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PHYSICO-CHEMICAL ANALYSIS OF TRIBHUVANA KEERTI RASA & VETTUMARAN GULIKA

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ABSTRACT

Background: Standardization of a compound is essential for establishing the authenticity, quality and efficacy of Ayurvedic medicines. This can be achieved only if herbal medicines are evaluated and analysed using modern techniques of standardization. Tribhuvana keerti Rasa and Vettumaran are the two preparations taken up in this study and had undergone the analytical tests that are essential for the establishment of the quality of the formulation. **Aim & Objective:** To study the organo leptic & physico-chemical characters of TBK and VTG. **Methods & Materials:** Organoleptic characters and physic-chemical analysis like pH, loss on drying, hardness test, disintegration test, friability etc. was done as per the standard protocol. **Results:** Analytical parameters results obtained were all within the permissible limit. The disintegration time was (36min) in VTG and (39mins) in TBK.

KEYWORDS: Standardisation, Tribhuvan Keerthi rasa and Vettumaran gulika.

INTRODUCTION

Rasashastra is a science that deals with preparations that include metals and minerals. Therefore standardization of these medicines is essential to avoid any untoward affects in the body. Tribhuvana keerti Rasa & Vettumaran Gulika are two rasa formulations that have been widely utilized for the treatment of Jwara. Samskara is an inevitable process in pharmaceutics; hence shodhana was done for the rasa dravyas in the preparation of Tribhuvana Keerti Rasa (TBK) and Vettumaran Gulika (VTG) to avoid impurities and

toxicity. The preparations were subjected to bhavana procedure as per the reference, which has helped not only in the vati preparation but also in enhancing the properties of both the yogas. Vati Kalpana plays an important role in Ayurvedic Pharmaceutics, with regards to the advantages like easy administration, prolonged shelf life and can be easily dispensed and transported. It is a dosage form wherein the powder of raw materials is mixed and triturated with juices or decoction prepared out of different herbal drugs. The medicine is then rolled into pills of required shape and size Both the medicines were in Vati form and the standardization was done following the standard protocol.

MATERIALS AND METHODS

Organoleptic Characters

- Colour
- Smell
- Touch
- Taste
- Consistency.

Physico – Chemical Analysis

1. Loss on drying at 105 C[1]

10 g sample was placed in tared evaporating dish. t was dried at 105 C for 5 hrs in hot air oven and weighed . The drying was continued until difference between two successive weights was not more than 0.001 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

2. Total Ash[2]

2g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450 C until free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

3. Acid Insoluble ash[3]

To the crucible containing total ash, add 25 ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators

for 30 minutes and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

4. Water soluble ash[4]

Boil the ash for 5 mins with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite for 15 minutes at a temperature not exceeding 450°. Subs tract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water soluble with reference to the air dried drug.

5. Alcohol soluble extractive[5]

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95 %). Shake occasionally for 6 hrs. Allow to stand for 18 hrs. Filter rapidly taking care not to lose any solvent. Pippette out 25 ml of the filtrate in a pre – weighed 100 ml beakers. Evaporate to dryness on a water bath. Keep it in an air oven at 105 C for 6 hrs; cool in desiccators for 30 mins and weigh .Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

6. Determination of pH[6]

The pH meter and electrode is operated according to the manufacturer's instructions. Standardise the meter and electrodes with 0.05 M potassium hydrogen phthalate (pH 4.00) when measuring an acid solution, or with 0.005 M sodium borate when measuring an alkaline solution. At the end of a set of measurements, take a reading of the solution used to standardise the meter and electrodes. This reading should not differ by more than 0.02 from the original value at which the apparatus was standardized.

7. Water soluble extractive[7]

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hrs. Allow to stand for 18 hrs. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre – weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in a pre – weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105 C for 6 hrs .Cool in desiccators and weigh. Repeat the experiment twice. Take the average.

8. Uniformity in weight[8]

Weighed individually 20 units selected at random or for single dose preparations in individual containers, the contents of 20 units, and calculate the average weight. Not more than two of the individual weight deviates from the average weight by more than the percentage shown in the table and none deviates by more than twice the percentage.

9. Hardness test[9]

5 tablets were taken and tested for hardness .The lower plunger was placed in contact with the tablet .The upper plunger was then forced against a spring by turning a threaded bolt until the tablet fractures. The force of fracture was recorded.

