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Research Article

ACCELERATED STABILITY STUDY OF MAJOONE FALASIFA (A UNANI HERBAL FORMULATION)

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ABSTRACT

Present study was conducted to evaluate shelf life of Majoone falasifa at accelerated storage conditions. In-house prepared Majoone falasifa packed in multiple air tight transparent PET containers was challenged with temperature and humidity at 40±2ºC/75±5%RH in photo-stability chamber for the period of six months. Packs were tested for various analytical parameters at zero, one, three and six month. Another two packs of MF was evaluated for photostability at overall illumination of 1.2 million lux hours with UV energy of 200Watt hours/square meter and 2.4 million lux hours with UV energy of 400Watt hours/square meter light intensity .No significant change in organoleptic characters of Majoone falasifa were observed at accelerated thermo-humidity condition till six month as well as in photostability at 2.4 million lux hours with UV energy of 400Watt hours/square meter light intensity. The changes in moisture content, ash value, pH, alcohol and water soluble matter, successive extractives, saponification value, iodine value, acid value and total alkaloid was less than 5%. HPLC showed minimum changes. Microbial studies were in confirmation to the WHO guideline. The present study showed that the shelf life of Majoone falasifa was up to 20 months.

Keywords: Accelerated Stability Study; Photostability; Shelf life; Majoone falasifa; Unani system of medicine.

INTRODUCTION

Nothing in this universe is immortal; all things that have form eventually decay. Medicinal products also degrade with time thus significance and indispensability of stability testing in development of dosage forms/formulation is well recognized. Stability testing is necessary to ensure that the product is of acceptable quality throughout its entire storage period.[1] The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of various environmental factors.[2] Recent efforts by the International Conference on Harmonization with regard to stability have brought an increased regulatory scrutiny at very low level.[3] Further Ministry of Health and Family Welfare, Dept. of AYUSH also admitted that the potency of ASU preparations looses/reduces after a certain period of time. Hence display of expiry date on container or package has been made mandatory.[4] Unani literature describes shelf life as that period within which the drug is able to keep its temperament, constituents and structural constitution in equilibrium, which may be judged by means of observing its organoleptic characteristics. Until these characteristics are intact the drug is said to be stable, and in case of any deviation it is assumed that the drug has lost its stability.[5]

Stability testing aims to document how environmental factors, such as humidity, temperature, light and UV visible radiation may alter the quality of a drug substances or product.[6] Stability study can be that of long term study, conducted for 12 months with storage conditions of 25°C±2°C/60% RH±5% or 30°C±2°C/65 RH±5%RH. In case of intermediate stability study, the storage conditions are set as 30°C±2°C/65%RH±5%RH, for the period of 6 months. Stability study can be that of accelerated type wherein storage conditions are: 40°C±2°C/75%RH±5%RH. This type of stability is carried out for six months. At the accelerated storage condition, minimum of three time points [e.g. 0, 3, and 6 months], including the initial and final time points is recommended for analysis. In case of expectation exists that the results from accelerated studies are likely to approach significant change criteria, increased testing should be done either by adding samples at the final time point or by including a fourth time point in the study design.[2,7] In case of our study we introduced fourth time point at the level of one month to monitor the status of results in more compact way.

ICH guidelines covering the stability testing of new drug substances and products noted that light testing should be an integral part of stress testing. The intrinsic photo stability characteristics of new drug substances and products should be evaluated to demonstrate, that an appropriate light exposure does not result in unacceptable changes. Normally, photostability testing is carried out on a single batch of material selected.[8] Test samples for photo stability were subjected to overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 Watt hours/square meter. A cool white fluorescent lamp designed to produce an output similar to that specified in ISO 10977(1993); and near UV fluorescent lamp

having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm and significant proportion of UV in both bands of 320 to 360 nm and 360 to 400 nm was used (Option 2).[8]

The study design was equipped with such parameters which could reflect the changes if any covering all the three components of stability i.e. physical, chemical and microbial stability. The important parameters included apart from organolaptic characters were moisture content, ash value, pH estimation, HPLC and microbial count analysis of test drug formulation. Rule of "less than 5% changes from the initial assay value, any specific degradient not exceeding its acceptance criteria, drug meeting the acceptance criteria for appearance and physical properties and pH not exceeding its acceptance criteria, means that the drug is meeting the specification on accelerated conditions of storage according to ICH guidelines" [9] was taken to establish the stability status of the majoone falasifa.

