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STANDARDIZATION OF AMRITAPRASHA GHRITA: A HERBAL GHEE BASED MEDICINAL PREPARATION

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Keywords:

Amritaprasha Ghrita, Under nutrition, karshya, Standardization, HPTLC

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ABSTRACT: Aim: To standardize Amritaprasha ghrita. **Materials and methods:** Physico-chemical studies like refractive index, specific gravity, acid value, saponification value, iodine value, determination of unsaponifiable matter, peroxide, viscosity, rancidity test and HPTLC were carried out as per the WHO guidelines, Indian Pharmacopoeia and Ayurvedic Pharmacopoeia. **Conclusion:** Standardization tests done on Amritaprasha ghrita helped in authenticating and ensuring the quality of the same.

INTRODUCTION: Standardization is necessary to make sure the availability of a consistent product and can assure a reliable product with definite constituents [1 - 2]. The standardization of herbal medicines is always challenging as it medicines contain more than one active principles and the active compound is frequently unknown [3 - 4]. Standardization of herbal formulations is essential to assess quality, consistency of active principles and therapeutic efficacy of drugs [5 - 6]. The quality assessment of herbal formulations is important to justify their acceptability and safety[7 - 8].

Amritaprasha ghrita [9] comprises of fifty drugs such as Jivaka (*Pueraria tuberosa* (Willd.) DC), [10] Rishabhaka (*Pueraria tuberosa* (Willd.) DC), [10] Veera (*Nardostachys jatamansi* (D. Don) DC.), [11] Jivanti (*Leptadenia reticulata* (Retz.) Wight & Arn),[10] Shunti (*Zingiber officinale* Roscoe), [12] Shati (*Hedychium spicatum* Sm. in A.Rees),[13] Shalaparni (*Desmodium gangeticum* (L.) DC.), [14] Prishniparni (*Uraria picta* (Jacq.) DC.), [15] Mudgaparni (*Phaseolus trilobus* Ait.), [16] Mashaparni (*Teramnus labialis* (L. f.) Spreng.), [16] Meda (*Asparagus racemosus* Willd.), [10] Mahameda (*Asparagus racemosus* Willd.), [10] Kakoli (*Withania somnifera* (L.) Dunal), [17] Ksheerakakoli (*Withania somnifera* (L.) Dunal), [18 - 19] Bruhati (*Solanum indicum.*), [16] Kantakari (*Solanum xanthocarpum* Schrad. & H. Wendl.), [14] Sweta Punarnava (*Boerhavia diffusa* L.), [10] Rakta Punarnava (*T. portulacastrum* L.), [20] Madhuka (*Glycyrrhiza glabra* L.), [21 - 24] Kapikachu (*Mucuna pruriens* (L.) DC.), [25] Shatavari (*Asparagus racemosus* Willd.), [10] Riddhi (*D. bulbifera* L.), [10] Vriddhi (*Dioscorea bulbifera* L.), [10] Parushaka (*Grewia asiatica* L.), [26] Bharangi (*Clerodendrum serratum*. (L)), [27] Mrudvika (*Vitis vinifera* L.), [28] Shringhataka (*Trapa bispinosa* Roxb.), [29] Tamalaki (*Phyllanthus niruri* L.), [10] Vidarikanda (*Pueraria tuberosa* (Willd.) DC), [30] Pippali (*Piper longum* L.), [31] Bala (*Sida cordifolia* L.), [10] Badara (*Ziziphus jujube* Mill.), [32] Akshotaka

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(*Juglans regia* L.), [33] Kharjura (*Phoenix dactylifera* L.), [34] Vatama (*Prunus amygdalus* Batsch), [35] Abhishuka (*Pistacia vera* L.), [36] Dhatri (*Phyllanthus emblica* L), [37] Ikshu (*Saccharum officinarum* L.), [38] Chaaga Mamsarasa (meat soup of goat fried with ghee), [39] Go ksheera, [40] Go ghrita, [41] Madhu (honey), [42] Sarkara (sugar), Maricha (*Piper nigrum* L.), [43] Twak (*Cinnamomum zeylanicum* Blume), [10] Ela (*Elettaria cardamomum* (L.) Maton), [44] Patra (*Cinnamomum zeylanicum* Blume), [45] Nagakesara (*Mesua ferrea* L.) [46]. Amritaprasha Ghrita is made use in the management of Karshya (Grade 1 & 2 under nutrition) in children. Literature survey did not reveal any standards for Amritaprasha ghrita and hence the current study was undertaken to standardize the same. The ingredients of Amritaprasha ghrita is detailed in **Table 1**.

