

A novel and rapid method for formulation and simultaneous evaluation of polyherbal tablet for hypothyroidism by HPTLC

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Abstract

Herbal Pharma industry is rapidly expanding its demand in national as well as international market. Due to lack of pharmaceutical and analytical validation of herbal products, quality of herbal products differs from batch to batch. Pharmaceutical and analytical validation can be achieved, if the herbal products are evaluated and analyzed using both herbal as well as modern techniques of standardization; in process and after preparation of finished product. A fast, rapid, sensitive and stability indicating high-performance thin-layer chromatographic (HPTLC) method is developed and validated for qualitative estimation of Patol (*Trichosanthes dioica*), Indrajav (*Holarrhena antidysenterica*), Kanchnar (*Bauhinia variegata*), Shigru (*Moringa oleifera*), Varun Chal (*Crataeva nurvala*) and Trikatu (*Piper longum*, *Piper nigrum* and *Zingiber officinalis*) simultaneously in pharmaceutical herbal formulation (Tablet). Chromatographic separation of the herbs is performed on aluminium backed plates coated with silica gel 60F₂₅₄ as the stationary phase and the solvent system consisted of *n*-Hexane: *n*-Butanol: Toluene: Ethyl Acetate (5:5:3:2). Samples were applied with Linomat V under nitrogen gas flow. The densitometric analysis was performed CAMAG TLC scanner 4 at 300nm. All the three samples prepared by this method shows identical characteristics and analytical parameters does not show significant difference. Also the observations of analytical study show similarity to those parameters which are already set in API for this drug.

Keywords: Herbal products, HPTLC, Hypothyroidism.

1. Introduction

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Medicinal plants are great importance to the health of individuals and communities in general [1]. Herbal formulations are becoming more and more popular amongst human population. Various classical and instrumental methods are available for determining the quality of the drug. However, instrumental methods are preferable as they are more reliable. The HPTLC is the most widely used quick, effective and low-cost instrumental method for the rapid separation, identification and quantification of the plant constituents, herbal drugs and herbal drug preparations. As TLC provides the chromatographic drug finger prints. It is therefore suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. TLC is widely

adopted for the rapid and positive analysis of plant drugs. The special advantage of TLC is its sensitivity, speed, versatility and low-cost per analysis. Sensitivity allows less than micrograms amount of the raw material and versatility for a great number of different organic and inorganic solvents available commercially. Also the time required for the screening Demonstration is very short. Hence, for identifying the ingredients present in the formulations, HPTLC technique is very suitable. If isolated markers are not available, the corresponding raw material or extract powder, used in the formulation can be used as working reference standard [2-3].

2. Material and Methods

2.1 Collection and Authentication of raw herbs:

Herbal ingredients were collected from the authentic source at Nagpur. All herbal raw materials were www.ssjournals.com

authenticated by pharmacognosy lab at Unijules Life sciences Limited Nagpur. Analysis was carried out by employing different physico-chemical parameters for raw materials. Three batches of this formulation were prepared for its authentication purpose viz. Batch A, Batch B and Batch C.

2.2 Ingredients of Hypothyroidism tablet and their Pharmacological properties

Each batch of 1000 Tablets was prepared which contains.

Contents	Latin name
Patol	: <i>Trichosanthes dioica</i>
Indrajav	: <i>Holarrhena antidysenterica</i>
Kanchnar	: <i>Bauhinia variegata</i>
Shigru	: <i>Moringa oleifera</i>
Varun Chal	: <i>Crataeva nurvala</i>
Trikatu	: <i>Piper longum, Piper nigrum, Zingiber officinalis</i>

2.2.1 Patola (*Trichosanthes dioica*):

Patola is known as *Trichosanthes Dioica*. Generally, it is famous with the name of pointed gourd. This is one of the most nutritious cucurbit vegetables that have a great, big reputation in the market of India, during rainy and summer seasons. The plant is of perennial, nectarous. Plant grows like a vine. Vines are of thick size, pencil like structure which contains ovate, oblong, cordate, unlobed, rigid, dark green color leaves. Flowers are of white color with a tubular appearance. The male flowers are not strobile. Fruits generally are of size 5 to 9 cm and of spherical orange red wine color after ripe.

