 6th month was 3.3%. It shows there was significant change in tannins content from 0 month to 6th month.[9,10]

Microbial study showed no bacterial load after 24 hours incubation in Agar Nutrient Media at 37^0C at 0, 3 and 6 month and also no fungal count after 5 days of incubation at room temperature. The zero viable count reflects that the drug is safe microbiologically. Its antimicrobial action may be the possible cause of getting zero viable counts in KGS.[2, 3]

HPTLC fingerprinting of KGS: Different compounds absorb light at different wavelength. In this case peaks were found at 280nm with maximum peak area, few peaks were detected with low intensity at UV 360nm. Peaks were not found at visible region (254nm). At 0 month 6 peaks were noticed i.e. at Rf 0.13 area was 23968.3 with height 763.5, at Rf 0.21 area was 3365.5 with height 146.5 , at 0.43 Rf area was 7435.9 with height 226.9 , at Rf 0.6 area was 2056.8 with height 90.2 and at Rf 0.66 area was 2056.8 with height 90.2, at Rf 0.77 area was 3204.2 with height 76.2, whereas in the 3rd month 4 peaks were noticed at Rf values 0.16 with area 23750 with height 681.3 , at Rf 0.23 area was 14551.3 with height 555.9, at Rf 0.64 area was 1725.1 with height 48.4, at Rf 0.81 area was 825.2 with height 28.6 This implies that at Rf 0.21 there was increase in height and area, which shows that there may be formation of degradation products , while at 0.13, 0.66, 0.77, there is decrease in area and height which shows that there is decrease in concentration of the constituent, while Rf, peak and area at 0.43 is not seen which shows that there is loss of constituent, while at 6th month 4 peaks were seen, at Rf 0.14 area was 23519.6 with height 661.6, at Rf 0.21 area was 16015.6 with height 566.2, and at Rf 0.66 area was 1373.4 with height 44.3 and at Rf 0.8 area was 422.7 with height 20.7, this implies that at Rf 0.21, there was formation of degradation products , and there was loss of one constituent at Rf 0.43 and there was decrease in concentration at Rf 0.14, 0.66, 0.8 , this study shows that there is significant change in Rf values, area and height from 0 month to 6th month[19] Results have been shown in table 2 and TLC plates have been shown from figure 1-4.

Stability study showed that there was significant difference in values from 3rd month to 6th month and all the readings were above limit of 5% degradation which is not in acceptance criterion for stability of the drugs but not much changes were noticed from 0 to 3rd month when compared with standard ICH guidelines, table for "Extension of the International Conference on Harmonization Tripartite Guideline for stability testing of new drug substances and products to Countries of Climatic Zone iii and iv" it has been stated that 6 month Accelerated study gives a provisional shelf life of 20 months at 30^0C.[18] As the study shows very little degradation between 0 to 3rd month but more that 5% degradation after 3rd month. Thus a provisional shelf life of 10 months to 12 months can be expected. Whereas Unani physicians have noticed the age of the Khamira for 3 years and in Gazzette of India draft rule 161B states that Stability of Khamira is 3 years [20,21], but the stability study disproves the claims. This does not validate the claim of Unani physician's empirical observations.

Table 1: Showing Ingredients of compound formulation

Ingredient	Botanical Name	Quantity
Gauzaban	*Onosma bracteatum*	50 gm
Gule Gauzaban	*Onosma bracteatum*	30 gm
Kishneez	*Coriandrum sativum* Linn.	10 gm
Abresham	*Bombyx mori*	10 gm
Behman Surkh	*Salvia haematodes* M.	10 gm
Behman Safaid	*Centaurea behen* Linn.	10 gm
Sandal Safaid	*Santalum album Linn*	10 gm
Tukhme Balangu	*Lallementia royleana* Benth.	10 gm
Tukhme Raihan	*Ocimum sanctum* Linn	10 gm
Badranjboya	*Melissa officinalis*	10 gm
Qand Safaid	*Saccharum officinarum*	1.5 kg

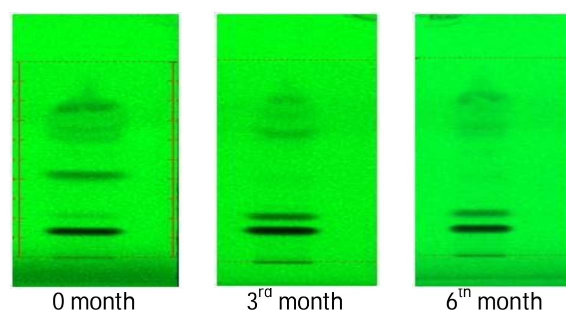

Figure 1: HPTLC profile of khamirae Gauzaban Sadaunder UV 254 nm.