10. Disintegration time[10]

The tank of the disintegration apparatus was filled with distilled water up to the mark .750 ml of distilled water in each of the 1000 ml beaker was taken. The timer of the instrument was set for 60 minutes. The temperature of water in beakers to 37˚C and that of water in the main tank to 37 .5˚C was maintained .One tablet was introduced into each tube and, added a disk to each tube. The assembly was suspended in the beaker containing water and the apparatus was operated. The time duration at which the tablet disintegrated was noted.

11. Friability[11]

The pills were selected randomly and weighed, placed in the tumbling chamber of the friabilator. The friabilator was then revolved for 100 revolutions at 25 rpm. During each revolutionthe pills fall from a distance of 6 " to undrgo shock , after 100 revolutions the pills were weighed again to see the loss in weight. The percentage of friability was calculated by applying the formula,

$$\% \text{ friability} = A-B/A \times 100$$

Where, A = Total weight of 20 pills before the friability.

B = total weight of 20 pills after the friability test

12. HPTLC[12]

1g of Vettumaran gutika and Tribhuvana Keerti Rasa was extracted with 10 ml of alcohol. 3 and 6 µl of the above extract wa applied on a pre – coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized under short UV, long UV and then derivatised with vanillin sulphuric acid, observed under white light and scanned

under UV 254 nm, 366 nm. Rf, colour of the spots and densitometric scan and 3-D chromatogram were recorded.

RESULTS

Table 1: Results of Organoleptic character.

	Parameters	Tribhuvana Keerti Rasa	Vettumaran Gulika
1	**Colour**	Dark red	Brick red
2	**Touch**	Hard, rough	Hard, slightly rough
3	**Smell**	Specific Smell	Ardraka smell
4	**Taste**	-	-
5	**Consistency**	Solid	Solid

Table No. 2: Results of Physicochemmical analysis.

Result n = 3 (% w/w)		
Parameters	**Vettumaran gutika (Average±SEM)**	**Tribhuvana keerti rasa (Average±SEM)**
pH	8.47	8.27
Loss on drying (%)	18.23	20.22
Total Ash	28.45±0.28	26.08±0.21
Acid Insoluble Ash	1.08±0.005	0.69±0.01
Water soluble ash	25.86±0.01	23.59±0.01
Alcohol soluble Extractive value	8.60±0.00	7.19±0.00
Water soluble extractive value	29.58±0.00	30.81±0.01
Uniformity of weight	0.756±0.002	0.065±0.004
Disintegration time(min)	36 mins	39 mins
Hardness (Kg/cm)	0.5kg/cm	1kg/cm
Friability	0.03%	0.01%

HPTLC

The Rf values of the samples in short wave, long wave and post derivatisation has been noted in the table below **(Table no 3).**

Table no 3.

Short UV		**Long UV**		**Post derivatisation**	
Vettumaran gutica	**Tribhuvana keerti rasa**	**Vettumaran gutica**	**Tribhuvana keerti rasa**	**Vettumaran gutica**	**Tribhuvana keerti rasa**
0.06 (L. green)	0.06 (L. green)	0.06 (F. blue)	0.06 (F. blue)	-	-
-	0.12 (L. green)	-	-	-	-
0.18 (D. green)	0.18 (D. green)	0.18 (F. blue)	0.18 (F. blue)	0.18 (yellow)	0.18 (yellow)
-	-	0.22 (F. blue)	0.22 (F. blue)	-	-
0.24 (L. green)	0.24 (L. green)	0.24 (F. blue)	-	-	-
-	-	0.27 (F. yellow)	0.27 (F. yellow)	0.27 (yellow)	-
0.32 (L. green)	0.32 (D. green)	-	-	-	-
-	-	0.35 (FD. blue)	-	-	-

0.39 (D. green)	0.39 (D. green)	0.39 (F. blue)	-	0.39 (Purple)	0.39 (Purple)
0.46 (L. green)	-	0.46 (FD. yellow)	-	-	-
0.54 (L. green)	-	0.54 (F aqua. blue)	0.54 (F. blue)	-	-
0.65 (L. green)	-	0.65 (F aqua. blue)	0.65 (F. blue)	-	-
0.69 (L. green)	-	-	-	-	-
0.84 (L. green)	-	-	-	0.84 (Purple)	-

RESULTS AND DISCUSSION

1. pH

The pH of TBK sample and VTG sample was 8.27, 8.47 respectively. Both the samples were observed to be basic in nature.

