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ANALYTICAL STANDARDIZATION OF RASNADI GHRITAM

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ABSTRACT

The term stroke is also known as cerebro vascular accident or cerebro vascular insult or brain attack. Annual incidence of stroke is estimated between 180 and 300 per 1000 persons. The incidence is rises steeply with age and adoption of less healthy life styles. According to Ayurveda CVA is compared with Pakshghata mentioned in 80 types Vata vyadhi. So samanya Vata vyadhi chikista sutras like snehana, swedana, sodhana(virecana) followed by vasti to be followed. In hemiplegia snehana can be done internally or externally with siddha ghritas or tailas having vata samaka drugs. One such ghritas is Rasanadi ghritam which contain Rasna.Sati, Pushkaramula, Mahoushada, Pippali, Chitraka.It is indicated in vataroga mentioned in (A.H .VATAVYADHI. CHI 21/57) and also having Vata hara properties. An attempt has been made in present study to prepare Rasnadi ghrita and analysis it for its organoleptic properties like Iodine value, Moisture value, Saponification value as well as Chromatographically for developing standard parameters.

KEYWORDS: Rasnadi ghritam, Pakshaghata, Snehapana.

INTRODUCTION

The importance of Ayurveda in global scenario is because of its holistic approach towards positive life style. Pancha karma therapy is an effective therapy in managing neurological, auto immune, psychiatric and chronic metabolic diseases. Pakshaghata is mentioned 80 types

of Vata vyadhi, and characterized by loss of function in the either half of the body sometimes associated with pain and impairment of speech. The treatment of Hemiplegia is focused on improving sensation, motor abilities allow the patients to better manage their activities of daily living. So Snehana, Swedana, Mridu Virecana, Vasti etc are consider the best way to treat a patient of hemiplegia. In hemiplegia Snehana can be done internally or externally with siddha ghritas or tailas having Vata shamaka drugs use in hemiplegia. These ghritas or tailas orally to lubricate the body system reduce dryness and aid in removing impurities. In condition of hemiplegia it mainly the oil is medicated with vata hara drugs in hemiplegia brain is damaged due to lack of blood supply and nutrition. For the repair of the damaged time and nutrition plays a very important role sneha mainly contain protein and fat metabolism of the body as nerve tissue are closely connected with the fat and protein metabolisam, snehana because of its similarity of constitution with the nerve fiber may be help in repair the structural degeneration and restore the lost function, Due to its Brimhana propertity of snehana dravya it normalize the Vata dosha, relieve from the pain in the body soothens and enable the nerve to the properly.

Sneha kalpana is a procedure where the active principles present in the drugs are extracted in to the sneha during pharmaceutical process.Rasnadi Ghrita is one such formulatio which mainly consist of Rasna,Sati,Pushkaramula,Mahoushada,Pippali ,Citraka are as kalka dravyas and as well as kashaya dravya and go ghritam as base.

Alpina galanga Rosacoe. known as **RASNA** part used is Rhizome having properties like Vāta Śūlahara, Kāsahara, Śophahara, Pācaka.

Hedydichium spicatum Buch part used rhizome known as **SATI** having properties like Śotha, Swāsa, Kāsa, Jvara, Sūla, Sidhma, Vrana, Hikka.

Inula racemosa Hook.f. as known as **PUSHKARAMULA** part used Mulam Kaphavata hara, Deepana.

Zingiber officinalis as known as **MAHOUSHADA** part used is Rhizome Ajīrna, Agnimāndya, Anahā, Sotha.

Piper Longum Linn as **PIPPALI** partused is fruit vatakapha hara,Pittakara,Deepana, Rasayana,Vrishya, Rechana, Medhya.

Plumbago zeylanica as known as **CITRAKA** part used is Mulam Agnimāndya, Ajīrna, Grahaṇī, Udara.

MATERIAL AND METHODS

A. PHARMA CEUTICAL STUDY

a. Procurement of raw material

All the ingredients were purchased from kerala (trivendrum) local market and foreign matter adhering to raw drugs was removed and clened. The base which was used for preparation this ghrita i.e go ghrita purchased from local market.

b. Raw drug standardization

HPTLC Study of ethanol extract of powder of Rasna, Sati, Pushkaramula, Mahoushada, Pippali, Citraka carried out at State Drug Testing Laboratory, Hyderabad.

c. Method

Hptlc analysis of the samples were carried out by loaded as 6.0mm band length of the sample in the 10x10cm silica gel 60f 254 HPTLC plates using Camag Linomat 5 instrument with a 100µl syringe. The plates were vertically positioned in the chambers saturated with respective organic. The chromatographic chamber left undisturbed till the completion of experiment. The samples were allowed to run under mobile phase, the plates are visualized in a photo documentation chamber Camag visualize 101017.Ethanol extract of drug sample was used for spotting. The mobile phase was run with 100nl/sec.

d. Results

The mobile phase was stopped and the plates were removed from the chromatographic chamber, the Hptlc plates are air dried then they were sprayed with anasaldehyde acid and vanillin sulphuric acid and left for developing the colour. The electrostatic attraction takes place in the absorption phenomena therefore the polar nature of solvent influence absorption. Generally absorption is maximal in non-polar solvent and decreases as the polarity is increase commonly solvents are used in the increasing order of polarity as follows:

Hexane<Bezene<Carbontetrachloride<Ethileethere<Chloroform<Acetone<Water.

The Rf value of the compound were determine the following formula:

$$\text{Rf value: } \frac{\text{Distance moved by the substance}}{\text{Distance moved by the solvent}}$$

B. PREPARATION OF RASNADI GHRITA

a) Preparation of kalka dravya

Dried drugs of Rasna(rhizome),Sati(rhizome), Pushkaramul(mula), Pippali (fruit), Citraka (mula)were taken into general rule of sneha kalpana and were pounded in a clean khalwa yantra to form coarse powder. Sufficient quantity of water was added to form kalka.

b) Preparation of kashaya dravya

Preparation of Decoction was based on Śārṅgadhara Samhitā's (Ma.Kh.9) general rule by mixing above said coarse powder of the drugs with water in the ratio of 1:4,which was there after heated at medium temperature, till it reduced to one fourth of its original quantity.

c) Method of preparation of drug

For Ghṛta-Pāka, drugs were taken in specific proportion as mentioned in Āyurvēdic classics,

Drugs		Quantity
Kalka Dravyas	-	1 part
Cow Ghee	-	4 parts
Kwātha	-	16 parts

At first Ghṛta was taken and then Kwātha was added to it. It was allowed to boil on Madhyam Agni until whole quantity of Kwātha was vaporised then Kalka was added and boiled. To confirm whether the water part was completely vaporised or not, 'Varti Parikṣā' and 'Kalka Parikṣā' was performed. Along with this, Snēha Siddhi Lakṣaṇas mentioned in our classics were assessed.When kalka was put on fire it did not produce any crackling sound and when rolled in between the fingers forms a varti.Phena santhi is observed then heating was stopped. Then the vessel was taken out of gas stove, siddha ghrita is filtered through double layered cloth in the warm stage its self.

