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Original Article

Accelerated Stability Study of Powder Formulation *Safoof Muqliyasa* and *Safoof Mulayyin*

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ABSTRACT

Background and Objectives: Evaluation of stability studies of Unani Medicine (UM) formulations determined on the basis of scientific data are the need of the time and also recommended in D and C Act. There is immense need to evaluate precise shelf life for each powder formulations. **Materials and Methods:** Accelerated Stability Study (ASS) of *Safoof Muqliyasa* (*S. Muq*) and *Safoof Mulayyin*. (*S. Mul*) was carried out with at elevated temperature and humidity conditions 40ºC±2ºC with Relative Humidity of 75%±5% RH according to ICH guidelines, by keeping in stability chamber in containers used by commercial UM. Pharmacy's (i.e. sealed HDPE containers). Testing frequency of samples was 0, 3 and 6 months and was subjected to various organoleptic, physical, microbiological and chemical tests parameters with HPLC quantification of active constituents/markers (Chebulic acid in *S. Muq* and Gingerol in *S. Mul*). Results were accessed as per API guidelines and are also statistically analyzed for 10% degradation, from liner regression equation using individual slope and Intercept. **Results:** Both *S. Muq* and *S. Mul* were suitable at accelerated condition upto 3 month storage on selected physical, microbiological and chemical parameters as per API evaluation guidelines, and it can be extrapolated that real time shelf life period (predicted stability) according to Grimm's statement, for zone III and IV, 3.3 as 10 month at room temperature and packaging method adopted in the study and by 10% degradation in different parameters by multiplying factor of 3.3 accelerated stability of *Safoof Muqliyasa* can be extrapoled as 2 years and 1 month and for *Safoof Mulayyin* 1 year and 5 months. **Conclusion:** According to physical and microbiological parameters both *S. Muq* and *S. Mul* are stable for 6 months in ASS but changes in selected chemical parameters was observed for after 3rd month making it stable approximately for 1 year. Stability of salt containing *S. Mul* was found to be comparatively lower than the non-salt containing *S. Muq* This observation/ shelf life may be exclusive to both the powders.

Keywords: Accelerated stability study, Powder, *Safoof Mulayyin*, *Safoof Muqliyasa*, Stability, Unani.

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INTRODUCTION

Quality control and stability data for traditional/herbal drugs are possible but difficult to achieve. Unani medicine literature describes shelf life as that period during which the drug retains its temperament, constituents and structural constitution intact, which may be judged by means of observing its organoleptic characteristics.[1] Specific shelf life for different Unani formulation is described in D and C act for each of the Pharmacopeal dosage form. Shelf life of Unani drug mentioned in the list may be altered due to combination of various types of traditional drug of plant, animal and mineral origin, it may not be always accurate or same for one type/category of different formulations such as for all the *Arq* (Distillate), *Safoof* (Powder) or *Qurs* (Tablets). Concepts of shelf life and Shelf life of compound formulation are described in several Unani classical texts such as *Firdous ul Hikmat*,[2] *Qarabadeene Qadri*[3] etc. Unani physician established the shelf life of various formulations in these texts according to their observations and expertise but shelf life for all the Unani compound formulation or dosage forms are not specified in these texts especially according to the current market setting, packaging and need. Chemical markers listed in several Pharmacopoeias are helpful for stability testing of proprietary products.[4] The first person to mention the shelf life of *Safoof* (powder) was Galen (*Jalinoos*) (131-201AD). In his book Kitabul Murakkabat, *Ghulam Jelani* mentioned a citation from *Jalinoss* that all powder retains its potency no more than two months.[5] *Safoof* (Powder) may have higher tendency of degradation due to higher surface area in comparison to other solid dosage form.[6] Shelf life mentioned for all the *Safoof* is 2 years and for *Safoof* containing salts is mentioned 1 year in D and C act and rule inferred from Unani

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text.[7] Shelf life of *Safoof* (Powder) is 2 month but according to *Arastatalees* shelf life of *Safoof* (Powder) is 1 year.[8]

In order to assess/validate the shelf life, six month accelerated stability test of Unani powder formulation *Safoof Muqliyasa*[9] and *Safoof Mulayyin*[10] was conducted by complying ICH (40°C±2°C/75% RH±5%)[11] and API guidelines for assessment. A minimum of three time points in the accelerated storage state, including the initial and final time points (e.g. 0, 3, and 6 months) from a 6-month study is recommended.[12] 'Significant change' at 40°C/75% RH is defined as failure to meet the specification. The data from accelerated testing condition may be used to assess the impact of short-term excursions outside the label storage conditions such as might occur during transport.[13]

Formulations selected are particularly susceptible to deterioration as per the observations. Both salt (*Safoof Mulayyin*) and non-salt variant (*Safoof Muqliyasa*) of *Safoof* has taken up for the study due to depiction of different shelf life in D and C act. Formulations selected are the commonly used *Safoof* formulation in Unani Medicine.

MATERIALS AND METHODS

Accelerated stability study of *Safoof Muqliyasa* (*S. Muq.*) and *Safoof Mulayyin* (*S. Mul.*) was conducted to establish the stability at accelerated thermal/humidity condition by analyzing the possible variation in the samples at predetermined time points.

Ingredients of *Safoof Muqliyasa* are Tukhme Teera Tezak (*Lepidium sativum* L.) 72 g, Zeera Siyah (*Carum carvi* L.) 21 g, Tukhme Alsi (*Linum usittatissimum* L.), Tukhme Gandana (*Allium ascalonicum* L.), Halela Siyah (*Terminalia chebula* Retz) 9 g and Mastagi (*Pistacia lentiscus* L.) 4.5 g.[9] Ingredients of *Safoof Mulayyin* Berg-e-Sana (*Cassia angustifolia* Vahl.), Post Halela Zard (*Terminalia Chebula* Rerz), Namak siyah (Sodium chloride), Badiyan (*Foeniculum vulgare* Mill.) and Zanjabeel (*Zingiber officinale* Roscoe) each 200 g.[10]

Procurement of raw drugs

Tukhme Alsi, Halela Siyah, Barg-e-Sana, Post Halela Zard, Badiyan, Zanjabeel was procured from Pharmacy of National Institute of Unani Medicine, Bangalore, and *Tukhme Teera* Tezak, *Zeera siyah, Tukhme gandana, Mastagi, Namak siyah*, was procured from local market in Bangalore and Sirka (Sugar cane) was procured from Amazon and the seller is Unani remedies, Ghati mamu Bhanja, Agra (U.P).

Raw drugs procured for preparation of *Safoof* formulation (*S. Muq.* and *S. Mul.*) were identified from Trans-Disciplinary University (TDU), Foundation for Revitalization of Local Health Tradition (FRLHT) Attur, Bengaluru. The drug authenticated/identified with accession numbers is as follows *Berge Sana* (*Cassia angustifolia* M. Vahl.)-5230, *Halela Zard* (*Terminalia Chebula* Retz.) - 5231, *Zeera siyah* (*Carum carvi* L.) - 5232, *Tukhme Alsi* (*Linum usittaissimum* L.) - 5233, *Zanjabeel* (*Zingiber officinale* Roscoe.) - 5234, *Mastagi* (*Pistacia lentiscus* L.) - 5235, *Badiyan* (*Foeniculum vulgare* Mill.) - 5236, *Tukhme teera tezak* (*Lepidium sativum* L.) - 5237, *Tukhme gandana* (*Allium ascalonicum* L.) - 5238, *Halela Siyah* (*Terminalia Chebula* Retz.) - 5239 and *Namak siyah* (Black salt) - 5397 (Annexure I.) A specimen of each plant material used was deposited in the drug museum of National Institute of Unani Medicine, Bangalore with voucher specimen no. 74/IS/Res/2020, for future reference.

Preparation of *Safoof Muqliyasa* and *Safoof Mulayyin*

All the drug were cleaned manually from impurities[14] and Powdering was done by super mixer-grinder while in case of *Sonth* it is firstly bruised in mortar and pastel then powdering was done by super mixer-grinder. Sieving of powder was done by using sieve # no. 80 as per UPI specification and stored in air tight HDPE container.[15] High-density polyethylene is the material most commonly used by the pharmaceutical industry for containers, and is likely to continue to be used for the next few years. Tadbire- advia of *Zeera siyah* (Detoxification of drug *Carum carvi*): *Zeera siyah* is soaked in *Sirka Naishakar* (Sugarcane vinegar).[16] The level of sugarcane vinegar was kept 5 cm above the level of drug. The drug was then removed and allowed to dry and then is roasted before use over low fire.[17]

Container and closure system

Stability tests were conducted on the dosage type packaged / stored in the proposed marketing container and closure system. The samples were filled in air tight, powder plastic jar (HDPE) containers with capacity of 100 g in which marketed formulations are available. Each container was filled with about 100 g of drug formulation. The procedure was carried with extreme care to avoid contamination.

Powder was accurately weighed and packed into a HDPE container (purchased from maruthi enterprises, Bengaluru) and sealed it by Smartpack HDPE Aluminium Foil. Each Aluminium foil was a multilayered foil of size 3 x 4 x 6 cm which was made up of two different plastic layer and one aluminium sheet in between the two plastic layers. The innermost plastic layer was photo resistant, thus preventing the harmful rays to the material during transportation.[18]

Methodology of accelerated stability testing

Each *Safoof* was divided in three different batches for testing to determine the drug's stability profile for 0 (base line) - 3 and 6 month. They were labeled properly including formulation name, date of preparation, date of commencement of thermal/humidity, date of withdrawal etc. Thermal/humidity challenge was carried out for a period of six months. Batch one (Base line/day 0) was tested for various analytical parameters just after the manufacture, other batch/packs i.e. for 3 month and 6 months were kept in stability chamber for accelerated stability analysis

and the temperature stress was controlled/regulated at 40±2°C and relative humidity at 75±5% RH.[13]

The second batch was removed from stability chamber at the completion of 3rd months and third batch was removed at completion of 6 months and studied for various parameters (physicochemical and microbial analysis).[19] The procedures were strictly followed according to ICH Tripartite Guidelines and the guideline for the stability studies as prescribed in Ayurvedic Pharmacopoeia of India, Part-I, Volume-VIII.[20]

Organoleptic Parameters: The colour, taste, odour, appearance was noted which provide firsthand information.

