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STANDARDIZATION STUDY OF GHRITAS

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Abstract. The standardization of ghritas such as amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita has been studied. These ghritas are the important Ayurvedic formulations used for peri-natal care of mother and child health. Standardization of ghritas were achieved by organoleptic study, physico-chemical analysis, qualitative analysis, thin layer chromatography (TLC), UV - visible spectrophotometry and high performance liquid chromatographic (HPLC) fingerprint studies. Qualitative analysis of alcoholic extracts of all the four ghritas shows the presence of glycosides and hexane extracts shows the presence of glycosides and steroids. TLC study of ghritas was carried out in toulene-ethyl acetate solvent system. Hexane extracts of ghritas were used for UV- visible spectrophotometry and qualitative HPLC fingerprint study.

Key words: Standardization, Ghritas, TLC and HPLC.

Introduction

Ghrita is one of the Ayurvedic drugs that contain ghee as the base to dissolve or extract or hold the active therapeutic principles from the ingredients. Ghritas are medicated ghee preparations containing the fat-soluble components of the ingredients used in these preparations. The principle of preparation is the protracted boiling of ghee with prescribed kashayas (decoctions) and kalkas (a fine paste of the drug/drugs) to dehydration or near dehydration thereby effecting the transference of the fat soluble principles to the ghrita, from the drug ingredients or kashayas or swarasas as the case may be according to the formulation.[1]

Studies on the standardization of ghritas such as triphala ghrita, dadimadi ghrita, jatyadi ghrita, sukumara ghrita, dhanvantara ghrita have been reported.[1-5]

The present communication deals with the standardization of ghritas such as amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita which are used for the perinatal care of mother and child health. In Ayurveda, amritaprasa ghrita is therapeutically indicated for nastasukra, ksataksina, vyadhikarsata, kasa, hikka, jvara, svasa, daha, trsna, raktapitta, etc., brahmi ghrita for unmada, kustha, apasmara, vandhyatva, etc., chagalyadi ghrita for kasa, uroroga, parsvasula, urakasta, ksaya, etc., phala ghrita for balaroga, balagraha, sukravikara, yonivikara, vandhyatva, garbhiniroga, etc. A few analytical standard values have been prescribed for amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita.

Materials and Methods

The authentic ingredients were procured from the local market of Hyderabad, Andhra Pradesh and were botanically identified. Amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita were prepared as per the procedure described in Ayurvedic Formulary of India.

Analytical study

The prepared samples were analyzed for the parameters such as organoleptic study, moisture content, total ash, acid insoluble ash, alcohol soluble extractive, hexane soluble extractive, qualitative organic analysis, free fatty acid, acid value, saponification value, iodine value and refractive index. [5]

Thin layer chromatography

TLC plates were prepared as per the procedure described by Stahl.[8] The 4% hexane extracts of the samples were prepared by soaking them for 18h in absolute hexane. Hexane extracts were filtered and concentrated. Respective concentrated hexane extracts were redissolved in toluene- ethyl acetate (93:7) and about 100µl was loaded on the TLC plate and eluted in toluene- ethyl acetate (93:7) solvent system.[9] The plates were sprayed with vanillin-sulphuric acid reagent and the spots were detected after heating at 110°C for 30min. Rf value of each spot was calculated.

Sample preparation

The 4% hexane extracts of the samples were prepared by soaking them for 18 h in hexane. The extracts were filtered through Whatman filter paper number 1 using high-pressure vacuum pump. The samples were used for UV- visible spectrophotometric and HPLC fingerprint study.

UV-visible spectrophotometric analysis

The samples were scanned over a range of 200-800nm using ELICO (SL-159) UV-visible spectrophotometer equipped with quartz cuvettes of 10mm path length and UV-visible spectrasoft software. Hexane was used as a reference.

HPLC Analysis

An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC- 10 AT *VP* pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), CTO-10AS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and normal phase Luna 5µ Silica (2) Phenomenex column (250mm X 4.6mm) was used. The HPLC system was equipped with software Class *VP* series version 6.1 (Shimadzu). The mobile phase components hexane-isopropanol were filtered through 0.2µ membrane filter before use and pumped from the solvent reservoir to the column at a flow rate of 2ml/min which yielded a column back pressure of 180 kgf/cm^2. The column temperature was maintained at 27°C. 20µl of sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

The data of organoleptic study and physico-chemical analysis of amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita is summarized in Table-1 The standard values for amritaprasa ghrita, acid value not more than 3, saponification value 220-232, iodine value 30-40 and moisture content not more than 5%; for brahmi ghrita, acid value not more than 4, saponification value 220-232, iodine value 30-40, moisture content not more than 1% and refractive index 1.4524-1.4545; for chagalyadi ghrita, acid value not more than 3, saponification value 204-212, iodine value 31-34, moisture content not more than 6% and refractive index 1.4540-1.4600; for phala ghrita, acid value not more than 3, saponification value 220-232, iodine value 30-40, moisture content not more than 1% and refractive index 1.4524-1.4545 have been reported.

