



## ANALYZING THE THREE STAGES OF SNEHA PAKA IN KSHEERSHATPALA GHRITA: AN EXPERIMENTAL STUDY USING MODERN ANALYTICAL TOOLS

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### ABSTRACT:

**Background-** *Sneha Paka* is a pharmaceutical process to prepare oleaginous medicaments from the substances taken in specific proportion. Standardization of its three stages viz. *Mridu*, *Madhyam* and *Khar Paka* is imperative to establish classical preparatory methods and examination techniques in more efficient way. Hence, proper scientific validation by pharmaceutical and analytical study should be done and documented. **Objectives-** The current study was conducted to differentiate the three stages of *sneha paka* using modern analytical tool w.s.r. to *ksheershatpal ghrita*. **Method-** The present study was carried out to differentiate the stages of *Sneha Paka* i.e. *Mridu Paka*, *Madhyam Paka* and *Khar Paka* in *Ksheershatpal Ghrita* (KSG) on the basis of classical and modern parameters, namely *Paka Pariksha*, organoleptic characters, physico-chemical parameters and chromatography. **Result-** pH of all the samples were mild acidic. Sp. Gravity of *Mridu Paka*, *Madhyam Paka* and *Khar Paka* are 0.910, 0.908 and 0.911 respectively. Loss on drying obtained is 0.0% for all the three samples. Iodine value obtained is 50.28, 17.65 and 18.97 respectively. Not much variation is observed in the Refractive Index of the samples. Saponification value of *Mridu Paka* is highest. Acid value and peroxide value of *Khar Paka* is more than the other two. Free fatty acid present is 2.28, 3.39 and 2.85 respectively. **CONCLUSION-** Thus an attempt has been made to create comparative database of the three stages of *Sneha Paka* with the help of modern analytical methods. It can be concluded that there were minimal comparative differences found among them but *Madhyam Paka* showed better results from standardization point of view.

**Keywords-** Standardization, *Sneha Paka*, *Mridu Paka*, *Madhyam Paka*, *Khar Paka*.

1.0 INTRODUCTION

Ayurvedic drugs are often chosen for chronic illness but there is no single best way for its documentation. Process standardization and its critical analysis has always been a daunting task. Recent advances in technologies are being used to analyze pharmaceutical products in a rational way. Sneha Kalpana/Paka may be defined as “A pharmaceutical process to prepare oleaginous medicaments from the substances like kalka [herbal paste of different parts of botanicals], kwatha [specifically prepared decoction in accordance of Ayurvedic principles] or Drava Dravya [any other liquid such as milk, self expressed juices, meat juice etc.] taken in specific proportion and by subjecting them to unique heating pattern and duration to fulfill certain pharmaceutical parameters, according to the need of therapeutics”[1]. This modality of sneha has better pharmacokinetic action in comparison to other dosage forms because of the lipoid nature of bio-membranes of our body. Hence, this mainstay has utmost importance over other dosage forms.

2.0 OBJECTIVES- The current study was conducted to differentiate the three stages of sneha paka using modern analytical tool w.s.r. to ksheershatpal ghrita.

Ksheershatpal Ghrita (KSG) is taken as the basis for this study [2]. The whole study can be observed under these stages-

Stage I- Stage of mixing all the ingredients into Sneha

Stage II- Stage of separation of oil

Stage III- Stage of appearance of different sneha paka lakshanas

Stage IV- Stage of analysis.

Table 1- Ingredients of KSG

Sr.N o.	Drug	Latin Name	Part Used	Quantity
1.	Pippali	<i>Piper longum</i> Linn.	Fruit	1 Pala
2.	Pippali Moola	<i>Piper longum</i> Linn.	Root	1 Pala
3.	Chavya	<i>Piper chaba</i> Hunter.	Root	1 Pala
4.	Chitraka	<i>Plumbago zelenica</i> Linn.	Root	1 Pala
5.	Nagara	<i>Zingiber officinalis</i> Roxb.	Rhizome	1 Pala
6.	Yavakshara	Salt of Tartar	-	1 Pala
7.	Go-Dugdha	Cow Milk	-	1 Prastha
8.	Go-Ghrita	Clarified butter	-	1 Prastha