Powder characterization - Bulk density

Known weight of powders was taken into a long measuring cylinder and the volume corresponding to the top level of powder in the cylinder was determined, from which the bulk density that is ratio of mass of the sample to the volume was calculated.[21] Bulk Density = Mass/Bulk Volume; Tapped density: Powder was carefully taken into a long measuring cylinder and subjected to 500, 750 and 1250 tapings till constant tapped volume was obtained, The volume of the sample was noted. And tapped density that is the ratio of mass of the sample to the volume was calculated. Tapped Density=Mass/Tapped volume; Compressibility index: For Carr's index determination, same process was followed as that of in tapped density. Smaller the Carr's index the better is the flow properties, so this method is also used to evaluate the flow ability of the powder sample as well as the rate at which it packs down. It was calculated by following formula.[21]

$$\text{Carr's index (\%)} = \frac{(\text{Unsettled apparent volume} - \text{Final tapped volume})}{\text{Unsettled apparent volume}} \times 100$$

Hausner's ratio

For determination of Hausner's ratio, same process was followed as in tapped density. Hausner's ratio, like Carr's index is considered to evaluate the flow ability of the powder substances. Since Hausner's ratio is related to inter particle friction, powder flow properties can be predicted. The finer is the powder lower is the flow ability, while larger and denser particle tend to flow freely. Hausner's ratio was calculated by the following formula.[21]

Angle of repose

Angle of repose is the maximum angle between horizontal plane and surface of powders was calculated with the help of fixed funnel and free-standing cone method. A funnel was fixed with its tip 2 cm above a graph paper was set on a flat horizontal surface. The powder was carefully passed through the funnel. Pouring is continued till the cone of powders just reached to the tip of the funnel. The diameters of cones of the powder were noted and mean value is calculated. And tangent of angle of repose was calculated by using following equation.[22,23] Tan Ø=2h/D, h=Height of powder (from graph paper to tip of funnel), D= Mean diameter of the powder.

pH values

(pH value of 1% and 10% solution)

1% and 10% solution of *Safoof-e-Muqliyasa* and *Safoof Mulayyin* was prepared in distilled water (w/v) and pH was determined by using digital pH meter.[24]

Determination of Moisture Content (Loss on Drying)

Done as per protocol for testing Ayurveda, Siddha and Unani Medicine.[25]

Ash values

Determination of Total Ash, Acid-insoluble Ash, Water-soluble Ash and Sulphated Ash was done as per Unani Pharmacopeia of India (UPI).[16]

Isolation of mucilage

For isolation of mucilage, fresh plant material was washed with distilled water to remove dirt and debris; dried material was powdered with the help of grinder, of which 12.5 g was soaked in 100 mL distilled water for 5-6 hr, boiled for 30 min, and allowed to stand for 1 hr. The material was then squeezed through muslin cloth to remove the marc from the solution. Three times volume of acetone was added to the squeezed material to precipitate the mucilage which was separated, dried in an oven at a temperature not more than 50°C for 4-5 hr, collected and the dried material was passed through a sieve no. 80 and then calculate the percentage of mucilage obtained.[26]

Extractive Values

Determination of Alcohol soluble, Water-soluble extractive value was done as per the Quality Control Manual for Ayurvedic, Siddha and Unani Medicine.[27] Non-Successive and Successive Extractive Value: was done as per the UPI.[15]

HPLC fingerprinting

These powder formulations have no standard fingerprint available so for, an attempt has been made to evolve preliminary chromatographic physico-chemical profile of these formulations. Analytical HPLC: Shimadzu LC20AT SPD 20A;

HPLC Quantification of Chebulagic acid in Safoof Muqliyasa

The HPLC quantitative analysis of *S. Muq* was done at Natural Remedies Pvt. Ltd., Samples was run twice for each batch.

Mobile phase preparation

Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 mL of HPLC grade water and add 0.5 mL of Orthophosphoric acid. Make up to 1000 mL with

water, filter through 0.45 µ membrane and degas in a sonicator for 3 min. (Solvent A) 2 Acetonitrile (Solvent B).

Gradient conditions: HPLC analysis was carried out by using a gradient elution in 0.01-18 min with 5-20% B, 18-25 min with 20-35% B, 25-28 min with 35% B, 28-35 min with 35-20% B, 35-40 min with 20-5% B and 40-45 min with 5% B.

Standard preparation: 0.1 mg/mL of Chebulic acid reference standard in HPLC Grade Water. Chromatographic Conditions-Column: Hibar, Prepacked column, LiChrospher 100, RP-18e (5 µm) (Merck) Phenomenex-Luna 5 µ C-18(2) Size: 250x 4.60 mm; Detector: Photo diode array detector or UV Detector; Wave length: 270 nm; Flow rate: 1.5 mL/min; Injection volume: 20 µL; Sample preparation: Weigh the sample quantity equivalent to 18.0 mg/mL of *Safoof Muqliyasa* in to a 100 mL volumetric flask., And add 40 mL of Hot HPLC Grade water and sonicate for 10 min, Cool and make up the volume to 100 mL with HPLC Grade Water. Mix well and filter the solution through 0.2 µ (or) 0.45 µ membrane filter paper.

Procedure: Set the instrument as per the chromatographic condition as prescribed above. Inject 20 µL of standard preparation and record the chromatogram. Inject another 3 times and calculate the mean area and the RSD. The RSD should not be more than 2.0%. Inject 20 µL of sample preparation and record the chromatogram.

Calculations: Calculate the content of Chebulic acid by using following formula:

$$\frac{\text{Area of the sample} \times \text{Weight of standard in mg} \times \text{Sample dilution (mL)}}{\text{Area of the standard} \quad \text{Standard Dilution (mL)} \quad \text{weight of the Sample (mg)}} \times \text{Purity of the Standard (\%)}$$

HPLC Quantification of Gingerol in *Safoof Mulayyin*

The HPLC quantitative analysis of *Safoof Mulayyin* was done at Natural Remedies Pvt. Ltd. Samples was run twice for each batch. Mobile phase preparation: 1) Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 mL of HPLC grade water and add 0.5 mL of Orthophosphoric acid. Make up to 1000 mL with water, filter through 0.45 µ membrane and degas in a sonicator for 3 min. (Solvent A). 2) Acetonitrile (Solvent B); Mobile phase: Solvent A (45): Solvent B (55); Standard preparation: 0.1 mg/mL of 6-Gingerol reference standard in HPLC Grade Methanol.

Chromatographic Conditions-Column

Hibar, Prepacked column, LiChrospher 100, RP-18e (5 µm) (Merck) Phenomenex-Luna 5µ C-18(2) Size: 250 × 4.60 mm; Detector: Photo diode array detector or UV Detector; Wave length: 278 nm; Flow rate: 1.3 mL/min; Injection volume: 20 µL; Sample preparation: 20.0 mg/mL of *Safoof Mulayyin* (Powdered) in HPLC Grade Methanol.

Procedure

Set the instrument as per the chromatographic condition as prescribed above. Inject 20 µL of standard preparation and record the chromatogram. Inject another 3 times and calculate the mean area and the RSD. The RSD should not be more than 2.0%. Inject 20 µL of sample preparation and record the chromatogram. Calculation: The above-mentioned active marker is calculated by the following formula:

$$\frac{\text{Area of Sample} \times \text{Weight of standard (mg)} \times \text{Sample Dilution (mL)}}{\text{Area of the standard} \quad \text{Standard Dilution (mL)} \quad \text{Weight of the Sample (mg)}} \times \text{Purity of the Standard (\%)}$$

Microbial contamination test and test for Total Saponin, Sugar, Tannin and Flavonoids Estimation

Tests was done as per Protocol for Testing of Ayurvedic, Siddha and Unani Medicine.[25]

Statistical Analysis

The result was analyzed by calculating MEAN±SEM (Standard error of mean) and data presentation will be done in the form of table, graph wherever necessary. The assessment of shelf life was carried out using G pad Instat version 3.06, 32 bit for windows, created Sep 11, 2003. Regression analysis was used to calculate the time for 10% reduction in each parameter.

RESULTS

There were no significant changes observed in organoleptic characteristics of *Safoof Muqliyasa*. Test drug preserved its powder form homogeneity with brownish colour (Pantone 168);[28] aromatic odour and pungent slight bitter and astringent taste that persists until the end of six month. There were no significant changes observed in organoleptic characteristics of *Safoof Mulayyin*. Test drug preserved its powder form homogeneity with greenish yellow colour (Pantone 117);[28] aromatic odour and salty taste that persists until the end of six month.

Physical Parameters including Powder characterization/flow property of *Safoof Muqliyasa* (*S. Muq.*) with analysis of data at day 0 (baseline) at 3rd and at 6th month is depicted in Table 1, Microbiological Parameters *S. Muq* is depicted in Table 2, Chemical Parameters of *S. Muq*. is depicted in Table 3. Physical Parameters including powder characterization/flow property of *Safoof Mulayyin* (*S. Mul.*) at day 0 (baseline) at 3rd and at 6th month is depicted in Table 4, Microbiological Parameters *S. Mul*. is depicted in Table 5, Chemical Parameters of *S. Mul*. is depicted in Table 6.

Physico-chemical profile of *S. Muq*. and *S. Mul*. at different intervals is depicted in Table 7, Intercept, slope and R2 of *S. Muq*. and *S. Mul* for different parameters is depicted in Table 8. Approximate period in months for 10% degradation of *S. Muq*.

and *S. Mul*. with condition: 40°C±2 and 75%±5 RH is depicted in Table 9. Extrapolation of Shelf life indicates shelf life of 2 years and 1 Month for *S. Muq*. and shelf life of 1 year and 5 months for *S. Mul* for Climatic zone III and IV. Table 10 Chebulagic acid % (w/w) quantitative estimation by HPLC in *S. Muq* at baseline 0.77 at 3rd month 0.77 and at 6th moth was 0. 27. Quantitative estimation by HPLC in *S. Mul* for 6-Gingerol, 8-Gingerol, 10-Gingerol % (w/w) at baseline was 0.038, 0.010 and 0.008 respectively at 3rd month 0.034, 0.032 and 0.00 respectively and at 6th month 0.02, 0.005 and 0.01 respectively. HPLC fingerprinting at baseline and other intervals for *S. Muq*. and *S. Mul* is depicted in (Tables 11-18 and Figures 1-12).

