

ANALYTICAL STUDY OF ASHTAMANGALA GHRITA

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ABSTRACT

Ghrita kalpana is a kind of formulation which are processed in a manner that both lipid soluble and water soluble active principles of the drugs are transferred into *Ghrita* which is used here as a base. *Ashtamangala ghrita* (AMG) is a polyherbal formulation prepared by using *Vacha*, *Kushta*, *Brahmi*, *Peetasarshapa*, *Sariva*, *Saindhava lavana*, *Pippali* and *Murchita ghrita*. Analytical study provides us the objective parameters for standardization. It helps in understanding and interpretation of a drug and its structure. These methods are intended to establish the purity, physical characteristics and potency of the drugs that we use. Analytical study for standardization of AMG was carried out on the basis of classically illustrated organoleptic tests and modern parameters i.e. Physico chemical properties were carried out where Refractive index at 25° c is 1.459, Specific gravity is 0.9013, Rancidity test shows no oxidation of fat. The crucial parameters like Acid value, Saponification value, Iodine value is 0.56, 210.19 and 31.37 respectively with zero Peroxide value. Chromatological technique like High Performance Thin Layer Chromatography (HPTLC) shows 5 spots in 254nm and 8 spots in 366nm wavelength.

Keywords: *Ashtamangala Ghrita*, Analytical study, HPTLC

INTRODUCTION

Ayurveda describes the use of numerous drugs as medicine. But to become beneficial for the sufferers these drugs should be converted into absorbable forms. Pharmaceutical processes convert *Dravya* into *Oushadha*. These pharmaceutical processes are mentioned in *Ayurveda* as *Samskaras*.

By applying these pharmaceutical techniques, number of *Kalpanas* were developed, one of them is *Sneha Kalpana*. It is a pharmaceutical process to get the oleaginous medicinal substances. The main advantage of *Sneha Kalpana* is that as *Jala* is also used

in the formulation both water soluble and fat soluble substances can be absorbed simultaneously.

AMG is a polyherbal formulation mentioned in our classics. The reference of this formulation can be traced in *Chakradatta*, *Bhaishajya Ratnavali* and in *Sahasrayoga*. The ingredients and indications of AMG ¹mentioned in *Chakradatta* and *Bhaishajya Ratnavali* is same which contains *Vacha*, *Kushta*, *Brahmi*, *Peetasarshapa*, *Sariva*, *Saindhava lavana* and *Pippali* along with *Murchita ghrita*, which is said to be medhya, enhances *Drida smriti*,

Kshipramedha and is indicated in *graha rogas*. The formulation mentioned in *Sahasrayoga* contains *Patola, Sariva, Musta, Madhuka, Katurohini, Usheera, Chandana, Pippali* and *Ghrita* and acts as *Sarva Jwarahara, Rakshoghna* and *Pushti Vardhaka*. In the present study the reference of AMG¹ has been taken from *Chakradatta, Balaroga Chikitsadhyaya*, 64th chapter.

The present study aims to analyse *Ashtamangala Ghrita* by qualitative and quantitative parameters. In our classics *Acharyas* has mentioned *Gandha Varna Rasotpatti* for prepared *Sneha* as *Siddhi lakshana*. To interpret the same there are few modern parameters has been explained in PLIM Govt. of India Gaziabad, among them tests like Refractive index², Specific gravity³, Rancidity⁴, Acid value⁵, Saponification value⁶, Iodine value⁷, Peroxide value⁸ and HPTLC⁹ were assessed.

AIM AND OBJECTIVE:

- Analytical standardization of *Ashtamangala Ghrita*
- To carryout Physico-chemical analysis of *Ashtamangala Ghrita*

MATERIAL AND METHODS:

Table 1: Showing Organoleptic characters of AMG:

Parameters	AMG
<i>Varna</i>	<i>Harita yukta peeta</i>
<i>Rasa</i>	<i>Tikta</i>
<i>Gandha</i>	Characteristic
<i>Sparsha</i>	<i>Snigdha</i>
<i>Shabdha</i>	Nil

Physico-chemical tests:

Acid value, refractive index, specific gravity, rancidity, saponification value, iodine value, peroxide value were conducted at Drug testing laboratory, Government central pharmacy, Jayanagar, Bengaluru and HPTLC was done at SDM Research Centre for Ayurveda and Allied Sciences, Udupi.