**Table 2 – Classical review of KSG**

Reference	Ingredients and their method of preparation	Indication
Charak Samhita Chikitsa 5/147-148	Kalka Dravya- Panchkola + Yavakshar-each 1 Pala(48 gms) Milk and ghee- each 1 Prastha (768 gms)	Gulma
Chakradutt 5/147-148	Kalka Dravya- Panchkola + Yavakshar-each 1 Pala(48 gms) Milk and ghee- each 1 Prastha (768 gms) Water- 3 times of Ghrita	Gulma
Sharangdhar Samhita Madhyam Khand 9/20	Kalka Dravya- Panchkola + Saindhav-each 1 Pala(48 gms) Milk and ghee- each 1 Prastha (768 gms)	Vishamjwara
Sushruta Samhita Chikitsa 15/14	Kalka Dravya- Panchkola + Saindhav-each 1 Pala(48 gms) Milk and ghee- each 1 Prastha (768 gms)	Udararoga
Ashtang Hridya Chikitsa 5/22-23	Kalka Dravya- Panchkola + Yavakshar- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Rajyakshama
Ashtang Hridya Chikitsa 14/26	Kalka Dravya- Panchkola + Yavakshar- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Gulma
AshtangSamgrahChikitsa 7/32-33	Kalka Dravya- Panchkola + Yavakshar- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Rajyakshama
Bhaishajya Ratnavali 5/1300-1301	Kalka Dravya- Panchkola + Saindhav- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Vishamjwara
Bharat Bhaishajya Ratnakar part 5/7751	Kalka Dravya- Panchkola+ Saindhav- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Vishamjwara
SodhalaChikitsakhand 25/67-68	Kalka Dravya- Panchkola + Yavakshar- each 1 Pala (48 gms)	Gulma

	Milk and ghee- each 1 Prastha (768 gms)	
Gada Nigrah Prayog khand 1/66-67	Kalka Dravya- Panchkola + Yavakshar- each 1 Pala (48 gms) Milk and ghee- each 1 Prastha (768 gms)	Gulma
YogratnakarKushtha Chikitsa- TiktashatpalGhrita	Kwath Dravya- Nimba, Patola, Darvi, Duralabha, Kutki, Triphala, Parpata, Trayamad- each 2 karsha (24 gms). Ghee- 6 pala (288 gms). Water- 4 prastha (3.72 lit.)	Kushtha

\*Chakradutt5/147-148 reference is taken for this study.



**Image 1: Raw drugs used in preparation of KSG**

**Table 3- Rasapanchak of ingredients of KSG**

Sr.no	Drugs	Rasa	Guna	Virya	Vipaka	Karma
	Pippali	Madhura, Katu, Tikta	Laghu, Snigdha	Anushna	Madhura	Dipana
	Pippalimula	Katu	Laghu, Ruksha	Ushna	Katu	Pachana
	Chavya	Katu	Laghu, Ruksha, Tikshna	Ushna	Katu	Pachana

	<i>Chitraka</i>	<i>Katu</i>	<i>Laghu, Ruksha, Tikshna</i>	<i>Ushna</i>	<i>Katu</i>	<i>Pachana</i>
	<i>Shunthi</i>	<i>Katu</i>	<i>Laghu, Snigdha</i>	<i>Ushna</i>	<i>Madhura</i>	<i>Anulomana</i>
	<i>Yavakshar</i>	<i>Katu</i>	<i>Ruksha, Mridu</i>	<i>Ushna</i>	<i>Katu</i>	<i>Lekhana</i>
	<i>Go- Ghrita</i>	<i>Madhura,</i>	<i>Guru, Snigdha, Mridu</i>	<i>Sheeta</i>	<i>Madhura</i>	<i>Agnidipana</i>
	<i>Go- Dugdha</i>	<i>Madhura,</i>	<i>Guru, Snigdha</i>	<i>Sheeta</i>	<i>Madhura</i>	<i>Ahaladkara</i>
	<i>Jala</i>	<i>Madhura,</i>	<i>Laghu</i>	<i>Sheeta</i>	<i>Madhura</i>	<i>Ahaladana</i>