C. ANALYTICL STUDY

Organoleptic characters

Rasnadighrita was inspected for Colour, Odour, Appereance, Touch. and Carity.

Physico-chemical properties

Iodine value[4]

The iodine value of an oil or fat is the weight of iodine absorbed by 100 parts by weight of the sample; it is determined bythe following method.

Iodine monochloride method (Wij's method)

Place the sample accurately weighed in a dry iodine flask of 250 ml capacity, add10 ml of carbon tetrachloride and dissolve. (The approximate weight in grams of the sample to be taken may be calculated by dividing 20 by the highest expected Iodine value). Add 10 ml of Chloroform and 20ml of Iodine mono-chloride solution, insert the stopper previously moistened with Potassium iodide solution and allow standing in a dark place at a temperature of about 17˚C for 30 minutes. Add 15 ml of Potassiumiodide solution and 100 ml of water shake and titrate with N/10 Sodium thio-sulphate using starch mucilage as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the sample being tested and note the number of ml N/10 Sodium thio – sulphate required (b).

$$\text{Iodine value} = \frac{(b\text{-}a) \times 0.01269 \times 100}{\text{Wt. of Sample in grams}}$$

Moisture value w/w[5]

Weighed quantity of oil was taken in a crucible, heated to 105 ˚ c for an hour. After cooling it was reweighed. The difference in the weight, before and after heating, indicated amount of moisture presents (loss on drying).

Saponification value[6]

The Saponification value of an oil or fat is defined as the number of milligrams of Potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1gram of the sample.

Procedure

Weigh 2 gram of the oil or fat into a conical flask and add exactly 25 ml of the alcoholic Potassium hydroxide solution. Attach reflex condenser and heat the flask in boiling water for 1hr along with shaking frequently. Add 1 ml of Phenolphthalein (1%) solutionand titrate excess alkali with N/2 Hydrochloric acid (titration=a ml) and carry out a blank at the same time (titration=b ml).

$$\text{Saponification value} = \frac{(b - a) \times 56.1}{\text{Wt. of sample in grams}}$$

RESULTS

Table no-1: Organoleptic changes as follows.

S.no	Parameters	Results
1.	Colour	Yellow
2.	Odour	Slightly pungent odour
3.	Appearance	Oily(after cooling it become solidiedfy)
4.	Clarity	Clear,transparent
5.	Touch	Snigdha
6.	Taste	Slightly madhura

Table -2: Physio chemical parameters.

S.no	Name of the test	Value
1.	Moisture value w/w	1.95
2.	Iodine value	35.98
3.	Saponification value	171.29

HPTLC CHROMATOGRAPHIC STUDY

RESULTS

RASNA

Before spraying: In track -1 are 0.27+0.68, in track -2 are 0.15+0.27, and in track-3 are 0.14+0.26.

After spraying: In track -1, 0.15,0.35, 0.68+0.91:track-2 0.15, 0.29,0.35,0.69+0.91 and in track-3: 0.14,0.34,0.48,0.69 and 0.90 are in close proximity as per reference of analysis API PART -2,VOLUME-5.

SATI

Hptlc chromatograms of alcoholic extract of sati with mobile phase. Toluene:Ethyl acetate(9;1) at wave length 254nm are carried out and obtained peacks in track-1,track-2, and track-3 are reported.

PUSHKARA MULA

Hptlc chromatograms of alcoholic extract of Pushkaramula is carried out and obtained peaks at RF VALUE at wave length 254nm in track-1 are 0.29, 0.35.0.38, 0.64.0.72, 0.95 in track-2 0.11,0.28,0.38,0.48,0.64 and 0.74 and in track-3, 0.11, 0.28, 0.37, 0.47, 0.74, 0.94 and at wavelength 366nm in track-10.29,0.38,0.48,0.63,0.72 and 0.95 intrack-2 0.11, 0.27, 0.35, 0.39, 0.49, 0.64 and 0.72 and in track-3 0.11, 0.28, 0.34, 0.49, 0.63, 0.73 and 0.95 are in close proxity as per reference of analysis.

PIPPALI

Hptlc study of alcoholic extract of Pippali is carried out and the obtained peaks at RF VALUES.

Before spraying:In track-1 are 0.15,0.27,in track-2 0.25,0.50 and in track-3 0.14,0.25,and 0.50.

After spraying: In track-1 are 0.04,0.35,0.43+0.82 in track-2 0.04 ,0.35,0.43+0.82 and in track -3 0.05,0.35,0.44+0.83 are in close proximity as per reference of analysis.

MAHOUSHADA

Hptic study of alcoholic extract of Mahoushada is carried out and the obtained peaks at RF VALUE.

The results at Rf 0.05, 0.08, 0.10, 0.12, 0.15, 0.18, 0.21, 0.24, 0.26, 0.29, 0.31, 0.33, 0.37, 0.38, 0.44, 0.46, 0.49, 0.52, 0.54, 0.56, 0.60, 0.63, 0.65, 0.71, 0.73, 0.76, 0.78, 0.80, 0.81.

CITRAKA

Hptlc result of piumbagin of sveta chitraka is 16.93.[2]

