Research Article

Accelerated Stability Study of Arqe Gulab

Firoz Khan*, Roohi Zaman*, Shariq Shamsi****

*PG Scholar, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.

**Lecturer, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.

***Reader and HOD, Dept. of Ilmul Saidla, National Institute of Unani Medicine, Bangalore, Karnataka, India.

***Corresponding author's E-mail:** firoznium@gmail.com

Received: 22-02-2017; **Revised:** 18-04-2017; **Accepted:** 10-05-2017.

ABSTRACT

Evaluation of Stability studies of various Unani formulations are need of the time. The keen observations of the Unani classical authorities need to be substantiated with empirical evidence using scientific methodology. This study will furnish the feasible methods for evaluating stability of Unani drugs. Accelerated study for six months was carried out to assess stability of Unani formulation Arqe Gulab. The drug was kept in containers used by Unani Pharmacy Units, and kept in stability chamber at 40ºC ± 2ºC and 75%RH ± 5%RH. The samples were analysed at 0, 3, and 6 months for evaluation of organoleptic characters, physical, chemical standards and microbiological status as outlined by ICH (International Conference on Harmonization) Guidelines. GCMS (Gas Chromatography-Mass Spectroscopy) was done at 0, 3 and 6 months and compared with zero month data. The finding showed that degradation changes were observed in the drug formulations between third and sixth month. The present study showed that the shelf life of AG (Arqe Gulab) was found to be six months to one year which validated the claims of Unani Physicians. The data collected at ASC (Accelerated Storage Conditions) can be extrapolated for Intermediate and Long term studies so that a better storage conditions for this essential drug distillate can be recommended. As well as Bracketing and Matrixing should also be incorporated in the studies, so that a complete profile of the best storage condition can be evaluated in future.

Keywords: Arqe Gulab, Accelerated stability study, Unani formulations, ICH guidelines, GCMS, Bracketing, Matrixing.

INTRODUCTION

Herbs are in the extensive use in the traditional practices of different countries since time immemorial.[1] Natural products including plants, animals and minerals have been the basis of treatment of human diseases.

Modern medicine has gradually developed over years of scientific and observational efforts of scientists however, the basis of its development remains in the roots of traditional medicine and therapies.[2] The consumption of Unani medicines has considerably increased in the recent years in India and their substantial production is also being organized for export.[3]

Every compound has got its own importance and the processing makes them to acquire a specific form.[4] All the non living substances that are grown or manufactured go through a life span in which they influence and are influenced by their environment.[10]

In order to do this, it is necessary to monitor compliance of the product with suitable quality specification throughout the shelf life. To ensure the quality of finished product and predict the expiration dates, stability studies are essential which will be evaluated according to the International Conference on Harmonization (ICH) guidelines.[5]

MATERIALS AND METHODS

In the present study one Unani Pharmacopeial preparation Arqe Gulab was screened for its shelf life by assessing the stability of the product. The study was Accelerated stability study. The physical, chemical and microbiological assays were evaluated as per the Guidelines of the ICH.

The *Rosa damascena* was cultivated in the herbal garden of NIUM (National Institute of Unani Medicine) by procuring the saplings from Nursery of Lalbagh Botanical Garden, Bangalore. When the plant matured and started flowering, these fresh flowers were plucked early in the morning and were identified and authenticated by Prof. Dr. Vasundhara, GKVK, Bangalore. Arqe Gulab was prepared in the Laboratory of Dept. of Ilmul Saidla, NIUM, Bangalore.