TABLE 1: SHOWING INGREDIENTS OF AMRITAPRAASHA GHRITA

S. no.	Sanskrit name	Substitute used	Botanical name	Part used
1	Jivaka	Vidarikanda	*Pueraria tuberosa* (Willd.) DC	Tuber
2	Rishabhaka	Vidarikanda	*Pueraria tuberosa* (Willd.) DC	Tuber
3	Veera	-	*Nardostachys jatamansi* (D.Don) DC.	Root
4	Jivanti	-	*Leptadenia reticulata* (Retz.) Wight & Arn	Root
5	Shunti	-	*Zingiber officinale* Roscoe	Rhizome
6	Shati	-	*Hedychium spicatum* Sm. in A. Rees	Rhizome
7	Shalaparni	-	*Desmodium gangeticum* (L.) DC.	Root
8	Prushniparni	-	*Uraria picta* (Jacq.) DC.	Root
9	Mudgaparni	-	*Phaseolus trilobus* Ait.	Root
10	Mashaparni	-	*Teramnus labialis* (L. f.) Spreng.	Root
11	Meda	Shatavari	*Asparagus racemosus* Willd.	Root tuber
12	Mahameda	Shatavari	*Asparagus racemosus* Willd.	Root tuber
13	Kakoli	Aswagandha	*Withania somnifera* (L.) Dunal	Root
14	Ksheerakakoli	Aswagandha	*Withania somnifera* (L.) Dunal	Root
15	Brihati	-	*Solanum indicum*	Root
16	Kantakari	-	*Solanum xanthocarpum* Schrad. & H. Wendl.	Root
17	SwetaPunarnava	-	*Boerhavia diffusa* L.	Root
18	RaktaPunarnava	-	*Trianthema portulacastrum* L.	Root
19	Madhuka	-	*Glycyrrhiza glabra* L.	Root
20	Kapikachu	-	*Mucuna pruriens* (L.) DC.	Seed
21	Shatvari	-	*Asparagus racemosus*Willd.	Root tuber
22	Riddhi	Varahikanda	*Dioscorea bulbifera* L.	Tuber
23	Vriddhi	Varahikanda	*Dioscorea bulbifera* L.	Tuber
24	Parushaka	-	*Grewia asiatica* L.	Fruit
25	Bharangi	-	*Clerodendrum serratum* .(L)	Root
26	Mrudvika	-	*Vitis vinifera* L.	Fruit
27	Brihati	-	*Solanum indicum*	Root
28	Shringataka	-	*Trapabi spinosa* Roxb.	Fruit
29	Tamalaki	-	*Phyllanthus niruri* L.	Whole plant
30	Vidarikanda	-	*Pueraria tuberosa* (Willd.) DC.	Tuber
31	Pippali	-	*Piper longum* L.	Fruit
32	Bala	-	*Sida cordifolia* L.	Root
33	Badara	-	*Ziziphus jujube* Mill.	Fruit
34	Akshotaka	-	*Juglans regia* L.	Seed
35	Kharjura	-	*Phoenix dactylifera* L.	Fruit
36	Vatama	-	*Prunus amygdalus* Batsch	Seed
37	Abhishuka	-	*Pistacia vera* L.	Seed
38	Dhatri	-	*Phyllanthus emblica* L	Fruit
39	Vidarikanda	-	*Pueraria tuberosa* (Willd.) DC.	Tuber
40	Ikshu	-	*Saccharum officinarum* L.	Stem
41	Chaaga Mamsarasa	-	Goat's meat	
42	Go Ksheera	-	Cow's milk	
43	Go Ghrita	-	Cow's ghee	
44	Madhu	-	Honey	
45	Sarkara	-	Sugar	
46	Maricha	-	*Piper nigrum* L.	Fruit
47	Twak	-	*Cinnamomum zeylanicum* Blume	Bark
48	Ela	-	*Elettaria cardamomum* (L.) Maton	Fruit
49	Patra	-	*Cinnamomum zeylanicum* Blume	Leaf
50	Nagakesara	-	*Mesua ferrea* L.	Flower

MATERIALS AND METHODS: Physico-chemical studies like refractive index, specific gravity, acid value, saponification value, iodine value, determination of unsaponifiable matter, peroxide, viscosity, rancidity test and HPTLC were carried out as per the WHO guidelines, Ayurvedic Pharmacopoeia and Indian Pharmacopoeia.

