ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Applicability of Physicochemical Parameters in Stability and Self-Life Estimation of Ayurvedic Semi-Solid Dosage Form '*Ashtamangal Ghrita*' at Accelerated Storage Conditions.

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ABSTRACT

The pharmaceutical products are considered to be stable up to the time as their important characteristics are found within the manufacturer's specifications. In herbal and ayurvedic preparations, numbers of constituents are available due to which single instrumental technique is insufficient for calculating the product age. In the present study, the stability study of '*Ashtamangal Ghrita*' was evaluated at accelerated storage conditions (Temperature: 40 °C ± 2 and Relative Humidity (RH): 75% ± 5). The changes in organoleptic characterstics and physicochemical parameters such as iodine value, saponification value, peroxide value, acid value, refractive index and specific gravity were studied at storage conditions. The 10% changes in the initial results of physicochemical parameters were considered to evaluate the age of the product. It was analyzed that during storage at accelerated conditions, these parameters were changed and the self-life of the S1 sample in climate zone I and II was 2.641 Year and in climate zone III and IV, it was 1.743 Year. The results of this study revealed that the changes in physicochemical parameters can be considered for assessing the self-life of ayurvedic products that it otherwise impossible due to quantification and identification of plenty of chemical components simultaneously in these preparations for self-life assessment.

Keywords: ghrita, relative humidity, unsaturated fat, degradation.

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ISSN: 0975-8585

INTRODUCTION

In *Ayurveda*, the medicines are given in five different forms (*Kalpna*) as *Swarasa* (juice), *Kalka* (paste), *Kwatha* (decoction), *Hima* (cold decoction) and *Fanta* (hot infusion); but *Swarasa* is the heaviest form to digest among all the above given *Kalpana. Kwatha Kalpana* is considered as one of the potent as well as easiest form to digest, hence; it is the most widely used form of medicine in *Ayurveda*. *Kwatha* and its subsequent preparations, *Tailapaka, Kshirapaka, Rasa prakriya, Ghritapaka, Arishta* are considered as the *Upkalpana's* of the *Kwatha* [1]. These *Kalpanas* (preparations) and *Upkalpanas* (subsequent preparations) are meant to convert the crude drugs into such a form which can be assimilated easily in the body, helps to preserve the potency of drug and make it palatable to the patient.

One of the best substances that easily assimilated in the body in its natural as well as in its medicated form is *"Ghrita"*. This dosage form is considered as the best preparation amongst all the other *Sneha*, as it is assimilated easily within the body of young as well as the old ones, achieves the properties of drug by which it gets medicated without losing its original properties.

'Snehakalpana' is one of the commonly prescribed *Ayurvedic* dosage form in day to day practice. Although, lot of varieties of oils and fats are described in classical *Ayurvedic* texts, the most common amongst them are *Taila* and *Ghritakalpana*. *'Snehakalpana'* is a pharmaceutical procedure to prepare oleaginous medicine from substances like *Kalka* (bolus of the drugs) and *Drava* (liquid material). They are prepared in specific proportions by subjecting them to uniform heating pattern and duration to fulfill certain pharmaceutical parameters as per the requirement of the therapeutics [2]. The rationality behind taking *Ghrita* as a base is presumably to extract or hold lipid soluble active fractions from the ingredients used. Classically, these formulations have mentioned to be having a longer shelf life in comparison to other *Ayurvedic* herbal medication forms. *Ghritakalpna* plays an important role in treatment of both internally and externally. If taken internally, it enters into the systemic circulation and can easily cross the blood-brain barrier, thereby; strengthens or stimulates the central nervous system. When used for topical application, it has the potential to diffuse locally into the soft tissues and produces the desired therapeutic action.

'Ashtamangal Ghrita' is widely used and highly effective in treating various mental illnesses [2]. According to the US Department of Health and Human Services, mental illness is characterized by alterations in thinking; mood and behavior associated with distress and impaired function. Mental illness is a global public health concern. According to the World Health Organization, one out of four (25%) persons is affected with some kind of mental illness. The prevalence of mental disorders is higher in developed countries, but the global burden of untreated mental disease is higher in developing nations. Eighty percent (80%) of the population suffering with mental illness lives in low-and middle-income (LAMI) countries. The presence of mental illness does not affect only the personal, social, educational and occupational life but also it makes the families to suffer from negative consequences. Considering the fact that India has 17.5% of the world's population, the number of people suffering with mental illness is assumed to be huge. It is highly crucial for India to have a true estimate of the number or prevalence of mental illness in the nation. The prevalence of mental illness appears higher in children due to behavioral and emotional disorders. Those are often associated with learning difficulties, poor parenting and schooling, while age-related dementia and other cognitive disorders increase with age.

In allopathic system of the treatment, a few drugs are available and the treatment time is more with less percentage of complete cures. Besides this, the prolonged treatment with these drugs affects the patient compliance in terms of economy, body functioning and the side/toxic effects of drugs. Keeping all these points for the treatment of mental illness, the herbal therapy with ayurvedic medicines is one of the best options. However, the most important challenges faced by ayurvedic medicines are the lack of identification and quantification of their constituents due to complex nature. Evaluation of the constituents is necessary for the assessment of purity, quality and stability of the formulations. The stability testing of the preparations provides a clue about the impact of environmental conditions such as temperature, humidity and light on their nature and it also helps in establishing the self-life of products with recommended storage conditions [3,4].

In the present study, the crude drugs components of '*Ashtamangal Ghrita'* were procured and analyzed for standardization as per compendial requirements. The formulation was prepared in accordance to traditional ayurvedic literature and tested subsequently. The pharmaceutical preparations are generally

studied for stability testing at accelerated conditions of temperature and humidity and the experimental results are applied to predict the reliable self-life or date of expiry at room temperature by using certain criterions and assumptions [3].The self-life estimation of the formulations was determined at accelerated stability conditions and the testing was performed by analyzing the testing variables as specific gravity, nature of preparation, sedimentation behavior, refractive index at different time intervals of the study at environmental conditions of humidity and temperature.