Preparation of Arqe Gulab

Ingredients Used

Arqe Gulab was prepared according to method described in Bayaaze Kabir (Unani Pharmacopeia), Vol-2.[6]

Table 1: Ingredients used in Arqe Gulab

S. No.	Ingredients	Botanical Name	Quantity
1.	Gulab	*Rosa Damascena*	Quantity
2.	Water		500 grams

Method of Preparation of Arqe Gulab (AG)

Sepals and petals weighing 500 grams were immersed in 8 litres of water and the Arq was prepared through Steam Distillation. This method of steam distillation almost simulates the Unani Classical method "*Hammame*

Nariya" for preparation of Arq from drugs having the essential oils. Out of 8 litres of water, 2 litres of Arqe Gulab was collected.[6]

Storage

Arq was prepared cleanly and cautiously. The prepared Arq was filled in the Low Density Poly Ethylene Terephthalate (LDPE) bottles keeping in view the sterilizing factors. This Arq was stored in 4 bottles each of 500 ml and the cap was sealed.

Evaluation

After that these bottles were placed in stability chamber for the study, each sample was analyzed for physical, chemical and microbiological status at 0, 3 and 6 months respectively. The first sample of AG was analyzed at zero month for the above mentioned properties and viable count was also calculated.

Organoleptic Characters and Physical Parameters of Ag

Determination of Colour of AG

The colour of the Arqe Gulab was tested at the time of manufacture that is at zero month.[4]

Determination of Odor

The description of this feature sometimes may not be accurate because it depends on individual perception. If the material is expected to be innocuous, a small portion of the sample can be examined by slow and repeated inhalation of air over the material. The strength of the odor like weak, distinct, strong is first determined and then the odor sensations like musty, mouldy, rancid, fruity, aromatic etc were determined.[3-4, 7-8]

Determination of Taste

Initially the depth of organoleptic capacity should be tested. This can be done by asking the tester to taste serial dilutions of drugs. It should be noted that the technicians are not taste testers in ordinary sense. In so doing they would have to score the degree of flavorings e.g. was it less than present originally, i.e., was the flavor being lost? They would also have to be able to describe the flavor well originally.[4]

Determination of pH of AG

The pH value of 1% solution

An accurately measured 1ml AG was mixed in accurately measured 100ml of distilled water, and pH was measured with a pH meter.[3]

The pH value of 10% solution

An accurately measured 10ml of AG was mixed in accurately measured 100ml of distilled water, and pH was measured with a pH meter.[3]

Refractive Index of AG

The refractive index of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.[5] Refractive index was measured using Abbe's Refractometer.[9]

Weight per ml or Specific Gravity of AG

Specific gravity, in general, is the ratio of the weight in air of a given volume of material at a stated temperature to the weight of the same volume of water (or other reference) at a stated temperature.[10]

Measurement of SG (Specific Gravity) by Pycnometer

A pycnometer is a bottle with a capacity of usually 10 ml to 100 ml, having a stopper. A pycnometer was weighed accurately, previously cleaned and dried, and the weight 'W' was noted.

The stopper was removed; the pycnometer was filled with AG, keeping at a temperature 25°C. The outside surface wiped thoroughly, weighed accurately, and the weight 'W1' was noted. The same procedure was performed, using the same pycnometer containing double distilled water, the weight 'W2' was noted at the 25°C temperature. The specific gravity was calculated by the formula.[3, 10]

$$Specific\ Gravity = \frac{Weight\ of\ AG - Weight\ of\ EP}{Weight\ of\ DDW - Weight\ of\ EP}$$

Chemical Stability

GC-MS (Gas Chromatography-Mass Spectrometry)

In Arqe Gulab, oil was the main part having a lot of components within it. The oil from the AG was separated from the whole distillate from one container and was analysed for various components present in it at zero month, similarly at third month and sixth month. This oil was separated from the distillery part with the help of the Clevenger apparatus. The essential oil separated from AG was further analyzed for GCMS study done at Bangalore Test House, Bangalore.