DISCUSSION

Powder characterization/flow property: There were no significant changes observed in powder characterization of *Safoof Muqliyasa* and *Safoof Mulayyin* in respect of angle of repose at 0, 3rd and 6th month and both powders display fair type of powder flow (Tables 1 and 4). Loss on Drying (LOD): Moisture value in both the *Safoof* can be considered "No significant change" as per API as it shall not vary beyond 25% of the initial value.[12] Where as in case of *Safoof Muq*. It was decreased 19.28%, where as in case of *Safoof Mul*. it increases 16.66% at 6th month. NaCl present in *S. Mul*. is hygroscopic and have desiccant property[29] this might be the reason of more moisture content in *S. Mul* (Tables 1 and 4). Ash values: All the Ash values in *S. Muq*. displays significant change

Table 1: Physical Parameters of *Safoof Muqliyasa* (*S. Muq*.) and analysis of Data.

Parameters	0 (Initial)	3rd Month	Difference (0-3) Month in %	6th Month	Difference (0-6) Month in %
	Powder characterisation				
Bulk Density	0.52±SEM 0.00	0.571±SEM 0.00	8.93	0.56±SEM 0.00	7.14
Tap Density	0.70±SEM 0.00	0.69±SEM 0.01	-1.42	0.70±SEM 0.00	0.00
Compress Index	25.40±SEM 1.41	18.09±SEM 1.90	-28.77	19.81±SEM 0.18	-22.00
Hausner's Ratio	1.34±SEM 0.02	1.22±SEM 0.02	-8.95	1.24±SEM 0.00	-7.46
Angle of repose	40.24±SEM 0.29	39.46±SEM 0.23	-1.93	38.92±SEM 0.46	-3.28
Loss on drying	6.12±SEM 0.18	5.62±SEM 0.08	-8.16	4.94±SEM 0.04	-19.28
	Ash Value				
Total Ash	4.71±SEM 0.04	2.54±SEM 0.10	-46.07	2.41±SEM 0.04	-48.83
Acid Insoluble Ash	2.12±SEM 0.18	0.713±SEM 0.03	-66.36	0.58±SEM 0.03	-72.64
Water Soluble Ash	2.26±SEM 0.07	1.16±SEM 0.06	-48.67	0.98±SEM 0.03	-56.63
Sulphated Ash	0.18±SEM 0.01	0.18±SEM 0.01	0	0.06±SEM 0.01	-66.66
	pH				
10%	5.14±SEM 0.02	5.13±SEM 0.02	-0.19	5.17±SEM 0.02	0.58
1%	5.63±SEM 0.04	5.86±SEM 0.04	3.92	5.60±SEM 0.09	-0.53
	Weight Variation				
Weight (gm)	100±SEM 0.00	100.48±SEM 0.06	0.47	99.68±SEM 0.19	-0.32

Table 2: *S. Muq* Microbiological Parameters.

Parameters	0 (Initial)	3rd Month	6th Month
Total Bacterial Count	450 cfu/g WPL	450 cfu/g WPL	23000 cfu/g WPL
Escherichia coli	Absent	Absent	Absent
Salmonella	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Total Fungal Count	Absent	Absent	Absent

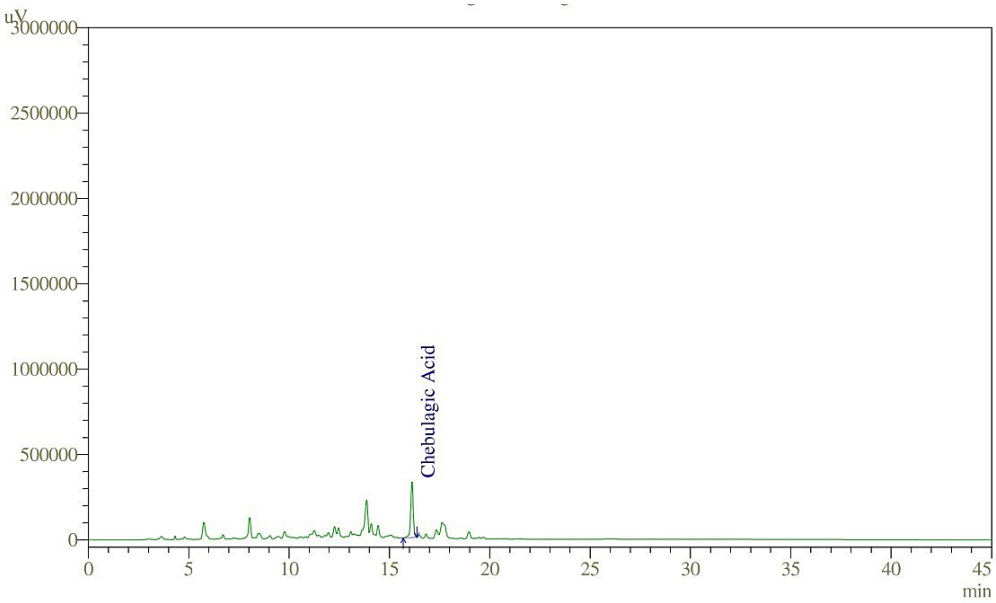

Figure 1: HPLC scan (Chebulagic acid) of *Safoof Muqliyasa* at 0 (Initial) (extract at 270 nm).

Table 3: ***Safoof Muqliyasa*** **Chemical Parameters.**

Parameters	0 Month	3rd Month	Difference (0-3) Month in %	6th Month	Difference (0-6) Month in %
	Mucilage				
Mucilage	2.36±SEM 0.06	2.29±SEM 0.02	-2.96	1.78±SEM 0.10	-24.57
	Cold Method Extraction				
Water	14.86±SEM 0.46	10.08±SEM 0.06	-32.16	9.26±SEM 0.03	-37.68
Ethanol	10.50±SEM 0.14	7.99±SEM 0.04	-23.90	6.33±SEM 0.03	-39.71
	Non-Successive Extractive Value (Soxhlet Method)				
Water	18.36±SEM 0.43	15.23±SEM 0.16	-17.04	14.86±SEM 0.02	-19.06
Pet. Ether	9.92±SEM 0.03	9.33±SEM 0.02	-5.9	8.19±SEM 0.02	-17.43
Chloroform	13.15±SEM 0.07	12.25±SEM 0.03	-6.84	9.73±SEM 0.05	-26
Ethanol	17.85±SEM 0.04	12.97±SEM 0.01	-27.33	11.23±SEM 0.01	-37.08
Benzene	23.6±SEM 0.62	22.80±SEM 0.04	-3.38	18.93±SEM 0.04	-19.78
	Successive Extractive Value (Soxhlet Method)				
Pet. Ether	10.66±SEM 0.06	9.31±SEM 0.03	-12.66	9.24±SEM 0.04	-13.32
Benzene	5.90±SEM 1.07	6±SEM 0.11	1.66	4.94±SEM 0.02	-16.27
Chloroform	3.34±SEM 0.58	3.25±SEM 0.26	-2.69	2.00±SEM 0.02	-40.11
Ethanol	6.93±SEM 0.05	6.52±SEM 0.05	-5.91	3.81±SEM 0.04	-45.02
	Quantitative Estimation of Functional group				
Total Saponins	30.29%w/w	14.34%w/w	-52.65	16.1%w/w	-46.84
Total Sugar	12.82%w/w	14.28%w/w	10.22	15.62%w/w	17.92
Tannins	1.41%w/w	2.76%w/w	48.91	3.78%w/w	62.69
Total Flavonoids	0.52%	0.89%	41.57	0.76%	31.57
	HPLC				
Chebulagic acid %(w/w)	0.77	0.77		0.27	-64.93

Table 4: *Safoof Mulayyin* Physical Parameters.

Parameters	0 Month	3rd Month	Difference (0-3) Month	6th Month	Difference (0-6) Month
	Powder characterization				
Bulk Density	0.43±SEM 0.02	0.44±SEM 0.01	2.27	0.47±SEM 0.00	8.51
Tap Density	0.61±SEM 0.03	0.58±SEM 0.00	-4.91	0.61±SEM 0.00	0.00
Compress Index	29.29±SEM 6.86	23.67±SEM 2.74	-19.18	22. 21±SEM 0.54	-24.17
Hausner's Ratio	1.44±SEM 0.13	1.31±SEM 0.04	-9.02	1.28±SEM 0.00	-11.11
Angle of repose	43.33±SEM 0.88	48.66±SEM 2.33	10.95	40.33±SEM 0.66	-6.9
Loss on drying	4.25±SEM 0.02	5.03±SEM 0.08	15.50	5.1±SEM 0.04	16.66
	Ash Value				
Total Ash	25.02±SEM 0.08	24.26±SEM 0.12	-3.03	23.09±SEM 0.07	-7.71
Acid Insoluble Ash	3.00±SEM 0.03	2.81±SEM 0.06	-6.33	2.55±SEM 0.04	-15
Water Soluble Ash	18.92±SEM 0.15	18.09±SEM0.02	-4.3	17.43±SEM 0.22	-7.87
Sulphated Ash	10.33±SEM 0.66	8.66±SEM 0.16	-16.16	8.16±SEM 0.16	-21
	pH				
10%	4. 85±SEM 0.04	5.74±SEM 0.05	15.50	5.82±SEM 0.01	16.66
1%	5.28±SEM 0.11	6.53±SEM 0.11	19.14	6.53±SEM 0.06	19.142
	Weight Variation				
Weight (g)	100±SEM 0.00	99.96±SEM 0.01	0.04	99.92±SEM 0.01	0.08

Table 5: *Safoof Mulayyin* microbial load Microbiological Parameters.

Parameters	0 Month	3rd Month	6th Month
Total Bacterial Count	450 cfu/g WPL	450 cfu/g WPL	25000 cfu/g WPL
Escherichia coli	Absent	Absent	Absent
Salmonella	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Total Fungal Count	Absent	Absent	Absent

at 3rd and 6th month and vary beyond 25% of the initial value at the end of 3rd and 6th month, but this change was reduction when compared to initial month. But the value of reduction was stabilized at 3rd and 6th month as no significant change was observed in 3rd and 6th month data. Reduction in inorganic content may be due to various factors which may be evaluated by further study, Many inorganic constituents like salts/sulphates/phosphates and Sulfur elements in the drug *Zeera siyah* (*Carum carvi*),[30] *Tukhme teera tezak* (*Lepidium sativum*),[31] *Mastagi* (*Pistacia lentiscus*),[32] *Alsi* (*Linum usitatissimum*),[33] was found on review, it may be volatilize during the course of accelerated stability conditions in stability chambers, it may be due to certain reaction and transformation process in the ingredient of *S. Muq*. Mechanism of volatilization and the decrease in ASH value/in-organics constituent need further investigations (Futher it can also be assumed that accelerated conditions may be responsible for this change and transformations, real time data for the said *Safoof* can also be generated for the confirmation (Table 1). All the Ash values in *S. Mul* can be considered "No significant change" as per API evaluation as it was not vary beyond 25 per cent of the initial value at the end of 6 month (Table 4). pH 10%: pH value in both the *Safoof* can be considered "no significant change" as it is not vary beyond 25% of the initial value, particularly in *S. Muq* there were less then 1% change but in case of *S. Mul*. it was less than 25% but it was normal pH i.e. 5.82 at 1% and 6.53 at 10% dilution, it is be to be bring in consideration that it contains NaCl whose pH is near about 7 (Tables 1 and 4).