2. Loss on drying

VTG and TBK contained 18.23 % and 20.22% of moisture respectively. This test was performed to determine the moisture and volatile content in the samples.

3. Total Ash

Ash value helps us to determine the amount of inorganic substance present in the samples. Total ash of the samples was 28.45±0.28 and 26.08±0.21.Comparing both the formulations, VTG has more ash value, may be because of the presence of less number of bhavana dravyas in its preparation.

4. Acid Insoluble Ash

This test is carried out to determine the amount of silica and sand particles. Acid insoluble ash of two samples was observed to be 1.08±0.005 and 0.69±0.01 respectively.

5. Water soluble ash

Water soluble ash is defined as the residue obtained after boiling the total ash and residue after treatment of total ash with water. The value was found to be 25.86±for VTG and 23.59±0.01 for TBK.

6. Alcohol soluble extractive value

The alcohol soluble extractive value of VTG and TBK were observed to be 8.60 ± 0.00and 7.19±0.01respectively.

7. Water Soluble Extractive

The water soluble extractive was more in case of TBK sample than VTG sample. This may due to the increase in bhavana dravyas in the preparation of TBK.

8. Uniformity of weight

20 pills were taken and weighed separately and the average weight was calculated. The variation in the weights may be due to the manual rolling of the pills.

9. Disintegration time

The disintegration time of VTG (36mins) is more compared to TBK (39cm) that indicates the probability of a quicker absorption rate.

10. Hardness

The hardness test was performed and the values were calculated. VTG had 0.5 kg/cm^2 and TBK had 1 kg /cm^2 of hardness. Hardness determines the stability of the vati prepared and it is found to be more in TBK as there is compactness of the particles due to the number of bhavanas.

11. Friability

The weights of vatis weighed before and after the revolution showed weight loss of 0.03% and 0.01% in VTG and TBK respectively. This shows that the ability to withstand the mechanical aberration is less in VTG sample.

HPTLC (High Performance Thin Layer Chromatography)

Rf value at short UV

HPTLC of TBK showed 6 spots at 0.06, 0.12, 0.18, 0.24, 0.32, 0.39 and Hptlc of VTG showed 10 spots at 0.006, 0.18, 0.24, 0.32, 0.39, 0.46, 0.54, 0.65, 0.69, 0.84.The alkaloid spot with RF value is common at 0.06, 0.18, 0.24, 0.32 and 0.39.

RF value at long UV

HPTLC of TBK showed 6 spots at 0.06, 0.18, 0.22, 0.27, 0.54, 0.65 and HPTLC of VTG showed 10 spots at 0.06, 0.18, 0.22, 0.24, 0.27, 0.35, 0.39, 0.46, 0.54, 0.65.The alkaloid spot with rf value is common at 0.06, 0.18, 0.24, 0.32 and 0.39.

Post derivation

HPTLC of TBK showed 2 spots at 0.18 and 0.39 and HPTLC of VTG showed 4 spots at 0.18, 0.27, 0.39 and 0.84.The alkaloids spot with Rf is common at 0.18 and 0.39.

Densitometric scan at 254 nm

TBK showed maximum area at Rf value 0.20 i.e. 25.82% and VTG showed maximum area at Rf value 0.16 i.e, 37.35%.

Densitometric scan at 254 nm

TBK showed maximum area at Rf value 0.04 i.e., 44.61% and VTG showed maximum area at Rf value 0.68 i.e, 61.94%.