Table 2: Showing Rf values, Peaks, Area and Height

Month	Under UV 254nm		Under UV 366nm		After spraying vanillin sulphuric acid.		No. of Peaks at 280nm and their peak area and height			
	Rf value	Colour	Rf value	Colour	Rf value	Colour	No. of peaks	Rf value	Area	Height
0 month	0.13	Dark	0.09	green	0.13	green	6 peaks	0.13	23968.3	763.5
	0.21	Light	0.17	Blue	0.49	pale violet		0.21	3365.5	146.7
	0.43	Dark	0.25	Blue	0.55	Violet		0.43	7435.9	226.9
	0.6	Light	0.55	green	0.62	Violet		0.6	2056.8	90.2
	0.66	Light	0.61	Blue	0.67	Pale violet		0.66	2056.8	90.2
	0.77	Dark	0.7	Blue	0.79	Violet		0.77	3204.2	76.2
3rd month	0.16	Dark	0.11	green	0.15	green	4 peaks	0.16	23750	681.3
	0.23	Light	0.20	Blue	0.80	Violet		0.23	14551.3	555.9
	0.64	Light	0.27	Blue				0.64	1725.1	48.4
	0.81	Dark	0.54	green				0.81	825.2	28.6
			0.62	Blue						
			0.67	Blue						
6th month	0.14	Dark	0.10	green	0.15	green	4 peaks	0.14	23519.6	661.6
	0.21	Light	0.18	Blue	0.82	Violet		0.21	16015.6	566.2
	0.62	Light	0.24	Blue				0.66	1373.4	44.3
	0.80	Dark	0.55	green				0.88	422.7	20.7
			0.67	Blue						
			0.82	Blue						

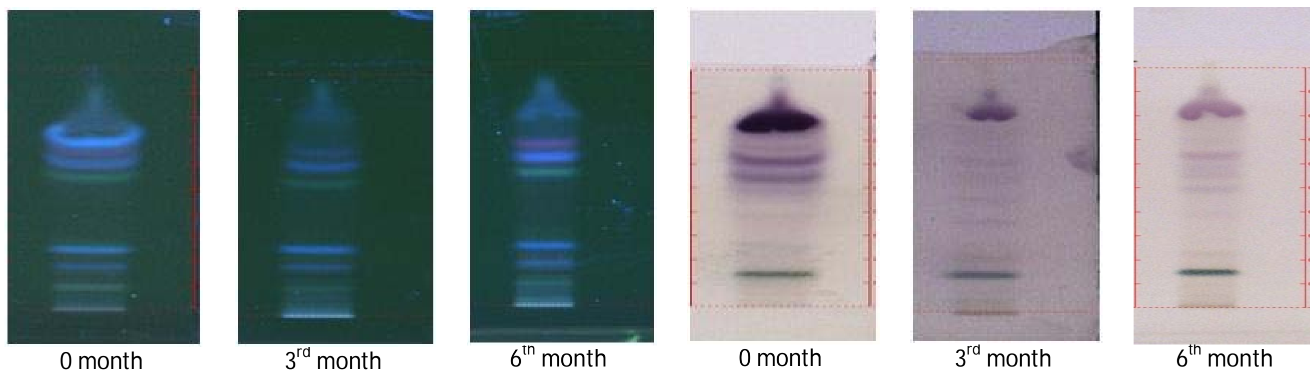

Figure 2: HPTLC profile of Khamirae Gauzaban Sada under UV 366nm.

Figure 3: HPTLC profile of Khamirae Gauzaban Sada after derivatised with vanillin sulphuric acid under white light

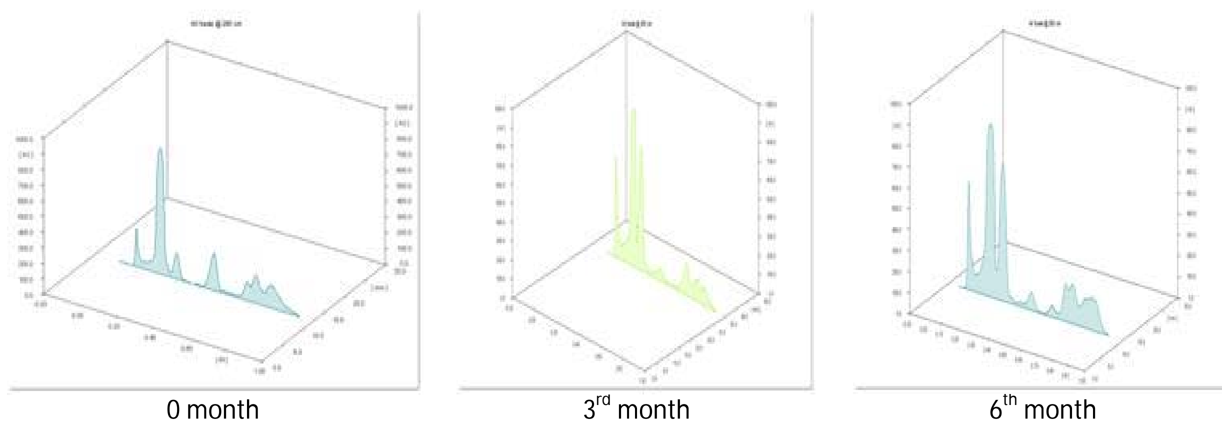

Figure 4: HPTLC densitometric scan of Khamirae Gauzaban Sada extract at UV 280nm

CONCLUSION

The study showed that the provisional shelf life of drug KGS may be from 10 months to 1 year, Physical parameters, Chemical parameters showed degradation more than 5 % which is more than the acceptance criteria according to ICH Guidelines.