Extractive Value

At 3rd month difference in extract value more than 25% in *S. Muq* was seen in only water (cold extractions) and ethanol only rest of the value in several solvent were below 25%, several

Table 6: *Safoof Mulayyin* Chemical Parameters.

Parameters	0 Month	3rd Month	Difference (0-3) Month	6th Month	Difference (0-6) Month
	Mucilage				
Mucilage	0.34±SEM 0.04	0.32±SEM 0.05	-5.88	0.33±SEM 0.00	-2.9
	Cold Method Extraction				
Water	30.45±SEM 0.67	29.56±SEM 0.03	-2.92	28.66±SEM 0.04	-5.87
Ethanol	11.15±SEM 0.06	11.01±SEM 0.01	-1.25	9.66±SEM 0.13	-13.36
	Non-Successive Extractive Value (Soxhlet Method)				
Water	49.35±SEM 0.37	44.34±SEM 0.01	-10.15	44.07±SEM 0.02	-10.69
Pet. Ether	6.26±SEM 0.03	5.01±SEM 0.01	-19.96	4.85±SEM 0.04	-22.52
Chloroform	5.76±SEM 0.03	5.47±SEM 0.01	-5.03	4.94±SEM 0.02	-14.23
Ethanol	20.21±SEM 0.04	17.3±SEM 0.02	-14.39	16.99±SEM 0.01	-15.93
Benzene	8.47±SEM 0.03	8.03±SEM0.04	-5.19	7.08±SEM 0.01	-16.41
	Successive Extractive Value (Soxhlet Method)				
Pet. Ether	6.06±SEM 0.03	5.01±SEM 0.03	-17.32	4.85±SEM 0.00	-19.96
Benzene	0.97±SEM 0.01	0.97±SEM 0.00	0	0.83±SEM 0.01	-14.43
Chloroform	0.21±SEM 0.01	0.20±SEM 0.01	-4.76	0.15±SEM 0.00	-28.57
Ethanol	8.43±SEM 0.04	8.02±SEM 0.02	-4.86	7.90±SEM 0.00	-6.28
	Quantitative Estimation of Functional group				
Total Saponins	17.65% w/w	1.65% w/w	-90.65	27.91% w/w	36.76
Total Sugar	17.28% w/w	16.37% w/w	-5.26	30.53% w/w	43.39
Tannins	14.39% w/w	6.09% w/w	-57.67	9.63% w/w	-33.07
Total Flavonoids	1.24%	1.84%	32.60	1.32%	6.06
	HPLC				
6-Gingerol %(w/w)	0.038	0.034	-10.52	0.02	-47.36
8-Gingerol %(w/w)	0.010	0.032	68.75	0.005	-50
6-Shogaol %(w/w)	0.024	0.159	90	0.03	25
10-Gingerol %(w/w)	0.008	Not detected		0.01	25
Total Pungent Compound	0.08	0.22	63.63	0.065	-18.75

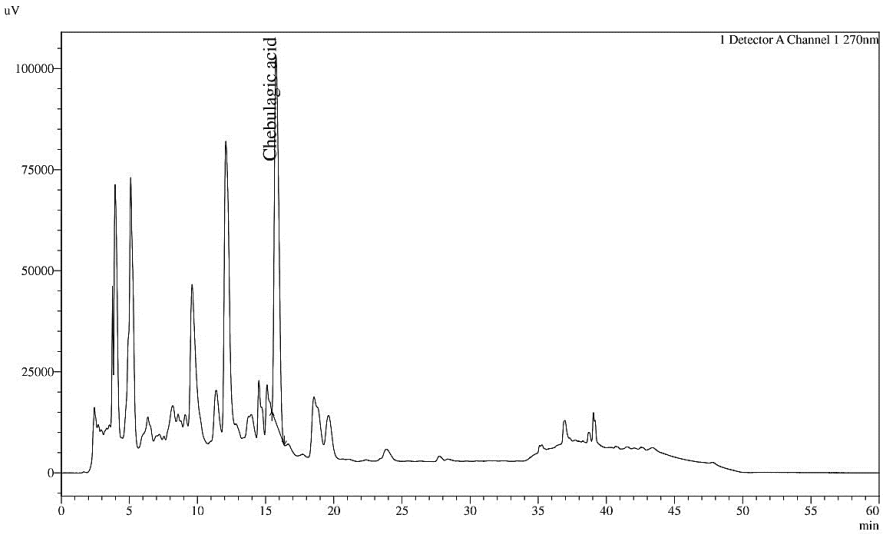

Figure 2: HPLC scan (Chebulagic acid) of *Safoof Muqliyasa* at 3rd Month (extract at 270 nm).

Table 7: Physico-chemical profile of *S. Muq.* and *S. Mul.* at different intervals.

Parameters	Initial		"3 Month"		"6 Month"	
	S. Muq	*S. Mul*	*S. Muq*	*S. Mul*	*S. Muq*	*S. Mul*
Loss on drying	6.12	4.25	5.62	5.03	4.94	5.1
Total Ash	4.71	25.02	2.54	24.26	2.41	23.09
Acid Insoluble Ash	2.12	3	0.713	2.81	0.58	2.55
Water Soluble Ash	2.26	18.92	1.16	18.09	0.98	17.43
S Ash	0.18	10.33	0.18	8.66	0.06	8.16
pH	5.63	5.28	5.86	6.53	5.60	6.53
CEV Water	14.86	30.45	10.08	29.56	9.26	28.66
CEV Ethanol	10.50	11.15	7.99	11.01	6.33	9.66
NSEV W	18.36	49.35	15.23	44.34	14.86	44.07
NSEV P Ether	9.92	6.26	9.33	5.01	8.19	4.85
NSEV Chloroform	13.15	5.76	12.25	5.47	9.73	4.94
NSEV Ethanol	17.85	20.21	12.97	17.3	11.23	16.99
NSEV Benzene	23.6	8.47	22.80	8.03	18.93	7.08
SEV Petroleum Ether	10.66	6.06	9.31	5.01	9.24	4.85
SEV Benzene	5.90	0.97	6	0.97	4.94	0.83
SEV Chloroform	3.34	0.21	3.25	0.20	2	0.15
SEV Ethanol	6.93	8.43	6.52	8.02	3.81	7.90
Mucilage	2.36	0.34	2.29	0.32	1.78	0.33
Total Saponins	30.29		14.34		16.1	
Total Sugar	12.82	17.28	14.28	16.37	10.22	30.53
Total Tannin	1.41		2.76		3.78	
Total Flavonoids	0.52	1.24	0.89	1.84	0.76	1.32
Chebulagic acid	0.77		0.77		0.27	
6-Gingerol		0.038		0.034		0.02
8-Gingerol		0.010		0.032		0.005
10-Gingerol		0.008		0.00		0.01

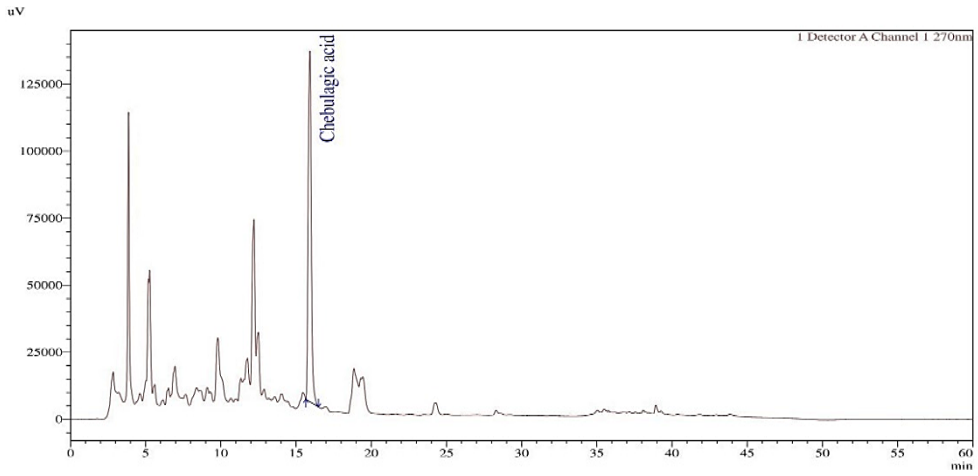

Figure 3: HPLC scan (Chebulagic acid) of *Safoof Muqliyasa* at 6th Month (extract at 270 nm).

Table 8: Intercept, slope and R^2 of *S. Muq.* and *S. Mul* for different parameters.