The crude drugs required in the preparation of final preparation were procured from local market of Haridwar, Uttarakhand. The chemicals and the reagents required in testing the crude drugs and the final preparations were purchased from Loba Chemie, Mumbai and Rankem, New Delhi.

Preparation and characterization of *Ashtamangal Ghrita*

About 120 g of *Murchan dravyas* in cleaned and dried form were made into coarse powder and soaked in sufficient amount of water for overnight and the *Kalkas* of crude drugs were obtained. Afterwards, *Go-Ghrita* (Cow Ghee) was poured into a big-wide mouth vessel and kept over heating to get rid of water i.e. up to subsiding the foams and sound. At that time, the temperature of the burner was noted down as 450^0F and the temperature of *Ghrita* was 140^oC. When a characteristic vapour having some fumes in heated *Ghrita* was observed, it was taken off from burner, and the *Kalkas* of crude drugs and water were added in *Ghee* and the whole mass was then again kept heating. Again the frothing appeared on the *Ghrita* and watery contents started evaporating. The *Ghrita* was allowed heating until the whole of the watery contents got evaporated along with the disappearance of frothing. The temperature remained constant at about 80^0C during whole process. This stage of *Ghrita* was expressed as the *Murchita Ghrita*. The whole procedure was also performed for the preparation of other two samples as S2 and S3 in which *Murchan Dravyas* were treated twice for getting *Kalkas* in similar quantity of water. In the next step of the formulation, *Kalka* from the ingredients of '*Ashtamangal Ghrita'* were prepared. The *Kalka Dravyas* were cleaned, dried and taken in equal amount (e.g. 250 g) and mixed with one fourth to the quantity of *Ghrita*. These contents were taken in dry form so that these could be crushed with sufficient amount of water to form *Kalka* and were kept in container for whole night. In the preparation of final formulation '*Ashtamangal Ghrita', Murchit Go-Ghrita* was poured into a big wide mouthed container and kept over burner for heating. *Ghrita* was heated until characteristic vapour having fumes on heated *Ghrita* was observed. The vessel was then removed from heating and *Kalkas* obtained previously and four times water was then added to the *Ghrita*. The whole mass was again kept over burner and heated on mild heat to evaporate the water content completely from *Ghrita* [2]. To avoid adhering of the material with the wall of the vessel, it was stir continuously with the help of ladle. This process was performed for 2 days on mild fire. After attaining the *Sneha Siddhi Lakshan,* the fire was withdrawn and the *Ghee* was filtered with the help of a previously washed and dried cloth. The similar procedure was performed two more times for preparation other two samples as S2 and S3. The crude drugs present in '*Ashtamangal Ghrita'* were *Haritaki (Terminalia chebula family Combretaceae), Bibhitak (Terminalia bellerica family Combretaceae), Amalaki (Emblica officinalis family Euphorbiaceae), Haridra (Curcuma longa family Zingiberaceae), Mustaka (Cyperus rotundus family Cyperaceae), Bija Puraka (Citrus medica family Rutaceae), Vacha (Acarus calamus family Araceae), Brahmi (Bacopa monnieri family Scrophulariaceae), Kutha (Saussurea lappa Family Compositae), Piper (Piper longum family Piperaceae), Sariva (Hemidesus indicus family Asclpiadaceae), and Sarshapa (Brassica compestris family Brassicaceae).* Besides these, Saindhava (sodium chloride) and Go-Ghrita were also used.

Estimation of appearance, colour, odour, taste and touch

The specific characters as mentioned in ayurvedic literature were also analyzed for evaluating the qualities of *Ghrita* by color, touch, fineness, taste and odor. The procedure was applied as per the official ayurvedic compendia [5]. Briefly, for assessing the colour and appearance, about 1 g of each sample was taken into a watch glass and placed against white tube light. The sample was observed for its colour by naked eyes. The odour of the samples was determined by smelling 2 g of sample. A pinch of each sample was taken and examined for its taste on taste buds of the tongue. The touch sensation of the samples was performed by taking 2 g of each among thumb, forefinger and middle finger and rubbed gently. The testing of the next sample was done after completing washing of the hands and assuring the complete washing of residuals of the first sample analyzed.

Determination of Specific Gravity

A specific gravity bottle of 25 mL capacity was cleaned thoroughly with freshly prepared chromic acid, distilled water, dried and weighed. It was filled up with distilled water and weighed again. After withdrawing the water from density bottle; it was dried and filled with sample of ghee and weighed. The weighing process was performed in triplicate and from these three successive readings, the specific gravity of the ghee samples was calculated by following expression. The similar procedure was followed for analyzing the specific gravity of the samples at 40^0C of the stability study [6].

$$\text{Specific gravity of ghee} = \frac{\text{Wt. of Ghee (in g)}}{\text{Wt. of equal Vol. of distilled water (in g)}}$$

Refractive Index determination

It was determined by Abbe refractometer (portable RA-130). For this, the sample of ghee was dropped over the prism after complete cleaning of the prism. The prism was filled with the liquid sample up to the line on sample stage. The measurement was made at that position on which the crossed horizontal line dissected the two-half contrasts of a circle and aligned with the scale on refractometer. The refractive index of the stability samples was performed at frequent time intervals as per stability guidelines in which the measurement was performed at 40^0C. For maintaining the thermal conditions, the pre-warmed water at 40^0C was circulated all-around the sample through tubing of the refractometer [6].