MATERIALS AND METHODS

The present study named Accelerated stability study of majoone falasifa was conducted to establish the stability of said formulation at accelerated thermal/humidity and photo stress conditions by analyzing the possible variation in the samples at predetermined time points.

Source of data: Data was collected out of experimental study at the laboratory of:
(1) Department of Ilmus Saidla, National Institute of Unani Medicine.
(2) Azymes Biosciences, Bangalore.
(3) Bangalore Test House, Bangalore.

Procurement of raw drugs: The ingredients of the formulation were procured from the herbalist/raw drug dealer at Bangalore, Karnataka, India during the month of May-June 2014. Bekhe baboona (*Matricaria chamomilla* L.) due to its non availability in the market was hand collected from Pampore, Kashmir. Ingredients of the formulation were identified and authenticated by the K. Ravi Kumar, Professor, Centre for Repository of Medicinal Resources (C-RMR), Transdisciplinary University (TDU) under FRLHT (accession numbers:3404, 3402, 3403, 3420, 3407, 3405, and 3406). As Bekhe baboona (Matricaria chamomilla L.) was collected fresh it was first cleaned under running tape water and then subjected to drying in hot air oven at 60^0C for 6 hours. All the ingredients were stored in cleaned air tight glass containers.

Ingredients of Majoone falasifa: [10]

S.no	Drug	Botanical Name	Dosage
1.	Maweez munaqqa	*Vitis vinefera*	450 gm
2.	Zanjabeel	*Zingiber officinale*	150 gm
3.	Filfil Siyah	*Piper nigrum*	150 gm
4.	Filfil Daraz	*Piper longum*	150 gm
5.	Darchini	*Cinnamommum zeylanicum*	150 gm
6.	Amla	*Emblica officinalis*	150 gm
7.	Post-e- Balela	*Terminalia belerica*	150 gm
8.	Sheetraj Hindi	*Plumbago zeylanicum*	150 gm
9.	Zarawand Madharaj	*Aristolochia rotunda*	150 gm
10.	Salab Misri	*Orchis latifolia*	150 gm
11.	Maghz-e- Narjheel	*Cocus nucifera*	150 gm
12.	Beikh –e- Babuna	*Matricaria chamomillia*	150 gm
13.	Maghz-e- Chilghoza	*Pinus gerardiana*	150 gm
14.	Tukhm-e- Babuna	*Anthemis nobilis*	75 gm
15.	Asl /Qand Safaid	Honey	7 kg

Preparation of majoone falasifa: All the ingredients of majoone falasifa were first washed with sterile water and then subjected to drying at 60 ºC in oven for 4 hours. Maweez munaqqa (*Vitis vinefera*) was kept in sterile water (1:3) for one night, and then maweez (*Vitis vinefera*) was crushed in the same water and passed through a cotton cloth so as to get rid of its seeds. In this paste of maweez (*Vitis vinefera*) sugar was introduced for *qiwam* formation. The rest of ingredients except maghze chilghoza (*Pinus gerardiana*) and maghze narjeel (*Cocus nucifera*) were powdered and passed through sieve no.80 and maghziyat were passed through sieve no.40 and introduced in the qiwam (consistency) of sugar. The sugar and total weight of all the ingredients was kept in the ratio of 3:1. All the steps were carried out in accordance of NFUM Part-1.[10] It is noteworthy that preparation of said formulation was carried out without adding any preservative.

Storage: The samples were filled in air tight, transparent polyethylene terephthalate (PET) containers with capacity of 250 ml. Each container was filled with about 200grams of drug formulation. The procedure was carried with extreme care to avoid contamination.

Methodology of accelerated stability testing: The formulation was filled in twelve stated packs. They were labeled properly including formulation name, date of preparation, date of commencement of thermal/humidity or photo challenge, date of withdrawal etc.