### Abbreviation of different samples-

1. MrKG-1 – *Mridu Ksheershatpal Ghrita*
2. MaKG-2 – *Madhyam Ksheershatpal Ghrita*
3. KhKG-3 – *Khar Ksheershatpal Ghrita*

## 3.0 MATERIAL AND METHOD-

### 3.1.0 Material-

All the 6 raw drugs were procured from the Goladinanath market, Varanasi. *Ghrita* (Anik) and milk (Amul) were collected from the nearby general store, Varanasi.

### 3.2.0 Method-

#### 3.2.1 Preliminary analysis of raw ingredients-

All the raw materials were taken based on their respective features from AFI. They were authenticated by subject experts from the *Rasa Shastra and Bhaishajya Kalpana* department and *Dravyaguna* department, Government PG Ayurvedic medical College and hospital, Varanasi.

#### 3.2.2 Drug preparation: *KsheershatpalGhrita*

### Preparation of *Ksheershatpal Ghrita*

was carried out according to the reference from *Chakradutta* and at every respective stage of *Sneha Paka* (traditional parameters of assessment), some of the *Ghrita* is filtered and stored separately. All the samples of KSG viz. MrKG, MaKG and KhKG were prepared in the laboratory of *Rasa Shastra and Bhaishajya Kalpana* Department, Government PG Ayurvedic medical college and hospital, Varanasi.

### 3.2.3 Drug analysis-

All the samples of KSG viz. MrKG, MaKG and KhKG were analyzed at VASU research centre, Vadodara, Gujarat. **Pharmaceutical study-**

### Equipment specifications-

Mortar and pestle, mixer- grinder, sieve, heating device - Gas burner with LPG cylinder, vessel stainless steel- diameter- 28.5 cm, depth-14.5 cm, wt –1664 gm , capacity- 12.5 lit., cotton cloth, measuring

flask, stainless steel ladle, digital thermometer.

#### Procedure-

All the six raw herbs were dried properly, pounded separately into *Yavkut* form (coarse powder form), mixed together and soaked in water to prepare *Kalka*. This *Kalka* was left undisturbed overnight. *Ghrita* was taken in a vessel and heated over mild flame. When *Ghrita* became mild hot, *Kalka Dravya* was added carefully along the sides of the vessel slowly to avoid splattering of the *Ghrita*. After *Kalka Dravya*, milk and then water was added simultaneously. The temperature was around 90°C (*Mandagni*)<sup>[3]</sup>. In the initial stage it was a light brown colour homogenous mixture with no fumes. The colour got dense with time. After nearly 4

hours, slight separation of *Sneha* and *Kalka Dravya* was seen. When *Mridu Paka* stage was observed, some of the *Ghrita* was filtered and kept separately in a closed container. Similarly, *Madhyam Paka* and *Khar Paka* stage was observed and filtered eventually.

#### Precautions-

Wide mouthed vessel should be taken. Too fine powder of the raw drug should be avoided as the powder settles down at the bottom and burns. *Ghrita* should be stirred carefully as bubbles starts popping up in later stages. In the later stage, frequent or continuous stirring was done as the *Kalka* particles readily started sticking to the bottom of the vessel. *Ghrita* should be filtered hot to minimize loss.

**Table 4: Organoleptic characters-**

Organoleptic characters	<i>Ghrita</i> before <i>Paka</i>	<i>Mridu Paka</i>	<i>Madhyam Paka</i>	<i>Khar Paka</i>
<b>Colour</b>	Mustered yellow	Dull yellow	Bright yellow	Light yellow
<b>Taste</b>	Pungent	Pungent	Pungent	Pungent
<b>Odour</b>	Smell of plain <i>Ghrita</i>	Pleasant medicated <i>Ghrita</i> like aroma	Pleasant medicated <i>Ghrita</i> like aroma	Little burnt <i>Ghrita</i> like smell
<b>Consistency</b>	Translucent+++	Translucent++	Translucent+	Translucent
<b>Texture</b>	Oily and grainy	Oily and grainy	Oily and grainy	Oily and grainy