RASNADI GHRITA INGREDIENTS

Rāsnā

Pippalī

Chitrakā mūla

Śaṭi

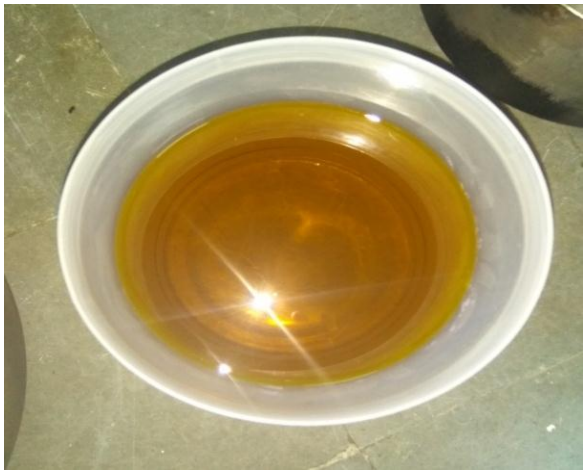
Śuṇṭi

puṣkara mūla

PREPARATION OF RASNADI GHRITA

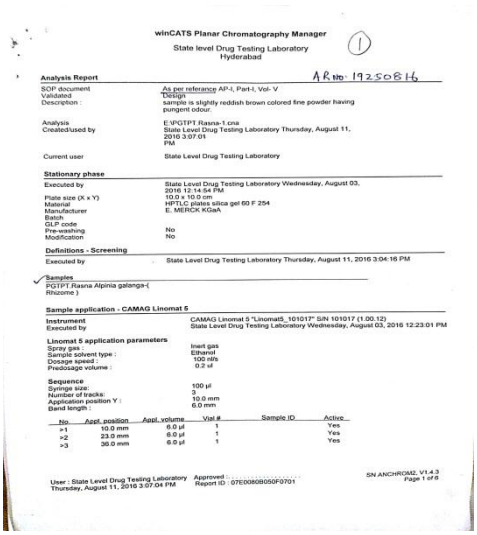
Mridu paka of Rāsnādi ghṛta

Madhyama paka of Rāsnādi ghṛta



Filtered Rāsnādi ghṛta

HPTLC RESULTS OF RASANADIGHRITA INGRIDENTS



winCATS Planar Chromatography Manager

State level Drug Testing Laboratory
Hyderabad

Analysis Report AR.No: 19250816

SOP document: As per reference AP-I, Part-I, Vol- V
Validated
Design
Description: sample is slightly reddish brown colored fine powder having pungent odour.

Analysis
Created/used by: E:\PGTPT Rasna-1.cna
State Level Drug Testing Laboratory Thursday, August 11, 2016 3:07:01 PM

Current user: State Level Drug Testing Laboratory

Stationary phase

Executed by: State Level Drug Testing Laboratory Wednesday, August 03, 2016 12:14:54 PM
Plate size (X x Y): 10.0 x 10.0 cm
Material: HPTLC plates silica gel 60 F 254
Manufacturer: E. MERCK KGaA
Batch
GLP code
Pre-washing: No
Modification: No

Definitions - Screening

Executed by: State Level Drug Testing Laboratory Thursday, August 11, 2016 3:04:16 PM

Samples
PGTPT Rasna Alpinia galanga-(Rhizome)

Sample application - CAMAG Linomat 5

Instrument: CAMAG Linomat 5 "Linomat5_101017" S/N 101017 (1.00.12)
Executed by: State Level Drug Testing Laboratory Wednesday, August 03, 2016 12:23:01 PM

Linomat 5 application parameters

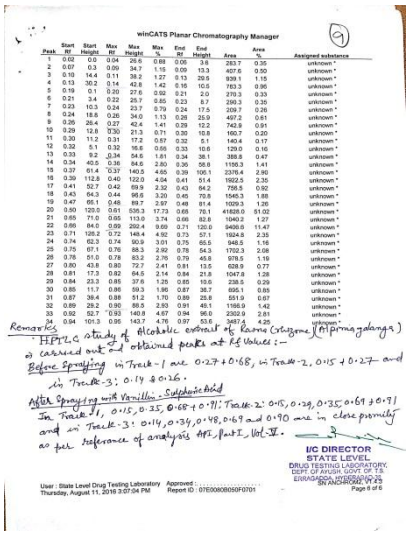
Spray gas: Inert gas
Sample solvent type: Ethanol
Dosage speed: 100 nl/s
Predosage volume: 0.2 µl

Sequence

Syringe size: 100 µl
Number of tracks: 3
Application position Y: 10.0 mm
Band length: 6.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	10.0 mm	6.0 µl	1		Yes
>2	23.0 mm	6.0 µl	1		Yes
>3	36.0 mm	6.0 µl	1		Yes

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 3:07:04 PM
Approved :
Report ID : 07E0080B050F0701
SN ANCHROM2, V1.4.3 Page 1 of 6



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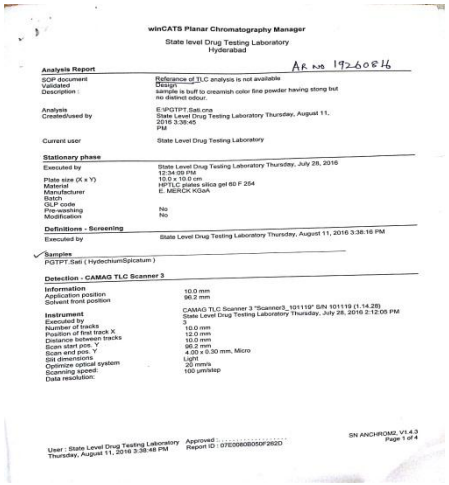
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.0	0.04	26.6	0.69	0.05	3.6	283.7	0.35	unknown *
2	0.07	0.3	0.09	34.7	1.15	0.09	13.3	407.6	0.50	unknown *
3	0.10	14.4	0.11	38.2	1.27	0.13	29.9	939.1	1.15	unknown *
4	0.13	30.2	0.14	42.8	1.42	0.15	10.5	783.3	0.96	unknown *
5	0.19	0.1	0.20	27.6	0.92	0.21	2.0	270.3	0.33	unknown *
6	0.21	3.4	0.22	25.7	0.85	0.23	8.7	290.3	0.35	unknown *
7	0.23	10.3	0.24	23.7	0.79	0.24	17.5	209.7	0.26	unknown *
8	0.24	18.8	0.26	34.0	1.13	0.28	25.9	497.2	0.61	unknown *
9	0.26	28.4	0.27	42.4	1.41	0.29	12.2	742.9	0.91	unknown *
10	0.29	12.6	0.30	21.3	0.71	0.30	10.8	160.7	0.20	unknown *
11	0.30	11.2	0.31	17.2	0.57	0.32	5.1	140.4	0.17	unknown *
12	0.32	5.1	0.32	16.8	0.56	0.33	10.6	129.0	0.16	unknown *
13	0.33	9.2	0.34	54.6	1.81	0.34	38.1	388.8	0.47	unknown *
14	0.34	40.8	0.36	84.6	2.80	0.36	58.8	1156.3	1.41	unknown *
15	0.37	61.4	0.37	140.5	4.65	0.39	106.1	2376.4	2.90	unknown *
16	0.39	112.8	0.40	122.0	4.04	0.41	51.4	1922.5	2.35	unknown *
17	0.41	52.7	0.42	69.9	2.32	0.43	84.2	786.5	0.92	unknown *
18	0.43	64.3	0.44	96.6	3.20	0.45	70.8	1545.3	1.88	unknown *
19	0.47	66.1	0.48	89.7	2.97	0.48	81.4	1029.3	1.26	unknown *
20	0.50	120.0	0.51	535.3	17.73	0.65	70.1	41828.0	51.02	unknown *
21	0.65	71.0	0.65	113.0	3.74	0.66	82.6	1040.2	1.27	unknown *
22	0.66	84.0	0.69	292.4	9.69	0.71	120.0	9469.6	11.47	unknown *
23	0.71	126.2	0.72	149.4	4.92	0.73	57.1	1924.8	2.35	unknown *
24	0.74	62.3	0.74	92.9	3.01	0.75	65.6	946.5	1.16	unknown *
25	0.75	67.1	0.76	88.3	2.92	0.78	54.3	1702.3	2.08	unknown *
26	0.78	51.0	0.78	83.2	2.76	0.79	45.8	978.5	1.19	unknown *
27	0.80	43.8	0.80	72.7	2.41	0.81	13.5	628.9	0.77	unknown *
28	0.81	17.3	0.82	64.5	2.14	0.84	21.5	1047.8	1.28	unknown *
29	0.84	23.3	0.85	37.6	1.25	0.85	10.6	238.5	0.29	unknown *
30	0.85	11.7	0.86	59.3	1.96	0.87	38.7	695.1	0.85	unknown *
31	0.87	39.4	0.88	51.2	1.70	0.89	25.8	551.9	0.67	unknown *
32	0.89	29.2	0.90	88.5	2.93	0.91	49.1	1166.9	1.42	unknown *
33	0.92	52.7	0.93	140.8	4.67	0.94	96.0	2302.9	2.81	unknown *
34	0.94	101.3	0.95	143.7	4.76	0.97	53.6	3487.4	4.25	unknown *