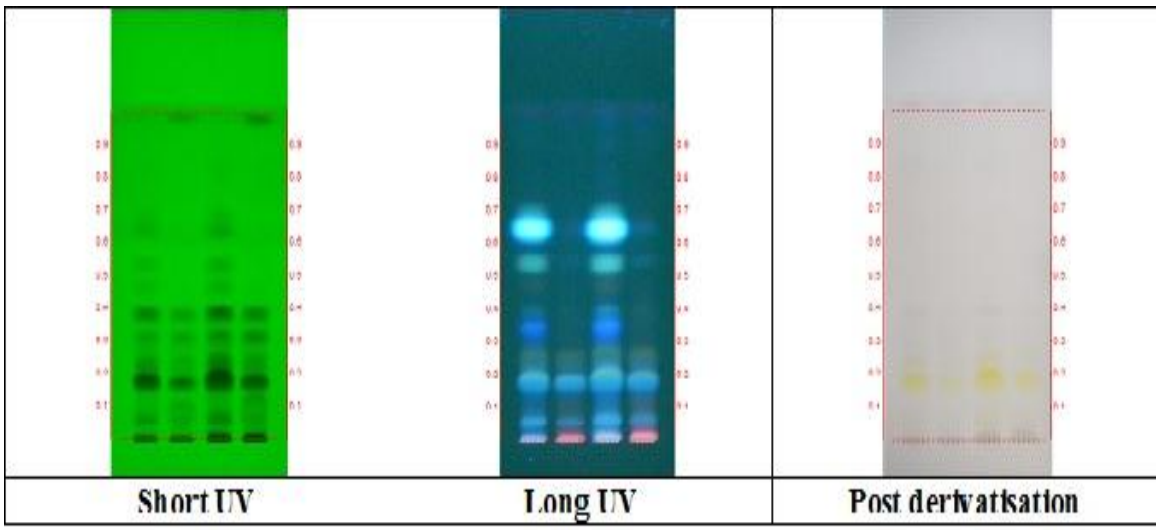
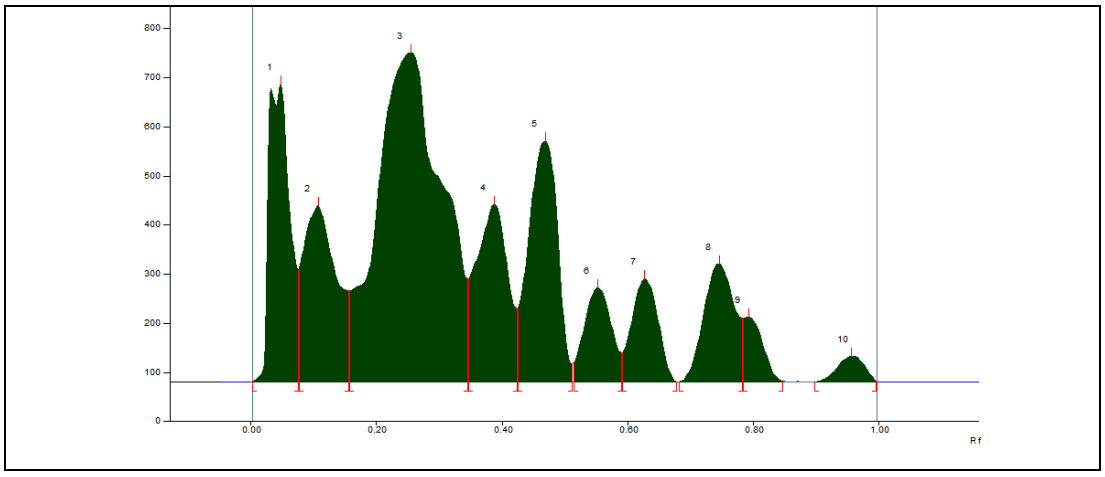


Fig 1: HPTLC of Tribhuvana Keerti Rasa & Vettumara.



Track 3, ID: Vettumaran gutica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.2 AU	0.05 Rf	605.8 AU	18.30 %	0.08 Rf	27.5 AU	14839.8 AU	11.17 %
2	0.08 Rf	230.7 AU	0.11 Rf	358.1 AU	10.81 %	0.16 Rf	86.3 AU	13596.6 AU	10.23 %
3	0.16 Rf	186.4 AU	0.25 Rf	670.1 AU	20.24 %	0.35 Rf	09.8 AU	49630.3 AU	37.35 %
4	0.35 Rf	210.3 AU	0.39 Rf	360.9 AU	10.90 %	0.42 Rf	49.5 AU	13141.5 AU	9.89 %
5	0.43 Rf	151.2 AU	0.47 Rf	490.2 AU	14.81 %	0.51 Rf	36.3 AU	16369.6 AU	12.32 %
6	0.51 Rf	38.2 AU	0.55 Rf	191.3 AU	5.78 %	0.59 Rf	59.4 AU	5967.9 AU	4.49 %
7	0.59 Rf	59.7 AU	0.63 Rf	209.7 AU	6.33 %	0.68 Rf	0.2 AU	6340.2 AU	4.77 %
8	0.68 Rf	0.4 AU	0.75 Rf	240.8 AU	7.27 %	0.78 Rf	30.2 AU	8488.5 AU	6.39 %
9	0.79 Rf	130.3 AU	0.79 Rf	131.8 AU	3.98 %	0.85 Rf	2.1 AU	2875.9 AU	2.16 %
10	0.90 Rf	0.0 AU	0.96 Rf	52.3 AU	1.58 %	1.00 Rf	1.7 AU	1634.8 AU	1.23 %