The variation in analytical values of ghritas may be due to several factors such as heat induced changes due to protracted boiling, oxidation changes due to open heating or due to presence of drugs during heating for a long time, changes brought about by the incorporation of fat soluble fractions from drugs, changes brought about by the mixing in of fats or oils other than ghee, changes caused by substitute drug ingredients.[7] The data of qualitative organic analysis of ghritas is summarized in Table-2 The data of TLC study of ghritas is summarized in Table -3 The UV-visible spectrophotometric data of ghritas is tabulated in Table-4.

TABLE 1 ANALYTICAL DATA OF GHRITAS

S. No.	Analytical Parameters	Amritaprasa ghrita	Brahmi ghrita	Chagalyadi ghrita	Phala ghrita
1.	Color	Light brown	Yellowish green	Yellow	Yellow
2.	Odor	Fragrant	Fragrant	Fragrant	Fragrant
3.	Taste	Sweet	Characteristic	Characteristic	Astringent
4.	Moisture (%)	1.09	0.014	0.182	0.155
5.	Total ash (%)	0.26	0.017	15.195	0.197
6.	AIA (%)	0.12	0.002	4.95	0.098
7.	Alcohol soluble extractive (%)	19.8	23.15	22.68	18.65
8.	Hexane soluble extractive (%)	14.9	97.4	50.35	72.4
9.	Free fatty acid (%)	1.63	2.49	5.09	1.69
10.	Acid value	3.23	4.06	2.91	3.37
11.	Saponification value	217.5	218.7	210	210.54
12.	Iodine value	30.5	35.5	32.17	33.15
13.	Refractive index	1.4553	1.4695	1.471	1.47

TABLE 2. QUALITATIVE ORGANIC ANALYTICAL DATA OF GHRITAS

S. No.	Analytical Parameters	Amritaprasa ghrita		Brahmi ghrita		Chagalyadi ghrita		Phala ghrita	
		A	H	A	H	A	H	A	H
1.	Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	Steroids	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
3.	Phenols	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4.	Tannins	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5.	Glycosides	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	Resins	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve
7.	Saponins	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
8.	Flavonoids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Where, A - Alcoholic extract H- Hexane extract

The qualitative HPLC fingerprint profiles of amritaprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita are shown in Figure –1 to 4 respectively. The HPLC chromatogram of amritaprasa ghrita showed four peaks at a retention time of 1.515min, 1.749min, 2.005min and 2.251min

with an area percentage of 71.54, 17.93, 2.23 and 4.71; brahmi ghrita showed two peaks at a retention time of 1.461min and 1.941min with an area percentage of 89.41 and 9.76; chagalyadi ghrita showed two peaks at a retention time of 1.461min and 2.051min with an area percentage of 91.09 and 5.2; phala ghrita showed four peaks at a retention time of 1.451min, 1.621min, 1.931min and 2.048min with an area percentage of 79.91, 11.59, 5.51 and 2.17 at a wavelength of 220 nm.

TABLE 3. DATA OF TLC STUDY OF GHRITAS

S. No.	Amritaprasa ghrita	Brahmi ghrita	Chagalyadi ghrita	Phala ghrita
	Rf value (color)	Rf value (color)	Rf value (color)	Rf value (color)
1.	0.06(V)	0.04(V)	0.06(V)	0.07(V)
2	0.08(V)	0.13(V)	0.14(V)	0.14(V)
3.	0.21(V)	0.22(LV)	0.23(V)	0.2(V)
4.	0.32(LV)	0.28(LV)	0.31(LV)	0.41(LV)
5.	0.38(V)	0.34(V)	0.4(LV)	0.55(RV)
6.	0.42(V)	0.39(LPV)	0.55(RV)	0.73(RV)
7.	0.47(V)	0.43(V)	0.7(V)	
8.	0.55(LV)	0.49(LV)	0.81(V)	
9.	0.68(LV)	0.57(LBV)		
10.	0.86(V)	0.64(RV)		
11.	0.93(RV)	0.82(RV)		