**Table 5: Analytical study-**

Sr.no.	Analytical tests as per API	<i>Mridu Paka</i>	<i>Madhyam Paka</i>	<i>Khar Paka</i>
1.	pH value	5.11	5.12	5.08
2.	Sp. gravity	0.910	0.908	0.911
3.	Loss on drying	0.0%	0.0%	0.0%
4.	R.I.	1.455	1.459	1.458
5.	Saponification value	250	242.17	217.3
6.	Iodine value	50.28	17.65	18.97
7.	Acid value	3.94	6.15	8.60
8.	Peroxide value	1.73	1.73	2.68
9.	FFA	2.28	3.39	2.85

**Table 6- HPTLC Test**

Chromatographic Conditions:	
1. Application Mode-	CAMAG Linomat 5 - Applicator
2. Filtering System-	Whatman filter paper No. 1
3. Stationary Phase-	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
4. Application (Y axis) Start Position-	10 mm
5. Development End Position-	80 mm from plate base
6. Sample Application Volume-	7.0 µL
7. Distance Between Tracks-	15 mm
8. Development Mode-	CAMAG TLC Twin Trough Chamber
9. Chamber Saturation Time-	30 minutes
10. Mobile Phase (MP)-	Petroleum Ether : Diethyl Ether : Acetic Acid (9 : 1 : 0.1 v/v)
11. Pre-chromatographic derivatization-	After sample spotting pre-chromatographic derivatization done with 5 % Alcoholic KOH (2.0 µL) followed by heating the plate for 10 minutes on TLC Plate Heater Preheated at 100 ± 50C.
12. Visualization-	@ 254 nm, @ 366 nm (after derivatization) and @ 540 nm (after derivatization)
13. Spray reagent-	Anisaldehyde – sulphuric acid reagent

14. Derivatization mode -	CAMAG – Dip tank for about 1 minute
15. Drying Mode, Temp. & Time-	TLC Plate Heater Preheated at 100± 50C for 3 minutes

#### Preparation of Test solution:

0.1 ml. of the sample was taken in a test tube and diluted it with 1 ml of Hexane. Mixed well and used the test solution thus obtained for HPTLC fingerprinting.

#### Preparation of Spray reagent

##### [Anisaldehyde - sulphuric acid reagent]:

5 ml. Anisaldehyde was mixed with 10 ml. Glacial acetic acid, followed by 85 ml. Methanol and 5 ml. Sulphuric acid (98%).

