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HPTLC Fingerprinting in the Standardization of *Panchavaktra Ras:* A Herbo-Mineral Preparation

Research article

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Abstract

High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements. Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One recent approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample. The assay combines the separation and quantification of the analyses on silica gel HPTLC plates with visualization under UV and scanning. Using this technique, the alkaloid content of different parts of the drug *Panchavaktra ras* finger print profile has been determined for the 3 experiments in the different times.

HPTLC of *Panchavaktra ras* is the preliminary quantitative analysis which shows the number of components present in the sample accurately and precisely on the basis of mild variations in Rf values, that can acceptable in this drug and it indicates the purity of drugs. The UV spectrum of the common peaks with large abundance appeared at Rf 0.46, 0.33, 0.28, 0.30, 0.41, 0.49, 0.56, 0.57, 0.59, 0.99 were found to be super imposable revealing the presence of the same constituents in all the 3 samples.

Keywords: Standardization, HPTLC, *Panchavaktra ras*, Herbo-Mineral Preparation.

Introduction:

The *Ayurveda*, *Unani* and *Sidha* system of medicines have been in practice since thousands of years. The need of quality control for those medicines is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of *Ayurvedic* pharmacy during past era (1).

The concept of quality in those days was based on physical aspects of the plant materials such as identification, colour, odour, size, type, age, etc. Today there are additional requirements, distinct in nature, for modern routine quality control of botanical raw materials, in addition to physical tests and identification i.e. chemical composition. The Govt. of India has adopted the "fingerprint" approach (2) for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication. The British Pharmacopoeia (3) has had an emphasis on using TLC and HPLC profiles to identify characteristic and active principles of herbal materials.

Panchavaktra ras, an *Ayurvedic* herbo-mineral formulation, consists of *Parada*, *Gandhaka*, *Tankana*, *Pippali*,

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Marica then *Krshna dhattura svarasa* as a *Bhavana dravya* and a final product as a Vati (tablet) form. It is one of the formulations mentioned in *Amavata* (rheumatoid arthritis). Therefore, an attempt has been made to standardize *Panchavaktra ras*, a *Ayurvedic* compound formulation based on their HPTLC fingerprint profile.

Figure 1: *Panchavaktra ras* preparation

Figure 2: HPTLC Apparatus

Material and Methods:

Drug:

The present study signifies the use of TLC, HPTLC fingerprint profiles for deciding the identity, purity and strength of the *Panchavaktra ras* a herbo-mineral formulation using HPTLC and also standardization and authentication for fixing standards for this *Ayurvedic* formulation.

Apparatus:

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttenz, Swizerland)

Syringe: 100µL Hamilton (Bonadug, Swizerland)

TLC chamber: Glass twin trough chamber (20× 10× 4).

Densitometer: TLC scanner 3 with CATS software; CAMAG

HPTLC Plate: 0.2 mm thickness and 10 cms length, 0.2 pre coated with silica gel $60F_{254}$; Merck, Germany

pH meter: Elico Ltd., Hyderabad, India.

Flame Photometer: Digital Biomed Flame Photometer, Hyderabad.

Muffle furnace: Dolphin Industries Ltd., Mumbai.

Place of Study: LAILA Impex, Vijayawada, Andhra Pradesh.

Herbo mineral materials:

Panchavaktra ras consists of equal parts of

1. *Purified Parada* (4) (Mercury)
2. Purified *Gandhaka* (5) (Sulphur)
3. Purified Tankana (6) (Borax)
4. *Barjita pippali* (dried fruit of Piper longum)

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5. *Barjita marica* (dried fruit of Piper nigrum)
6. *Bavana* (maceration) with the leaf juice of *krshna dhattura* (Black coloured leaf of Datura metel)

All these ingredients were procured from the local market of Vijayawada, Andhra Pradesh, India and all the herbal and mineral material were thoroughly screened by experts of *Rasasastra*, Dr.N.R.S.Govt.Ayurvedic College, Vijayawada based on the *Grahya lakshanas* mentioned in the classics.