Where, V- Violet; LV – Light Violet; RV – Reddish Violet; LBV – Light Brownish Violet; LPV – Light Pinkish Violet

TABLE 4. DATA OF UV-VISIBLE SPECTROPHOTMETRIC STUDY OF GHRITAS

S. No.	Amritaprasa ghrita		Brahmi ghrita		Chagalyadi ghrita		Phala ghrita	
	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.	W.L.	O.D
1.	238	2.203	234.5	2.217	238	2.245	238	2.226
2.	250	2.242	244.5	2.202	250	2.263	250	2.266
3.	270	2.641	251	2.299	285	2.775	278	2.716
4.	336	1.695	257.5	2.368	-		411	0.396
5.	-		268	2.615	-		-	
6.	-		299	1.719	-		-	

Where, W.L.- Wavelength (nm), O.D.- Optical density

FIGURE 1. HPLC CHROMATOGRAM OF AMRITAPRASA GHRITA

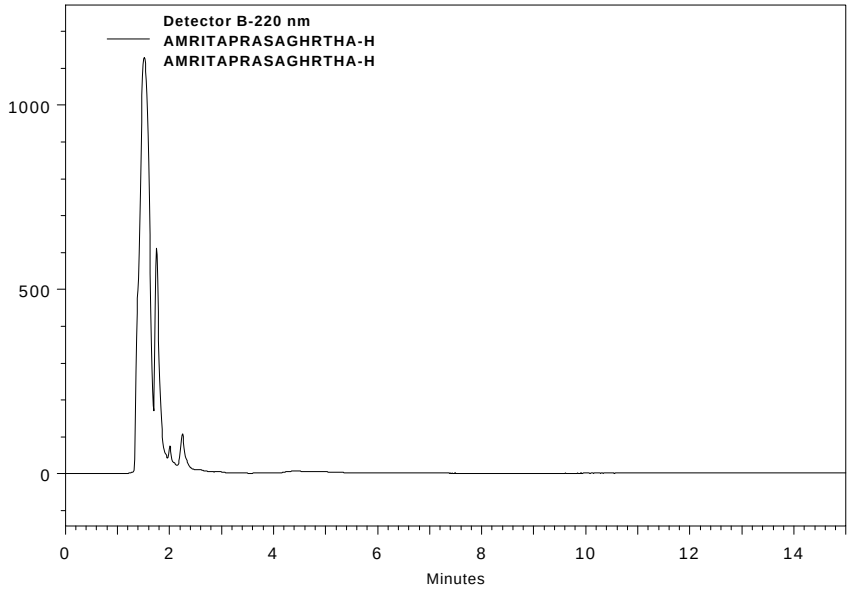


FIGURE 2. HPLC CHROMATOGRAM OF BRAHMI GHRITA

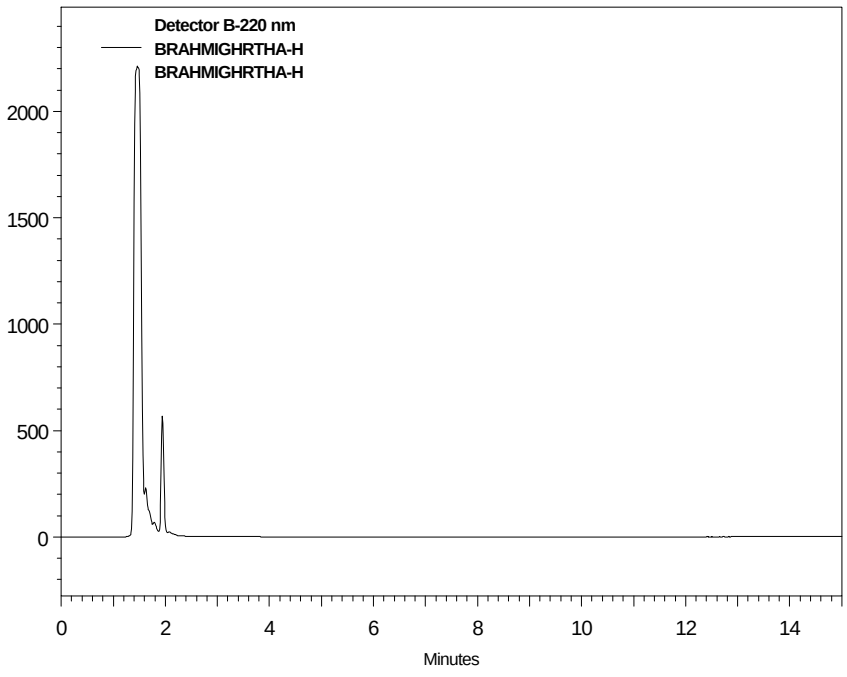


FIGURE 3. HPLC CHROMATOGRAM OF CHAGALYADI GHRITA

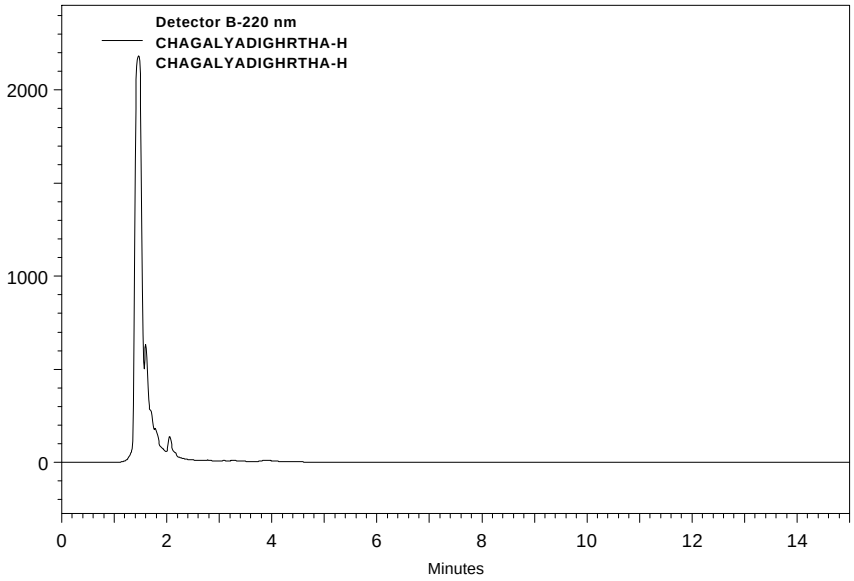
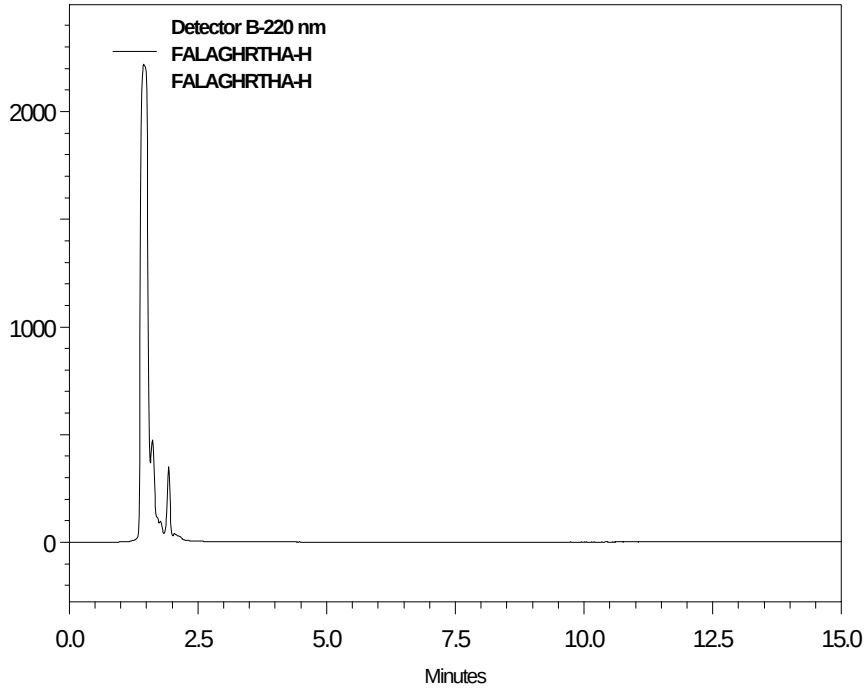


FIGURE 4. HPLC CHROMATOGRAM OF PHALA GHRITA



Conclusion

The analytical data, TLC, UV-visible spectrophotometric and HPLC fingerprint profiles evolved can be considered as viable parameters which will go a long way for prescribing a dependable standard to these preparations.

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