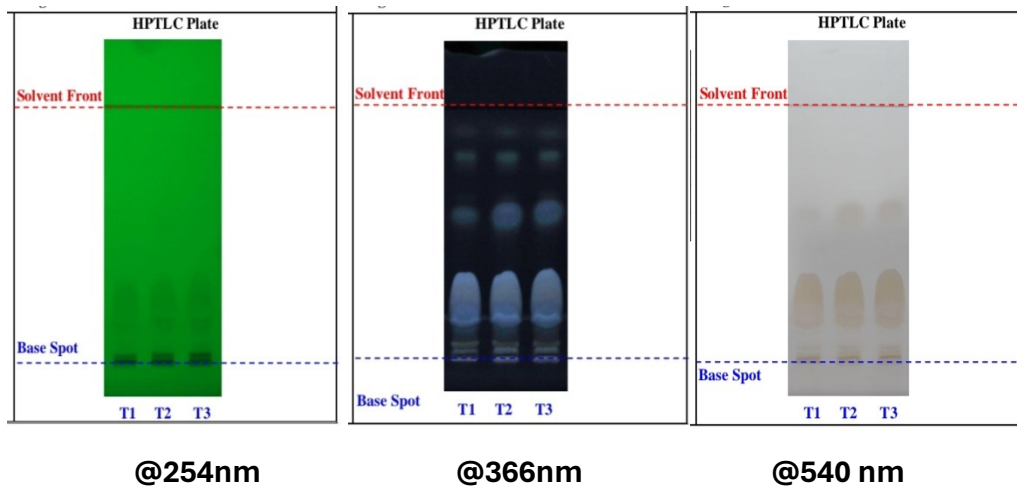


Image 2: HPTLC plate at different wavelengths

Table 7: HPTLC reports-

Sr. no.	Visualization	Tracks	No. of spots	R <sub>f</sub> values
1.	254 nm	T1	4	0.14, 0.17, 0.25, 0.35
		T2	6	0.14, 0.17, 0.25, 0.55, 0.65, 0.69
		T3	5	0.14, 0.17, 0.25, 0.55, 0.65
2.	366 nm	T1	7	0.12, 0.14, 0.21, 0.33, 0.58, 0.65, 0.81
		T2	5	0.14, 0.33, 0.58, 0.65, 0.81
		T3	6	0.12, 0.17, 0.21, 0.33, 0.58, 0.65, 0.81
3.	540 nm	T1	5	0.12, 0.14, 0.29, 0.58, 0.81
		T2	5	0.14, 0.29, 0.38, 0.58, 0.81
		T3	4	0.14, 0.29, 0.58, 0.81

## 4.0 RESULTS



**4.1.0 Pharmaceutical-** Total time taken to complete *Sneha Paka* is 8 hours 20 mins. Obtained amount of *Mridu*, *Madhyam* and *Khar Sneha Paka* is 220, 245 and 265 grams; total being 730 grams. Total amount of residue obtained is 638 grams. Loss observed is 38 grams. Loss in percentage is 4.9%.

**4.2.0 Analytical-** pH of all the samples were mild acidic. Sp. Gravity of *Mridu Paka*, *Madhyam Paka* and *Khar Paka* are 0.910, 0.908 and 0.911 respectively. Loss on drying obtained is 0.0% for all the three samples. Iodine value obtained is 50.28, 17.65 and 18.97 respectively. Not much variation is observed in the Refractive Index of the samples. Saponification value of *Mridu Paka* is highest. Acid value and peroxide value of *Khar Paka* is more than the other two. Free fatty acid present is 2.28, 3.39 and 2.85 respectively.

## 5.0 DISCUSSION-

Before preparation for this study, few pilot batches were prepared to find out the possible difficulties in preparation and to maintain the uniformity. For this study, the ratio of *Kalka*, *Sneha* and *Dravya* was taken in an explicit ratio of 1:4:16. Here, in *Drava Dravya*, milk was taken 1 part and water 3 parts to *Sneha*<sup>[4]</sup>. The *Paka* was continued for 2 days (around 5 hours in a day) as milk was used in the preparation. This blend was

cooked in *Mandagni*(mild flame) until the respective *Sneha Paka* stages appeared. The *Mridu*, *Madhyam* and *Khar Paka Lakshanas* were observed on the basis of *Kalka*<sup>[5]</sup>. Specific *Sneha Paka Pariksha* of *Kalka* was done for *Paka* in which *Niryasa* (resin) like *Kalka* was observed for *Mridu Paka*; *Samyava* (pudding) like *Kalka* in *Madhyam Paka*, while in *Khar Paka*, *Kalka* was getting powdered on rubbing and *varti* (wick) could not be formed<sup>[6-9]</sup>.

Temperature reached approximately 70±2 °c during completion of *Mridu Paka*, 78±2 °c during *Madhyam Paka* and 77±2 °c during *Khar Paka*. Average duration between *Mridu Paka* and *Madhyam Paka* was 1 hr ±5 mins and between *Madhyam Paka* and *Khar Paka* was 1 hr ±35 mins. Since, some of the *Ghrita* was extracted out after each stage of *Paka*, the amount of *Ghrita* obtained in all the three stages is random. Total loss observed was 6.9% on an average.

Organoleptic characters shows congruence in taste and odour of the samples. The colour of MrKG is dull yellow, MaKG is bright yellow and KhKG light yellow.

The pH of all the three samples is in acidic range. There is not any marked difference observed between MrKG, MaKG and KhKG but MaKG was more towards neutral. Decrease in pH in KhKG may be

because of overcooking resulting in an increment in acidity.

