Research Article

Accelerated Stability Study of Arqe Ajwain

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Received: 02-06-2018; **Revised:** 14-07-2018; **Accepted:** 05-08-2018.

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 Ajwain. 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 findings showed that degradation changes were observed in the drug formulations between third and sixth month. The present study showed that the shelf life of AA (Arqe Ajwain) 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 Ajwain, Accelerated stability study, Unani formulations, ICH guidelines, GCMS, Bracketing, Matrixing.

INTRODUCTION

Medicinal plants are the local heritage with global importance.[1] The history of herbal medicine is as old as human civilization.[3] Traditional medicine can serve as a cheap and culturally acceptable form of healthcare.[4] World Health Organization (WHO) has recognized the effectiveness of traditional system of medicine and its safety. [1] Unani system of medicine employs single drugs, simple preparations and compound preparations (using herbs, minerals and animal products in one and the same medicine) so the task of evolving standards and shelf life is enormous. 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.[5] 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.[4]

MATERIALS AND METHODS

In the present study one Unani Pharmacopeial preparation Arqe Ajwain 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.

Preparation of Arqe Ajwain

Ingredients Used

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

Table 1: Ingredients Used in Arqe Ajwain.

S.No	Ingredients	Botanical Name	Quantity
1	Ajwain	*Trachyspermum ammi*	500 grams
2	Water		6 litres

Procurement of the Raw Drugs

Ajwain seeds were procured from the market, identified and authenticated by the expert in the Dept. of Ilmul Advia, NIUM, and Bangalore.

Method of Preparation of Arqe Ajwain

500 gms of Ajwain seeds were soaked in 6000 ml of water overnight. Arq was prepared by simple distillation method and 2000 ml of distillate was collected.

Storage

Arq was cleanly and cautiously prepared. 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 after that these bottles were placed in stability chamber for the study, each sample was analysed for physical, chemical and

microbiological status at 0, 3 and 6 months respectively. The first sample of AA was analyzed at zero month, for the above mentioned properties and microbiological count was also calculated.

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 AA was analyzed at zero month, for the above mentioned properties and microbiological count was also calculated.

Organoleptic Characters and Physical Parameters of AA

Determination of Colour of AA

The colour of the Arqe Ajwain was tested at the time of manufacture that is at zero month.[5]

Determination of Odor

The description of this feature sometimes may not be accurate because it depends on individual perception. If the material is expected to be innocuous, a small portion of the sample can be examined by slow and repeated inhalation of air over the material. The strength of the odor like weak, distinct, strong is first determined and then the odor sensations like musty, mouldy, rancid, fruity, aromatic etc were determined.[5, 7-9]

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.[10]

Determination of pH of AA

The pH Value of 1% Solution

An accurately measured 1ml AA was mixed in accurately measured 100ml of distilled water, and pH was measured with a pH meter.[4]

The pH value of 10% Solution

An accurately measured 10ml of AA was mixed in accurately measured 100ml of distilled water, and pH was measured with a pH meter.[4]

Refractive Index of AA

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.[10] Refractive index was measured using Abbe's Refractometer.[11]

Weight Per mL or Specific Gravity of AA

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.[12]

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 AA, 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.[4, 12]

$$\textbf{Specific Gravity} = \frac{\text{Weight of AA} - \text{Weight of EP}}{\text{Weight of DDW - Weight of EP}}$$

Chemical Stability

GC-MS (Gas Chromatography-Mass Spectrometry)

In AA, oil was the main part having a lot of components within it. The oil from the AA was separated from the whole distillate from one container and was analyzed 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 AA was further analyzed for GCMS study done at Bangalore Test House, Bangalore.

Procedure

Sample Preparation Application

AA 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 AA

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 AA was taken into the laminar flow. Nutrient agar media (25 ml) kept into a sterilized Petridish and 1ml AA 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 and observations were recorded. The total number of viable organisms was noted in terms of colony forming units.[5]

RESULTS AND DISCUSSION

The observations and results that were recorded during the stability study of AA 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 AA was liquid and showed no changes in the third and the sixth month. Colour change was observed in AA in the study, the yellowish colour of AA had increased in third and sixth month. The odour of AA 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. AA was found to be pungent throughout the study. No precipitates were found in AA throughout the study. The minimal changes which were found in the organoleptic properties were probably due to alteration in the composition of the test drug between third and sixth month.