Preparation of *Panchavaktra ras*:

Panchavaktra ras was a *Khalviya rasayana* which was mentioned in the classical text of *bhasavarajiyam* 6th chapter of *Vataroga nidana lakshana cikitsa adhyaya* (7) and indicated for the *Amavata* (Rheumatoid arthritis). The ingredients number 1 to 3 was purified with the authentic mentioned. The ingredients number 4 and 5 fried an earthen pan on a mild flame and powdered individually and passed through 80# sieve. In the first *kajjali* (black sulphide of mercury) prepared with the equal parts of purified *Parada* and *Gandhaka* in *Khalva yantra* (mortar pestle apparatus).

All the ingredients were mixed thoroughly in specified ratio (1 part each) and ground in the *Khalva yantra* with the leaf juice of *Krshna dhattura* to obtain a homogeneous blend. The blended mass was dried in shade. Then added starch, binding agents and lubricants (quantity mentioned in table no.1) according to the drug quantity and made tablets through the punch machine fitted with suitable die. The rolled tablets were dried in a tray-dryer at a temperature not exceeding 60°C. It was packed in a tightly closed glass containers for further use. The final product of *Panchavaktra ras* was found to be a dark gray coloured. The same above the process this drug was prepared by the three times in different seasons.

Table. No 1: Preparation and Observation of *Panchavaktra Ras*

Ingredients	Expt-1	Expt-2	Expt-3
Shodhita Parada	300 gm	250 gm	200 gm
Shodhita Gandhaka	300 gm	250 gm	200 gm
Shodhita Tankana	300 gm	250 gm	200 gm
Pippali powder	300 gm	250 gm	200 gm
Maricha powder	300 gm	250 gm	200 gm
Total Qty. of Drug before *bhavana*	1500 gm	1250 gm	1000 gm
Qty. of *Dhattura patra* juice used for *bhavana*	2000 ml	1600 ml	1245 ml
Total Qty. of drug after *bhavana*	2520 gm	2100 mg	1700 gm
Total Qty. Of drug after drying	1925 gm	1600 gm	1300 gm
Quantity of Starch added	50 gm (2.6 %)	121 gm (7.6%)	98 gm (7.6%)
Quantity of binding agents added	192 gm (10%)	64 gm (4 %)	65 gm (5 %)
Quantity of Lubricants added (magnesium stearate, Talc, Aerosol)	77 gm (4%)	64 gm (4 %)	52 gm (4%)
Total weight of Drug mass	2245 gm	1849 gm	1515 gm
Weight of the tablet	150 mg	150 mg	150 mg
Total no of Tablets	15000	12000	10000
Date of commencement	13-11-2006	26- 02-2007	25-02-2008
Date of completion	17-11-2006	01-03-2007	31-02-2008

High Performance Thin Layer Liquid Chromatography (HPTLC):

Russian botanist Tswett discovered chromatography. In 1903 he succeeded in separating leaf pigments using a solid polar stationary phase. Chromatography (Greek means "Writing in colours") is a method used basically for the separation of the components in a sample. Chromatography is an analytical method that finds wide application for the separation, identification and determination of chemical components in complex mixtures. This technique is based on the separation of components in a mixture (the solute) due to the difference in migration rates of the components through a stationary phase by a gaseous or liquid mobile phase.

HPTLC Methodology:

HPTLC is an advanced versatile chromatography technique. It provides chromatography drug finger print. It is therefore suitable for monitoring the identity and purity of drugs.

In HPTLC various steps involved are 1. Sample application 2. Chromatographic development 3. Detection of spot 4. Quantification 5. Documentation

Sample Application:

One gram of *Panchavaktra ras* dissolved in a 20 ml methanol solvent and the sample is applied on pre-coated TLC plates with the automatic applicator.

Chromatographic development (separation):

The chromatogram is developed after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers were used for TLC developments.

Detection of spots:

The spots are detected in U.V at wavelength 254 nm by spraying the dragendroff reagent and Sodium nitrite. The spots of non fluorescent compounds are also observed - fluorescent stationary phase used by the silica gel GF.