Procedure

Sample Preparation Application

AG measuring 100 microlitres was dissolved in 1ml of Methanol, injected to GC/MS with following instrument conditions

GC/MS Analysis

Gas chromatography/mass spectrometry (GC/MS) analysis was carried out using a Hewlett-Packard 6890/5975 gas chromatograph. The separation was achieved by capillary column having dimensions 30m x 0.25 mm i.d. x film thickness, 250 micron. The column temperature was kept at 70°C at the rate of 5°C/min upto a temperature of 120°C. Then the temperature was increased to 280°C with a rate of 10°C/min and the same was maintained for 20 minutes. The constant flow rate of Helium (the carrier gas) was 2.0 ml/min and split ratio, 1: 60. The MS ion source temperature was 230°C.

Microbiological Stability of Ag

Viable Count

The basic principle of this method is that viable spores grow if they are provided with normal growth conditions and that each organism grow and multiply ultimately forming a visible mass of organism called colony.

Each organism grows into a separate colony with characteristic type of growth. Observation of their specific growth characteristics often provides useful information for identification.

The number of colonies present in a nutrient agar medium plate indicates the number of organisms present in the original sample.

Nutrient agar medium (25 ml) was prepared in a conical flask and sterilized in a pressure cooker.

Nutrient agar medium was cooled to 50°C after removing it from pressure cooker. Sterilized nutrient agar medium and 1ml of AG was taken into the laminar flow.

Nutrient agar media (25 ml) kept into a sterilized Petridish and 1ml AG was spread upon the sterilized nutrient agar medium already present in two different Petri dishes. These Petri dishes kept for incubation into Incubator for 24 hours. It was observed after 24 hours observations were recorded.

The total number of viable organisms was noted in terms of colony forming units.[4]

RESULTS AND DISCUSSION

The observations and results that were recorded during the stability study of AG showed that there were some minimal changes in the organoleptic properties and these changes were noted in third and sixth month when compared with standard parameters of zero month.

The appearance of AG was liquid and showed no changes in the third and the sixth month.

No colour change was observed in AG in the study.

The odour of AG was strongly aromatic at zero month, no changes were noted in the third month but the odour of the test drug had reduced in the sixth month.

The AG was found to be tasteless throughout the study. No precipitates were found in AG throughout the study.

Estimation of 1% pH of AG at zero month was 6.81 at 25°C and it decreased to 6.74 at third month and 5.65 at sixth month.

The change in 1% pH of AG at third month was found to be 1.02% which was insignificant change according to ICH, whereas at sixth month it was 17.03% which was highly significant according to ICH.

Estimation of 10% pH of AG at zero month was 7.70 at 25°C and it increased to 7.53 at 25°C at third month and it fell to 5.12 at 25°C at sixth month.

The change in 10% pH of AG at third month was found to be 2.20% which was insignificant change according to ICH, whereas at sixth month it was 33.50% which was highly significant according to ICH. The change between the third and sixth months were highly significant and indicates degradation changes, this difference exceeds the 5% limit set by ICH.[11-12]

Estimation of RI (Refractive Index) of AG at zero month was 1.3239, at third month the RI was 1.3234 and at sixth month the RI was 1.3233.

The difference between zero and third month was 0.0377% and between third and sixth month was 0.06%. This change was insignificant; it shows that the RI of AG was not influenced by ASC.

Estimation of SG or weight per ml of AG at zero month was 0.9935, at third month the SG was 0.9997 and at sixth month the SG was 1.000.

The difference between zero and third month was 0.6240% and between third and sixth month was 0.6542%.

This change was insignificant; it shows that the RI of AG was not influenced by ASC (Accelerated Storage Conditions).

Finally AG was subjected to GCMS study for identification of its constituents. AG was extracted from Gule Surkh which is rich in essential oil, this essential oil contains a number of components and simulates all the components present in the drug, hence the essential oil produced at the time of preparation of Arq was separated and this oil was subjected for GCMS analysis at zero, third and sixth month for identification of its components.