Estimation of 1% pH of AA at zero month was 6.70 at 25 ºC and it decreased to 6.52 at third month and 5.32 at sixth month. The change in 1% pH of AA at third month was found to be 2.68% which was insignificant change according to ICH, whereas at sixth month it was 20.59 % which was highly significant according to ICH.

Estimation of 10% pH of AA at zero month was 6.73 at 25 °C and it decreased to 6.70 at 25 ºC at third month and it fell to 4.60 at 25 °C at sixth month. The change in 10% pH of AA at third month was found to be 0.44% which was insignificant change according to ICH, whereas at sixth month it was 31.64% which was highly significant according to ICH. The changes between the third and sixth months was highly significant and indicate degradation changes, this difference exceeds the 5% limit set by ICH.[13-14]

Estimation of RI of AA at zero month was 1.3225, at third month the RI was 1.3221 and at sixth month the RI was 1.3220. The difference between zero and third month was 0.0302% and between third and sixth month was 0.0378%. This change was insignificant, it shows that the RI of AA was not influenced by ASC.

Estimation of SG or weight per ml of AA at zero month was 0.9987, at third month the SG was 0.9967 and at sixth month the SG was 0.9988. The difference between zero and third month was 0.2002% and between third and sixth month was 0.0100%. This change was insignificant; it shows that the SG of AA was not influenced by ASC.

Finally AA was subjected to GCMS study for identification of its constituents. AA was extracted from Ajwain which is rich in essential oil, this essential oil contains maximum 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 Ajwain, at zero month at different run times are as follows:

The component at RT 6.586 was terpene-4-ol and its quantity was 0.597%, at RT 6.944 was p-menth-1-en-8-ol and it was 0.376%, at RT 8.343 was p-Cymene-2,5-dione and it was 0.576%%, at RT 9.543 the component was Carvacrol and it was 0.646%, at RT 9.686 was Thymol and it was 96.802 %. These are the components which are found in the oil naturally.[15] The total quantity of these components in the oil at zero month was 98.997%.

Few other compounds were also detected by GCMS at RT 17.466 was Cyclononasiloxane, octadecamethyl- and it was 0.103%, at RT 19.038 was Cyclodecasiloxane, eicosamethyl- and it was 0.134%, at RT 25.017 was Tetratetracontane and it was 0.181%.

These are not the natural components which are found in the oil; they were found in traces which may have formed during preparation. The total quantity of these components was 0.418%. There were more components in traces in the oil and could not be detected in the chromatogram because of low peak heights and peak areas.

Gas Chromatography-Mass Spectroscopy

Arqe Ajwain chromatogram at zero month

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

The component at RT 6.586 was Terpene-4-ol; and it was 0.451%, at RT 6.944 was p-menth-1-en-8-ol and it was 0.376%, at RT 8.343 was p-Cymene-2, 5-dione and it was 0.576%, at RT 9.427 was Thymol and it was 95.536 %, at RT 9.543 was Carvacrol and it was 0.541 %.