Quantification and Documentation:

Camag TLC scanner scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode scanning speed is selectable up to 100 mm/s. The portion of the scanned peaks on the recorder charts is related to Rf values of the spots on the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

HPTLC finger printing profile:

Sample preparation:

1 gm of prepared sample of *Panchavaktra ras* tablets into a 50 ml round bottom flask. Add 20 ml methanol and reflux on water bath (80°C) for 30 mints. Filter (what's man filter paper No. 41) and evaporate the filtrate on a water bath to a soft paste and reconstitute in 10 ml of methanol.

Instrument method:

Absorbent – Pre-coated Silica Gel 60 F_{254} plate, 0.2mm thickness and 10 cms length (Merck, Germany)

Mobile phase – Toluene Ethyl Acetate Formic acid (50:15:5)

Visualizations – Five dark spots are observed between Rf 0.25 to 0.98 under UV light 254 nm

Development – Twin through chambers

Application position – 10 ml

Solvent front position – 76.7 mm

Volume of the sample applied – 5 micro liters and 10 micro liters

Detection – By U.V Detection at wavelength 254 nm by spray with the dragendroff reagent + $NaNo_2$

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Results and Discussion:

***Panchavaktra ras* sample-1:**

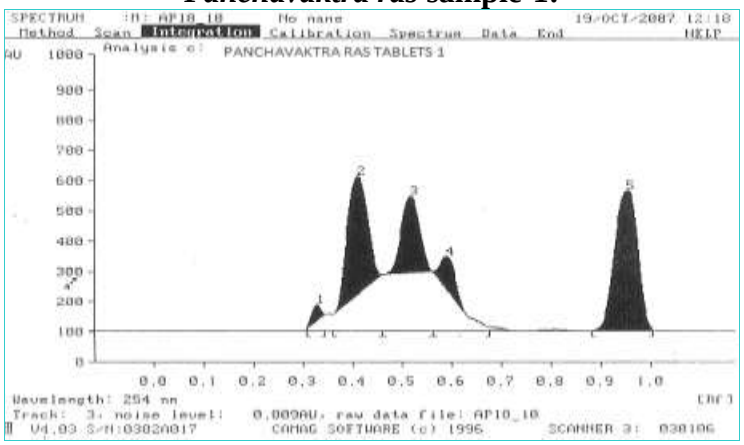

Figure 3: HPTLC fingerprinting of *Panchavaktra ras* sample 1

peak	start		Max			End		Area	
	R_f	H	R_f	H	%	R_f	H	F	%
1	0.31	00	0.33	54.5	4.32	0.35	0.0	506.1	1.88
2	0.36	0.0	0.41	387.0	30.69	0.46	0.0	8468.9	31.45
3	0.46	0.0	0.52	249.7	19.80	0.57	0.0	4865.5	18.07
4	0.57	0.0	0.60	112.1	8.89	0.68	0.0	1802.6	6.69
5	0.89	0.0	0.96	457.7	36.30	1.01	0.0	11286.6	41.91

Total height – 1261.1 Total area = 26929.7

Under the 254 nm wavelength- 5 spots were detected and starts with respect to retardation factor 0.31, 0.36, 0.46, 0.57, 0.89. These 5 spots were maximally reached to retardation factor 0.33, 0.41, 0.52, 0.60, 0.96 and these spots end at the Rf 0.35, 0.46. 0.57, 0.68, 1.01.

***Panchavaktra ras* sample-2:**

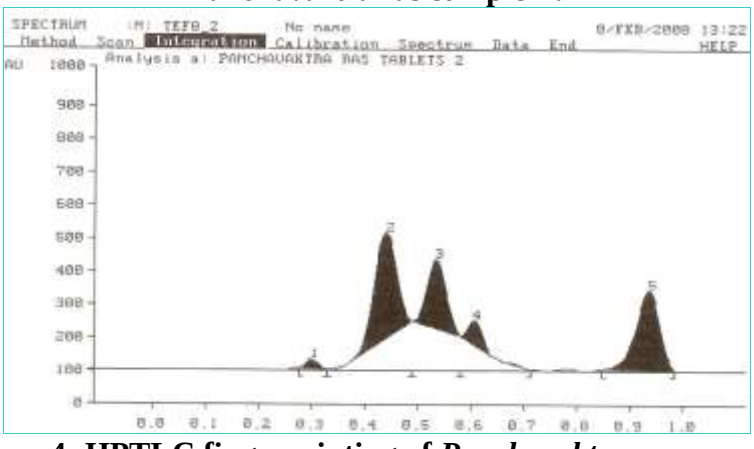

Figure 4: HPTLC fingerprinting of *Panchavaktra ras* sample 2

peak	start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.28	3.7	0.30	28.2	3.30	0.33	0.0	358.0	1.91
2	0.33	0.0	0.44	314.2	36.68	0.49	0.0	7393.9	39.35