Acknowledgements: We extend sincere thanks to Prof Nagarajan, Research officer, CPPP FRLHT, Madhuri Research fellow, CPPP FRLHT , Dr. Sumathi, Dr. Suma T.S and Dr. Noorunnisa Repository of Medical Resources , FRLHT, Mr. Mahesh, Azyme bio sciences, for there sincere help in accomplishing my research work.

REFERENCES

1. Carstensen JT, Rhodes CT, Drug Stability- principles and practices, Marcel DekkerInc, New York, 2(3), 2000, 3, 4, 5, 331, 262-325.
2. Thakur AK, Prasad NA, Laddha KS, Stability Testing of Herbal Products, Pharma Review, Kongposh publications, 2008, 109-114. www.kppub.com/articles/.../stability-testing-herbal-products.
3. Anonymous, WHO Regional Guideline for Mediterranean region: Stability testing of Active substances and pharmaceutical products, Draft 2.0, 2006. http://www.who.int/medicines.
4. Anonymous, National formulary of Unani medicine, Part 1, New Delhi, CCRUM, MOHFW, Govt. of India, Ramko Press, 2006, 110.
5. Anonymous, WHO Guideline on stability of pharmaceutical products, 2007.
6. Chemical Spot Test Kits for Preliminary Identification of Drugs of Abuse, National Law Enforcement and Corrections Technology Center and NIJ Standard–0605.00 Rockville, 1981. www.ncjrs.gov/pdffiles1/nij/183258.pdf.
7. Munsell color system, irtel.uni-mannheim.de/colsys/Munsell_A0.pdf
8. Ahmed H, Stability Studies of sharbath & safoof, Thesis, NIUM, Dept of IlmulAdvia Bangalore, Karnataka, 2007.
9. ICH Q6B, Specifications: Test Procedures and Acceptance Criteria for Biotechnological /Biological Products. http://www.ich.org/LOB/media/MEDIA432.pdf
10. ICH Q6A, Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances. http://www.ich.org/LOB/media/MEDIA430.pdf.
11. Anonymous, WHO, Quality control methods for medicinal plant materials, World Health Organization, Geneva, 34-35,36,50,71-75
12. Gracelin HS, Qualitative and quantitative analysis of phytochemicals in five pteris species, International Journal of Pharmacy and Pharmaceutical Sciences, 5(1), 2012, 105-107. www.ijppsjournal.com
13. Vivek D, Pharmacognostical and Pharmaceutical studies of *Achyranthes Aspera*, World journal of Pharmacy and Pharmaceutical sciences, 1(4), 2012, 1316-1331. www.wjpps.com
14. Meena R, Evaluation of an Unani compound formulation *Majoone Sandal*, International journal of pharma sciences and research (IJPSR), 1(5), 2010, 238-242. www.ijpsr.info/ijpsr-v1n5.php
15. Aneja KR, Experiments in Microbiology, Plant Pathology and Biotechnology, New Age International Pvt Ltd Publishers, 2009, 69-72, 192.
16. Banker GS, Rhodes Ct, Modern Pharmaceutics, Marcel dekker Inc, New York, Basil, 4, 2002, 215-251. https://www.federalregister.gov
17. Thakur I, Umang G, Patil N, Dhabi N, Novel approaches for stability improvement in natural medicines, Review article, 5(9), 2011, 48-49. http://www.phcogrev.com/text
18. Grim W, Extension of International Conference on Hormonization Tripartite Guideline for stability testing of New Drug Substances and products to countries of climatic zones III and IV, Drug Development and Industrial pharmacy, 24(4), 1998, 313-325. www.ncbi.nlm.nih.gov/pubmed
19. Kathrin K, Eike R, Anne B, Validation of standardized high performance thin layer chromatographic methods for quality and stability testing of medicines, Journal of AOAC International, 86(5), 2003, 909-915. www.ijpsr.com
20. Multani HC, Khan FNK, Hindustan wa Pakistan ki jadi bootiyan, Delhi Central dawakhana, Lahore, YNM:Preface V111.
21. Govt. Of India, Department of Ayush Drugs and Cosmetics (Amendment) Rules, Ministry Of Health and Family Welfare 2005, The principal rules were published in the Gazette of India, vide notification number F.29-10/45-II dated the 21st December, 1945 and last amended vide number G.S.R. 651(E), dated the 9th September, 2009, New Delhi, the 24th November, 2005. amam-ayurveda.org/pdf.

Source of Support: Nil, **Conflict of Interest:** None.