This plant contains various important phytochemical constituents that help plant patola to combat several diseases. Roots of the plant contain hentriacontane, trichosanthin, saponin, fixed oil; starch Colocynthin, essential oil containing terpens, reducing sugar and trace of tannin. Stems of the patola contain fatty acids like linoleic, oleic and oleo stearic or trichosanic acid and Cucurbita-5, 24-dienol which also found in mature plant [4].

2.2.2 Indrajav (*Holarrhena antidysenterica*):

Holarrhena Antidysenterica is a medicinal plant that has antidysenteric, antidiarrheal, and anti-amoebic properties. In Ayurveda, it is used for the treatment of amoebic dysentery, diarrhoea, irritable bowel syndrome, bleeding piles, and liver disorders [5].

Digestive Disorders: Both the bark and fruits of this plant are very useful in relieving digestive disorders like constipation, dysentery, diarrhea, stomach aches, gas etc [6-7].

2.2.3 Kanchnar (*Bauhinia variegata*):

All parts of tree are used in Ayurveda for treatment of wide variety of ailments and especially for **Hypothyroidism** and glandular enlargements [8]. *Bauhinia*

variegata or *kachnar* is used as astringent and for treating a number of skin conditions such as ulcer, rashes, leprosy and its bark can be taken as decoction. The herb works in case of bleeding piles or blood in urine. It is used as natural treatment for Hypothyroidism problems as it can cure goiter [9]. It is also a specific herb for hypo/hyper Hypothyroidism. It has a balancing activity on the thyroxine production; increasing any deficient production and decreasing any excess. It also clears swellings in the neck and goiter [10].

2.2.4 Shigru (*Moringa oleifera*):

Moringa oleifera belongs to family Moringaceae. It is also commonly known as *sahjana*, *sainjna* in Hindi, *sajina* in Bengali, *ben oil tree*, *miracle tree* and *mothers best friend*, 'Drumstick' and *horse radish tree* in English. It is medium sized tree about 10-12m height. It is considered to be rich in proteins, vitamins, minerals, folic acid and β -carotene. Every plant part such as leaves, roots, fruits, flowers, bark is used as food having high nutritional value. *Moringa oleifera* is used as a drug in the treatment of asthma [11] and possess the antidiabetic [12] antianaphylactic [13], antimicrobial [14], antioxidant [15] anti-bacterial, antibiotic, anti-inflammatory, anti-Hypothyroidism, antiulcer, antispasmodic, cholesterol-lowering, anti-HSV, antifungal, diuretic, antihypertensive, hepatoprotective, antitumor [16] activity.

2.2.5 Varun chal (*Crataeva nurvala*):

Crataeva Nurvala is a medium sized deciduous tree. It belongs to the family Capparidaceae. Its bark is smooth and brown in color. Its bark contains horizontal wrinkles. Its branches processes white patches with purple and yellow lines. The leaves of Varuna are trifoliate. Leaves grow up to 12 cm with oval leaflets [17]. *Crataeva nurvala* improves the functioning of kidney and is believed to be effective in dissolving stones and getting rid of infections in the urinary system. It can also reduce pain and inflammation. Researchers believe it can improve the suppleness of tissues and promote blood circulation to provide natural treatment for Hypothyroidism problems. It can be taken to prevent disorders of prostate glands. *Acorus calamus* is another herb used in the treatment of Hypothyroidism [18].