Acid-value estimation

About 5 g of each sample was weighed accurately and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed KOH solution was determined and the titration of each sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression. Previously, KOH aqueous solution used in this study was standardized for estimation its actual strength. Briefly, a 20 mL of 0.1 N aqueous oxalic acid was taken in a 250 mL conical flask in which 1 or 2 drops of phenolphthalein indicator was added. It was titrated against KOH taken in a burette. The appearance of pink color indicated the end point. From the volume of KOH solution consumed taken in burette, the normality of KOH was calculated [6].

Acid value = (Volume of KOH X Normality of KOH X Eq. wt X 1000) / Weight of *Ashtamangal Ghrita* sample (g)

Iodine value

About 10 g of the fatty sample was dissolved in chloroform taken in an iodination flask and labeled as test. To this ample, a 20 mL of iodine monochloride reagent was added and mixed thoroughly. Afterwards, the flask was maintained in dark condition for half an hour for incubation. The blank was also prepared by applying the similar method using 10 mL of chloroform. To the blank, 20 mL of iodine monochloride reagent was added and the contents of the flask were mixed homogenously. Afterwards, the blank was also incubated for 30 min. After incubation, 10 mL of potassium iodide was added to the flasks containing test and blank. The stopper and the walls of the flasks were rinsed by adding 50 mL of the distilled water. The test solution was titrated against sodium thiosulphate until a pale straw colour was observed. About one mL of starch solution was added to the flask and a purple colour was developed. The titration was continued until the purple colour of the flask was turned into colourless and it indicated the endpoint of the titration. Similarly, the end point for the blank was also determined [7]. The actual volume of sodium thiosulphate consumed by the sample was calculated: volume of sodium thiosulphate consumed by blank (mL)- thiosulphate consumed by test (mL). The iodine value of the sample was calculated by applying the following expression-

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

ISSN: 0975-8585

Saponification value

A 1 g of each of the sample was taken in beaker and dissolved in 3 mL of ethanol. Quantitatively, the contents of the beaker were transferred by washing successively three times with 7 mL of solvent. A 25 mL of 0.5N alcoholic KOH was also added, mixed well and attached to a reflux condenser. Other reflux condenser set was also used for the blank prepared as above in which all the contents were added except the fatty material. These flasks were placed in a boiling water bath for 30 min. These were cooled down at room temperature and phenolphthalein indicator was added. The contents of the flasks were titrated with 0.5 N HCl. The endpoint of sample and the blank were noted down and the difference between the blank and test readings provided the number of milliliters of 0.5N KOH required to saponify the fatty material [8]. The weight of potassium hydroxide (mg) consumed by 1g of fatty sample indicated the saponification value of the sample.

Peroxide value

A 2 g of the sample was taken into a 100 mL glass stoppered Erlenmeyer flask and to it; 12 mL of the acetic acid-chloroform solution was added. The contents of the flask were agitated vigorously until the sample was dissolved completely. To the flask, about 0.2 mL of saturated potassium iodide solution was added. The contents of flask were swirled for one minute. Afterwards, 12 mL of the distilled water was added and mixed homogenously to liberate the iodine from chloroform layer. The solution of the flask was titrated with 0.1N sodium thiosulphate solution taken in a burette. The titrant was added slowly to the flask until the colour of the titrand was turned into light colour [9]. With the help of dispenser, 1 mL of starch indicator was added. The titration was continued until the deep grey colour was disappeared from the upper aqueous layer. The peroxide value of the sample was determined by following expression-

$$Peroxide\ value = \frac{(S-B)\times normality\ of\ sodium\ thiosulphate}{weight\ of\ the\ sample}\times 1000$$

Where, S is the volume of thiosulphate consumed in titration of sample and B the volume of sodium thiosulphate consumed in titration of blank.

Stability testing

The accelerated stability study was performed as per ICH guidelines Q1.A.(R2) for the estimation of self-life of Ghrita samples [3]. The storage conditions in humidity cabinet (NSW-175) were set as temperature on 40±2^{0}C and relative humidity (%RH) on 75±5. The changes in properties of the samples were observed during 6 month for accelerated study. The sampling was done at 0, 1, 3 and 6 month. The evaluation of degradation during storage at accelerated conditions was studied and 10% changes in properties of the samples were set to extrapolate the stability data. Real time aging factor 5 and 3.3 was used for extrapolation of self life for climate Zone I & II countries and climate Zone III & IV countries respectively. As per ICH guidelines, ambient temperature and humidity for Zone I and II countries are 21^{0}C/45% RH and 25^{0}C/60% RH respectively and for Zone III & IV countries 30^{0}C/35% RH an 30^{0}C/70% RH respectively. India comes under zone III and IV [10]. The parameters considered for evaluation of stability were organoleptic characters like appearance, colour, odour, taste and touch; physicochemical parameters such as specific gravity, refractive index, acid value, saponification value, iodine value and peroxide value.

The number of months when 10% degradation occurred, was calculated by using following expression-

$$Number\ of\ months\ when\ 10\%\ degradtion\ occurs = \frac{\left[0\ month\ assay\ value - \left\{\frac{0\ month\ assay\ value\times 10}{100}\right\}\right] - intercept}{slope}$$

RESULTS AND DISCUSSION

The organoleptic parameters of the preparation were found as prescribed in ayurvedic literature that revealed the effectiveness of the manufacturing process. The appearance, colour, odour, taste and touch of all

ISSN: 0975-8585

samples were similar to the characteristics as mentioned in official compendia [5]. The organoleptic properties and physicochemical parameters of S1, S2 and S3 are shown in Table.1.