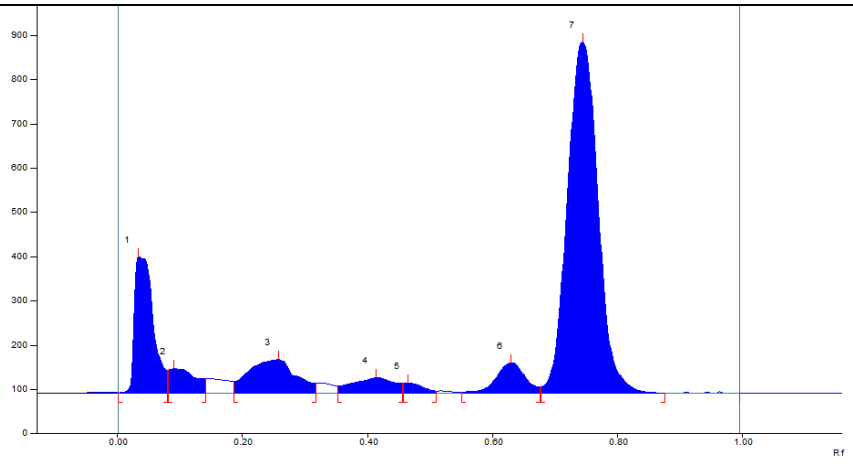
Fig 2a. Vettumaran gutica

Track 4, ID: Tribhuvanakeerti rasa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.0 AU	0.05 Rf	616.6 AU	21.61 %	0.08 Rf	10.4 AU	14998.4 AU	16.27 %
2	0.08 Rf	210.6 AU	0.11 Rf	243.2 AU	8.52 %	0.14 Rf	82.9 AU	7810.6 AU	8.47 %
3	0.14 Rf	183.8 AU	0.18 Rf	282.0 AU	9.88 %	0.19 Rf	50.0 AU	8252.0 AU	8.95 %
4	0.20 Rf	251.8 AU	0.24 Rf	578.4 AU	20.27 %	0.28 Rf	83.7 AU	23809.4 AU	25.82 %
5	0.29 Rf	284.0 AU	0.31 Rf	298.6 AU	10.47 %	0.34 Rf	62.1 AU	9305.1 AU	10.09 %
6	0.34 Rf	163.8 AU	0.39 Rf	382.3 AU	13.40 %	0.42 Rf	30.5 AU	13338.4 AU	14.47 %
7	0.43 Rf	131.8 AU	0.46 Rf	382.3 AU	13.40 %	0.51 Rf	20.4 AU	12047.3 AU	13.07 %
8	0.51 Rf	20.4 AU	0.54 Rf	29.9 AU	1.05 %	0.60 Rf	0.5 AU	1056.9 AU	1.15 %
9	0.62 Rf	2.3 AU	0.64 Rf	12.7 AU	0.45 %	0.66 Rf	6.8 AU	266.1 AU	0.29 %
10	0.67 Rf	5.4 AU	0.71 Rf	27.4 AU	0.96 %	0.80 Rf	0.0 AU	1311.3 AU	1.42 %

Fig 2b. Tribhuvanakeerti rasa

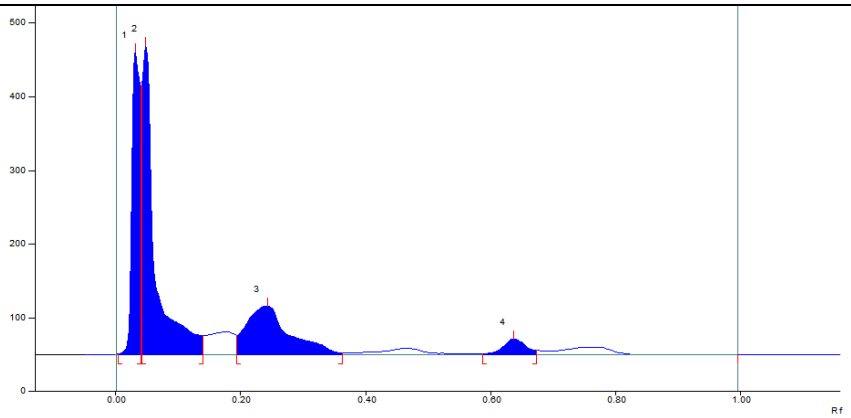
Fig 2: Densitometric Scan of the Samples AT 254NM.