2.2.6 Trikatu (*Piper longum, Piper nigrum, Zingiber officinalis*):

Trikatu powder contains three spices in equal proportions. Black pepper – *Piper nigrum* Long pepper fruit – *Piper longum* Ginger – *Zingiber officinalis*. If these spices are available, the Trikatu powder can be made at home, under hygienic conditions, using a mixer [19]. Trikatu Churna is an herbal formulation used in ayurveda for enhancing effects and increasing the absorption of ayurvedic medicines. It has hot potency, which means its

intake results in production of heat in the body and increase digestive juices and bile salt secretion. Trikatu Churna has primary effect on stomach and increases digestive juices, which helps stimulating digestion. It also has influence on liver and increases production of bile salts. It stimulates secretion of bile from the gall bladder. It stimulates pancreas functions. Therefore, it affects overall digestive system [20].

2.7 Formulation procedure:

All the above ingredients of table No. 1 were mixed homogenously in mass mixer. Excipients were added

to the mixture for proper binding of the tablets. Buff colored circular Hypothyroidism tablet 600mg was prepared in automatic tablet making machine. Weight after each pharmaceutical process was noted to observe the processing loss. About total 1000 to 1100 tablets were obtained from each sample batch A, B and C. Tablets were packed in air tight bottle and stored in cool dry place. All hygienic conditions were maintained during preparation of Hypothyroidism Tablet. Above detailed procedure was adopted for preparation of three different (sample A, sample B, sample C) batches of Hypothyroidism Tablets.

Modern parameters:

Test	Sample A	Sample B	Sample C
Description	Buff colored circular, compressed flat uncoated tablets.	Buff colored circular, compressed flat uncoated tablets.	Buff colored circular, compressed flat uncoated tablets.
Average weight	600.0mg \pm 5%	600.0mg \pm 5%	600.0mg \pm 5%
Uniformity of weight	Complies	Complies	Complies
Diameter	12.2 mm	12.3 mm	12.2 mm
Thickness	4.14mm	4.17 mm	4.11 mm
Hardness	3.00Kg/sq.cm	2.87Kg/sq.cm	2.82Kg/sq.cm
Friability	0.22%w/w	0.43%w/w	0.59%w/w
HPTLC	Complies	Complies	Complies
Disintegration	11 min to 12 min	10min to 11 min	11 min to 13 min

2.8 HPTLC Instrumentation:

2.8.1 Apparatus:

The HPTLC system CAMAG, Switzerland consisted of Limomat V auto-sprayer connected to a nitrogen cylinder, a twin trough chamber (10 \times 10 cm), a derivatization chamber, and a plate heater. The HPTLC plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. The samples were spotted in the form of bands 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland), Precoated silica gel 60 F254 TLC plates (10 \times 10 cm, layer thickness 0.2mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. The length of solvent front position was 90.0mm from the base. After that TLC plates were dried in a current of air, followed by heating on Camag HPTLC plate heater III at 60°C for about 5minutes. Densitometric analysis was carried out using a TLC scanner III with winCATS software. The detector used was a Deuterium lamp emitting a continuous UV spectrum between 200-400 nm.

2.9 Samples preparation:

2.9.1 Reference Samples Preparation:

Take and reflux all raw herbs (separately) equivalent of labeled claim of Hypothyroidism Tablet in 250ml iodine flask with 30ml methanol for 45minutes, cool and filter. The remaining residue was refluxed again twice with 15 ml of methanol for 25 min. Again cool, filter and combine the washing. Evaporate on water bath and dissolve the residue with 5ml methanol in volumetric flask.

2.9.2 Test Sample Preparation:

Weigh and powder 20 tablets. Weigh accurately about 600.0 mg of powder tablets in 250 ml of iodine flask add 30ml of methanol and reflux for 30mins. Then allow it to cool & filtered; further reflux the content of the flask with 2x20 quantity of methanol; each time filter and combine methanol washing and allow it to dry on water bath. Dissolve the residue with 5 ml of methanol in volumetric flask.