Parameters	Intercept		Slope		R^2	
	S. Muq	*S. Mul*	*S. Muq*	*S. Mul*	*S. Muq*	*S. Mul*
Loss on drying	6.150	4.368	0.196	0.141	0.753	0.450
Total Ash	4.370	25.088	0.383	0.321	0.908	0.662
Acid Insoluble Ash	1.908	3.012	0.256	0.0750	0.905	0.720
Water Soluble Ash	2.107	18.892	0.213	0.248	0.938	0.663
S Ash	0.200	10.135	0.020	0.361	0.90	0.768
pH	5.712	5.488	0.005	0.208	0.603	0.425
CEV Water	14.200	30.452	0.933	0.298	0.877	0.647
CEV Ethanol	10.358	11.352	0.695	0.248	0.898	0.704
NSEV W	17.900	48.560	0.583	0.880	0.752	0.686
NSEV P Ether	10.012	6.078	0.288	0.235	0.738	0.778
NSEV Chloroform	13.420	5.800	0.570	0.136	0.799	0.713
NSEV Ethanol	17.327	19.777	1.103	0.536	0.880	0.728
NSEV Benzene	24.112	8.555	0.778	0.231	0.752	0.730
SEV P Ether	10.447	5.912	0.236	0.201	0.707	0.759
SEV Benzene	6.093	0.993	0.160	0.023	0.720	0.710
SEV Chloroform	3.533	0.216	0.223	0.010	0.862	0.811
SEV Ethanol	7.313	8.382	0.520	0.088	0.890	0.650
Mucilage	2.433	0.335	0.096	0.001	0.783	0.622
Total Saponins	27.338		2.365		0.862	
Total Sugar	13.740	14.768	0.433	2.208	0.723	0.120
Total Tannin	1.465		0.395		0.030	
Total Flavonoids	0.603	1.427	0.040	0.013	0.293	0.491
Chebulagic acid	0.855		0.083		0.899	
6-Gingerol		0.039		0.003		0.776
8-Gingerol		0.018		0.000		0.287
10-Gingerol		0.005		0.000		0.105

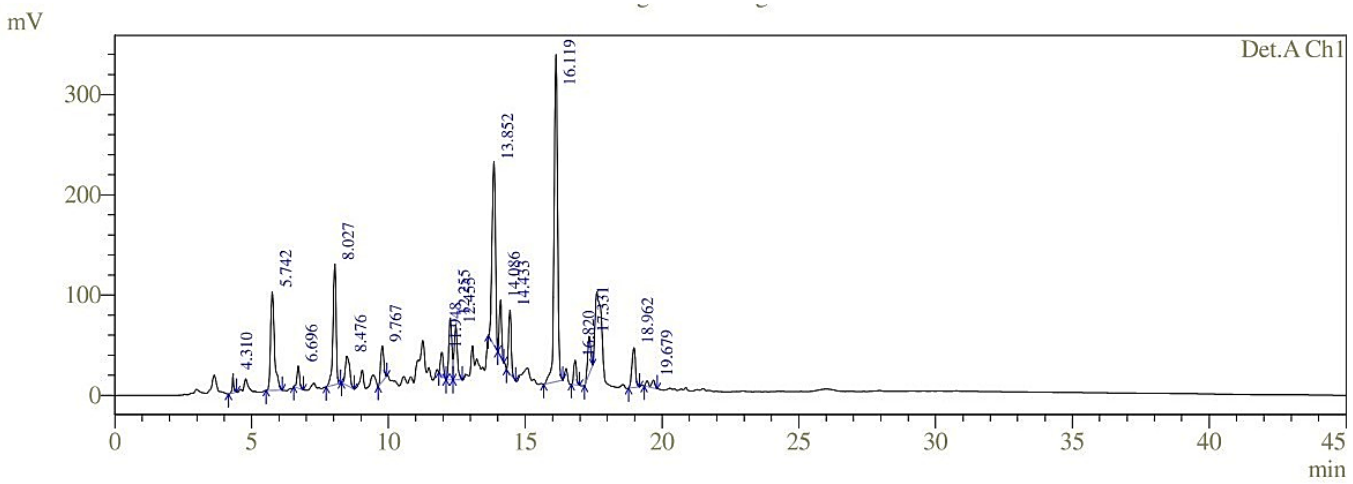

Figure 4: HPLC Finger print of *Safoof Muqliyasa* at 0 (Initial) (extract at 270 nm) .

Table 9: Approximate period (In months) for 10% degradation of *S. Muq.* and *S. Mul.* with condition: 40°C±2 and 75%±5 RH.

Parameters	Initial		10% Degradation		Months	
	S. Muq	*S. Mul*	*S. Muq*	*S. Mul*	*S. Muq*	*S. Mul*
Loss on drying	6.12	4.25	5.508	3.825	3.264	2.165
Total Ash	4.71	25.02	4.239	22.518	0.342	7.991
Acid Insoluble Ash	2.12	3	1.908	2.7	0.001	4.156
Water Soluble Ash	2.26	18.92	2.034	17.028	0.341	7.505
S Ash	0.18	10.33	0.162	9.297	1.900	2.317
pH	5.63	5.28	5.067	4.752	128.933	3.534
CEV Water	14.86	30.45	13.374	27.405	0.885	10.212
CEV Ethanol	10.50	11.15	9.45	10.035	1.307	5.302
NSEV W	18.36	49.35	16.524	44.415	2.359	4.710
NSEV P Ether	9.92	6.26	8.928	5.634	3.758	1.891
NSEV Chloroform	13.15	5.76	11.835	5.184	2.781	4.507
NSEV Ethanol	17.85	20.21	16.065	18.189	1.144	2.958
NSEV Benzene	23.6	8.47	21.240	7.623	3.690	4.023
SEV P Ether	10.66	6.06	9.594	5.454	3.603	2.269
SEV Benzene	5.90	0.97	5.310	0.873	4.896	5.157
SEV Chloroform	3.34	0.21	3.006	0.189	2.361	2.767
SEV Ethanol	6.93	8.43	6.237	7.587	2.070	8.996
Mucilage	2.36	0.34	2.124	0.306	3.200	17.400
Total Saponins	30.29		27.261		0.033	
Total Sugar	12.82	17.28	11.538	15.552	5.082	0.341
Total Tannin	1.41		1.269		0.218	
Total Flavonoids	0.52	1.24	0.468	1.116	0.783	4.700
Chebulagic acid	0.77		0.693		1.924	
6-Gingerol		0.038		0.0342		1.822
8-Gingerol		0.010		0.0099		9.920
10-Gingerol		0.008		0.007		6.600
Mean months					7.60	5.27

Note: 6 Shogaol, Total Saponin and Total Tannin, these are outlier from Safoof Mulayyin.

Table 10: Extrapolation of Shelf life in *S. Muq.* and *S. Mul* for Climatic zone III and IV.

Drug	Months	Multiplication factor	Shelf life (months)	Shelf-life years
S. Muqliyasa	7.60	3.3	25.08	2 years and 1 Month
S. Mulayyin	5.27	3.3	17.39	1 year and 5 months

Table 11: HPLC scan of *S. Muq* at 0 (Initial), 3 and 6 month (extract at 270 nm).

HPLC scan of *Safoof Muqliyasa* at	Peak#	Ret. Time	Name	Area	Area %
0 (Initial)	1	16.119	Chebulagic acid	2826306	100.000
	Total			2826306	100.00
3rd Month	1	15.762	Chebulagic acid	2088927	100.000
	Total			2088927	100.000
6th Month	1	15.923	Chebulagic acid	1893500	100.000
	Total			1893500	100.000

Table 12: HPLC Finger print of *Safoof Muqliyasa* at 0 (Initial) (extract at 270 nm).

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.310	76310	19033	0.767	1.585
2	5.742	969427	98323	9.749	8.190
3	6.696	140305	22077	1.411	1.839
4	8.027	964099	120301	9.696	10.021
5	8.476	322899	28749	3.247	2.395
6	9.767	279887	35707	2.815	2.974
7	11.948	165248	23278	1.662	1.939
8	12.255	510964	61667	5.139	5.137
9	12.455	416097	53962	4.185	4.495
10	13.852	1568125	182308	15.770	15.185
11	14.086	349583	55629	3.516	4.634
12	14.433	431146	63046	4.336	5.251
13	16.119	2826306	326022	28.424	27.156
14	16.820	173421	24861	1.744	2.071
15	17.331	292963	38131	2.946	3.176
16	18.962	358245	39709	3.603	3.308
17	19.679	98443	7747	0.990	0.645
Total		9943466	1200552	100.000	100.000

Table 13: HPLC Finger print of *Safoof Muqliyasa* at 3rd Month (extract at 270 nm).

Peak#	Ret. Time	Name	Area	Area %
1	5.107	RT:5.107	1429253	18.638
2	9.607	RT:9.607	961750	12.542
3	11.372	RT:11.372	251311	3.277
4	12.086	RT: 12.086	1673962	21.830
5	14.508	RT: 14.508	232237	3.029
6	15.106	RT: 15.106	157179	2.050
7	15.762	RT: 15.762	2088927	27.241
8	18.548	RT: 18.548	447294	5.833
9	19.613	RT: 19.613	218248	2.846
10	36.962	RT: 36.962	85826	1.119
11	38.694	RT: 38.694	30030	0.392
12	39.050	RT: 39.050	92268	1.203
Total			7668287	100.000

Table 14: HPLC Finger print of *Safoof Muqliyasa* at 6th Month (extract at 270 nm).

Peak#	Ret. Time	Name	Area	Area %
1	3.866	RT: 3.866	786610	14.561
2	5.268	RT: 5.268	538777	9.973
3	6.950	RT: 6.950	157215	2.910
4	9.807	RT: 9.807	266104	4.926
5	11.776	RT: 11.776	93193	1.725
6	12.197	RT: 12.197	645068	11.941
7	12.495	RT: 12.495	133540	2.472
8	15.923	RT: 15.923	1893500	35.051
9	18.853	RT: 18.853	417081	7.721
10	19.442	RT: 19.442	348588	6.453
11	24.249	RT: 24.249	87990	1.629
12	38.925	RT: 38.925	28433	0.526
13	39.265	RT: 39.265	6040	0.112
Total			5402138	100.000

Table 15: HPLC scan (Gingerol/Shogaol) of *S. Mul* at 0, 3 and 6 Month (extract at 278 nm).

Peak#	Ret. Time	Name	Area	Area %
At 0 (Initial)				
1	5.735	6-Gingerol	18713	47.365
2	11.031	8-Gingerol	5090	12.883
3	13.663	6-Shogaol	11514	29.145
4	24.553	10-Gingerol	4191	10.608
Total			39508	100.000
At 3rd Month				
1	6.404	6-Gingerol	27768	14.830
2	16.360	8-Gingerols	27591	14.736
3	18.303	6-Shogaol	131883	70.434
Total			187242	100.000
At 6th Month				
1	5.936	6-Gingerol	36426	24.668
2	11.795	8-Gingerols	8624	5.840
3	14.680	6-Shogaol	50542	34.228
4	27.343	10-Gingerol	37406	25.332
Total			132998	90.068

content of *S. Muq* contains Mucilage namely Tukhme teera tezak (*Lepidium sativum*),[34] Tukhme Alsi (*Linum usitatissimum*).[35] and there can be variation in mucilage extraction after exposure to elevated temperature as study showed on other mulilages that mucilage became water repellent after drying,[36] But at 6th month extract value in several solvent and procedures shout difference more than 25% (Table 3). Different extract value in case of *S. Mul* showed no more than 25% difference at 3rd month only Chloroform successive extract showed difference more than 25% at 6th month (Table 6). Mucilage: Mucilage content for both the *Safoof* displayed less than 25% difference at 3rd and 6th month (Tables 3 and 6).

Table 16: HPLC Finger printing of *Safoof Mulayyin* at 0 (Initial) (extract at 278 nm).