B. Modern parameters:

1. Organoleptic characters

The raw materials for *Ghrita murchana* and for the preparation of *Ashtamangala Ghrita* like *Hareetaki, Vibheetaki, Amalaki, Musta, Haridra, Vacha, Kushta, Peetasarshapa, Sariva Saindhava lavana, Pippali* and *Ghrita* were procured from *Amrut kesari depot*, Bengaluru and *Brahmi* was procured from Sirsi (Uttara Kannada dist, Karnataka). Initially *Ghrita murchana* was done as per *Bhaishajya Ratnavali jwaradhikara* reference. *Ashtamangala ghrita* was prepared as per the reference *Chakradatta balarogadhikara. Paka lakshanas* were assessed according to *Sharangdhara samhita* reference and the preparation was carried out in the practical hall of PG Dept. of *Rasashastra & Bhaishajya Kalpana*, Government Ayurvedic Medical College, Bengaluru. Physico chemical analysis was carried out with ancient and modern parameters.

ANALYTICAL STUDY:

To assess the quality of prepared *Ashtamangala Ghrita* it was subjected to both classical and modern parameters.

A. Classical parameters

The ancient parameters like *Varna, Gandha* etc., were carried out in *Rasashastra and Bhaishajya kalpana* Dept., GAMC, Bengaluru.

Colour, odour, taste of the given sample was tested using sensory organs and the same were noted in table no.2

2. Refractive index:

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects and separate it exactly at the centre. Reading was noted. Distilled water has a refractive index of 1.3325 at 25°C. The difference between the reading

and 1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28°C.

3. Specific gravity:

Specific gravity bottle was cleaned by shaking with acetone and then with ether. Dried the bottle and noted the weight. Sample solution was cooled to room temperature. Specific gravity bottle was carefully filled with the test liquid, inserted the stopper, surplus liquid was removed and weight was noted. Procedure was repeated using distilled water in place of sample solution.

4. Rancidity:

Mixed 1.0ml of melted fat and 1.0ml of conc. HCl in a test tube, add 1.0ml of 1% Phloroglucinol in diethyl ether and mixed thoroughly with the fat acid mixture. A pink colour indicates that the fat is slightly oxidized, while a red colour indicates that the fat is definitely oxidized

5. Acid value:

Weighed 2- 10g of ghee was taken in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Experiment was repeated twice to get concordant values.

6. Saponification value:

Weighed 2g of the Oil / Fat into a 250 ml RB flask fitted with a reflux condenser. 25ml of 0.5M alcoholic potash was added. It was refluxed on a water bath for 30 minutes. Later cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). The procedure was repeated omitting the substance being examined

(blank) (b ml). Experiment was repeated twice to get concordant values.

7. Iodine value:

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4 , 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17°C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

8. Peroxide value:

5g of the *Ghrita* was weighed accurately into a conical flask, added 30 ml of mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow colour disappears. Add 0.5ml of starch indicator continued the titration until blue colour disappears.

Peroxide value = $10(a-b)/W$

Where W = weight in g of the substance

9. Determination of Unsaponifiable matter

Weighed 5g of the *Ashtamangala Ghrita* into the flask. Added 50ml alcoholic KOH into the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to

settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shook vigorously. After each wash the alcohol-water layer was drawn off. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Flask was placed in an air oven at 85°C for about 1 hour to remove the last traces of ether. A few ml of acetone

was added and evaporated to dryness on a water bath. Cooled in a desiccator to remove last traces of moisture and then weighed.

10. High Performance Thin Layer Chromatography:

3, 6, 9 μ l of the above sample of *Ashtamangala Ghrita* was applied on a precoated silica gel F254 on aluminium plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized under short UV, long UV, and after derivatization in vanillin-sulphuric acid spray reagent it was visualized under white light and scanned under UV 254nm, 366 nm. R_f, colour of the spots and densitometric scan were recorded.