Remarks
HPTLC study of Alcoholic extract of Rasna (Alpinia galanga) is carried out and obtained peaks at Rf values :-
Before Spraying in Track-1 are 0.27 + 0.68, in Track-2, 0.15 + 0.27 and in Track-3: 0.14 & 0.26.
After Spraying with Vanillin - Sulphuric Acid in Track #1, 0.15, 0.35, 0.68 + 0.91; Track-2: 0.15, 0.29, 0.35, 0.69 + 0.91 and in Track-3: 0.14, 0.34, 0.48, 0.69 and 0.90 are in close proximity as per reference of analysis API, Part I, Vol-V.

I/C DIRECTOR
STATE LEVEL
DRUG TESTING LABORATORY,
DEPT. OF AYUSH, GOVT OF T.S.
ERRAGADDA, HYDERABAD-38.

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 3:07:04 PM
Approved :
Report ID : 07E0080B050F0701
SN ANCHROM2, V1.4.3 Page 6 of 6



winCATS Planar Chromatography Manager

State level Drug Testing Laboratory
Hyderabad

Analysis Report AR.No 19260816

SOP document: Reference of TLC analysis is not available
Validated
Design
Description: sample is buff to creamish color fine powder having stong but no distinct odour.

Analysis
Created/used by: E:\PGTPT Sati.cna
State Level Drug Testing Laboratory Thursday, August 11, 2016 3:38:45 PM

Current user: State Level Drug Testing Laboratory

Stationary phase

Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 12:34:09 PM
Plate size (X x Y): 10.0 x 10.0 cm
Material: HPTLC plates silica gel 60 F 254
Manufacturer: E. MERCK KGaA
Batch
GLP code
Pre-washing: No
Modification: No

Definitions - Screening

Executed by: State Level Drug Testing Laboratory Thursday, August 11, 2016 3:38:16 PM

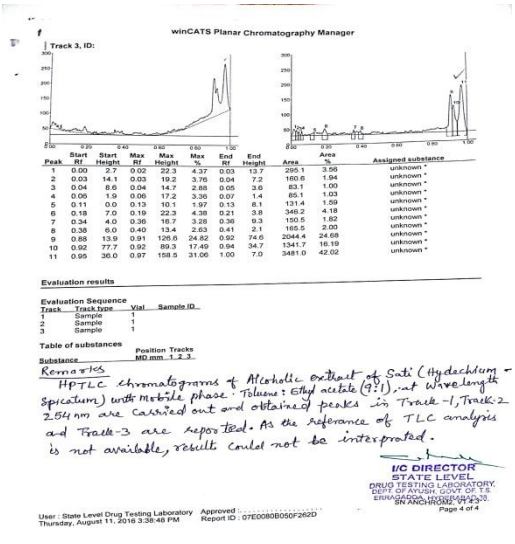
Samples
PGTPT Sati (Hydechium Spicatum)

Detection - CAMAG TLC Scanner 3

Information
Application position: 10.0 mm
Solvent front position: 96.2 mm

Instrument
Executed by: CAMAG TLC Scanner 3 "Scanner3_101119" S/N 101119 (1.14.28)
State Level Drug Testing Laboratory Thursday, July 28, 2016 2:12:05 PM
Number of tracks: 3
Position of first track X: 10.0 mm
Distance between tracks: 12.0 mm
Scan start pos. Y: 10.0 mm
Scan end pos. Y: 96.2 mm
Slit dimensions: 4.00 x 0.30 mm, Micro
Optimize optical system: Light
Scanning speed: 20 mm/s
Data resolution: 100 µm/step

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 3:38:48 PM
Approved :
Report ID : 07E0080B050F262D
SN ANCHROM2, V1.4.3 Page 1 of 4



winCATS Planar Chromatography Manager

Track 3, ID:

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	2.7	0.02	22.3	4.37	0.03	13.7	295.1	3.56	unknown *
2	0.03	14.1	0.03	19.2	3.76	0.04	7.2	160.6	1.94	unknown *
3	0.04	8.6	0.04	14.7	2.88	0.05	3.8	83.1	1.00	unknown *
4	0.05	1.9	0.06	17.2	3.36	0.07	1.4	85.1	1.03	unknown *
5	0.11	0.0	0.13	10.1	1.97	0.13	8.1	131.4	1.59	unknown *
6	0.18	7.0	0.19	22.3	4.38	0.21	3.8	346.2	4.18	unknown *
7	0.34	4.0	0.36	16.7	3.28	0.36	9.3	150.5	1.82	unknown *
8	0.38	6.0	0.40	13.4	2.63	0.41	2.1	165.5	2.00	unknown *
9	0.88	13.9	0.91	126.6	24.82	0.92	74.6	2044.4	24.68	unknown *
10	0.92	77.7	0.92	89.3	17.49	0.94	34.7	1341.7	16.19	unknown *
11	0.95	36.0	0.97	158.5	31.06	1.00	7.0	3481.0	42.02	unknown *

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	
2	Sample	1	
3	Sample	1	

Table of substances

Substance	Position MD mm	Tracks 1	2	3

Remarks
HPTLC chromatograms of Alcoholic extract of Sati (Hydechium Spicatum) with mobile phase: Toluene : Ethyl acetate (9:1), at Wavelength 254 nm are carried out and obtained peaks in Track-1, Track-2 and Track-3 are reported. As the reference of TLC analysis is not available, results could not be interpreted.