Thermal/humidity challenge was carried out for a period of six months. One pack (first pack) was tested for various analytical parameters at the time of manufacture i.e. zero month/day, other packs were kept in stability chamber for accelerated stability study. For thermal/humidity stress, temperature and humidity was regulated at 40±2ºC temperature and relative humidity at 75±5%. The second pack was removed from stability chamber at the completion of 1st month and third pack was removed from the chamber at the completion of 3 months and fourth was removed at completion of 6 months and studied for various parameters.[2] The procedures were strictly followed according to ICH Tripartite Guidelines.

Photostability testing: Two packs of majoone falasifa were subjected to photostability testing at different light potential. One pack was exposed to overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 Watt

hours/square meter. Another pack was exposed to 2.4 million lux hours with an integrated near ultraviolet energy of 400 Watt hours/square meter i.e. double to the light intensity given to pack one. Option 2 was used in this study. A cool white fluorescent lamp designed to produce an output similar to that specified in ISO 10977(1993) and near UV fluorescent lamp having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm and significant proportion of UV in both bands of 320 to 360 nm and 360 to 400 nm was used.[8]

Assessment of parameters: The following parameters were assessed of the sample packs of majoone falasifa for the estimation of its shelf life.

Organoleptic characters: Parameters/characters like appearance, color, odour, and taste were checked out for organoleptic analysis.

1. Appearance: Appearance was recorded according to the consistency whether semisolid, semiliquid etc.[11]
2. Colour: The colour of the drug formulation was noted by using Pentone color chart. If any changes occurred were noted according to color number given in the chart.[12]
3. Odour: Odour of any material is extremely criteria based on individual's perception. Therefore, the description of this feature may sometimes cause some difficulties. A small portion of the sample was examined by slow and repeated inhalation of air over the material. The strength of the odour like weak, distinct, strong was first determined and then the odour sensation like musty, mouldy, rancid, fruity, aromatic etc. were determined.
4. Taste: First of all the depth of organoleptic capacity was tested. This was done by asking the tester to taste the sample and categorize as weak, distinct or strong in terms of taste and then quality of taste was determined.[11]

Physical analysis

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 source of heat was an electric heater with temperature control. The upper portion of the flask and the connecting tube was insulated.
Receiving tube and the condenser of the apparatus were thoroughly cleaned, rinsed with water and dried. 75ml of toluene 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 to cool for about 30 minutes and reading of the volume of water to an accuracy of 0.05 ml (first distillation) was done.
Again accurately 10 gm of drug material was weighed, a few pieces of porous porcelain were added and the flask was gently heated for 15 minutes. When boiling began, 2 drops per second was distilled until most of the water had 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 was rinsed with toluene. The distillation was continued for 5 more minutes, removed from heat, the receiving tube was allowed to cool at room temperature and any 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.[11]
Content of water was calculated as percentage using the formula:

$$\text{Content of water} = 100\ (\eta 1 - \eta) / w$$

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

Ash value
Total ash: About 2 gm of the drug, weighed accurately, in a previously ignited and tarred silica crucible was taken. The 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. Ash was cooled in a desiccators and weighed. The content of total ash was calculated in mg per gm of drug.[13]
Acid insoluble ash: The total ash was boiled with 25ml of dilute hydrochloric acid for 5 minutes. Solvent was filtered through the ash less filter paper and the insoluble matter collected on the filter paper was washed with hot water and ignited to the constant weight. The residue was allowed to cool in a desiccator for 30 minutes and weighed without delay. The content of acid-insoluble ash was calculated in mg per g of material.
Water soluble ash: The total ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited. The weight of insoluble ash was subtracted from the weight of the total ash, giving the weight of the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug.

Chemical analysis

pH
pH of 1% solution: One gram of drug was accurately weighed and dissolved in accurately measured 100 ml of water and filtered. pH of the solution was checked with a standardized glass electrode.
pH of 10% solution: Ten gram of drug was accurately weighed and dissolved in accurately measured 100 ml of water, filtered and pH was checked with a standardized glass electrode.

Extractive values
Cold extraction: Cold extraction was carried out using chloroform water in capacity of 0.25% v/v while for alcohol soluble matter 100 ml of absolute alcohol was used. The solution was filtered using filter paper (Whatman No.1) and after evaporation of the solvents on water bath, the extractive values were determined with reference to the weight of drug.[13]
Successive extraction: The successive extractive values of majoone falasifa in different solvents viz. petroleum ether, chloroform and ethyl alcohol, were carried out by percolation in soxhlet apparatus. The heat was applied for six hours on water bath. After complete extraction solvent was evaporated on water bath and extractive value was calculated with reference to the total weight of drug taken.