RESULTS:

Table 2: Showing Organoleptic characters of AMG:

Parameters	<i>Ashtamangala ghrita</i>
Colour	Greenish yellow
Taste	Acrid
Odour	Characteristic
Appearance	Semisolid and greasy
Texture	Smooth

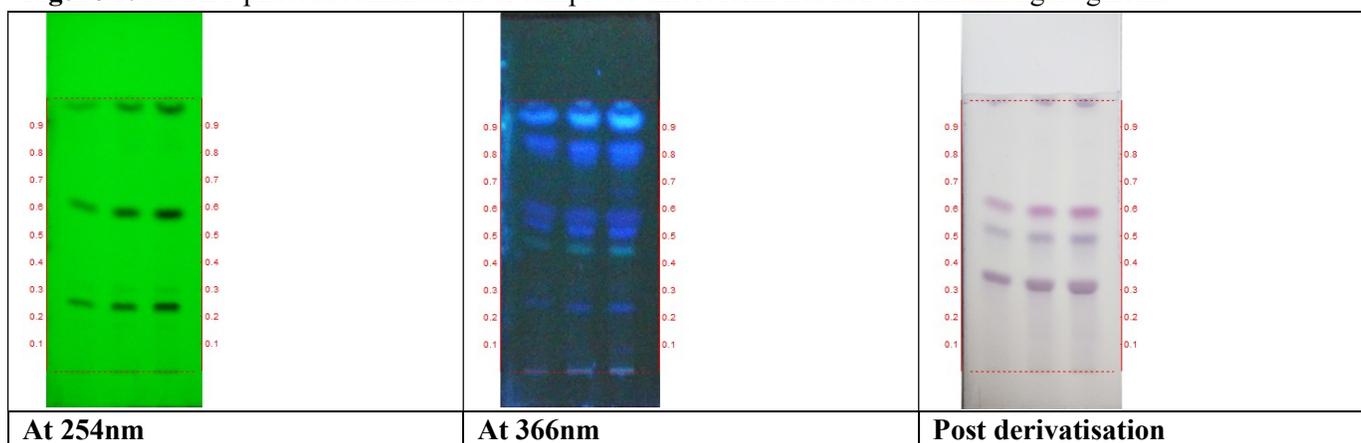
Table 3: Showing results of standardization parameters

Parameter	Results n = 3 %w/w
	<i>Ashtamangala ghrita</i>
Refractive index	1.459
Specific gravity	0.9013
Rancidity	Fat is not oxidized
Acid value	0.56
Saponification value	210.19
Iodine value	31.37
Peroxide value	0.00

Table 4: Unsaponifiable matter of *Ashtamangala ghrita*

	<i>Ashtamangala ghrita</i>
Unsaponifiable matter (%)	1.10

Figure 1: HPTLC photodocumentation of sample of chloroform fraction of Ashtamangala ghrita



Track 1: *Ashtamangala ghrita* - 3µl

Track 2: *Ashtamangala ghrita* - 6µl

Track 3: *Ashtamangala ghrita* - 9µl

Solvent system- Toluene: Ethyl acetate (9.0:1.0)

Table 5: Rf value of sample of *Ashtamangala ghrita*

At 254nm	At 366nm	Post derivatisation
0.25 (D. green)	0.25 (F. blue)	-
0.30 (L. green)	-	-
-	-	0.32 (D. purple)
-	0.45 (F. green)	-
-	-	0.50 (D. purple)
-	0.52 (F. blue)	-
0.58 (D. green)	0.58 (F. blue)	0.58 (D. pink)
-	0.68 (F. blue)	-
-	0.79 (F. blue)	-
0.83 (L. green)	0.83 (F. blue)	-
-	0.93 (F. blue)	-
0.96 (L. green)	-	-