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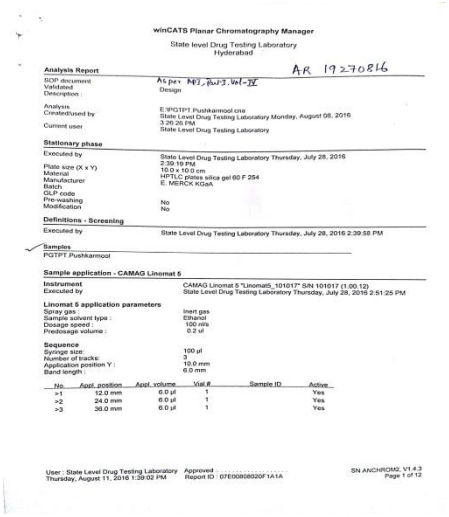
DEPT. OF AYUSH, GOVT OF T.S.
ERRAGADDA, HYDERABAD-38.

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 3:38:48 PM
Approved :
Report ID : 07E0080B050F262D
SN ANCHROM2, V1.4.3 Page 4 of 4



winCATS Planar Chromatography Manager

State level Drug Testing Laboratory
Hyderabad

Analysis Report AR 19270816

SOP document: As per MP, Part-I, Vol-IV
Validated
Description: Design

Analysis
Created/used by: E:\PGTPT.Pushkarmool.cma
State Level Drug Testing Laboratory Monday, August 08, 2016 3:26:26 PM
Current user: State Level Drug Testing Laboratory

Stationary phase

Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 2:39:18 PM
Plate size (X x Y): 10.0 x 10.0 cm
Material: HPTLC plates silica gel 60 F 254
Manufacturer: E. MERCK KGaA
Batch
GLP code
Pre-washing: No
Modification: No

Definitions - Screening

Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 2:39:58 PM

Samples

PGTPT.Pushkarmool

Sample application - CAMAG Linomat 5

Instrument: CAMAG Linomat 5 "Linomat5_101017" S/N 101017 (1.00.12)
Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 2:51:25 PM

Linomat 5 application parameters

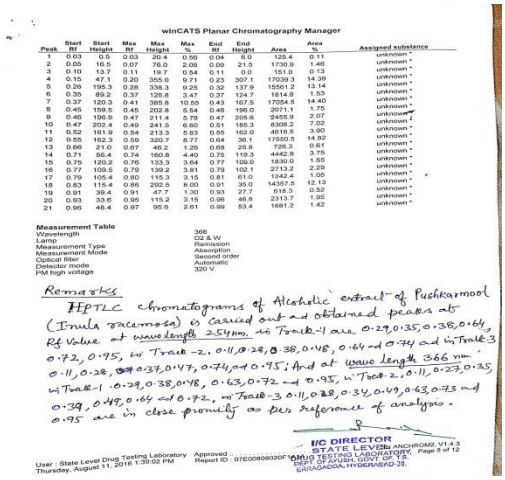
Spray gas: Inert gas
Sample solvent type: Ethanol
Dosage speed: 100 nl/s
Predosage volume: 0.2 µl

Sequence

Syringe size: 100 µl
Number of tracks: 3
Application position Y: 10.0 mm
Band length: 6.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	12.0 mm	6.0 µl	1		Yes
>2	24.0 mm	6.0 µl	1		Yes
>3	36.0 mm	6.0 µl	1		Yes

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 1:39:02 PM
Approved :
Report ID : 07E008080F1A1A
SN ANCHROM2, V1.4.3 Page 1 of 12



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Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.5	0.03	20.4	0.56	0.04	9.0	125.4	0.11	unknown *
2	0.05	16.5	0.07	76.0	2.08	0.09	21.5	1730.9	1.46	unknown *
3	0.10	13.7	0.11	19.7	0.54	0.11	0.0	151.0	0.13	unknown *
4	0.15	47.1	0.20	355.0	9.71	0.23	307.1	17039.3	14.39	unknown *
5	0.26	195.3	0.28	358.3	9.25	0.32	137.9	15561.2	13.14	unknown *
6	0.35	89.2	0.37	126.8	3.47	0.37	124.7	1814.8	1.53	unknown *
7	0.37	120.3	0.41	385.8	10.55	0.43	167.5	17054.5	14.40	unknown *
8	0.45	159.5	0.45	202.8	5.54	0.46	196.0	2071.1	1.75	unknown *
9	0.46	196.9	0.47	211.4	5.78	0.47	205.6	2455.9	2.07	unknown *
10	0.47	202.4	0.49	241.5	6.60	0.51	185.3	8308.2	7.02	unknown *
11	0.52	181.9	0.54	213.3	5.83	0.55	162.0	4616.5	3.90	unknown *
12	0.55	162.3	0.59	320.7	8.77	0.64	36.1	17550.5	14.82	unknown *
13	0.66	21.0	0.67	46.2	1.26	0.68	25.8	726.3	0.61	unknown *
14	0.71	56.4	0.74	160.8	4.40	0.75	119.3	4442.9	3.75	unknown *
15	0.75	120.2	0.76	133.3	3.64	0.77	109.0	1830.9	1.55	unknown *
16	0.77	109.5	0.79	139.2	3.81	0.79	102.1	2713.2	2.29	unknown *
17	0.79	105.4	0.80	115.3	3.15	0.81	61.0	1242.4	1.05	unknown *
18	0.83	115.4	0.86	292.5	8.00	0.91	35.0	14357.5	12.13	unknown *
19	0.91	39.4	0.91	47.7	1.30	0.93	27.7	618.3	0.52	unknown *
20	0.93	33.6	0.95	119.2	3.15	0.96	46.8	2313.7	1.95	unknown *
21	0.96	48.4	0.97	95.6	2.61	0.99	53.4	1681.2	1.42	unknown *