Estimation of alkaloid: Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract 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 is the alkaloid, which was dried and weighed.[14]

Estimation of saponification value: Majoone falasifa 2 gm was taken in a 200 ml glass flask, 25 ml of 0.5 M ethanolic potassium hydroxide and few glass beads was added to it. Then a condenser was attached to the flask while keeping the flask on water bath and boiled for 30 minutes. Now 1.0 ml dilute phenolphthalein was added and titration was done with 0.5 M hydrochloric acid. The amount of hydrochloric acid required to neutralize the alkali was noted and same procedure was repeated without the drug. The saponification number was calculated using this formula:

$$\text{Saponification value} = (b - \alpha) \times 0.02805 \times 1000 / \text{weight of drug in grams}$$

Where
a= value of hydrochloric acid required to neutralize alkali with drug
b= value of hydrochloric acid required to neutralize the alkali without drug

Estimation of iodine value: Drug sample 2 gram, was mixed in 10 ml of carbon tetrachloride in a dry iodine flask; 20 ml of iodine monochloride solution was added to it and a stopper previously moistened with potassium iodide was inserted. The mixture was allowed to stand in a dark place at room temperature of about 17°C. After 30 minutes 15 ml of potassium iodide solution and 100 ml of distilled water was added to it. Now, the mixture was shaked well and titrated with 0.1 N aqueous sodium thiosulphate solution, using starch solution as an indicator. The volume of thiosulphate solution required to neutralize the above mixture was noted and same procedure was repeated without drug. Iodine value was calculated using formula:

$$\text{Iodine value} = (b - \alpha) \times 0.01269 \times 100 / \text{weight of drug in grams}$$

Where
a=volume of sodium thiosuphate needed to neutralize the mixture with drug
b= volume of sodium thiosulphate needed to neutralize the mixture without the drug

Estimation of acid value: Two gram of drug was dissolved in 50 ml mixture of ethanol and ether taken in 1:1 ratio. One ml phenolphthalein was added as indicator and then this mixture was titrated with 0.1N aqueous potassium hydroxide shaking constantly until a pink color which persists for 15 seconds was obtained. The acid value was calculated using formula:

$$\text{Acid value} = \alpha \times 0.00561 \times 1000 / b$$

Where
a= volume of potassium hydroxide required to neutralize the mixture
b= weight of the drug taken.[15,16]

HPLC (High performance liquid chromatography)**:** Ten mg of test drug sample was mixed in 20 ml of methanol and subjected to filtration through 0.22 mic membrane to leach out sugar. The filtrate was then mixed with acetonitrile and water in the ratio of 7:3. HPLC of filtrate was run on an ultrafast liquid chromatography system (Waters 510) with stationary phase column C_{18}, length 25×4.6 cm using Rheodyne manual injector. Injection volume was 20 µl. Flow rate was adjusted at 1 ml /minute using mobile phase Acetonenitrile**:**Water (7:3 ratio) under pressure of 1200 PSI with run time of 10 minutes. Analysis was carried out at wavelength 256 and 366 nm using UV detector. IRIS-HPLC SPECTRAL PROCESSING SOFTWARE 32 was used to control all parameters.

Microbial analysis: The following tests are designed for the estimation of the number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in MF. The term 'growth' is used to designate the presence and presumed proliferation of viable micro-organisms. In the present study microbial analysis was done for total bacterial count, total fungal count and presence of specific pathogenic viz; E.coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa in the samples of test drug formulation.

Pretreatment of sample: Ten g of MF was dissolved in buffered sodium chloride-peptone solution pH 7.0 and volume was adjusted to 100 ml with the same medium. In case of sample to be examined for the specific pathogenic count lactose broth was used in place of buffered sodium chloride-peptone solution.

Procedure for total bacterial/fungal count: Membrane filtration method was used for total bacterial/fungal count. Membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm were used.