Specific gravity of a sample can be correlated to the ratio of mass/volume. Here, it is relative to time. MaKG has lowest Sp. Gravity (0.908) of the other two samples (MrKG- 0.910 and KhKG-0.911). However, there is only slight difference in specific gravity among all the three samples.

Moisture presence is 0.0% in all the three samples. Refractive index is mostly applied to identify a particular substance or to measure its concentration. It also indicates the presence of turbid materials. There is negligible difference among Refractive indices of the samples which indicates similar concentration of turbid materials.

Saponification value represents the no. of milligrams of KOH required to saponify 1gm of fat under the conditions specified<sup>[10]</sup>. It provides initial evidence of rancidity in unsaturated fats and oils. Saponification value of MrKG, MaKG and KhKG samples are 250, 242.17, and 214.3 mg KOH/g respectively.

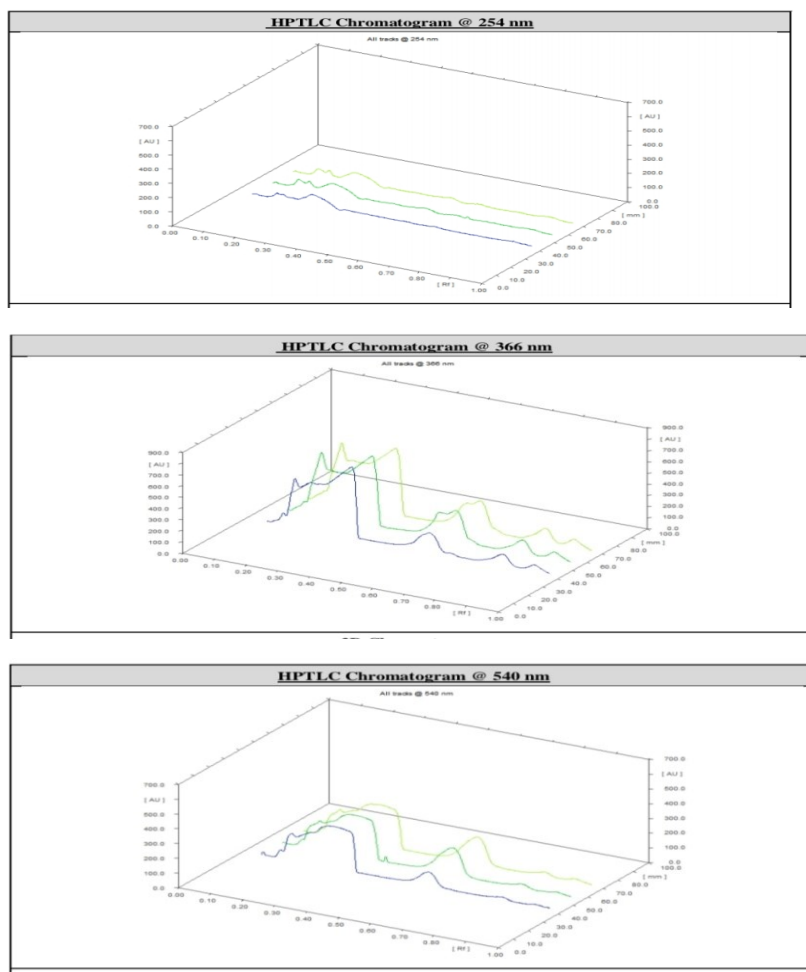
Iodine value determines the amount of unsaturated fatty acid in the form of double bond which reacts with iodine<sup>[11]</sup>. The higher the iodine value, the more reactive and less stable the oil is. Thus sample MaKG(17.65) is better than the other two

(MrKG- 50.28 and KhKG-18.97). Acid value indicates the presence of free fatty acid present in it which further indicates early rancidity. Acid value should get increase with increase in duration of heat and or intensity of heating. Here, gradual increment in Acid value is observed from MrKG, MaKG to KhKG (3.94, 6.15, 8.60), which could be time related.