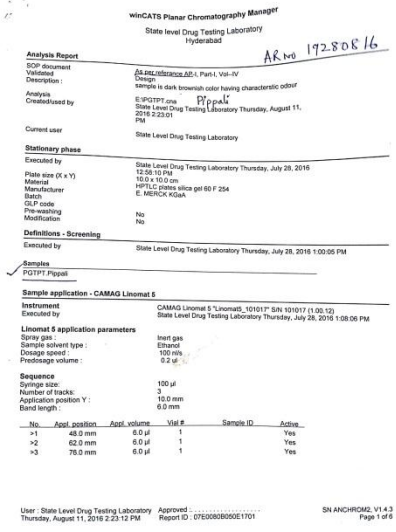
Measurement Table

Wavelength	366
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	320 V

Remarks
HPTLC chromatograms of Alcoholic extract of Pushkarmool (Inula racemosa) is carried out and obtained peaks at Rf Value at wavelength 254nm. in Track-1 are 0.29, 0.35, 0.38, 0.64, 0.72, 0.95, in Track -2, 0.11, 0.28, 0.38, 0.48, 0.64 and 0.74 and in Track-3 0.11, 0.28, 0.37, 0.47, 0.74 and 0.95; And at wave length 366 nm in Track-1 0.29, 0.38, 0.48, 0.63, 0.72 and 0.95, in Track-2, 0.11, 0.23, 0.35 0.39, 0.49, 0.64 and 0.72, in Track-3 0.11, 0.28, 0.34, 0.49, 0.63, 0.73 and 0.95 are in close proximity as per reference of analysis.

I/C DIRECTOR
STATE LEVEL
DRUG TESTING LABORATORY,
DEPT. OF AYUSH, GOVT. OF T.S.
ERRAGADDA, HYDERABAD-38.

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 1:39:02 PM
Approved :
Report ID : 07E008080F1A1A
SN ANCHROM2, V1.4.3 Page 5 of 12



winCATS Planar Chromatography Manager

State level Drug Testing Laboratory
Hyderabad

Analysis Report ARM 19280816

SOP document: As per reference AP-I, Part-I, Vol-IV
Validated
Description: Design
sample is dark brownish color having characteristic odour

Analysis
Created/used by: E:\PGTPT.cma Pippali
State Level Drug Testing Laboratory Thursday, August 11, 2016 2:23:01 PM
Current user: State Level Drug Testing Laboratory

Stationary phase

Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 12:58:10 PM
Plate size (X x Y): 10.0 x 10.0 cm
Material: HPTLC plates silica gel 60 F 254
Manufacturer: E. MERCK KGaA
Batch
GLP code
Pre-washing: No
Modification: No

Definitions - Screening

Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 1:00:05 PM

Samples

PGTPT.Pippali

Sample application - CAMAG Linomat 5

Instrument: CAMAG Linomat 5 "Linomat5_101017" S/N 101017 (1.00.12)
Executed by: State Level Drug Testing Laboratory Thursday, July 28, 2016 1:08:06 PM

Linomat 5 application parameters

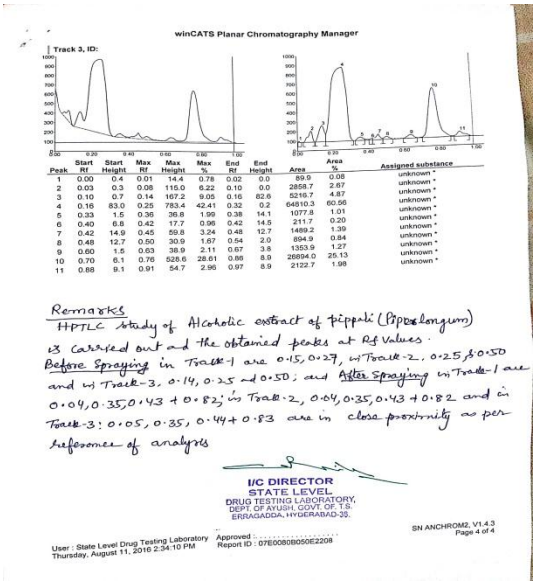
Spray gas: Inert gas
Sample solvent type: Ethanol
Dosage speed: 100 nl/s
Predosage volume: 0.2 µl

Sequence

Syringe size: 100 µl
Number of tracks: 3
Application position Y: 10.0 mm
Band length: 6.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	48.0 mm	6.0 µl	1		Yes
>2	62.0 mm	6.0 µl	1		Yes
>3	76.0 mm	6.0 µl	1		Yes

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 2:23:12 PM
Approved :
Report ID : 07E008080050E1701
SN ANCHROM2, V1.4.3 Page 1 of 6



winCATS Planar Chromatography Manager

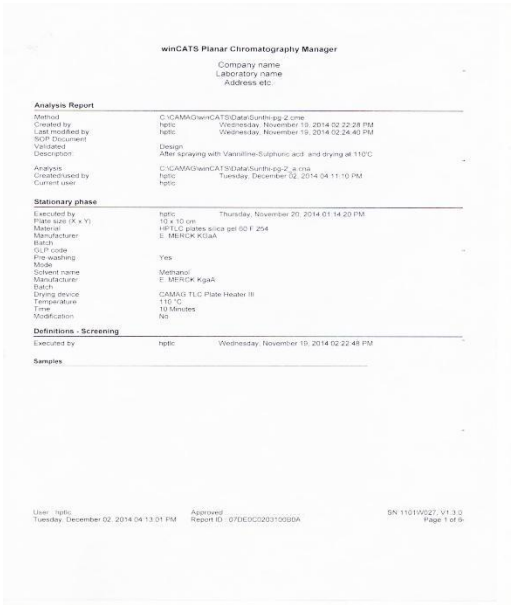
Track 3, ID:

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.4	0.01	14.4	0.78	0.02	0.0	89.9	0.08	unknown *
2	0.03	0.3	0.08	115.0	6.22	0.10	0.0	2858.7	2.67	unknown *
3	0.10	0.7	0.14	167.2	9.05	0.16	82.6	5216.7	4.87	unknown *
4	0.16	83.0	0.25	783.4	42.41	0.32	0.2	64810.3	60.56	unknown *
5	0.33	1.5	0.36	36.8	1.99	0.38	14.1	1077.8	1.01	unknown *
6	0.40	6.8	0.42	17.7	0.96	0.42	14.5	211.7	0.20	unknown *
7	0.42	14.9	0.45	59.8	3.24	0.48	12.7	1489.2	1.39	unknown *
8	0.48	12.7	0.50	30.9	1.67	0.54	2.0	894.9	0.84	unknown *
9	0.60	1.5	0.63	38.9	2.11	0.67	3.8	1353.9	1.27	unknown *
10	0.70	6.1	0.76	525.6	28.61	0.86	8.9	26894.0	25.13	unknown *
11	0.88	9.1	0.91	54.7	2.96	0.97	8.9	2122.7	1.98	unknown *

Remarks
HPTLC study of Alcoholic extract of pippali (Piper longum) is carried out and the obtained peaks at Rf values. Before Spraying in Track-1 are 0.15, 0.29, in Track-2, 0.25, 0.50 and in Track-3, 0.14, 0.25 and 0.50; and After Spraying in Track-1 are 0.04, 0.35, 0.43 + 0.82, in Track-2, 0.04, 0.35, 0.43 + 0.82 and in Track-3: 0.05, 0.35, 0.44 + 0.83 are in close proximity as per reference of analysis

I/C DIRECTOR
STATE LEVEL
DRUG TESTING LABORATORY,
DEPT. OF AYUSH, GOVT. OF T.S.
ERRAGADDA, HYDERABAD-38.