Table. 1. Physicochemical properties of 'Ashtamangal Ghrita' prepared by ayurvedic traditional method

Physico-chemical parameter	Name of the sample		
	S1	S2	S3
Organoleptic characteristics (e.g. appearance, colour, odour, taste and touch)	viscous, light yellow, unctuous, sweet and pleasant	viscous, yellowish, unctuous, pleasant	viscous, whitish yellow, sweet and pleasant
Specific gravity	0.991	0.99	0.991
Refractive index	1.462	1.463	1.466
Iodine Value (mg iodine/g)	38.496	35.58	38.681
Peroxide value (meq/kg)	3.5	3.6	3.5
Acid Value (mg KOH/g)	1.383	1.695	1.22
Saponification value (mg KOH/g)	131.835	126.225	129.03

Iodine value determination

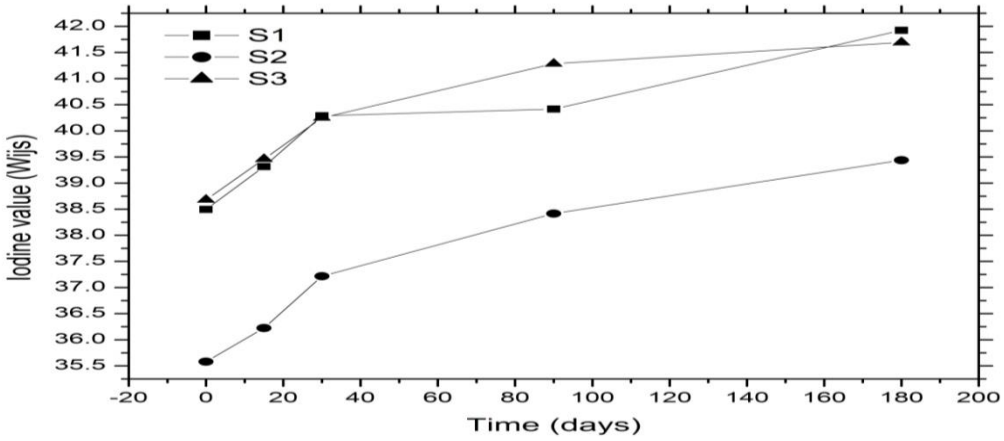

Fig.1. Changes in Iodine value of samples during stability study

Iodine value is related to the fatty materials specially fixed oils. It is also called Iodine Number and measures the degree of unsaturation of an oil, fat or wax. It is the amount of iodine in grams that is taken up by 100 gram of the oil, fat or wax. Saturated oils, fats and waxes take up no iodine; therefore their iodine value is zero; but unsaturated oils, fats and waxes take up iodine. Unsaturated compounds contain molecules with double or triple bonds which are very reactive toward iodine. The more iodine is attached, the higher is the iodine value and the more reactive, less stable, softer and more susceptible to oxidation and rancidification is the oil, fat, or wax. Drying oils are used in those preparations that are dried very fast after applying/using. These oils are used in the paint and varnish industry and have relatively high iodine values (about 190). Semidrying oils such as soybean oil have intermediate iodine values (about 130). Non-drying oils such as olive oil, linseed oil etc. used for soap making and in food products have relatively low iodine values (about 80). The fatty materials that are solid on 14.5-15.5^{0}C are considered as fat while those having liquid consistency at this temperature are known as oils. The fatty materials due to very less amount of unsaturation are solid. The location and number of double bonds are important because they influence reactions that can occur to destabilize the fatty acid chains. The interaction of oxygen molecules with the fatty acid chain called "oxidation" is the chemical mechanism that destabilizes oil. After oxidation, hydroperoxides (one hydrogen atom and 2 oxygen atoms) are attached to the fatty acid chain. In food oil, this leads to rancidity. The presence of fatty material in more content provides the solid consistency to the "ghee". However, the availability of many crude drugs affects this consistency of "Ghrita preparation" due to number of active constituents extracted. The extracted constituents may be alkaloid, terpenoids, flavonoids, glycosides etc. and it depends upon the nature of crude drug as well as extraction ratio. Sometimes, the concentrations of active constituents increase as the time for extraction increases. But the changes are very less as the amount of unsaturated fats is

very less. A low degree of unsaturation in oil/fat makes it less susceptible for rancidity. The rancidity of the fatty materials is related to unpleasant smell or taste. Specifically, it is the hydrolysis and/or autoxidation of fats into short-chain aldehydes and ketones which are objectionable in taste and odor [11]. When these processes occur in food, undesirable odors and flavors can result.

In this study, 'Ghrita' preparations were studied for stability at 40±2^{0}C /75±5%RH in stability chamber for 6 month under accelerated stability protocol [12]. The results of iodine value for all samples are shown in graphical form in Fig.1. The samples as S1, S2 and S3 were analyzed for Iodine value by Wij's method at starting (0 days), 15 day, 1 month, 3 month and 6 month. It was observed that the iodine value of all samples was increased as the time for storage was increased. For S1 sample, the iodine value at above time intervals was found as 38.496, 39.316, 40.289, 40.416 and 41.923 (mg iodine/g) respectively. The iodine values for S2 samples were 35.58, 36.223, 37.216, 38.416 and 39.439 (mg iodine/g) respectively. However, the observations for S3 samples indicated the iodine values as 38.681, 39.461, 40.25, 41.285 and 41.69 (mg iodine/g) respectively. The changes in iodine values could be due to formation of free fatty acids that could be unsaturated fatty acids. The temperature might have impact on generation of free fatty acids. The degradation profile of fatty materials in all Ghrita preparations was not different statistically ($p>0.05$). The iodine values of preparations at different time intervals were also found within the recommended range. However, the iodine value of the fresh formulation used in the study was found different statistically from stability data of all concerned samples ($p<0.05$).