The components detected in the oil, extracted from the sample of Arqe Gulab, at zero month at different run times are as follows:

The component at RT(run time) 4.972 was Linalool and it was **1.622%,** at RT 5.197 was Rosoxide and it was **0.186%,** at RT 5.587 was Phenyl ethyl Alcohol and it was **71.209%,** at RT 6.892 was à-Terpineol and it was **0.483%,** at RT 7.838 was (R)-(+)-á Citronellol and it was **14.900%,** at RT 8.373 was cis Geraniol and it was **2.754%,** at RT 10.627 was Citronellol acetate and it was **0.269%,** at RT 10.721 was Eugenol and it was **1.538%,** at RT 11.679 was Benzene,1,2-dimethoxy-4-(2-propenyl)- and it was **2.233%,** at RT 11.900 was Caryophyllene and it was **0.247%,** at RT 12.247 was à-Guaiene and it was **0.311%,** at RT **15.919** was Heptadecane and it was **0.340%,** at RT 17.865 was 9-Nonadecene and it was **1.745%,** at RT 18.129 was Eicosane and it was **1.123%,** at RT 20.075 was Heneicosane and it was **1.295%.**

The total percentage of these compounds which were found naturally in rose oil is **100.255%.** Other natural constituents that were detected at zero month have not been considered as the peak height and peak areas were less.

Gas Chromatography-Mass Spectroscopy

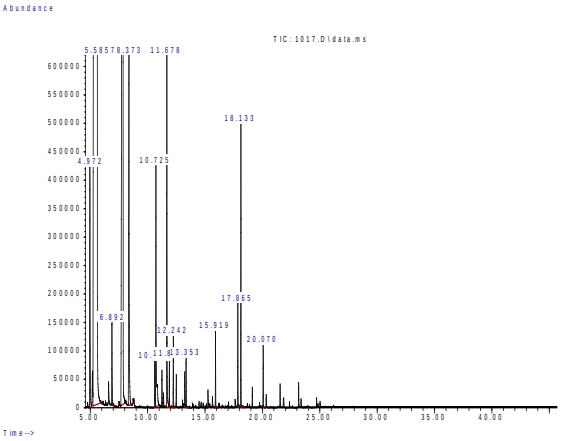

Figure 1: Arqe Gulab Chromatogram at Zero Month

The components detected in the oil, extracted from the sample of Arqe Gulab, at third month at different run times are as follows:

The component at RT 4.987 was Linalool and its quantity was **1.49%,** at RT **5.197** was Rosoxide and it was **0.180%,** at RT 5.734 was Phenylethyl Alcohol and it was **70.295%,** at RT 6.935 was à-Terpineol and it was **0.400%,** at RT 7.933 was(R)-(+)-á Citronellol and it was **14.500%,** at RT 8.438 was cis-Geraniol and it was **2.585%,** at RT 10.637 was Citronellol acetate and it was **0.253%,** at RT 10.721 was Eugenol and it was **1.432%,** at RT 11.258 was Nerol acetate and it was **0.224%,** at RT 11.700 was Benzene, 1,2-dimethoxy-4-(2-propenyl)- and it was **2.036 %,** at RT 11.850 was Caryophyllene and it was **0.235%,** at RT 12.007 was à-Guaiene and it was **0.300%,** at RT 12.499 was à-Caryophyllene and it was **0.178%,** at RT 15.459 was Heptadecane and it was **0.330%,** at RT 17.869 was 9-Nonadecene and it was **1.267%,** at RT 18.145 was Eicosane and it was **1.111%,** at RT 20.075 was Heneicosane and it was **1.215%.**

The total quantity of the compounds which were found in the rose oil at zero month, were also present at the end of third month and their percentage was **97.523%.** It was observed that there was a reduction in the quantity of the compounds by approximately 3%. But this change was insignificant according to ICH guidelines.[12-13]

Some new compounds were also detected at the end of third month, these may be the degradation compounds, the percentage of these degradation compounds was **0.982%,** following are the components which were detected:

The component at RT 13.236 was Hexadecane, 1-chloro- and its quantity was **0.194%,** the component at RT 13.352 was à-Bulnesene and it was **0.244%,** at RT 15.252 was Phenethyl butyrate and it was **0.151%,** at RT 21.538 was Phenylethylacetal and it was **0.125%,** at RT 23.158 was 1-Hexene, 3-methyl-6-phenyl-4-(1-phenylethoxy)- and it **0.119%,** at RT 25.010 was Benzothiophene-3-carbonitrile,4,5,6,7-tetrahydro-2-(3ethoxy-4 hydroxybenzylidenamino)- and it was **0.149%.**

Gas Chromatography-Mass Spectroscopy

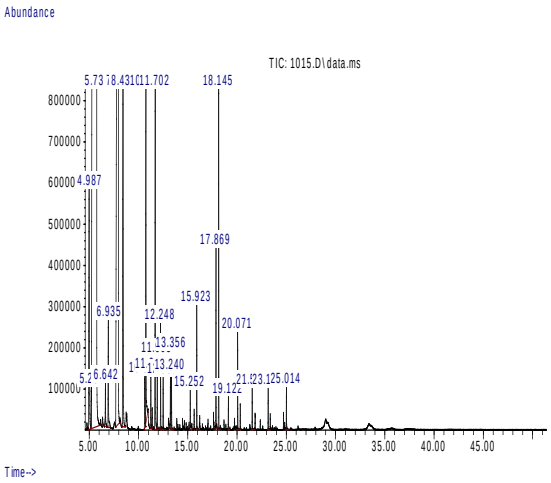

Figure 2: Arqe Gulab Chromatogram at Third Month

The components detected in the oil, extracted from the sample of Arqe Gulab, at sixth month at different run times are as follows:

The component at RT 5.100 was Linalool and its quantity was **1.201%,** at RT 5.965 was Phenylethyl Alcohol and it was **23.065%,** at RT 8.100 was Citronellol and it was **12.016%,** at RT 10.890 was Eugenol and it was **0.989%,** at RT 11.813 was Benzene, 1,2-dimethoxy-4-(2-propenyl)- and it was **1.202%,** at RT 12.349 was à-Guaiene and it was **0.222%,** at RT 16.004 was Heptadecane and it was **0.190%,** at RT 17.948 was 9-Nonadecene and it was **0.884%,** at RT 18.227 was Eicosane and it was **0.767%,** at RT 20.224 was Heneicosane and it was **1.177%.**

These compounds are the natural compounds of the rose oil and these were also present at zero and third month. The total quantity of these compounds had reduced to **41.713%** from **100.282%** that was present at zero month.

This reduction may be due to degradation changes or may due to escape of PEA into the distillate.[13] As evident from various researches, PEA (Phenyl Ethyl Alcohol) dissolves in the distillate and therefore it cannot be claimed that PEA had escaped in the Arq or its constituents had degraded, the quantity of the PEA can be calculated by other analytical procedure.

So, it was assumed that PEA had degraded at the end of sixth month though it was not confirmed.

If it is obtained from the distillate than no significant change in the Arq had occurred and it can withstand ASC but if PEA could not recovered from the distillate by any other analytical technique than significant change had occurred and the distillate have to be assessed at Intermediate or Long term storage conditions to observe the changes.

By these data we can say that AG can have a shelf life of 6 month to 1 year.[14]

Some new compounds were also detected at the end of sixth month, these may also be the degradation

compounds, the percentage of these degradation compounds was **59.365%,** following are the components which were detected:

The component at RT 20.935 was Silane, 1,8-octanediylbis[trimethyl- and its quantity was **3.283%**, at RT 27.052 was Disiloxane, 1,1,3,3-tetramethyl-1,3-dioctadecyl- and it was **0.660%**, at RT 27.346 was Disiloxane, 1,1,3,3-tetramethyl-1,3-dioctadecyl- and it was **2.254%**, at RT 27.830 was Disiloxane, 1,1,3,3-tetramethyl-1,3-dioctadecyl- and it was **2.108%**.