User : State Level Drug Testing Laboratory Thursday, August 11, 2016 2:34:10 PM
Approved :
Report ID : 07E008080050E2208
SN ANCHROM2, V1.4.3 Page 4 of 4



winCATS Planar Chromatography Manager

Company name
Laboratory name
Address etc.

Analysis Report

Method: C:\CAMAG\winCATS\Data\Sunthi-pg-2.cme
Created by: hptlc Wednesday, November 19, 2014 02:22:28 PM
Last modified by: hptlc Wednesday, November 19, 2014 02:24:40 PM
SOP Document
Validated
Description: Design
After spraying with Vanillin-Sulphuric acid and drying at 110°C

Analysis
Created/used by: C:\CAMAG\winCATS\Data\Sunthi-pg-2_a.cma
hptlc Tuesday, December 02, 2014 04:11:10 PM
Current user: hptlc

Stationary phase

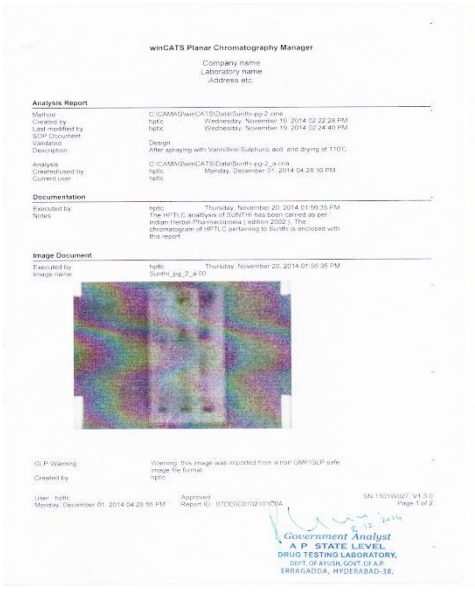
Executed by: hptlc Thursday, November 20, 2014 01:14:20 PM
Plate size (X x Y): 10 x 10 cm
Material: HPTLC plates silica gel 60 F 254
Manufacturer: E. MERCK KGaA
Batch
GLP code
Pre-washing: Yes
Mode
Solvent name: Methanol
Manufacturer: E. MERCK KgaA
Batch
Drying device: CAMAG TLC Plate Heater III
Temperature: 110 °C
Time: 10 Minutes
Modification: No

Definitions - Screening

Executed by: hptlc Wednesday, November 19, 2014 02:22:48 PM

Samples

User : hptlc Tuesday, December 02, 2014 04:13:01 PM
Approved :
Report ID : 07DE0CD2031009DA
SN 1101W027, V1.3.0 Page 1 of 6



winCATS Planar Chromatography Manager

Company name
Laboratory name
Address etc.

Analysis Report

Method: C:\CAMAG\winCATS\Data\Sunthi-pg-2.cme
Created by: hptlc Wednesday, November 19, 2014 02:22:28 PM
Last modified by: hptlc Wednesday, November 19, 2014 02:24:40 PM
SOP Document
Validated
Description: Design
After spraying with Vanillin-Sulphuric acid and drying at 110°C

Analysis
Created/used by: C:\CAMAG\winCATS\Data\Sunthi-pg-2_a.cma
hptlc Monday, December 01, 2014 04:28:10 PM
Current user: hptlc

Documentation

Executed by: hptlc Thursday, November 20, 2014 01:56:35 PM
Notes: The HPTLC analysis of SUNTHI has been carried as per Indian Herbal Pharmacopoeia (edition 2002). The chromatogram of HPTLC pertaining to Sunthi is enclosed with this report.