*F – fluorescent; D – dark; L – light

Figure 2: Densitometric scan of the sample of *Ashtamangala ghrita* at 254nm

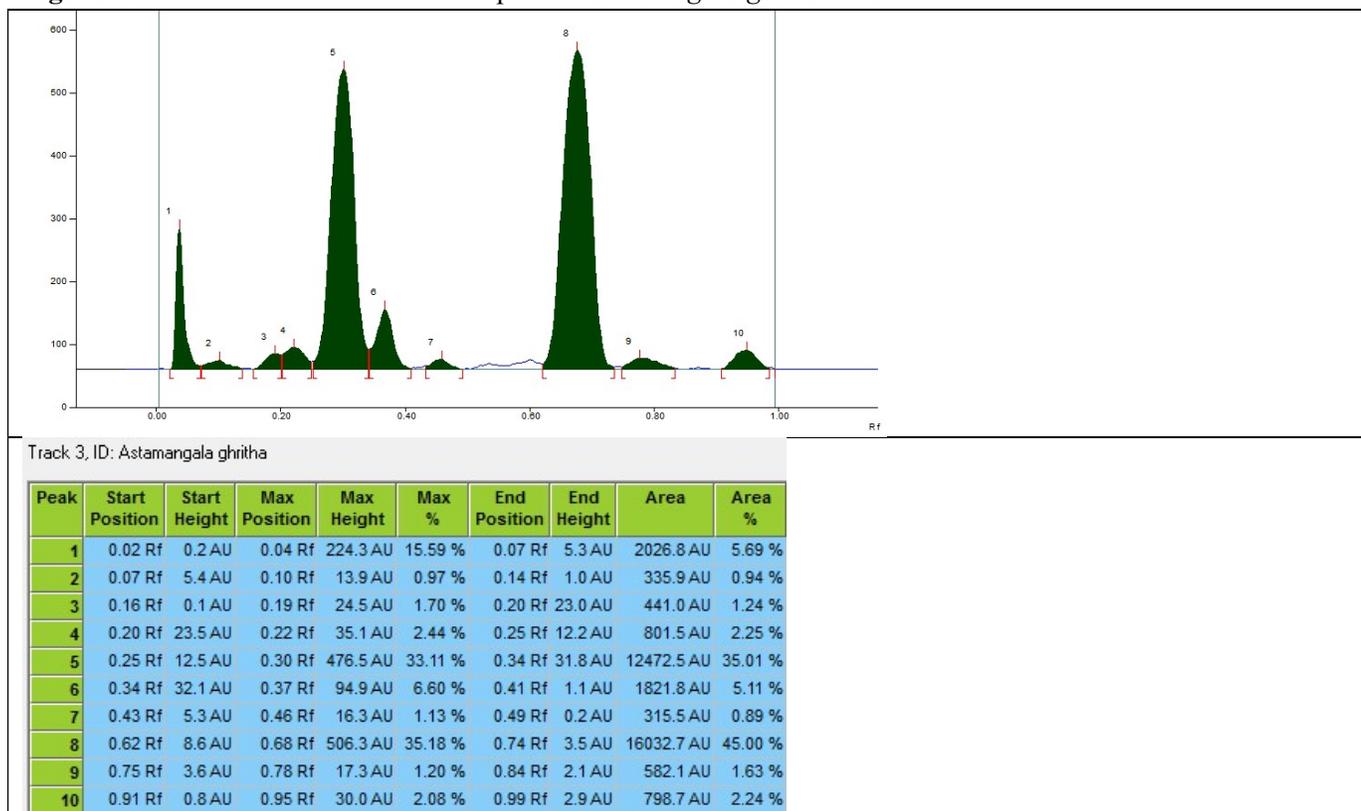


Fig 2a. At 254nm

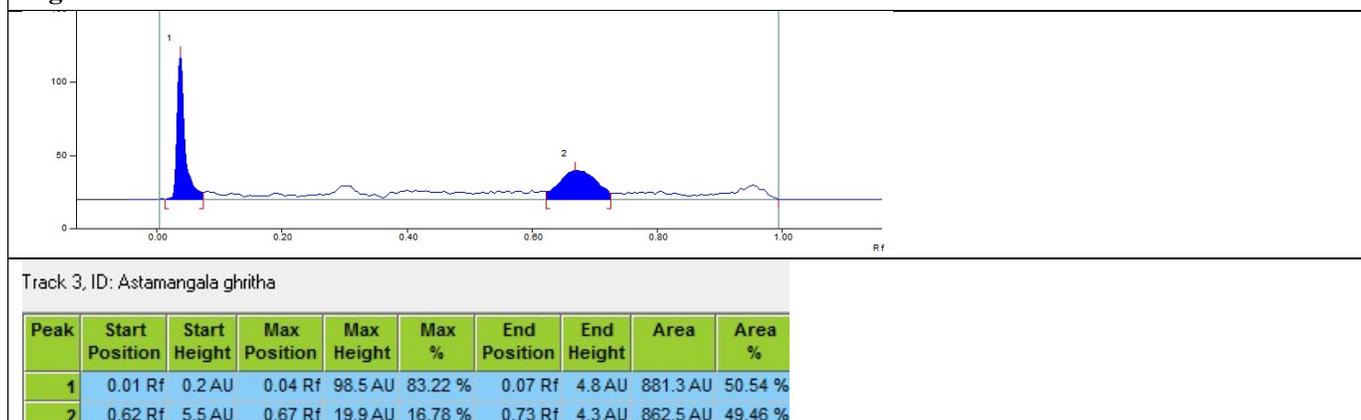


Fig 2b. At 366nm

DISCUSSION

Physico-chemical parameters:

Refractive index-It is the ratio of the velocity of light in a vacuum to its velocity in the substance. It is a fundamental physical property of a substance often used to identify a particular substance, confirm its purity, or measure its concentration. More will be

Refractive Index, there will be more concentration of light which facilitates rancidification of *Ghrita* i.e., decomposition of *Ghrita*.

Refractive index of the *Ashtamangala Ghrita* was 1.459

Specific gravity– It indicates the solid to liquid ratio in the *Ghrita*. Specific gravity of the *Ashtamangala Ghrita* was 0.9013.

Rancidity– Oils and fat with higher degree of unsaturation will pick up oxidative rancidity earlier. Volatile products which are produced by complex chemical changes due to high peroxide level are responsible for rancid taste and odour.

Fat is not oxidized in *Ashtamangala Ghrita*.

Acid value–It is a measure of the amount of Carboxylic acid groups in a chemical compound, such as fatty acid, or in a mixture of compounds as oil-fats rancidify, triglycerides are converted into fatty acids and glycerol, causing an increase in acid. Less acid value denotes the less chance of decomposition of *Ghrita* thus increasing both life span and therapeutic value.

The acid value of the *Ashtamangala Ghrita* was 0.56.

Saponification value –Saponification value gives an idea about the molecular weight of an oil / Fat. The saponification value and molecular weight of oil are inversely proportion. It is helpful in determining adulteration of given fat by one of the lower or higher saponification value.