Plant Material: The constituents of Amritaprasha Ghrita were collected from the local market of Hassan District, Karnataka State, India in the month of March 2017. The collected drug was identified and authenticated (no: SDMCAH-DG/2017/16) at the teaching pharmacy of Department of Dravyaguna (Ayurveda Pharmacology), SDM College of Ayurveda and Hospital, Hassan, Karnataka State, India.

Methodology: The studies were done at SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka State, India as per standard procedure.

Refractive Index: Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundry line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.33217 at 28˚C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3320, 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.

Specific Gravity: Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Acid Value: Weighed 2-10 g of ghritha 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. Repeated the experiment twice to get concordant values.

Saponification Value: Weighed 2 g of the Amritaprasha ghritha into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

Iodine Value: The sample was accurately weighed in a dry iodine flask. Dissolved with 10 ml of CCl_4, 20 ml 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 ºC for 30 min. 15 ml of potassium iodide and 100 ml 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.

Determination of Unsaponifiable Matter: Weighed 5 g of the Amritaprasha ghritha into the flask. Added 50 ml alcoholic KOH into the sample. Boiled gently but steadly under reflux condenser for one hour. The condensor 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, 50 ml of water was added to the separating funnel followed by an addition of 50 ml petroleum ether. The stopper was inserted and shaken vigorously for 1 min 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

50 ml 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 25 ml of aqueous alcohol and shaked vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25 ml 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. Placed the flask in an air oven at 85 °C for about 1 h 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 desicator to remove last traces of moisture and then weighed.

Peroxide Value: 5 g of the Amritaprasha 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 0.5 ml of potassium iodide, allowed it to stand for 1 minute, add 30 ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5 ml of starch indicator continued the titration until blue color disappears.

$$\text{Peroxide value} = 10(a-b) / W$$

Where W= weight in g of the substance

Viscosity: The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula:

$$\eta 1 = \frac{\rho 1 t 1 \text{ X } \eta 2}{\rho 2 t 2}$$

η1 – Viscosity of sample
η2 - Viscosity of water
t1 and t 2- time taken for the sample and water to pass the meniscus
ρ1 and ρ2 – Density of sample and water
X= Specific gravity of sample x 0.9961/specific gravity of water
Π= Xx Time for samplex1.004/specific gravity of water x70sec

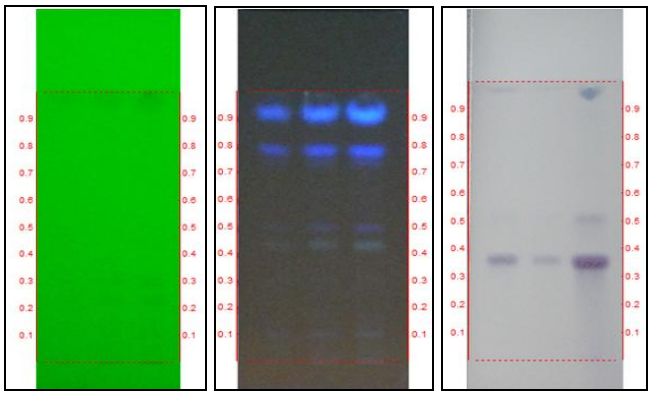
Rancidity Test: 1 ml of melted fat was mixed with 1ml of conc. HCl and 1 ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Sample Preparation for HPTLC: Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform this was followed for all the sample of Amritaprasha ghritha, and chloroform soluble portion was used for HPTLC.

HPTLC: 4, 8 and 12 µl of the above sample of *Amritaprasha ghrita* was applied on a precoated silica gel F254 on aluminum 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 derivatisation in vanillin-sulphuric acid spray reagent it was visualized under white light and scanned under UV 254 nm, 366 nm and 620 nm. R_f, colour of the spots and densitometric scan were recorded.