Track 3, ID: Vettumaran gutica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.3 AU	0.03 Rf	307.3 AU	22.62 %	0.08 Rf	52.2 AU	6766.9 AU	15.03 %
2	0.08 Rf	52.5 AU	0.09 Rf	55.4 AU	4.08 %	0.14 Rf	32.1 AU	1705.2 AU	3.79 %
3	0.19 Rf	26.9 AU	0.26 Rf	75.7 AU	5.58 %	0.32 Rf	22.2 AU	4145.0 AU	9.21 %
4	0.35 Rf	16.4 AU	0.41 Rf	34.8 AU	2.56 %	0.46 Rf	21.9 AU	1695.5 AU	3.77 %
5	0.46 Rf	21.9 AU	0.47 Rf	22.5 AU	1.66 %	0.51 Rf	4.8 AU	520.9 AU	1.16 %
6	0.55 Rf	2.7 AU	0.63 Rf	69.1 AU	5.08 %	0.68 Rf	14.2 AU	2304.0 AU	5.12 %
7	0.68 Rf	14.4 AU	0.75 Rf	793.5 AU	58.42 %	0.88 Rf	0.0 AU	27884.5 AU	61.94 %

Fig 3a. Vettumaran gutica



Track 4, ID: Tribhuvanakeerti rasa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.3 AU	0.03 Rf	410.9 AU	44.78 %	0.04 Rf	63.6 AU	3966.2 AU	28.13 %
2	0.04 Rf	366.5 AU	0.05 Rf	419.0 AU	45.66 %	0.14 Rf	25.8 AU	6289.1 AU	44.61 %
3	0.19 Rf	26.4 AU	0.24 Rf	66.3 AU	7.22 %	0.36 Rf	2.3 AU	3280.5 AU	23.27 %
4	0.59 Rf	1.2 AU	0.64 Rf	21.4 AU	2.33 %	0.67 Rf	5.7 AU	563.3 AU	4.00 %

Fig 3b. Tribhuvanakeerti rasa

Figure 3: Densitometric Scan of the Samples AT 366NM.

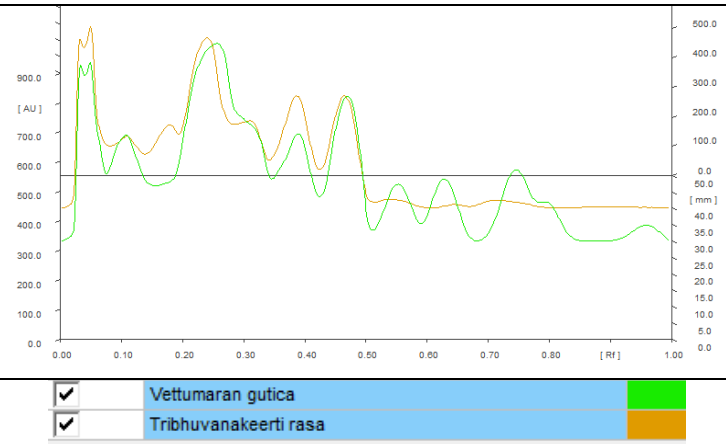


Fig 4a. At 254nm

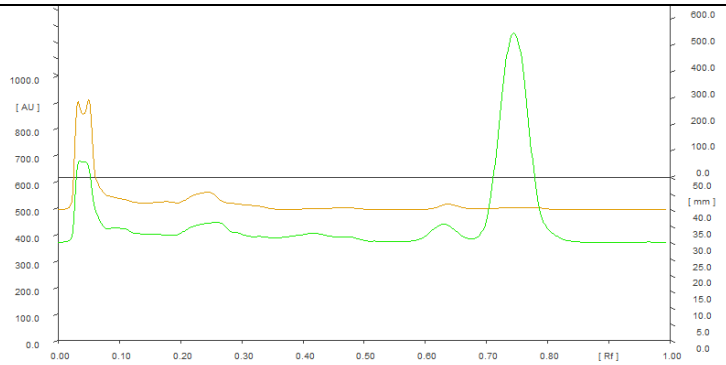


Fig 4b. At 366nm

Figure 4: 4: 3-D Chromatogram.

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