Ten ml of each dilution containing 1 g of the preparation being examined were transferred to each of two membrane filters and filtered immediately. Each membrane was washed by filtering through it three or more successive quantities, each of about 100 ml, of buffered sodium chloride-peptone solution pH 7.0. One of the membrane filters, intended for the enumeration of bacteria, was transferred to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, was transferred to the surface of a plate of Sabouraud dextrose agar with antibiotics. Plates were incubated for 5 days, at 30°C in the test for bacteria and 20°C in the test for fungi. Number of colonies that formed were counted and number of micro-organisms was calculated per g of the preparation being examined.

Tests for specific pathogens

***Escherichia coli*:** One gram of sample was placed in a sterile screw-capped container, 50 ml of nutrient broth was added, shaked, allowed to stand for 1 hour and shaked again. The cap was loosen and incubated at 37°C for 18 to 24 hours.

Primary test: One ml of the enrichment culture was added to a tube containing 5 ml of MacConkey broth and incubated in a water-bath at 36°C for 48 hours. There was no formation of acid and gas in the tube thus there was no need to carry out the secondary test and absence of Escherichia coli was thereby confirmed.

Salmonella: The pretreated preparation of Majoone falasifa containing one g of the product was transferred to 100 ml of nutrient broth in a sterile screw-capped jar, shaked, allowed to stand for 4 hours and shaked again. The cap was loosened and incubated at 35°C for 24 hours. 1.0 ml of the enrichment culture was added to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubated at 36° C for 48 hours. From each of these two cultures subculture was carried out on bismuth sulphate agar and brilliant green agar media. The plates were incubated at 36°C for 20 hours. Upon examination, if the selected media does not meet the description of colony (color change) then there is absence of salmonella in the sample under investigation.

***Pseudomonas aeruginosa*:** Hundred ml of fluid soyabean-casein digest medium was inoculated with 1 g of pretreated sample preparation being examined. It was mixed and incubated at 35ºC for 30 hours. Medium was examined for growth. No growth in the medium confirms the absence of Pseudomonas aeruginosa in the sample of MF.

***Staphylococcus aureus*:** Same process as carried in case of *Pseudomonas aeruginosa* was followed and instead of soyabean-casein digest medium, Vogel-Johnson agar medium was used. Upon examination of the incubated plates, none of them contained colonies having the characteristics colonial morphology i.e. black spot surrounded by yellow zones, thus confirming the test is negative for "staphylococcus aureus."[17]

RESULTS AND DISCUSSION

The observation from the stability study of majoone falasifa showed that there were < 5% changes in the parameters evaluated at different time points. In case of physical analysis the organoleptic characteristics showed no significant changes. Test drug preserved its semisolid consistency; homogeneity without any phase separation, sedimentation, crystallization, or caking or gas formation; with blackish brown colour; pleasant odour and sweet taste tending to bitter that persists until the end of six month. Stability in appearance of majoone falasifa may be due to the fact that interaction between sugar and water and sugar's high affinity to control the level of moisture helps to slow down the moisture loss and prevent drying out and distorting appearance of product, thereby extending its shelf life.[18]

Moisture content gradually reduced in Accelerated stability sample (ASS) and Photo stability sample (PSS) of majoone falasifa. Maximum change in moisture content in ASS was observed when baseline value (14.96±0.03%) was compared with the sixth month value (14.82±0.01%). However, the percentage of change was only 0.93%. Likewise in the case of PSS moisture content gradually reduced from base line (14.96±0.03%) to ninth day (14.83±0.033%) and changes observed was only 0.86%. As these results of moisture content displayed less than 5% change in between various samples of drug examined at different time points from the initial assay value, it confirms to the ICH guideline for stability study.

In present study total ash value in ASS of majoone falasifa at baseline was 1.78±0.016% which increased to 1.80±0.000% at first month but gradually reduced further to 1.79±0.006 and 1.77±0.005% in third and sixth month respectively, however percentage of change was only 1.66% when maximum and minimum ash value was compared. Ash values also fluctuate in case of PSS of majoone falasifa but percentage of change was only 0.02 from baseline to 4th day sample but there was no change in between 4th day and 9th day sample. Maximum variation in acid insoluble ash value in case of ASS and PSS of MF from base line was 2% only. Maximum change in water-soluble ash in ASS was observed at the end of third month i.e. was 1.59%, this percentage of change reduced to 0.79% at the end of six month. While in PSS water-soluble ash gradually reduced and maximum change was seen at the end of 8th day i.e. was 3.45%.