Acid value determination

The acid number and/or acid value is a measure of the content of free fatty acids in the vegetable oil and describes the quantity of caustic potash solution which is necessary for the neutralization of the free fatty acids. The acid number depends strongly on the refining degree and the aging degree of oil. By water in the oil as well as micro organisms, enzymes and other chemical constituents, a hydrolytic splitting of the triglyceride can occur and lead to a rise of the acid number. Acid number and oxidation stability are characteristic values for the ageing of fatty material and oils used in different industries. These describe different procedures in the oil which are affected partially by the same parameters as temperature, light, water and storage conditions. A high portion of free fatty acids in the oil possibly leads to a short induction period with the measurement of oxidation stability. Water in the oil is the condition for hydrolytic splitting of triglycerides and thus for the occurrence of free fatty acids. The water content would therefore have the correlation with the acid number which measures the portion of free fatty acids to correlate. Also, the humidity in the environment also plays an important role in changing the acid value of the fatty materials.

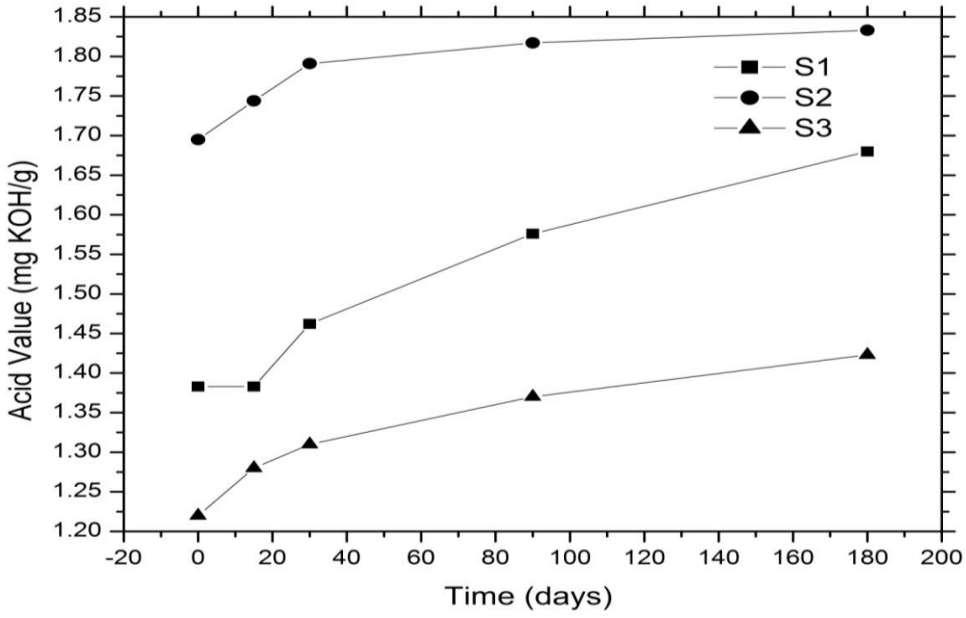

Fig.2.Acid values of the samples during stability study

The acid values of all samples are shown in Fig.2.The acid values of all the samples as S1, S2 and S3 were studied at starting (0 days), 15 day, 1 month, 3 month and 6 month. The acid values were represented as mg KOH/ g of the sample. In all the samples, it was observed that the acid values were increased. The acid values for S1 sample were found as 1.383, 1.383, 1.462, 1.576 and 1.68 (mg KOH/g) respectively at starting (0 days), 15 day, 1 month, 3 month and 6 month of stability study. However, at these time intervals, the acid values for S2 were found as 1.695, 1.744, 1.791, 1.817 and 1.833 (mg KOH/g) respectively. It was analyzed that for S3 sample, the acid values were 1.22, 1.28, 1.31, 1.37 and 1.423 (mg KOH/g) respectively. In all the samples, the increment in acid values was speculated due to the formation of small chain free fatty acids that consumed more KOH for neutralization and it resulted into more acid values. However, the increment pattern in acid values for all the samples was not different statistically ($p>0.05$). It indicated the resemblance of all test samples towards acid values characteristics. The formation of free acid in test samples might be due to the influence of crude drugs present in Ghrita. It was observed that the acid values of the samples were similar to the starting Ghrita but as the stability study was performed up to 6 month, the observed values became different significantly from the initially prepared fresh sample ($p<0.05$).

Estimation of Peroxide value

Lipid peroxidation is a major deteriorative change commonly encountered in oil and the extent of lipid peroxidation depends on different factors which include the fatty acid composition viz-a-viz level of unsaturation; packaging material and storage condition [13]. Amongst other functions, packaging serves to protect food materials against contamination and adverse environment conditions that can initiate deterioration of food material. Fat and oil are usually packaged in a material that will protect them against atmospheric oxygen, light, heat and metal contamination. These factors are known to accelerate lipid peroxidation and the development of rancidity in oil. Glass, metals and different kinds of plastic bottles have been used to package oil, each with its advantages and limitations. The result of storage stability shows that the oil samples may be relatively stable to oxidation and hydrolysis when properly stored in a good packaging material. Oil samples stored in plastic transparent bottle recorded highest lipid peroxidation values while amber glass bottle gave the maximum protection against lipid peroxidation [14,15].