Saponification value of the *Ashtamangala Ghrita* was 210.19

Iodine value- It indicates the degree of unsaturation. Greater degree of unsaturation indicates the possibility of the ghee becoming rancid due to atmospheric oxidation. And the iodine value of the *Ashtamangala Ghrita* was 31.37.

Peroxide value- it is the most widely used analytical method. It gives a measure of the extent to which an oil/ghee sample as undergone primary oxidation, extent of secondary oxidation may be determined from p-anisidine test. Peroxide value of *Ashtamangala Ghrita* was 0.

Discussion on HPTLC

HPTLC is the sophisticated analytical parameter for the evaluation of the herbal drugs. HPTLC can also serves as Fingerprinting technique for identification and quantification of the herbal and Herbo-mineral

formulations. Through HPTLC technique major phytochemical present the drug or formulation can be estimated. It helps to find out the adulteration in the formulation and is used as a standard for the herbal compounds. The prepared drug was subjected to HPTLC fingerprinting at different wavelengths (254nm & 366nm). The colour spots observed indicates the presence of different components in the sample.

CONCLUSION

Physical test shows AMG is greenish yellow in colour with acrid taste and characteristic odour. Quantitative chemical analysis shows that in *Ashtamangala Ghrita*, Refractive index-1.459, Specific gravity-0.9013, Rancidity-fat is not oxidized, Acid value-0.56, Saponification value-210.19, Iodine value-31.37 and Peroxide value- zero. HPTLC study of *Ashtamangala Ghrita* confirms the presence of different components in the sample and there is no any degradation in the final product. It acts as the fingerprint of the used sample, which can be used as the reference for the preparation of same kind of *Ghrita*

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