The results of total ash, water soluble ash and acid insoluble ash (Table 1) displayed less than 5% change in between the samples examined at various time points from the initial assay value both in case of accelerated stability samples as well as in case of photostability samples. Thus it confirms to the ICH guidelines.

Table 1: Total, acid insoluble and water soluble ash of ASS of Majoone Falasifa

S. No.	Total Ash (%)				Water Soluble Ash (%)				Acid Insoluble Ash (%)			
	0 M	1st M	3^{d} M	6th M	0 M	1st M	3rd M	6th M	0 M	1st M	3rd M	6th M
1.	1.8	1.8	1.8	1.76	0.99	0.98	0.97	0.98	0.38	0.37	0.37	0.37
2.	1.75	1.8	1.78	1.77	0.99	0.95	0.96	0.97	0.38	0.37	0.38	0.37
3.	1.8	1.8	1.8	1.78	0.97	0.96	0.95	0.96	0.37	0.37	0.37	0.37
Mean ± SEM	1.78± 0.016	1.80± 0.000	1.79± 0.006	1.77± 0.005	0.98± 0.006	0.96± 0.000	0.96± 0.005	0.97± 0.005	0.376± 0.003	0.376± 0.003	0.37± 0.000	0.373± 0.003

The probable factors as to why the test drug formulation was able to maintain its physical stability can be many, first, results in case of moisture content revealed that drug had less tendency to absorb moisture which in turn can be due to less hygroscopic nature of the molecules of the drug, secondly as the container closure system of high quality was used, it may not have allowed the moisture to enter into the drug, besides preventing the drug from direct exposure to air that may cause oxidation.

Regarding pH it was all time slightly acidic ranging from 4.46±0.06 to 4.40±0.05 in 1% solution and 5.6±0.05 to 5.53±0.03 in 10% solution in of ASS. While in PSS in 1% solution pH was 4.53±0.06 to 4.46±0.03 while in case of 10% solution the values were static to 5.6±0.05 in all samples (Figure 1 & 2). These results of pH value among various samples of test drug at different time points displayed less than 5% change from initial assay value in case of both ASS and PSS.

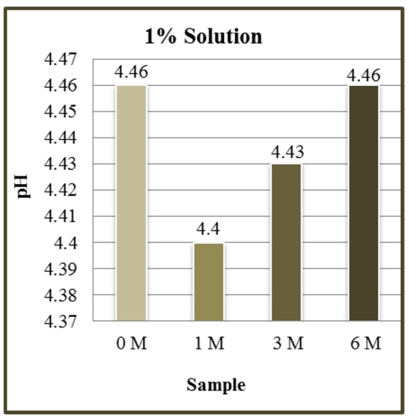

Figure 1

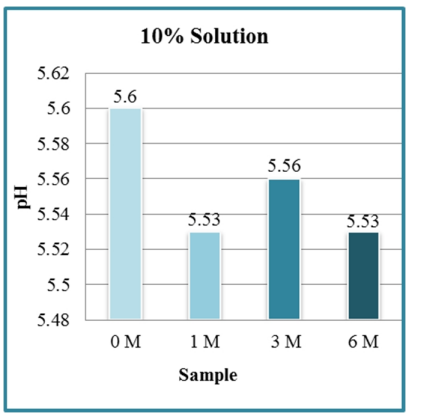

Figure 2

pH values in 1% and 10% solution of ASS of majoone falasifa

After temperature the second most important player in drug degradation is pH.[19] The degradation of many drug in solution accelerate or decelerate exponentially as the pH is increased or decreased over the specific range of pH value. Most of the degradation pathways are hydrolysis or oxidation reaction.[20]

According to Abba et al. it is the pH which takes care of a number of chemical and microbiological reactions. He also stated that in case of low pH, the microbial count could be less, whereas at neutral or higher pH, the level of microbial load could observe to be higher. This suggests that alkaline pH favors high contamination levels of the herbal preparations while acidic pH favors low microbial contamination.[21]

The pH of the test formulation was acidic and the microbial count was under limit, thus it supports what is suggested by Abba et al. The pH values both in case of ASS and PSS of MF displayed less than 5% change from initial assay value, it shows that there were no further chemical reactions. Thus it can be stated that the drug formulation meets the specification in case of stability of pH is concerned.
In ASS mean percentage of alcohol soluble matter of majoone falasifa was 67.73±0.33% at baseline which gradually increase to 68.40±0.03% at the end of 6th month, while water soluble matter showed fluctuation, 55.41±0.28% at base line, slightly reduced in 1st month and gradually increased in 3rd and 6th month with maximum value 55.64% at the end of 6th month.