One of the concerns with using rendered fats such as blended fats or other fat-containing animal by-products is that they may be of poor quality due to oxidative rancidity and consequently pose a threat to the performance. Two major forms of rancidity exist, namely hydrolytic and oxidative, both of which cause unpleasant flavors and odors in fats and feeds that contain added fat. Hydrolytic rancidity occurs when triglycerides are hydrolyzed into fatty acids and glycerol. More specifically, this type of rancidity occurs in the presence of moisture and an enzymatic catalyst and results in the liberation of free fatty acids which have a lower flavor threshold than the parent triglycerides. This is why hydrolytic rancidity causes a distinct rancid flavor which is contributing 12 factor to the decreased quality and acceptability of the fat. The most effective methods to reduce the occurrence of hydrolytic rancidity would be to reduce the amount of moisture in the fat source or store the fat at cold temperatures. Other than the formation of off-flavors and odors, another reason to avoid hydrolytic rancidity is that the reactions of hydrolysis supply free oleic, linoleic and linolenic acids that could then undergo further oxidative rancidity [16]. Oxidative rancidity also termed autoxidation is certainly the most complex type of rancidity and causes the greatest level of concern among producers. Although all fats are made up of a variety of fatty acids, fats that contain a high level of unsaturated fatty acids tend to be more susceptible to autoxidation, as rancidity typically takes place at a double bond [16]. Oxidation proceeds at different rates for each of the abundantly occurring unsaturated fatty acids. The order of reactivity generally remains as linolenic > linoleic > oleic [16]. The process of autoxidation consists of three main phases: initiation, propagation and termination. A rancid taste is often noticeable in many oils when the peroxide value is between 20 and 40 mEq/kg oil [17]. Initiation takes place when two free radicals are formed through the cleavage of a hydrogen atom from a triglyceride, a reaction that usually requires a heavy metal catalyst such as copper or energy from heat or light [18]. At this point, oxidation occurs at a relatively slow, uniform rate of speed during what is known as the induction period. Next, in the first of two propagation reactions, a peroxy radical is formed when the triglyceride free radical reacts with an oxygen molecule. In the second propagation reaction, the peroxy radical reacts with another triglyceride, forming a hydroperoxide and regenerating a new free radical that is then available to react with another oxygen molecule, causing an accelerated chain-reaction to occur [16]. The hydroperoxide concentration can be measured, providing a peroxide value.

The peroxide value is increased on storage at room temperature as well as on higher temperature. Generally, the oils not exposed to the light as well as air have very little peroxide value.

The presence of free fatty acids also has an impact on peroxide value. The presence of heat and water breaks up triglycerides through hydrolysis to form free fatty acids [19]. Hence, the storage conditions and containers used for this purpose also influence the quality of fatty formulations.

It was observed that the peroxide values of S1, S2 and S3 were changed with time on storage during stability. The changes in peroxide values could be due to storage temperature and humidity. As in preparation of the 'Ashtamangal Ghrita', all the water content was evaporated through continuous heating, the changing pattern of peroxide value in samples could only be due to storage conditions of accelerated stability study. The changes in peroxide values of all the samples during stability study have been shown in Fig.3.

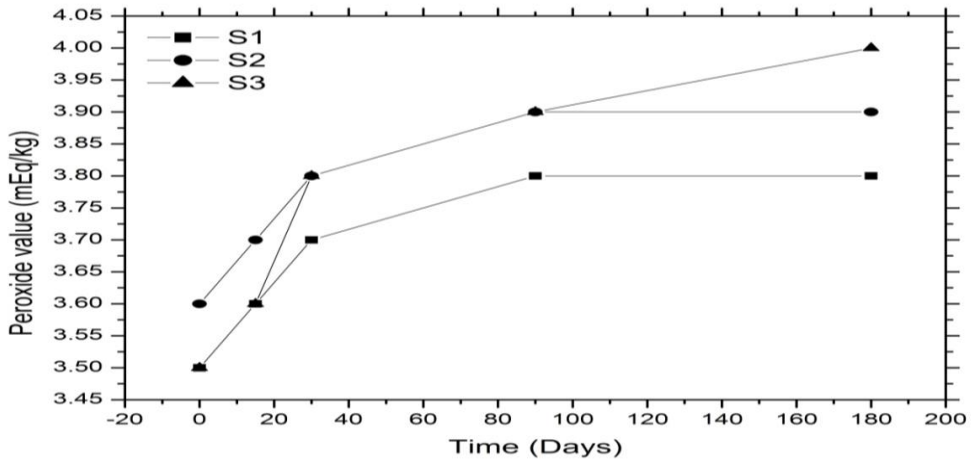

Fig.3. Peroxide values of the samples during stability study

Saponification value determination

Saponification value is expressed by potassium hydroxide in mg required to saponify one gram of fat. It depends on the kind of fatty acid contained in the fat. The changes in saponification values of all the samples were observed during the stability study. As it is amount of potassium salt of free fatty acids, this parameter is indicator of free acidic groups available in the fatty matter. It was observed that the saponification value of S1, S2 and S3 was found 131.835, 126.225 and 129.03 (mgKOH/g sample) respectively in the fresh samples and after 6 month of the stability study, the data for this parameter was 135.235, 133.98 and 132.75 (mgKOH/g sample) respectively. The saponification values of all the samples during stability analysis are shown in Fig.4.

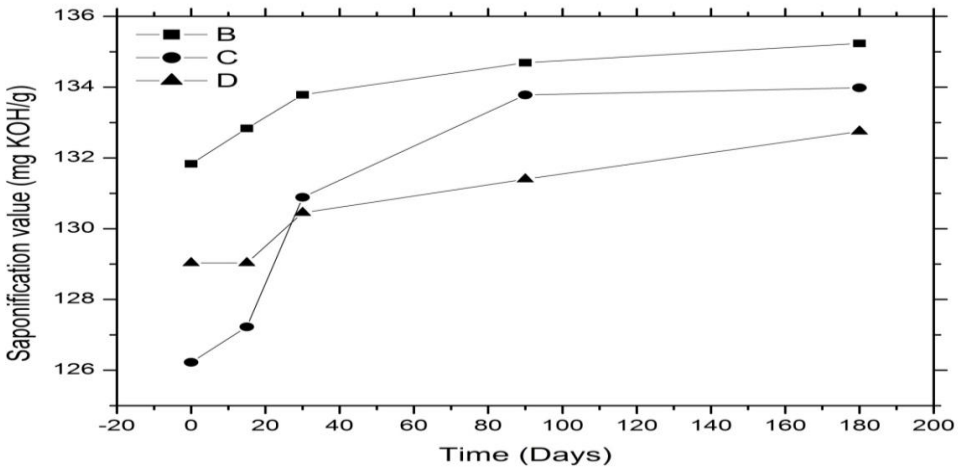

Fig.4. Saponification values of samples (B=S1, C=S2, D=S3)