Peak#	Ret. Time	Name	Area	Area %
1	4.401	RT: 4.401	104751	44.425
2	5.735	RT: 5.735	18713	7.936
3	6.214	RT: 6.214	6225	2.640
4	7.137	RT: 7.137	4336	1.839
5	11.031	RT: 11.031	5090	2.159
6	13.663	RT: 13.663	11514	4.883
7	15.574	RT: 15.574	59116	25.071
8	18.724	RT: 18.724	21855	9.269
9	24.553	RT: 24.553	4191	1.777
Total			235792	100.000

Table 17: HPLC Finger printing of *Safoof Mulayyin* at 3rd Month (extract at 278 nm).

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.813	178098	15551	40.963	57.816
2	6.404	27768	2491	6.387	9.261
3	6.856	21857	1021	5.027	3.795
4	8.204	13013	905	2.993	3.366
5	12.986	5880	334	1.352	1.240
6	16.360	27591	1195	6.346	4.441
7	18.303	131883	4447	30.334	16.534
8	22.776	28687	954	6.598	3.546
Total		434777	26898	100.000	100.000

Table 18: HPLC Finger printing of *Safoof Mulayyin* at 6th Month (extract at 278 nm).

Peak#	Ret. Time	Name	Area	Area %
1	4.448	RT: 4.448	230017	46.048
2	5.936	RT: 5.936	36426	7.292
3	6.054	RT: 6.054	14666	2.936
4	6.453	RT: 6.453	11298	2.262
5	7.443	RT: 7.443	16146	3.232
6	11.795	RT: 11.795	8624	1.726
7	12.555	RT: 12.555	4968	0.995
8	14.680	RT: 14.680	50542	10.118
9	16.217	RT: 16.217	33527	6.712
10	20.162	RT: 20.162	41176	8.243
11	24.292	RT: 24.292	14718	2.946
12	27.343	RT: 27.343	37406	7.488
Total			499515	100.000

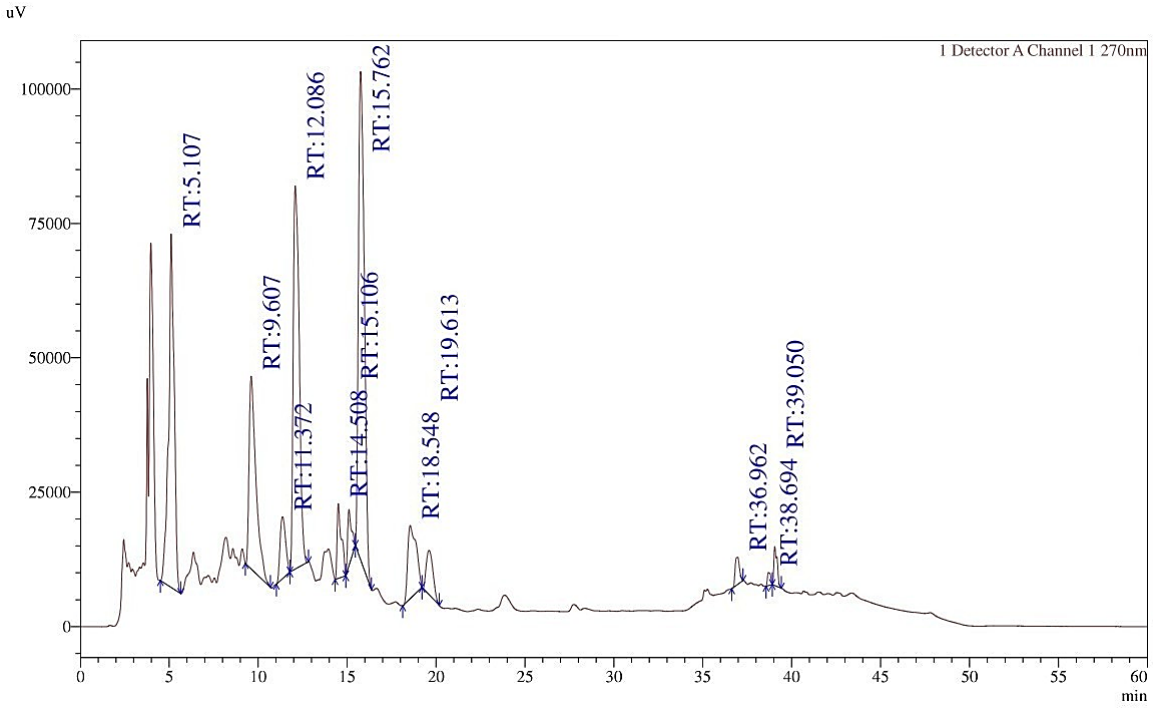

Figure 5: HPLC Finger print of *Safoof Muqliyasa* at 3rd Month (extract at 270 nm).

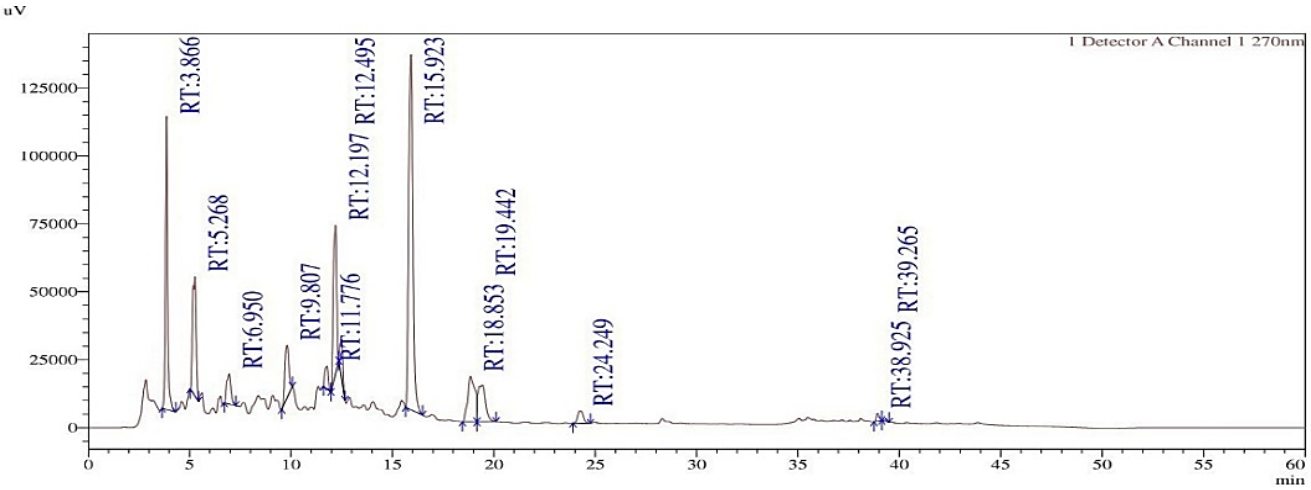

Figure 6: HPLC Finger print of *Safoof Muqliyasa* at 6th Month (extract at 270 nm).

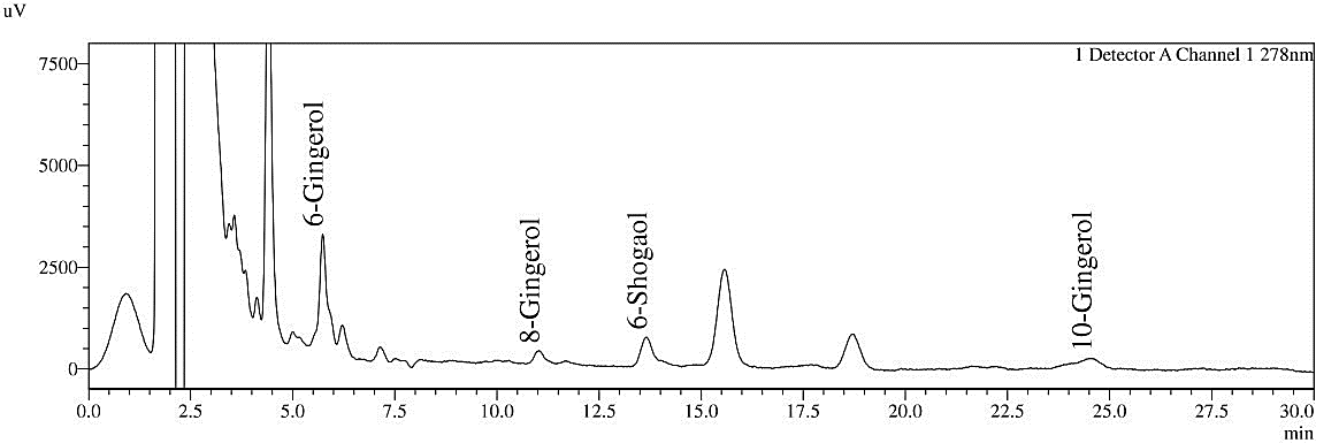

Figure 7: HPLC scan (Gingerol/Shogaol) of *Safoof Mulayyin* at 0 (Initial) (extract at 278 nm).

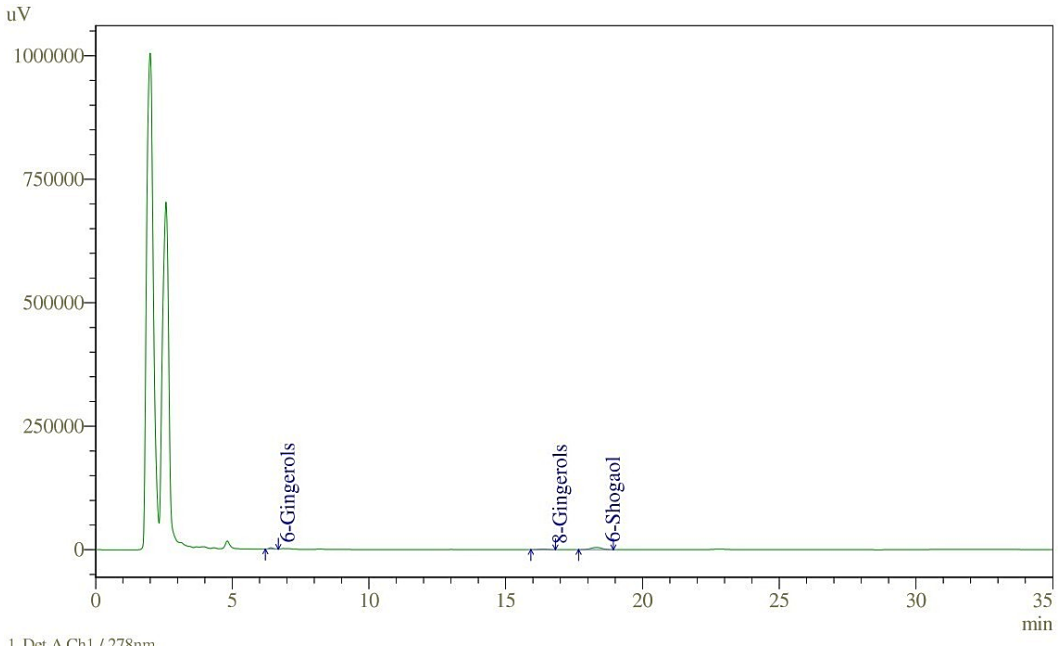

Figure 8: HPLC scan (Gingerol/Shogaol) of *Safoof Mulayyin* at 3rd Month (extract at 278 nm).