TABLE 2: SHOWING RESULTS OF STANDARDIZATION PARAMETERS

Parameter	Results n = 3 %w/w
	Amritaprasha ghrita
Refractive index	1.45783
Specific gravity	0.9551
Acid value	5.66
Saponification value	124.96
Iodine value	47.71
Unsaponifiable matter (%)	1.03
Peroxide value	0.19
Rancidity	Fat is not oxidised



Short UV Long UV Post derivatisation

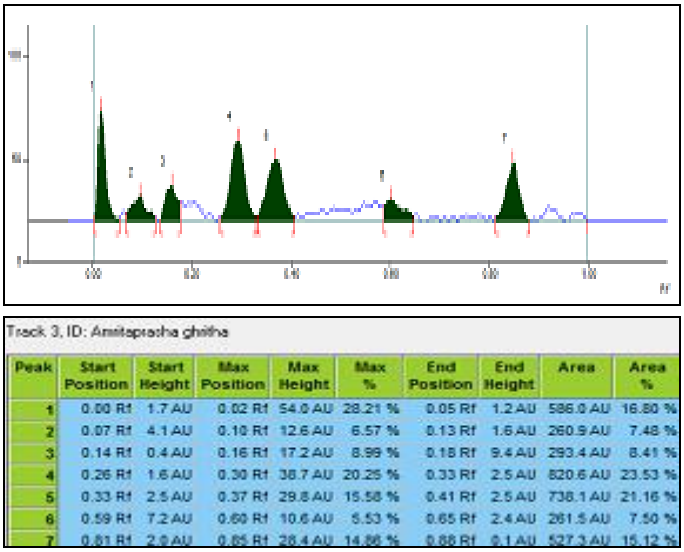
Track 1- Chloroform fraction of Amritaprasha ghrita- 4 µl
Track 2 - Chloroform fraction of Amritaprasha ghrita - 8 µl
Track 3- Chloroform fraction of Amritaprasha ghrita - 12 µl
Solvent system- Toluene: Ethyl acetate (9.0:1.0)

FIG. 1: TLC PHOTO DOCUMENTATION OF CHLORO-FORM FRACTION OF AMRITAPRASHA GHRITA

TABLE 3: R_f VALUES OF THE SAMPLE OF AMRITAPRASHA GHRITA

Short UV	Long UV	Post derivatisation
-	0.10 (FL. blue)	-
0.24 (L. green)	-	-
0.30 (L. green)	-	-
-	-	0.36 (D. purple)
-	0.43 (FL. blue)	-
-	0.50 (FL. purple)	0.50 (L. purple)
-	0.74 (F. blue)	-
-	0.78 (F. blue)	-
-	0.92 (F. blue)	-

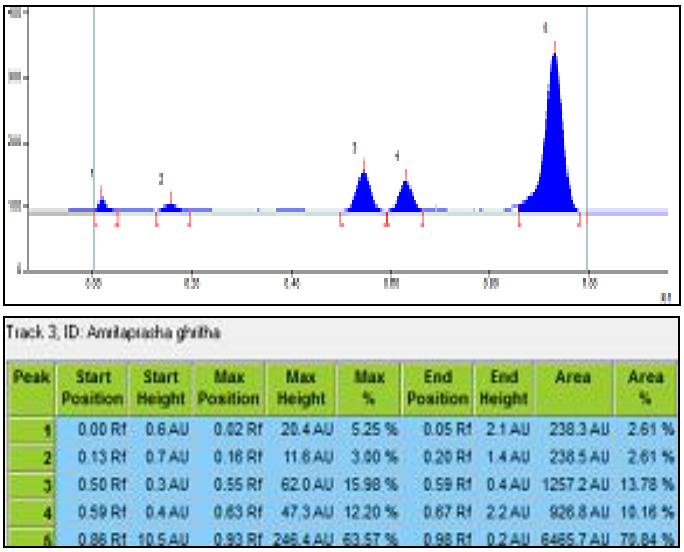
*F-fluorescent; D-dark; L-light



Track 3, ID: Amritaprasha ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.7 AU	0.02 Rf	54.0 AU	28.21 %	0.05 Rf	1.2 AU	586.0 AU	16.80 %
2	0.07 Rf	4.1 AU	0.10 Rf	12.6 AU	6.57 %	0.13 Rf	1.6 AU	260.9 AU	7.48 %
3	0.14 Rf	0.4 AU	0.16 Rf	17.2 AU	8.99 %	0.18 Rf	9.4 AU	293.4 AU	8.41 %
4	0.26 Rf	1.6 AU	0.30 Rf	38.7 AU	20.25 %	0.33 Rf	2.5 AU	820.6 AU	23.53 %
5	0.33 Rf	2.5 AU	0.37 Rf	29.8 AU	15.58 %	0.41 Rf	2.5 AU	738.1 AU	21.16 %
6	0.59 Rf	7.2 AU	0.60 Rf	10.6 AU	5.53 %	0.65 Rf	2.4 AU	261.5 AU	7.50 %
7	0.81 Rf	2.0 AU	0.85 Rf	28.4 AU	14.86 %	0.88 Rf	0.1 AU	527.3 AU	15.12 %