3	0.49	0.0	0.54	204.1	23.83	0.59	0.0	4024.8	21.42
4	0.59	0.0	0.61	72.4	8.45	0.72	0.0	1122.8	5.98
5	0.85	0.0	0.94	237.6	27.74	0.99	0.0	5891.5	31.35

Total height – 856.6 Total area = 18790.9

Under the 254 nm wavelength- 5 spots were detected and starts with respect to retardation factor 0.28, 0.33, o.46, 0.49, 0.59, 0.85. These 5 spots were maximally reached to retardation factor 0.30, 0.44, 0.54, 0.61, 0.94 and these spots end at the Rf 0.33, 0.49. 0.59, 0.72, 0.99.

***Panchavaktra ras* sample-3:**

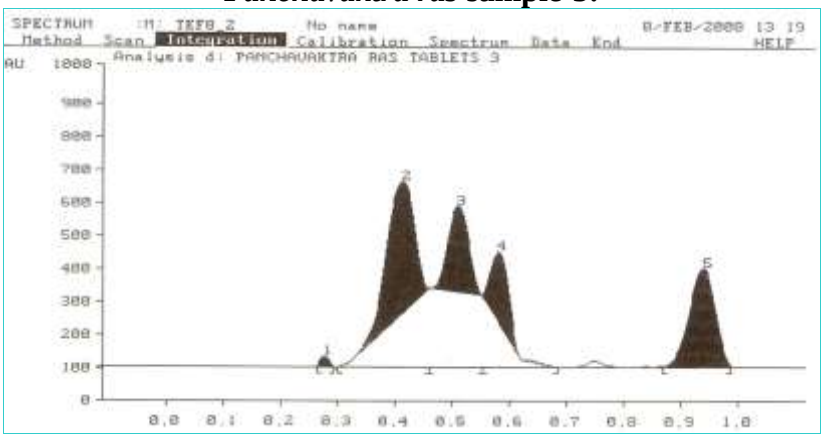

Figure 5: HPTLC fingerprinting of *Panchavaktra ras* sample 3

peak	start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.27	0.0	0.28	30.0	2.53	0.29	0.0	232.1	0.87
2	0.30	0.0	0.41	394.8	33.22	0.46	0.0	10601.8	39.53
3	0.46	0.0	0.51	255.3	21.49	0.56	0.0	5067.1	18.89
4	0.56	0.0	0.59	216.0	18.18	0.69	0.0	3663.0	13.66
5	0.88	0.0	0.95	292.1	24.58	0.99	0.0	7257.7	27.06

Total height = 1188.3 Total area = 26821.8

Under the 254 nm wavelength- 5 spots were detected and starts with respect to retardation factor 0.27, 0.30, 0.46, 0.56, 0.88. These 5 spots were maximally reached to retardation factor 0.28, 0.41, 0.51, 0.59, 0.95 and these spots end at the Rf 0.29, 0.46. 0.56, 0.69, 0.99.

Conclusion:

HPTLC of *Panchavaktra ras* is the preliminary quantitative analysis which shows the number of components present in the sample accurately and precisely on the basis of mild variations in Rf values, that can acceptable in this drug and it indicates the purity of drugs.

The UV spectrum of the common peaks with large abundance appeared at Rf 0.46, 0.33, 0.28, 0.30, 0.41, 0.49, 0.56, 0.57, 0.59, 0.99 and were found to be super imposable revealing the presence of the same constituents in all the samples (Figure 1-3). The small variation in non-matching of the spectra may be due to the changes in the concentration of the spot in the powder samples. *Panchavaktra ras*, a *Ayurvedic* herbo mineral formulation of 5 ingredients was prepared in the clinical study and three samples HPTLC finger

print profile developed from the study can be considered for pharmacopial standards.

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