With the increase in the run time, Disiloxane, 1,1,3,3-tetramethyl-1,3-dioctadecyl- was the only component detected till the end.

Gas Chromatography-Mass Spectroscopy

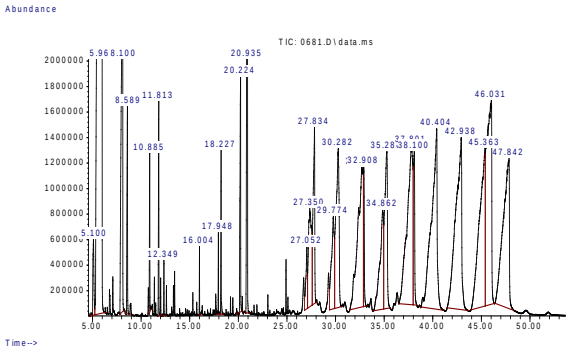

Figure 3: Arqe Gulab Chromatogram at Sixth Month

Table 2: Percent report of Arqe Gulab components at zero month

Peak No.	R.T. (in min)	First scan	Max scan	Last scan	PKTY	Peak Height	Correction Area	Correction Area %	% of Total
1	4.976	31	38	52	BV	415402	11342027	2.01%	1.622%
2	5.197	53	59	61	VV	98919	2749780	0.26%	0.186%
3	5.587	63	96	131	VB	4034200	564470500	100.00%	71.209%
4	6.892	211	220	239	BB	145075	3677346	0.65%	0.483%
5	7.838	290	310	329	BV	1723446	110298452	19.54%	14.900%
6	8.373	352	361	383	BB2	610962	19662072	3.48%	2.754%
7	10.627	567	575	581	BV	76505	1920815	0.34%	0.269%
8	10.725	581	584	609	VB	403415	11700117	2.07%	1.538%
9	11.678	668	675	692	BV	680951	15487890	2.74%	2.233%
10	11.900	692	696	708	VB	82765	1785398	0.32%	0.247%
11	12.242	723	729	735	BB	119590	2364007	0.42%	0.311%
12	15.919	1073	108	1093	BB	282408	5021484	0.48%	0.340%
13	17.865	1258	1263	1279	BB	187006	3548477	1.51%	1.745%
14	18.133	1282	1288	1308	BB	466352	8542098	1.51%	1.123%
15	20.070	1460	1473	1492	BB	228045	4363249	0.41%	1.295%

Table 3: Percent report of Arqe Gulab components at third month.

Peak No.	R.T. (in min)	First scan	Max scan	Last scan	PKTY	Peak Height	Correction Area	Correction Area %	% of Total
1	4.987	29	39	53	PV	581660	23950215	2.27%	1.49%
2	5.197	54	56	60	VB	98919	3749780	0.56%	0.180%
3	5.734	61	110	145	VV 2	5016590	105281249	100.00%	70.295%
4	6.935	215	224	246	BB	257276	8343409	0.79%	0.400%
5	7.933	294	319	333	PV	2497573	221360059	21.03%	14.500%
6	8.438	356	367	386	BB	1193086	40675197	3.86%	2.585%
7	10.637	568	576	582	BV	122336	3972985	0.38%	0.253%
8	10.721	592	599	613	VB	403415	43200117	1.07%	1.432%
9	11.258	628	635	643	BV 2	133739	3303515	0.31%	0.224%
10	11.700	668	677	692	BV	1273594	32981304	3.13%	2.036 %
11	11.850	695	699	701	VV	169012	3648066	0.35%	0.235%