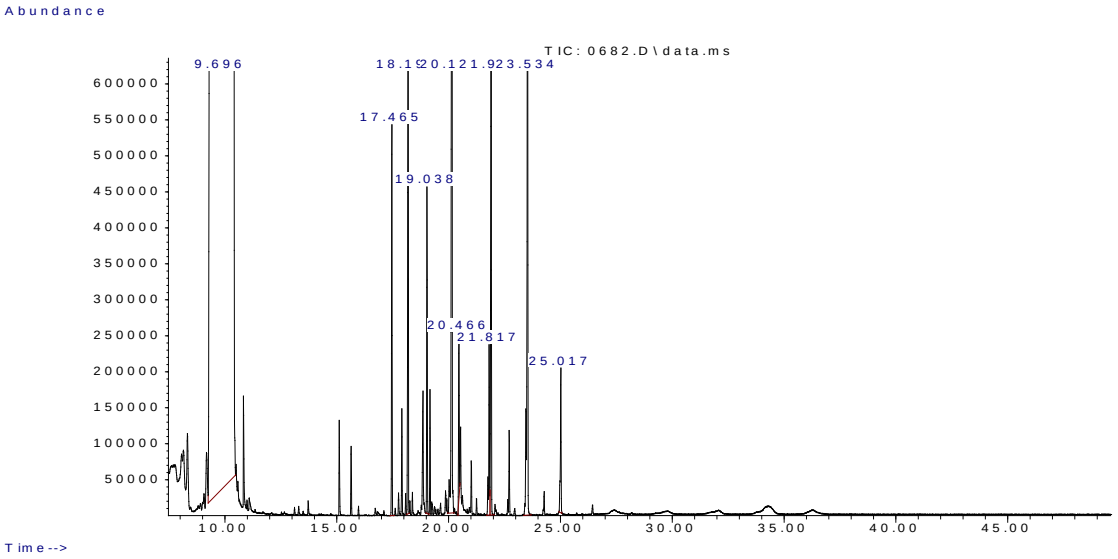

Figure 1

Table 2: Percent Report of Arqe Ajwain 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	6.586	185	191	203	BB 3	87081	4255084	0.93%	0.597%
2	6.944	209	225	243	BB 2	74953	3207914	0.70%	0.376%
3	8.343	355	358	374	M6	29712	1329841	0.29%	0.576%
4	9.543	469	472	491	VB	102037	3063098	0.67%	0.646%
5	9.686	495	546	561	VV	44149006	2407226619	100.00%	96.802%
6	17.466	1217	1225	1232	BB	532588	10227140	0.42%	0.103%
7	19.038	1369	1374	1381	BV	422298	8470450	0.35%	0.134%
8	25.017	1935	1943	1947	M	207329	4604827	0.19%	0.181%

These are the natural components which were also found at zero month. The total quantity had reduced **97.147%** showing the difference of **1% to 2%** which is insignificant, probably this may be due some degradation changes in the product.

The component at RT 5.250 was Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, (1R-endo)- and its quantity was **0.552%.** This may be the degradation compound that was formed during the storage, which is also insignificant.