ISSN: 0975-8585

Refractive index

The refractive index of the fatty materials is also characteristic feature of the fatty materials as well as the formulations containing fatty materials. In some conditions, the atmospheric conditions as well as light have influence on refractive index. It is well known that the refractive index is deceased with the molecular weight of the fatty materials. It is also related with low iodine value since refractive index decreases with un-saturation. Also, the presence of free fatty acids appreciably lowers the refractive index of an oil or fat. The low refractive index might be related to high proportion of fatty acids of low molecular weight resulting higher saponification value. In freshly prepared samples of the formulation, the refractive index was found 1.46269, 1.46369 and 1.46668 for S1, S2 and S3 respectively. However, after 6 month of stability study, these samples revealed the refractive index as 1.46148, 1.46145 and 1.46372 respectively. The changes in refractive index values might be related to the chemical changes occurring in samples due to storage conditions of temperature and humidity. The changes in refractive indices with respect to time during stability are shown in Fig.5.

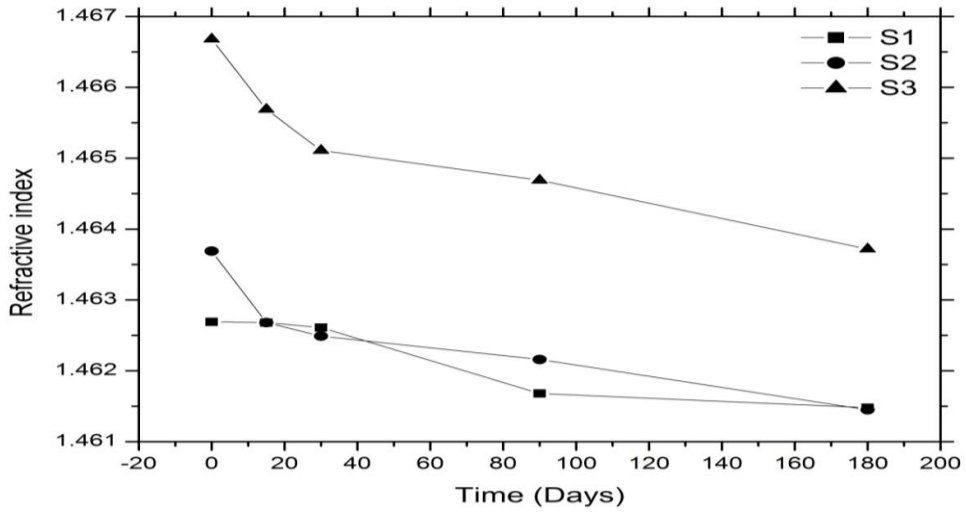

Fig.5. Refractive indices of the samples during stability

Specific gravity estimation

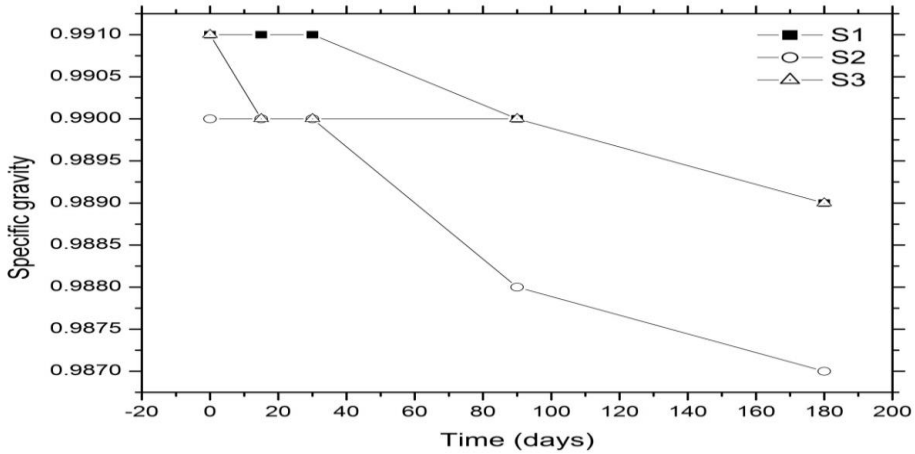

Fig.6.Specific gravity of the samples

The specific gravity is the inherent characteristic of any pharmaceutical product. In case of liquid and semi-solid preparations, the weight depends upon solid and liquid contents and as the time passes on, the chemical processes convert the solid contents into liquid or vice-versa and these components undergo

sedimentation and consequently change the consistency. Any remarkable changes with respect to time in specific gravity of such products can be related to going on physicochemical processes. These phenomenons directly/indirectly influence the self-life of the product. Hitherto, this parameter was used to assess its impact on expiration of *'Ashtamangal Ghrita'*. The changes in specific gravity of the samples studied during storage under stability study are shown in Fig.6. It was observed that in initial state, the specific gravity of S1, S2 and S3 was 0.991, 0.99 and 0.991 respectively and after 6 month of the stability study, it was changed into 0.989, 0.987 and 0.989 respectively.

Self-life estimation

The self-life of the product can be defined as the length of time under specific environmental conditions of storage under which the product remains within specific prescribed limits of all its important characteristics [12, 20, 21, 22]. The estimation of '*Ashtamangal Ghrita'* was performed to ensure the efficacy and quality of this preparation and to establish self-life or expiration time.