12	12.007	726	733	756	BV	252353	4956579	0.47%	0.300%
13	12.499	748	753	769	BB	121036	2628277	0.25%	0.178%
14	13.236	814	823	829	BV 2	128457	2865677	0.27%	0.194%
15	13.352	829	834	854	PB	176880	3604520	0.34%	0.244%
16	15.252	1011	1015	1034	BB	79667	2223869	0.21%	0.151%
17	15.459	1067	1075	1089	BB	562489	6821484	0.48%	0.330%
18	17.869	1250	1263	1268	BV	408515	7595819	0.72%	1.267%
19	18.145	1283	1290	1297	BV	898725	18181066	1.73%	1.111%
20	20.071	1460	1473	1492	BB	228045	4363249	0.41%	1.215%
21	21.538	1606	1612	1629	BB	94167	1842493	0.18%	0.125%
22	23.158	1762	1766	1772	BV	95194	1758945	0.17%	0.119%
23	25.010	1931	1942	1960	VB	98125	2200673	0.21%	0.149%

Table 4: Percent report of Arqe Gulab components at sixth month

Peak No.	R.T. (in min)	First scan	Max scan	Last scan	PKTY	Peak Height	Correction Area	Correction Area %	% of Total
1	5.100	16	50	63	BV	591909	43569618	2.73%	1.201%
2	5.965	63	123	161	VB	45057618	159801684	100.00%	23.065%
3	8.100	307	335	344	BB	2859865	305802553	19.14%	12.016%
4	10.890	563	660	666	PB	1234316	38014356	2.38%	0.989%
5	11.813	679	688	702	BV	1592742	48919528	3.06%	1.202%
6	12.349	733	739	756	BB	2430502	9292108	0.58%	0.222%
7	16.004	1081	1086	1093	BB	488839	9121644	0.57%	0.190%
8	17.948	1255	1271	1286	VB	2640903	14000585	0.88%	0.884%
9	18.227	1290	1297	1315	BB	21253748	32543731	2.04%	0.767%
10	20.224	1446	1487	1502	BV	1837446	89601164	5.61%	1.177%
11	20.935	1537	1555	1569	BB	23847374	249982910	15.64%	3.283%
12	27.052	2113	2136	2143	VV	482940	50280477	3.15%	0.660%
13	27.346	2143	2164	2188	VV	763174	171650608	10.74%	2.254%
14	27.830	2188	2210	2225	VV	1376990	160528767	10.05%	2.108%
15	29.776	2357	2395	2408	VV	722534	146305040	9.16%	1.921%
16	30.282	2431	2443	2475	VB	1244844	267550229	16.74%	2.513%
17	32.723	2555	2675	2688	BV	41093560	420046188	26.29%	4.516%
18	32.912	2691	2693	2715	VB	21082405	70666704	4.42%	0.928%
19	34.859	2786	2878	2887	BV	777954	199451433	12.48%	2.619%
20	35.279	2887	2918	2950	VB	21226997	23470649	14.69%	2.083%
21	37.805	3036	3158	3175	BV	41244422	509629912	31.89%	5.692%
22	38.099	3175	3186	3210	VB	21196039	122380533	7.66%	1.607%
23	40.404	3245	3405	3473	BB	1404705	571553776	35.77%	5.505%
24	42.939	3507	3646	3706	BB	21350171	581314259	36.38%	5.634%
25	45.359	3739	3876	3879	BV	1225961	493097436	30.86%	5.475%
26	46.033	3888	3940	3968	VB	21599868	584588410	36.58%	6.677%
27	47.842	3973	4112	4160	BB	31170352	524708454	32.83%	5.890%

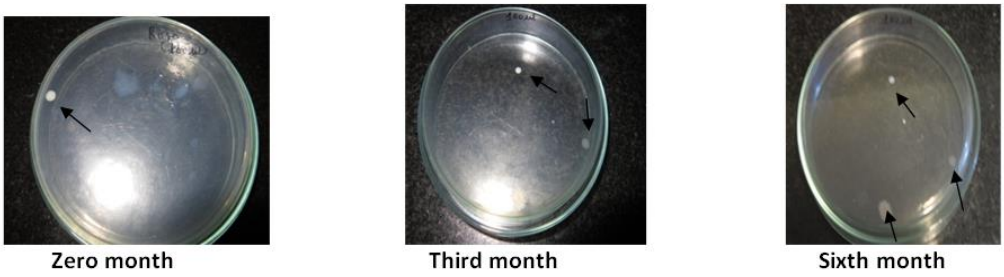

Figure 4: Petridish showing colony in AG at zero month, third and sixth months respectively.