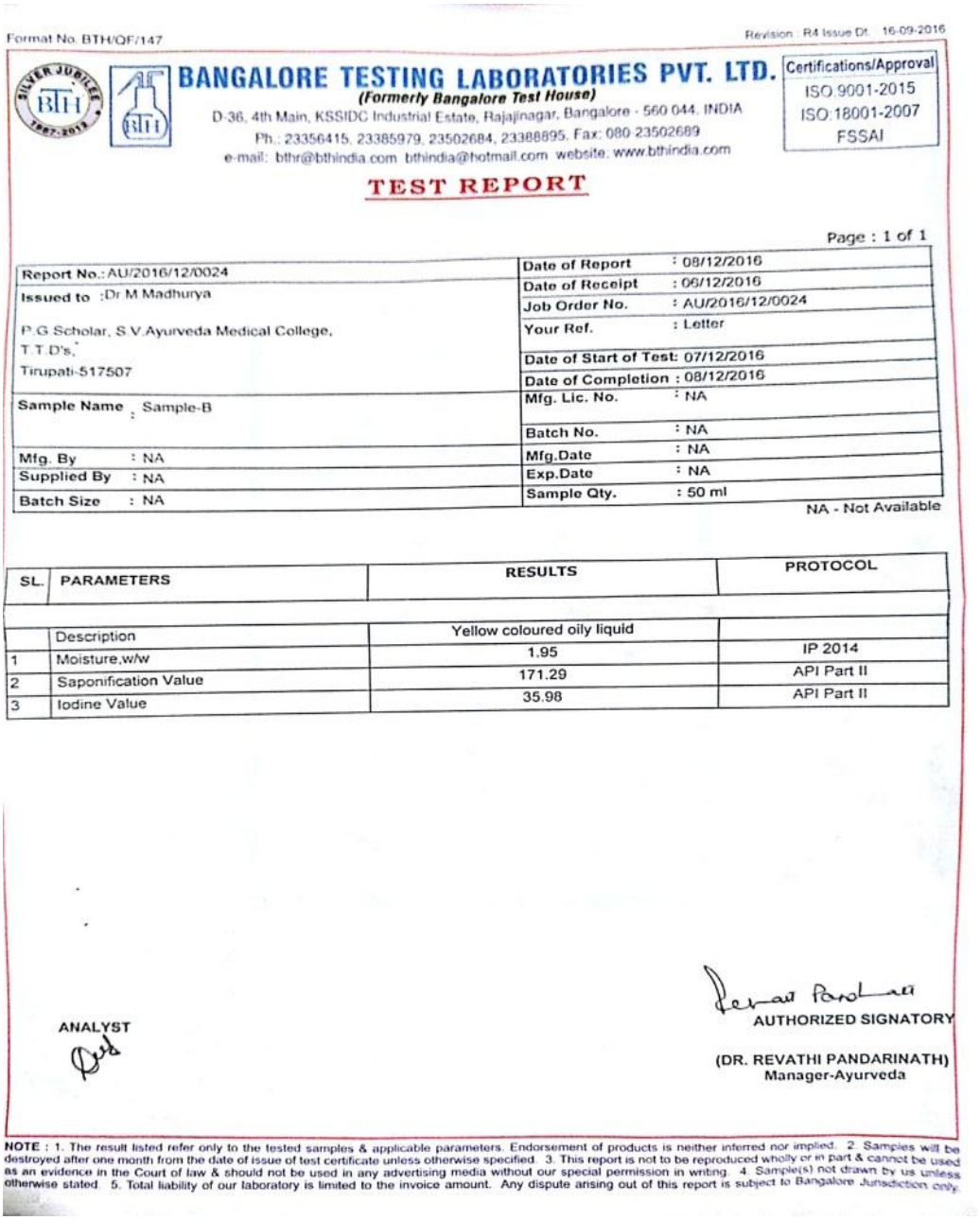
Image Document

Executed by: hptlc Thursday, November 20, 2014 01:56:35 PM
Image name: Sunthi_pg_2_a.00

GLP Warning: Warning: this image was imported from a non GMP/GLP safe image file format.
Created by: hptlc

User : hptlc Monday, December 01, 2014 04:28:56 PM
Approved :
Report ID : 07DE0C01021010DA
SN 1101W027, V1.3.0 Page 1 of 2

Government Analyst
A.P. STATE LEVEL
DRUG TESTING LABORATORY,
DEPT. OF AYUSH, GOVT. OF A.P.
ERRAGADDA, HYDERABAD-38.



Format No. BTH/QF/147 Revision : R4 Issue Dt. 16-09-2016

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Certifications/Approval
ISO 9001-2015
ISO 18001-2007
FSSAI

TEST REPORT

Page : 1 of 1

Report No.: AU/2016/12/0024	Date of Report : 08/12/2016
Issued to :Dr M Madhurya	Date of Receipt : 06/12/2016
P.G Scholar, S.V.Ayurveda Medical College, T.T.D's, Tirupati-517507	Job Order No. : AU/2016/12/0024
	Your Ref. : Letter
	Date of Start of Test: 07/12/2016
	Date of Completion : 08/12/2016
Sample Name : Sample-B	Mfg. Lic. No. : NA
	Batch No. : NA
Mfg. By : NA	Mfg.Date : NA
Supplied By : NA	Exp.Date : NA
Batch Size : NA	Sample Qty. : 50 ml

NA - Not Available

SL.	PARAMETERS	RESULTS	PROTOCOL
	Description	Yellow coloured oily liquid	
1	Moisture,w/w	1.95	IP 2014
2	Saponification Value	171.29	API Part II
3	Iodine Value	35.98	API Part II

ANALYST

AUTHORIZED SIGNATORY

(DR. REVATHI PANDARINATH)
Manager-Ayurveda

NOTE : 1. The result listed refer only to the tested samples & applicable parameters. Endorsement of products is neither inferred nor implied. 2. Samples will be destroyed after one month from the date of issue of test certificate unless otherwise specified. 3. This report is not to be reproduced wholly or in part & cannot be used as an evidence in the Court of law & should not be used in any advertising media without our special permission in writing. 4. Sample(s) not drawn by us unless otherwise stated. 5. Total liability of our laboratory is limited to the invoice amount. Any dispute arising out of this report is subject to Bangalore Jurisdiction only.

DISCUSSION

1. Saponification value

The amount of alkali needed to saponify a given quantity of fat will depend up on the number of –COOH group present. The long chain fatty acids found in fats have low saponification value because they have relatively less number of Carboxylic Functional groups per unit mass of the fat as compared to short chain fatty acids. Saponification value is directly proportional to the fatty matter content. More the fatty matter content there will be the more chances of rancidity factor and less will be the shelf life and therapeutic value. In this study, Rasnadi ghritam Saponification value was-171.29.

2. Iodine value

The determination of Iodine number is useful in determining the quality of oil or whether it is free from adulteration. Iodine number is also a measure of the degree of unsaturation of fat. The more Iodine number, more are unsaturated fatty acid bonds present. This indicates that more number of double bonds in the ghrita. The more iodine is attached, the higher is the value of its being more reactive, less stable, softer and more susceptible to oxidation and rancidification with Taila. The result reported by this study Rasnadi ghritam is -35.98.

3. Moisture value w/w

An excess of moisture content in medicinal plant material will encourage microbial growth, the presence of fungi or insect and deterioration following Hydrolysis. Limits for moisture content should therefore be set for every given material. The moisture value of Rasnadi ghritam-1.95.

CONCLUSION

Rasnadi ghritam was subjected to standardization methods to check its shelf life,rate of decomposition and stability. Iodine value is-35.98 Moisture content W/W value is 1.95, Saponification value is 171.29.

REFERENCES

1. Vāgbhaṭaś Aṣṭāñga Hṛdayam translated by k.R Srikantha Murthy volume -2cikistā sthana chapter num-21verse num- 57page num- 507 Chaukambha Krishnadas academy Varanasi-2006.
2. A Comparative Pharmacognostical study of Sweta chitraka and Neela chitraka W.s.r to Madhumeha by Dr.P.Veena 2010 Dr. Brkr Govt Ayurvedic Medical college.
3. Bhaisajya Kalpana Vijnanam by Dr.K.Rama Chandra Reddy – Sneha Kalpana Adhyaya Page no. 332 & 335; Choukambha Sanskrit Pratistana, Delhi.
4. Quality control & Standardisation of Ayurvedic Medicines by Dr.Devendra Joshi & Dr.Geetha Joshi- page no. 177; Choukambha Orientalia, Varanasi.
5. Quality control & Standardisation of Ayurvedic Medicines by Dr.Devendra Joshi & Dr.Geetha Joshi- page no.133; Choukambha Orientalia, Varanasi.
6. Quality control & Standardisation of Ayurvedic Medicines by Dr. Devendra Joshi & Dr.Geetha Joshi- page no. 178; Choukambha Orientalia, Varanasi.