In case of PSS of majoone falasifa alcohol soluble matter values showed fluctuation of 67.73±0.33% at baseline 67.40±0.53% at fourth day and again 68.03±0.27% at ninth. For water-soluble matter, the values were 55.41±0.28% at base line, slightly decreased at 4th day (54.92±0.39%) and again reduced to 54.71±0.37% at 9th day.

Mean percentage of pet ether successive extractive value of ASS of majoone falasifa at zero month was1.766±0% which slightly decreased at 1st month (1.743±0.00%) but again returned to baseline value in 3rd and 6th month. In case of PSS it was 1.766±0% at base line which gradually reduced to 1.73±0.011% at 9th day. The mean percentage of chloroform extractive value of ASS and PSS samples was all time stable at 0.35±0.00%. The mean percentage of alcoholic extractive value of ASS sample was 10.48±0.02% at baseline which increases to 10.49±0.0% in first month and same persisted till the end of 6th month. In case of PSS alcoholic extractive value was 10.48±0.020% at base line which gradually reduced and at the end of 9th day it was 10.46±0.008%.

Mean percentage of total alkaloids in ASS gradually reduced from 2.18±0.012% at baseline to 2.12±0.011% at 6th month, while in case of PSS total alkaloids value reduced to 2.14±0.008% at the end of 9th day.

The results in case of cold extraction, successive extraction and total alkaloids displayed changes of less than 5% from initial assay value in both ASS and PSS.

The extractive/alkaloid value which a drug yields in a particular solvent is often an approximate measure of the amount of certain active constituents or a group of related constituents, the drug contains.[15] This suggests that until the drug shows variation which is less than 5% , the drug can be said to have retained its potency. In case of test drug, the extractive and total alkaloid values of both ASS and that of PSS displayed less than 5% changes from initial assay value, so it can be concluded that the test drug meets the ICH Guideline specifications as far as extractive and total alkaloid values are concerned.

Mean percentage of acid value in ASS was 14.4±0.07% at baseline which slightly increased at first month (14.11±0.08%) but gradually declined in later time point and reduced to 13.91±0.01% at six month. In case of PSS of majoone falasifa the acid values was always near to base line i.e. at 14.4±0.07%.

Acid value test is conducted to see whether there is presence of free acids or not in oil/fatty substance. Usually the free acids should be minimum or absent in case of fresh substances. The acid content in an alimentary fat or oil is given by the quantity of free fatty acid driving from the hydrolytic deterioration (rancidity) of triacylglycerols. This type of degradation takes place due to inappropriate processing and storage condition of oil containing materials. If there is presence of free acids beyond acceptance criteria the substance is said to have affected by microbes, light or heat. Therefore, acid value corresponds to the genuineness and stability of product.

Water contenting products are more vulnerable to hydrolysis and get altered more easily and develop unpleasant smell and taste.[15,22]
Mean percentage of saponification value in ASS at baseline was 214 ±1.00% which increased in 1st month (215±1.00%) and again

gradually reduced to 213±0.88% at the end of the study i.e. 6th month. In case of PSS of MF the values were slightly but insignificantly reduced on 9th day.

The lower saponification value suggest that the mean molecular weight of fatty acids is lower and that the number of ester bonds is less. Pandurangan *et al*. found that the saponification value of the substance decreases with thermal challenge.[23] However Ngassapa *et al.* suggests that this could be due to the chemical reactions giving degenerate products other than free fatty acids.[24]

Mean percentage of iodine value of ASS of majoone falasifa was 92.20±0.74% at base line which persist for whole study period with very less changes, while in case of PSS iodine values gradually reduced to 90.82±0.44% at ninth day.