Microbiological Status

Microbiological status of AG shows that it had one colony at zero month, this can be seen in the herbal preparations and this is within limit according to European Pharmacopoeia.[15] At third month it was 2 and at the end of sixth month it became three. The count was within the limit according to the Limits mentioned by the regulatory authorities.

Table 5: Microbial Contamination of different samples of AG during Accelerated Study.

S. No.	At Month	Total Bacterial Count
1.	Zero Month	1 cfu/ml
2.	Third Month	2 cfu/ml
3.	Sixth Month	3 cfu/ml

CONCLUSION

The Physical, Chemical parameters and Microbiological status was assessed at zero month and this data was used as the reference for the future study at third and sixth month respectively.

The assessment of parameter of AG at third month showed no significant changes and the Microbiological status also remained within the limits.

But the effect of ASC showed significant changes in AG at the sixth month. The physical and chemical changes were significant but the microbiological status was within limit. According to the acceptable limits proposed by regulatory authorities, AG had a shelf life of 6 months to 1 year.

REFERENCES

1. Culpeper N. The complete Herbal and English Physician Enlarged, New Delhi, Logos Press, Prologue vii, 1987, 153.
2. Purohit SS and Vyas SP. Medicinal Plant Cultivation- A Scientific Approach, Jodhpur, Agrobios, Agro house, 2008, pg-1-3, 180.
3. Anonymous. Physicochemical Standards of Unani Formulations, Part 1, New Delhi, CCRUM, Ministry of Health and Family Welfare, Govt. of India, Preface, 1986, pg-A-47, A-49, A-50.
4. Husain A. Evaluation of Stability Study on Safoof and Sharbat of Unani System of Medicine, Thesis, NIUM, Dept of Ilmul Advia, Bangalore, Karnataka, 2007, pg-2-3, 20, 22, 27-41, 46.
5. Leon L, Lieberman A,Herbert and Kanig L, Joseph. The Theory and Practice of Pharmacy, Indian-Edition, 4th edition, Mumbai, Varghese Publishing House, 1991, pg-849.
6. Kabeeruddin HM, Bayaze Kabeer, Vol 2, 4th ed, Hyderabad, Hikmat Book Depot, 1938, pg-95, 100.
7. Sina Ibne, *Alqanoon*, 5th Vol, New Delhi, Idara Kitab us Shifa, (YNM), pg-1455-1458.
8. Khan HAS, Misbah ul Advia, 2nd edition, Delhi, Aijaz Publishing House, 2000, pg-191.
9. http://www.noise.physx. u szeged.hu /MP/Education/PhysicsBiophysics/Practicals/RefractiveIndex.pdf
10. http://www.particletesting.com/docs/density_determinations.pdf
11. ICH Q1A: Stability Testing of New Drug Substances and Products, 2003.
12. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1F/Stability_Guideline_WHO.pdf
13. Rusanov EK, Kovacheva NM and Atanassov II. Comparative GC/MS Analysis of Rose flower and distilled oil volatiles of the oil bearing rose Rosa damascena. *Biotechnol. & Biotechnol,* 25(1), 2011, pg-2210-2216.
14. http://www.ikev.org/haber/stabilite/kitap/Stability Workshop Baisc Principles.pdf
15. http://193.218.17.133 /ex/downloads/datasheets/pheur/dok4.pdf

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