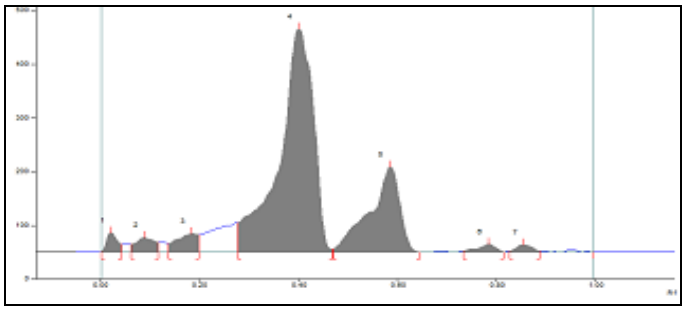
Fig 2a. At 254nm



Track 3, ID: Amritaprasha ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.6 AU	0.02 Rf	20.4 AU	5.25 %	0.05 Rf	2.1 AU	238.3 AU	2.61 %
2	0.13 Rf	0.7 AU	0.16 Rf	11.6 AU	3.00 %	0.20 Rf	1.4 AU	236.5 AU	2.61 %
3	0.50 Rf	0.3 AU	0.55 Rf	62.0 AU	15.98 %	0.59 Rf	0.4 AU	1257.2 AU	13.78 %
4	0.59 Rf	0.4 AU	0.63 Rf	47.3 AU	12.20 %	0.67 Rf	2.2 AU	926.8 AU	10.16 %
5	0.86 Rf	10.5 AU	0.93 Rf	246.4 AU	63.57 %	0.98 Rf	0.2 AU	6465.7 AU	70.84 %

Fig 2b. At 366nm



Track 3, ID: Amritaprasha ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.2 AU	0.02 Rf	36.3 AU	5.16 %	0.04 Rf	15.0 AU	561.8 AU	1.88 %
2	0.06 Rf	15.0 AU	0.09 Rf	27.4 AU	3.92 %	0.12 Rf	18.2 AU	759.3 AU	2.46 %
3	0.14 Rf	15.6 AU	0.19 Rf	34.3 AU	4.89 %	0.28 Rf	32.6 AU	1083.5 AU	3.51 %
4	0.28 Rf	55.0 AU	0.40 Rf	415.0 AU	59.23 %	0.47 Rf	5.2 AU	20717.2 AU	67.05 %
5	0.47 Rf	5.6 AU	0.59 Rf	158.9 AU	22.67 %	0.65 Rf	0.0 AU	7022.0 AU	22.73 %
6	0.73 Rf	2.2 AU	0.79 Rf	14.8 AU	2.11 %	0.82 Rf	0.4 AU	398.5 AU	1.29 %
7	0.83 Rf	2.4 AU	0.86 Rf	14.0 AU	2.00 %	0.89 Rf	2.7 AU	335.8 AU	1.09 %

Fig 2c. At 620nm

FIG. 2: DENSITOMETRIC SCAN OF AMRITAPRASHA GHRITA

RESULTS AND DISCUSSION: The standardization parameters of Amritaprasha ghrita are detailed in **Table 2**. The TLC photo documentation of chloroform fraction of Amritaprasha ghrita is shown in **Fig. 1**. The Rf values of sample of Amritaprasha ghrita is detailed in **Table 3**. The Densitometric Scan of Amritaprasha ghrita is shown in **Fig. 2**. The physicochemical standards would serve as preliminary test for the standardization of the

formulation. Tests such as refractive index, specific gravity, acid value, saponification value, iodine value, determination of unsaponifiable matter, peroxide, viscosity, rancidity test and HPTLC, results of TLC photo documentation, the unique R_f values, densitometric scan and densitogram obtained at different wavelengths can be used as fingerprint to identify the herbal drug of Amritaprasha ghrita.

CONCLUSION: Amritaprasha ghrita has been standardized using diverse scientific quality parameters. The results obtained can be used as reference while setting the pharmacopoeial standards for Amritaprasha ghrita for the benefit of the end user without any unwarranted complications.

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CONFLICT OF INTEREST: Nil

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