Iodine value is an indication of the degree of unsaturation in the oily and fatty matter and is a measure of vulnerability to the oxidation. Higher the iodine value, more will be the vulnerability of the oil to oxidation. The iodine value increase at elevated temperature could be attributed to destruction of double bonds in the oils upon heating.[24]

As our test drug displayed less than 5% changes both in case of ASS and PSS as far as acid, saponification and iodine values are concerned, it can be concluded that the test drug meets the ICH guideline specification, in other words we can say that test drug is stable as far as above parameters are concerned.

In present study HPLC (High performance liquid chromatography) was also done**.** The method development and selection of a suitable mobile phase involved several trials because of the complexity of the chemical composition of the majoone falasifa and the affinity of the components towards various solvents. The mobile phase proportions were adjusted to obtain a rapid and simple assay method with reasonable run time, suitable retention time and sharp peak. Under optimized conditions HPLC with C18 column and UV detector at 254 and 366 nm using mixture of acetonitrile and water in the ratio of 7**:**3 as mobile phase gave well resolved symmetric peak for MF. However HPLC study of ASS and PSS showed that tested samples were not identical to base line as some new peaks appeared, retention time and area under the peak varied in later day samples (Figure 3 & 4). However, if overall physico-chemical parameters applied on the test drug are kept in mind, these variations creates suspicion that weather these changes are due to drug degradation or deviation from uniform protocol to be followed in each sample of majoone falasifa analysed.

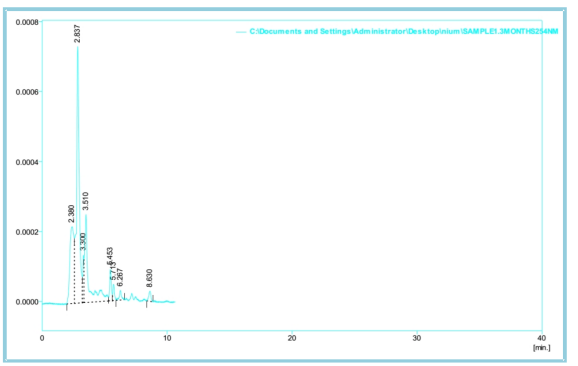

Figure 3. HPLC scan of Majoone falasifa at three month (extract at 254 nm)

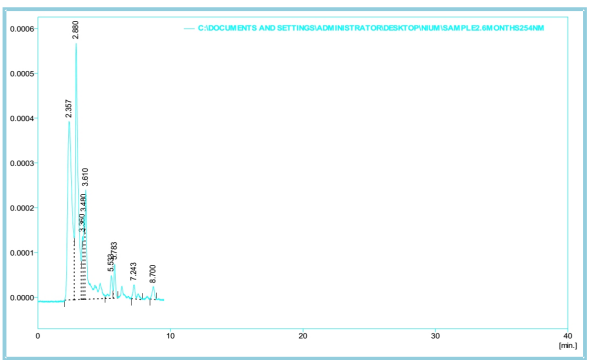

Figure 4. HPLC scan of Majoone falasifa at six month (extract at 254 nm)

Thus, it is worthwhile to follow uniform/identical protocol while carrying out stability study of herbal preparation by means of HPLC. Further, it is advisable to identify the new peaks as it may be the toxic substance and likewise missing peaks should be confirmed whether these are of pharmacologically active component.

Microbial analysis was done by evaluation of total bacterial, total fungal and specific pathogenic count in test drug samples at predetermined time points. Total bacterial count in ASS at zero, one, third and six month was 305, 250, 3300 and 2450 Cfu/gm/ml respectively, while in case of PSS it was 305, 895 and 8050 Cfu/gm/ml at zero, fourth and ninth day respectively. Total fungal count in ASS and PSS at all study time point were 10Cfu/gm/ml. Specific pathogenic count was absent in all samples. These values of bacterial and fungal estimation fall under the acceptance limit i.e. 10^5/gm for total bacterial count and 10^3/gm for total fungal count according to WHO.[25]

CONCLUSION

From the above discussion, it can be concluded that the test drug formulation majoone falasifa has shelf life of 20 months, if Grimm's statement is taken into account, which seems to be more judicious as Grimm has stated of zone IV to which India is included. However, this shelf life of 20 months cannot be implied to all majoon formulations, as formulations differ in terms of their respective ingredients and manufacturing process which define their shelf life.

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