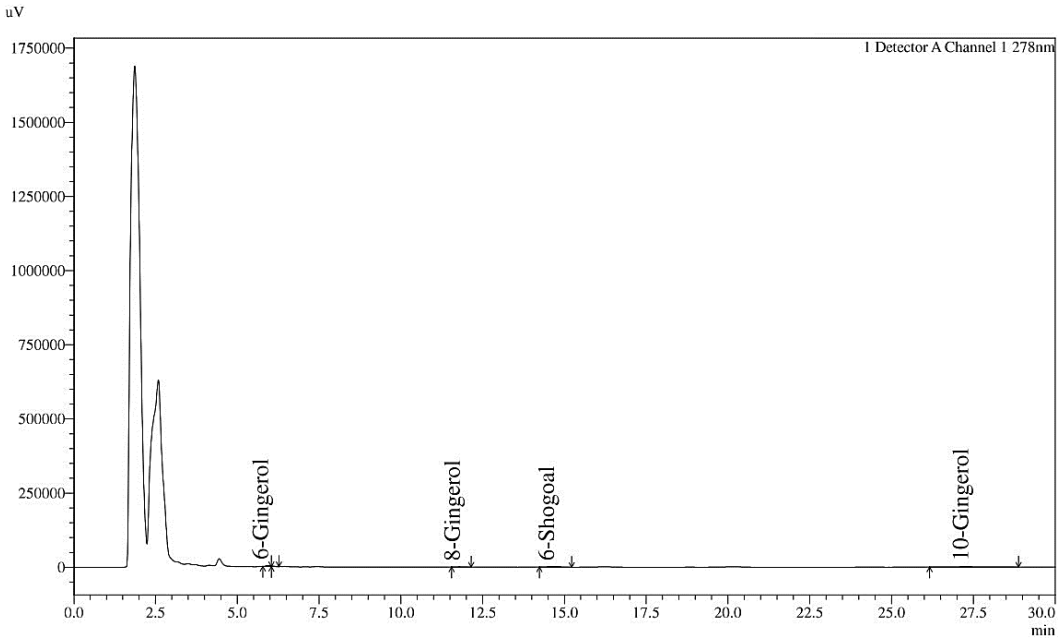

Figure 9: HPLC scan (Gingerol/Shogaol) of *Safoof Mulayyin* at 6th Month (extract at 278 nm).

Microbial Analysis

All the microbial analysis was in confirmation to World Health Organization (WHO) guideline, API, protocol for testing of ASU drug for both *S. Muq* and *S. Mul* (Tables 2 and 5).[25,37]

Total Saponins

Variation in saponin content can also occurs due to processing, hydrolysis, simple leaching of water soluble saponin, chemical alteration and degradation.[38] Decrease in saponin content in *Safoof Muq*. may be attributed to various factors like hydrolysis and chemical alteration, that might be the case for this change and that can be justified by increase value of sugar, tannins and flavonoids, as aglycon moiety can be any of the increases component, these changes need further detailed study.[39] The reading of saponin values in case of *S. Mul*. looks erratic which needs further investigated hence this value is not considered for assessing as per API parameters and other statistical analysis as out layered (Tables 3 and 6). Total Tannins: The reading of total values in case of *S. Mul.* looks erratic which needs further investigated hence this value is not considered for assessing as per API parameters and other statistical analysis as out layered. Total Flavonoids is depicted in Table 2.

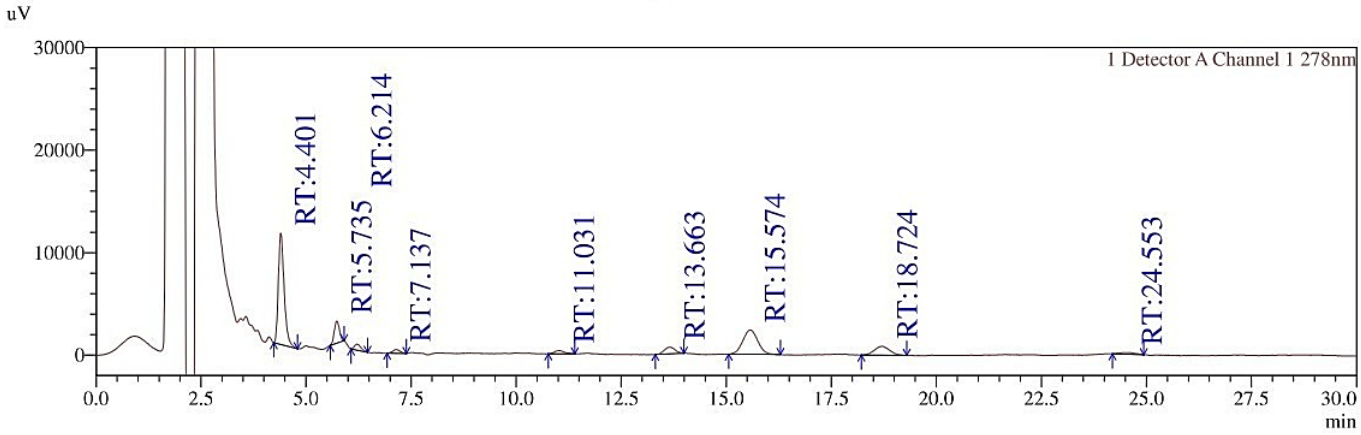

Figure 10: HPLC Finger printing of *Safoof Mulayyin* at 0 (Initial) (extract at 278 nm).

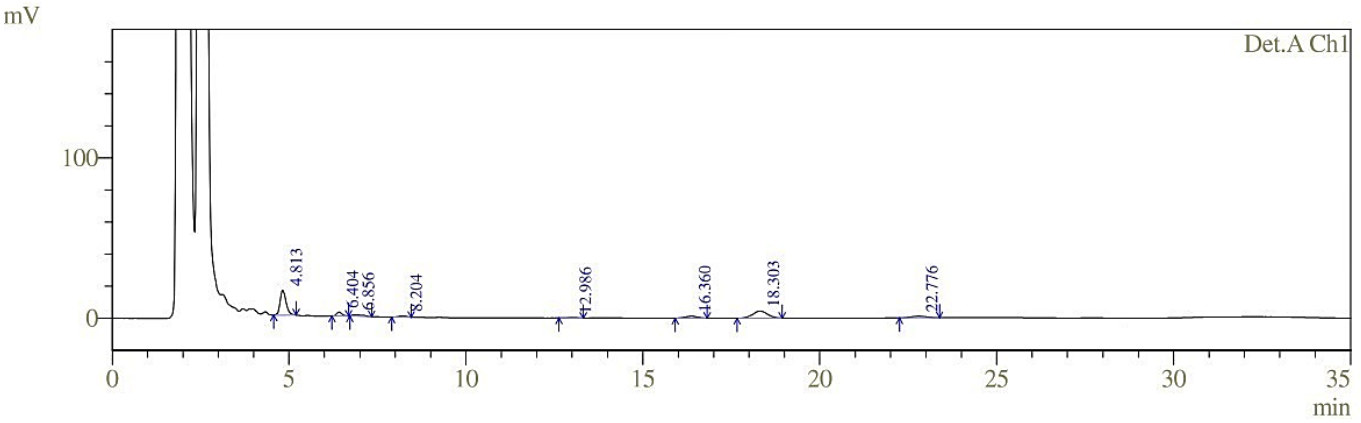

Figure 11: HPLC Finger printing of *Safoof Mulayyin* at 3rd Month (extract at 278 nm).

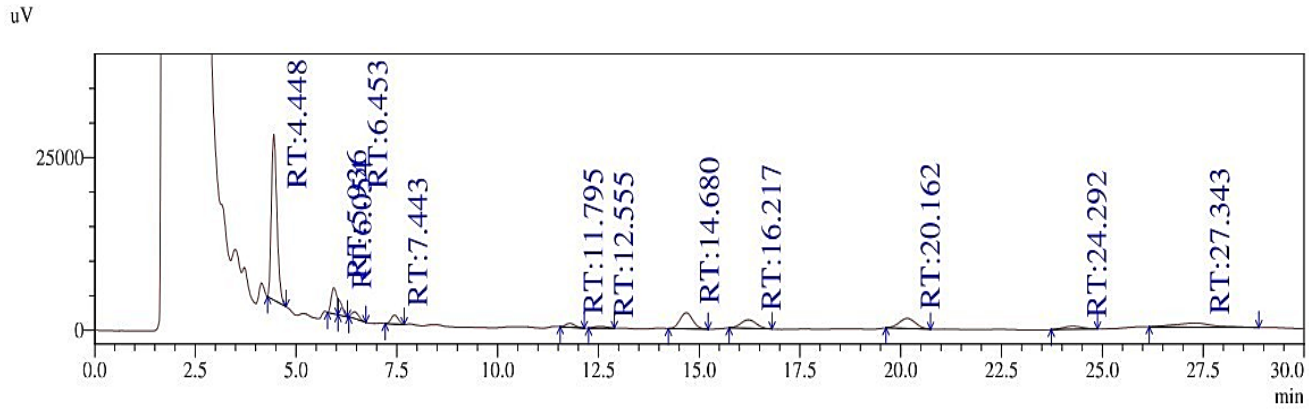

Figure 12: HPLC Finger printing of *Safoof Mulayyin* at 6th Month (extract at 278 nm).