During stability profile study of '*Ashtamangal Ghrita'*, all the samples were maintained at temperature 40±2^{0}C and relative humidity (RH%) at 75±5%. These conditions were set upto 6 month of the study and at different time intervals such as 0, 15, 30, 90 and 180 day, these samples were studied for estimation of appearance, colour, odour, taste, touch and physicochemical parameters such as iodine value, refractive index, peroxide value, saponification value, acid value and specific gravity. When the appearance, colour, odor, taste and touch were assessed at different time intervals of the study, no remarkable changes were noticed in these parameters. As all these organoleptic characteristics were studied manually for qualitative estimation, the chances of variations might be in observation. Hence, the changes in *Ghrita* samples were studied by physiochemical parameters and quantitative results were taken into account for impacts of humidity and temperature on samples in accelerated storage condition.

Table.2. Estimation of self-life of S1 sample by accelerated stability study

Parameter	Results at initial month	Intercept	Slope	Results at 10% degradation	Months at 10%degradation
Acid value (mgKOH/g)	1.383	1.383	0.0017	2.82	0.235
Saponification value (mg KOH/g)	131.835	132.63	0.0166	842.078	28.069
Peroxide value (meq/kg)	3.5	3.588	0.0015	292	9.733
Iodine value (mg iodine/g)	38.496	39.063	0.016	270.957	9.0319
Specific gravity	0.991	0.991	0.001	99.3	3.31
Refractive index	1.462	1.462	0.001	146.279	4.875
Mean months at accelerated conditions					6.339 (0.528 Year)
Climate Zone I & II					31.696 (2.641 Year)
Climate Zone III & IV					20.919 (1.743 Year)

The results of all these parameters discussed as above indicated the significant differences from initial values of the samples ($p<0.05$). The results of different parameters obtained during stability study and the calculated self-life of the samples in different climatic zones have been shown in Table.2, 3 & 4. It revealed the impact of humidity and temperature on the physicochemical properties of the samples studied with respect to time profile of stability assessment. For the assessment of self-life of these samples as S1, S2 and S3, ±10% changes in the results of these parameters were considered as these parameters might be related to the efficacy of the samples. According to ICH guidelines, upto 90% potency of the drugs is within the acceptable limit [12]. The results indicated that the time required for 10% degradation of S1 sample in terms of physicochemical parameters studied were 6.339 months (0.528 Year). However, the time required for 10% changes in these parameters in climate zone I & II was 31.696 months (2.641 Year) and in climate zone III & IV, it was 20.919 months (1.743 Year). The results for S2 sample were 7.2005 months (0.600 Year) for 10 % changes in parameters. For climate zone I and II, the time required for 10% changes was 36.0025 months (3.00 Year) and for climate zone III and IV, the result was 23.761 month (1.980 Year). In case of S3 sample, the mean months required for 10% degradation was 5.875 month (0.489 Year). For climate zone I & II, the mean time requirement for 10% changes in physicochemical parameters such as iodine value, acid value, saponification value, peroxide value, specific gravity and refractive index was 29.378 months (2.44 Year) and for climate zone

III & IV, it was found 19.389 months (1.615 Year). The results for 10% changes in S1, S2 and S3 samples were found more than 2 years for climate zone I & II. It has been reported elsewhere that the self-life of Ghrita preparations varies from 6 month to 2 year [12]. Hitherto, the self-life in terms of time required for 10% changes in physicochemical parameters was found within the acceptable official limit.

Table.3. Estimation of self-life of S2 sample by accelerated stability study

Parameter	Results at initial month	Intercept	Slope	Results at 10% degradation	Months at 10%degradation
Acid value (mgKOH/g)	1.695	1.7357	0.0006	227.833	7.594
Saponification value (mg KOH/g)	126.225	127.79	0.0618	229.571	7.652
Peroxide value (meq/kg)	3.6	3.688	0.0015	298.667	9.955
Iodine value (mg iodine/g)	35.58	36.093	0.0204	345.147	11.504
Specific gravity	0.99	0.9902	0.002	49.6	1.653
Refractive index	1.463	1.4631	0.001	145.379	4.845
Mean months at accelerated conditions					7.2005 (0.600 Year)
Climate Zone I & II					36.0025 (3.00 Year)
Climate Zone III & IV					23.76165 (1.980 Year)

Table.4. Estimation of self-life of SIII sample by accelerated stability study

Parameter	Results at initial month	Intercept	Slope	Results at 10% degradation	Months at 10%degradation
Acid value (mgKOH/g)	1.22	1.2571	0.001	227.833	7.594
Saponification value (mg KOH/g)	129.03	129.22	0.0508	257.7362	8.591
Peroxide value (meq/kg)	3.5	3.6021	0.0025	180.84	6.028
Iodine value (mg iodine/g)	38.681	39.31	0.0153	228.242	7.608
Specific gravity	0.991	0.9905	0.006	16.433	0.547
Refractive index	1.466	1.4666	0.001	146.588	4.886
Mean months at accelerated conditions					5.875 (0.489 Year)
Climate Zone I & II					29.378 (2.44 Year)
Climate Zone III & IV					19.389 (1.615 Year)

CONCLUSION

The results of the study revealed that '*Ashtamangal Ghrita*' preparation can be formulated effectively by applying traditional ayurvedic method. The organoleptic properties of this formulation such as appearance, colour, odour, taste and touch were found as prescribed in official compendium. During storage, these properties were also changed to an extent but being qualitative parameters, these parameters could not be taken into account for self-life estimation. The physicochemical parameters such as specific gravity, refractive index, iodine value, saponification value, peroxide value and acid value were found within the acceptable limit of ayurvedic literature for freshly prepared samples. However, the changes were also analyzed during storage at accelerated temperature and humidity conditions of stability study. The self-life of all samples was found more than 2 year for countries of climate zone I and II and less than 2 year in climate zone III and IV. The recommended self-life of Ghrita preparations has been mentioned from 6 month to 2 year in various ayurvedic literatures. Moreover, it can be concluded that these parameters can be applied for self-life estimation of ayurvedic 'Ghrita' formulation.

ISSN: 0975-8585

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