Gas Chromatography-Mass Spectroscopy

Arqe Ajwain Chromatogram at Third Month

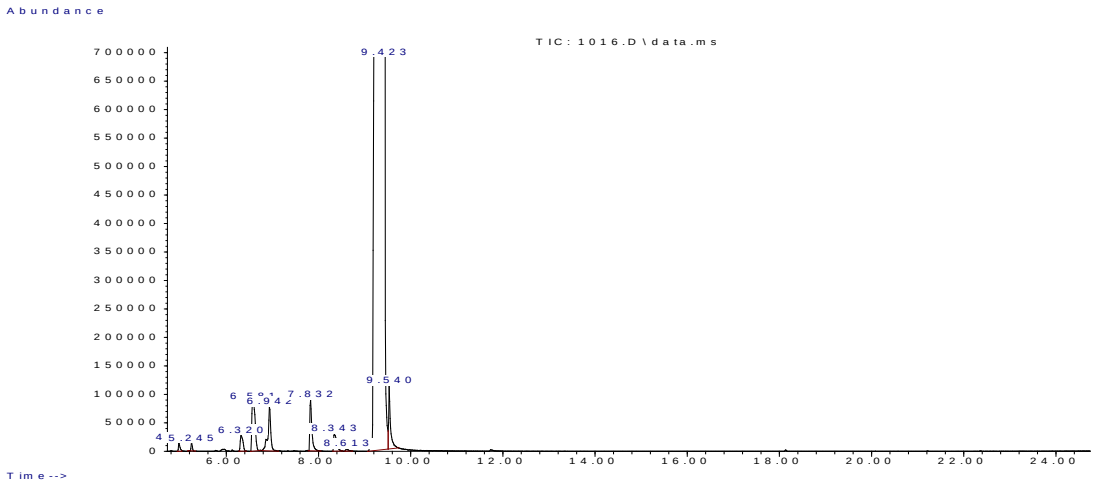

Figure 2

Table 3: Percent Report of Arqe Ajwain 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	5.250	56	64	71	M2	12832	248552	0.05%	0.552%
2	6.586	185	191	203	BB 3	87081	4255084	0.93%	0.451%
3	6.944	209	225	243	BB 2	74953	3207914	0.70%	0.376%
4	8.343	355	358	374	M6	29712	1329841	0.29%	0.576%
5	9.427	427	461	469	BV	4546286	457980212	100.00%	95.536%
6	9.543	469	472	491	VB	102037	3063098	0.67%	0.541%

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

The component at RT 6.586 was Terpene-4-ol; and its quantity was 0.337%, at RT 7.028 was p-menth-1-en-8-ol and it was 0.291%, at RT 8.459 was p-Cymene-2, 5-dione and it was 0.273%, at RT 9.522 was Thymol and it was 94.926%, at RT 9.606 was Carvacrol and it was 0.766%. These are the natural components which were also found at zero month. The total quantity had reduced showing that probably this may be due some degradation changes in the product.

The component at RT 5.250 was Fenchol and its quantity was 0.781%. This may be the degradation compound that was formed during the storage. These degradation could occur as a result of processing or storage (e.g., by deamidation, oxidation, aggregation, proteolysis, etc.).[16] The results and observations shows that AA did not show high levels of degradation at the end of third and sixth month, and the quantity of the degradation components was within the acceptable limits of ICH i.e. 5%.[17]

Gas Chromatography-Mass Spectroscopy

Arqe Ajwain Chromatogram at Sixth Month

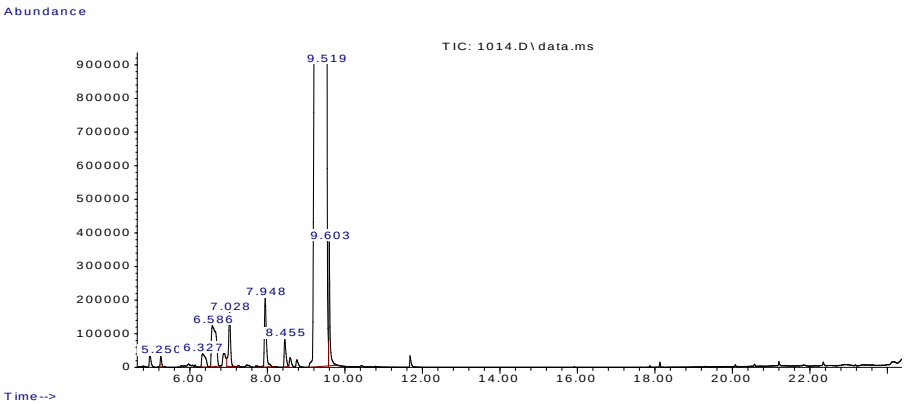

Figure 3

Table 4: Percent Report of Arqe Ajwain 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	5.250	61	64	71	M2	31791	713316	0.08%	0.781%
2	6.586	186	191	210	BB 3	118689	10151408	1.20%	0.337%
3	7.028	225	233	251	VB	160798	5616294	0.67%	0.291%
4	8.459	363	369	377	BV 2	79480	2575438	0.31%	0.273%
5	9.522	425	470	475	BV	5576453	843615470	100.00%	94.926%
6	9.606	476	478	498	VB	340400	6740155	0.80%	0.766%

Microbiological status of AA shows that it had no colony formation at zero month. At third month also there was no colony and at the end of sixth month there was one colony. The count was within the limit according to the Limits mentioned by the regulatory authorities.[18]

Table 5: Microbial Contamination of Different Samples of AA during Accelerated Study

S. No.	At Month	Total Bacterial count
1.	Zero Month	0 cfu/ml
2.	Third Month	0 cfu/ml
3.	Sixth Month	2 cfu/ml

Figure 4: Petridish having no colony in AA at zero and third months and its showing two colonies at sixth month.

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 AA 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 AA 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, AA had a shelf life of 6 months to 1 year.

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