HPLC Quantification of Chebulagic acid as a active constituent in *Safoof Muqliyasa* displayed Total chebulagic acid which displayed no significant change at 3rd month as per API, but displayed more then 15% reduction in 6th month thereby restricting its shelf life up to 3 month in ASS. HPLC Quantification of Gingerol in *Safoof Mulayyin*: This result displays no significant change at 3-month interval as per API evaluation guideline for marker/active component whereas change was observed at 6-month interval. All the results showed no significant change as it does not display more than -20% reduction as per API at 3rd month reduction but 6- Gingerol displayed more than -20% reduction at 6th month but its justification is that it simultaneously showed increase of shagol content, it was due to conversion of gingerol to shagol in the due course of time which is a normal phenomenon in dried ginger (Tables 3 and 6).[40]

HPLC fingerprinting data obtain during study of quantitative estimation of Chebulic acid in case of *S. Muq*. Reveals few

A

Figure 13: Test drugs in Stability Chamber.

missing peaks in 3rd month and formation of new/additional peaks in 6th month. where as in case of *S. Mul.* no significant missing peak at 3rd month only one peak was missing but there are 4 new additional peak formation at 6th month (Tables 11-18 and Figures 1-12). For detail regarding finger printing data appropriate chromatographic method with appropriate solvent system displaying maximum peaks can be studied for further detail assessment. The chromatogram of samples degraded with acid, base, hydrogen peroxide, and heat showed well-separated spots as well as some additional peaks. The number of degradation product with their R_f values, can be calculated. These chromatograms particularly with HPTLC can give stability indicating property. The variation determination of common peaks/regions in a set of chromatographic fingerprints can provide useful qualitative information on the characteristic components of herbal medicines studied. Chromatographic fingerprint analysis serves as a promising quality control tool for herbal medicines.[41] As per ICH guideline, countries come under climatic zones II and IV having climatic condition 30°C/35% RH and 30°C/70% RH. India comes under climatic zone III and IV. Climate of Zone IV is hot, humid climate. Temperature of Zone IV is 30°C and Relative Humidity is 70%.[13] According to WHO to determine the shelf-life of finished herbal products, strong emphasis should also be placed on tests such as moisture content, microbial contamination and general dosage form control tests.[42]

On the basis of API guidelines both the powder *S. Muq* and *S. Mul* complies Physical, Microbiological stability parameters upto 6 month and quantitative estimation of selected active constituent for chemical stability upto 3 months with justification of few constituents in quantitative estimations. As far as HPLC quantitative estimation of Chebulagic acid as a active constituent considered in case of *Safoof Muqliyasa as* per API suggestion i.e. "A + or – 15% change from the initial assay value (If the drug is analyzed for its active compound)" it complies ASS criteria for 3 month and "No significant change" was observed at 3 months but significant change occurs at 6 months. Overall shelf life of *S. Muq*. as per consideration of ICH guidelines with evaluation criteria as per API is 3 months. And when we applied Grimm concept to extrapolate real time period as according to Grimm's statement, predictive factor for zone IV is 3.3 of the accelerated study periods. Then shelf life of *S. Muq* can be extrapolated as 10 month and in round figure 1 year.

And for *S. Mul*. As per API "A + or – 20% change from the initial assay value (If the drug is analyzed for its marker)" It also complies ASS criteria for 3 month and "No significant change" was observed at 3 months but significant change occurs at 6 months. Marker selected in case of *S. Mul*. Gingerol have one disadvantage that normally there is heat-induced conversion of gingerols to shogaols and it is very difficult to assess the chemical stability of the powder with this marker, it can be further studied with other appropriate marker and active constituent to establish accurate chemical stability/shelf life of the product.

It is preliminary shelf-life study, advance study is needed for both the *Safoof* preparation with testing frequency including one month interval. Appropriate sophisticated constituent study to comment on chemical changes occurs in both the powders is also needed. Consideration of acceptance criteria as per individual monographs or specification if developed for the studied drug can also be considered. In case of *S. Muq* ingredients National Formulary of Unani Medicine states that the stability of Sufoof containing Maghziyat (kernels) is <6 months.[43] Generally in Unani medicine shelf-life mention for powder formulation are off shorter duration such as according to *Arzani*, Sufoof has the shelf life of only 3 months[3] and according to *Arastatalees* shelf life of *Safoof* is one year.[8] Obtained result also support Unani physician's claims for shorter shelf life of powders. Powder formulations might have reduced shelf life due to increase in surface area and its ability to absorb moisture is increased.[44,45]

As far as physical and microbiological parameters is concern both *S. Muq* and *S. Mul*. are stable for 6 months in ASS as on extrapolation into Zone IV 3.3 formula 20 month (2 Years round

figure) for the packed condition provided without inclusion of any preservatives/excipients. (As per WHO guidelines "Samples used for stability studies should be stored in the containers intended for marketing" and is followed in this work in respect of using HDPE container and sealing it).[42]

S. Mul is a *Safoof* containing salt and *S. Muq* does not, Present study suggest that shelf-life data of salt and non-salt containing *Safoof* should be assesses scientifically irrespective of its contents when studied in sealed condition. The 10% reduction was also predicted from liner regression equation using individual slope and Intercept not forced through zero. The r^2 value (Table 8) was also calculated to ascertain the linearity and its deviation from linearity. The values calculated are tabulated in (Table 8). The shelf life for individual parameters was assessed multiplying factor of 3.3 with the time required to reduce the initial value to 90%. On the basis of available data from accelerated stability study of *S. Muq* and *S. Mul*, it can be extrapolated that shelf life of *Safoof Muqliyasa* is 25.08 months (2 years and 1 Month) and *Safoof Mulayyin* 17.39 months (1 year and 5 months) for countries under climatic zone III and IV. It was calculated with consideration of 10% degradation rate in different physicochemical parameters,[46-48] No significant change was observed in organoleptic characters (physical parameter) and microbial load (Microbiological parameter) at 6 months.

Accelerate study for both the *Safoof* was done under "Study conditions for drug substances and formulations intended to be stored under general conditions. As per Drug and Cosmetic act", the optimal condition for the storage of medicine in ASS was 40°C±2°C/75% RH±5% RH for 6 months in stability chamber (Figure 13). If we can maintain general storage condition for the both the *Safoof* then shelf life of *Safoof Muqliyasa* will be 2 years and 1 Month and *Safoof Mulayyin* will be 1 year and 5 months. These results matched with the implemented rule of The Drug and Cosmetic act Rule 161 B (Shelf life or date of expiry of medicine, Unani Medicine), i.e. 2 years for *Safoof* and 1 year for *Safoof* containing salt which is the case of *Safoof Mulayyan* as it is less than 2 year (1.5 months). As per amendment in the act it is now mandatory to display the date of expiry of the ASU drugs and Unani medicine defined under clause (h) of section 3 of the Act for Unani Patent and Proprietary Drugs have to submit data based shelf life or date of expiry of medicine based on the real time stability studies of medicines in accordance with the guidelines prescribed in API (Ayurvedic Pharmacopea of india),[7] even this rules prescribed for Unani medicine (Pharmacopea) as per clause (a) of section 3 of D and C act, Rule 161 B to follow the list given for shelf life of different Unani formulation but with a quote unless otherwise determined on the basis of scientific data and propose shelf of both the *Safoof* matches the list in respect of 10% degradation study. As per GO. Government of India letter no T.13011/3/2019-DCC (AYUSH), Ministry of AYUSH dated: 29th July 2019 state that "under section 33P of the Drug and Cosmetics Act, 1940 and in consultation with Pharmacopoeia Commission of Indian Medicine and Homoeopathy, Government of India in the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy (AYUSH) directs all state Licensing Authorities of Ayurveda, Siddha and Unani (ASU) Drugs to also consider and accept the accelerated stability study data for fixing the shelf life of ASU drugs under Rule 161-B of the Drugs and Cosmetics Rules 1945 for the purpose of grant of license and renewal of license in reference to GSR No. 789(E) dated 12.8.2016".[49] This order is permissible to submit the accelerated stability data for license of patent and proprietary ASU drugs. In view of this recent development studied data became more relevant and can be utilized as a reference.

As per WHO guidelines "The stability of preservatives and stabilizers should be monitored. When these are not used, alternative tests should be done to ensure that the product is self-preserving over its shelf-life"[42] and this formulation can be further evaluated in future with preservative. It can be evaluated for its shelf life with variable of appropriate testing frequency, excipients, preservatives and powder sterilization techniques in future work on the same formulations. This was a preliminary study. Long-term testing covering a minimum of 12 months duration and or real time shelf-life study for a period of time sufficient to cover the proposed shelf life can be attempted. It should be done simulating production and packaging according to ASU industrial pharmacy for the drug substance or the formulation.

CONCLUSION

It can be concluded that shelf life of *Safoof Muqliyasa* is 2 years and 1 month and of *Safoof Mulayyin* is 1 year and 5 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. Values of physicochemical parameters/standardization data of both *Safoof Mqliyasa* and *Safoof Mulayyin* was also set in at initial period (Base line) of the study. Further sophisticated study is required for the status comment on degradation of constituent and evaluation of other functional/active constituents and markers and by various other testing frequency and study model.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ASS: Accelerated stability study; ***S. Muq***: *Safoof Muqliyasa*; ***S. Mul***: *Safoof Mulayyin*; **D and C:** Drugs and Cosmetics; **RH:** Relative Humidity; **ICH:** International Council for Harmonisation; **UM:** Unani Medicine, **HDPE:** High-density polyethylene; **HPLC:** High-performance liquid chromatography; **API:** Ayurvedic Pharmacopoeia of India; **UPI:** Unani Pharmacopoeia of India; **UV:** Ultraviolet; **cfu:** Colony-forming unit; **WPL:** Within permissible limit.

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SUMMARY

The study focuses on evaluating the stability of two Unani Medicine (UM) powder formulations, *Safoof Muqliyasa* (*S. Muq*) and *Safoof Mulayyin* (*S. Mul*), through Accelerated Stability Studies (ASS) conducted at 40°C±2°C and 75%±5% relative humidity, following ICH guidelines. The formulations were stored in sealed HDPE containers and tested at 0, 3, and 6 months for organoleptic, physical, microbiological, and chemical parameters, including HPLC quantification of active markers (Chebulic acid in *S. Muq* and Gingerol in *S. Mul*). Results, analyzed using API guidelines and statistical methods, indicated that both formulations remained stable under accelerated conditions for up to 3 months. Extrapolating using Grimm's statement, the predicted shelf life at room temperature for zone III and IV was estimated to be 10 months. Specifically, *S. Muq* showed a predicted stability of 2 years and 1 month, while *S. Mul* had a predicted stability of 1 year and 5 months. Although both formulations were physically and microbiologically stable for 6 months in ASS, chemical changes were observed after 3 months, suggesting an approximate real-time stability of 1 year. *S. Mul*, which contains salt, exhibited lower stability compared to *S. Muq*. The findings highlight the need for precise shelf-life determination for UM formulations, with the observed stability being specific to these two powders.

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