Peroxides are the intermediate products of fat oxidation. It is used to assess the stability or rancidity of fats by measuring lipid peroxides and hydro-peroxides formed during the initial stage of oxidation. American oil chemists' society considers peroxide value and active oxygen method are the standard methods for stability analysis of oils and fats. High peroxide value is the index of rancidity. KhKG sample (2.68) has comparatively higher peroxide value of the three samples (MrKG- 1.73 and MaKG- 1.73). Free fatty acid content of *Ghrita* depends upon the quality of butter used, method of manufacturing, temperature during processing, duration of heat, residual moisture content and number of ingredients added to it. Free fatty acid value of MrKG, MaKG and KhKG samples are 2.28, 3.39 and 2.85. There is some alteration in desired values which may be due to market *Ghrita* taken for this study.

HPTLC was done at three wavelengths. At 254 nm and 540 nm, Track 2 i.e. MaKG shows max spots with 6 rf values and at 366 nm, Track 1 i.e. MrKG shows max spots. This means that during *Madhyama Paka* stage *Ghrita* was present with

maximum active constituents. This also means some of the constituents would have been imperceptible during *Mridu Paka* stage and some might have got burnt due to excess duration of heating for *Khar Paka* stage.



**Image 3: HPTLC chromatogram at 254nm, 366nm, 540nm wavelengths**

Specific gravity, Acid value and peroxide value of KhKG are maximum and saponification value and iodine value of MrKG is highest. Most of the values of MaKG were in between MrKG and KhKG. The results of parameters of medicated *Sneha Paka* are particular for specified formulation. In present study, there was

neither significant change in range of highest temperature given for *Sneha Paka* nor significant change in duration of *Sneha Paka*. Therefore, no any significant difference is observed in results of these tests but support significance of Ayurvedic principles of stages of *Sneha Paka*. The main difference between these stages is

attributed to further additional duration of heat. These little changes can change the nature of prepared *Sneha Paka*; hence classical texts has mentioned different types of *Sneha Paka* (e.g. *Ama*, *Mridu*, *Madhyam*, *Khara*, *Dagdha Paka* etc) along with their *Siddhi Lakshanas* (parameters to identify optimum end points for state of *Sneha Paka*).

## 6.0 CONCLUSION-

The study was performed considering the explanations given by the *Acharyas* pharmaceutically and analytically. Therapeutic indications of all the three stages of *Paka* are advocated for different routes of administration like *Nasya*, *Pana*

and *Abhyanga*. The conclusion of the study was difficult to draw out as the evaluation showed no major difference. The physico-chemical analysis shows minimal comparative difference among values of the three samples. Time factor also affects the physico- chemical profile which can be related with their respective therapeutic indication. HPTLC study showed maximum number of active phyto- constituents in the MaKG sample. Thus an attempt has been made to create a comparative database of the three stages of *Sneha Paka* by various evaluation parameters viz. organoleptic, physico- chemical and chromatography study.



Khar Paka Kalka



Filtered Ghrita



Obtained residue



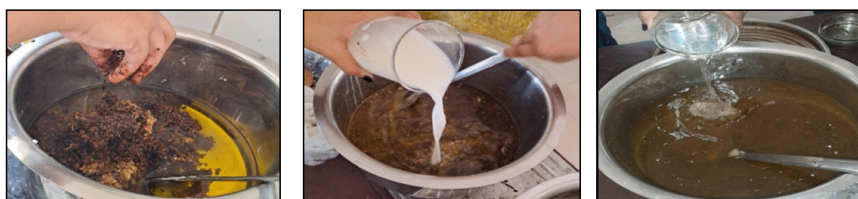
Obtained Ghrita

**Image 3: Steps involved in pharmaceutical study**

### DIAGRAMATIC REPRESENTATION OF THE PHARMACEUTICAL STUDY PERFORMED



**Ghrita**



**Addition of Kalka, milk and water**



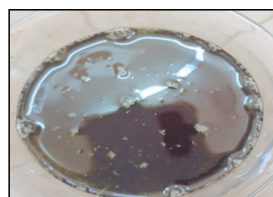
**Mridu Paka Kalka**



**Filtered Ghrita**



**Madhyam Paka Kalka**



**Filtered Ghrita**

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