2.9.3 Chromatographic condition:

Stationary phase	- HPTLC Precoated, silica gel 60, F ₂₅₄ (Merck)
Thickness	- 0.2 mm
Mode of application	- Band
Sample Applicator	- Linomat 5
Band width	- 6 mm
Solvent front pos.	- 85 mm
Solvent volume	- 10 ml
Drying device	- TLC plate Heater 3
Slit dimension	- 5.0 x 0.45 mm
Scanning speed	- 10 mm/s
Data resolution	- 100 μ m/step
Scanning wavelength	- 300 nm
Visualization Aid	- Through UV-Cabinet under 254nm & 366 nm and under day light also.
Measurement mode	- UV absorbance/reflectance
Separation technique	- Ascending
Scanning mode	- Single level
Sample applied	- 10 μ l

2.9.4 Mobile phase Composition:

Mobile phase - *n-Hexane: n-Butanol: Toluene: Ethyl Acetate (5:5:3:2)*.

2.9.5 Preparation of Post Chromatographic Derivatization agent:

1% Ethanolic vanillin and 10% Ethanolic sulphuric Acid were prepared separately and mixed just before spraying.

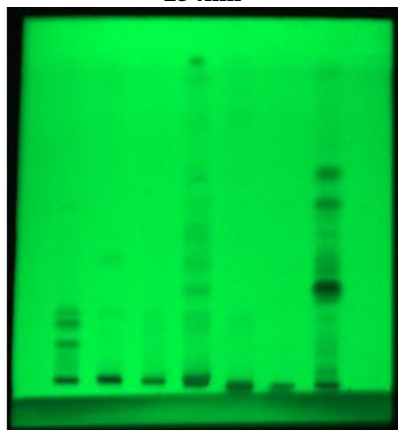
2.10 HPTLC of hypothyroidism tablet:

All three samples batches of this formulation were studied for HPTLC study and spots were observed. For Study 10µl layer was applied on HPTLC plate and the plate was developed to a distance of 9cm using *n-Hexane: n-Butanol: Toluene: Ethyl Acetate (5:5:3:2)* ratio as mobile phase. After development the plate allowed to dry air and examine under UV light. It shows following results.

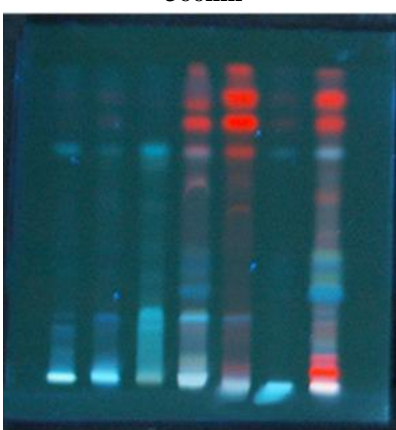
S.N	Sample name	Rf Value												
1.	Patol		0.01	0.09	0.15	0.20			0.44	0.49	0.69	0.77		0.88
2.	Indrajav		0.01	0.07	0.13	0.18		0.34		0.54	0.65	0.71		0.89
3.	Kanchnar				0.14	0.19					0.67		0.82	
4.	Hypothyroidism tablet	0.00	0.03	0.09	0.14	0.18	0.24	0.32	0.43	0.49	0.61	0.77	0.82	
5.	Shigru		0.05		0.14	0.19				0.49	0.70	0.78	0.81	0.87
6.	Varun Chal			0.15		0.20	0.24	0.32	0.38		0.70		0.82	
7.	Trikatu		0.03	0.07	0.14	0.17	0.22	0.32	0.43	0.48	0.60	0.69	0.80	

HPTLC images of Hypothyroidism Tablets at:

After Development Wavelengths 254nm



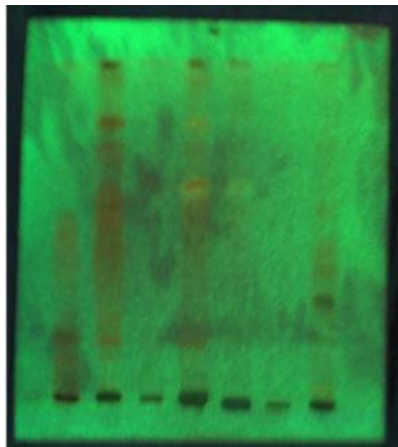
After Development Wavelengths 366nm



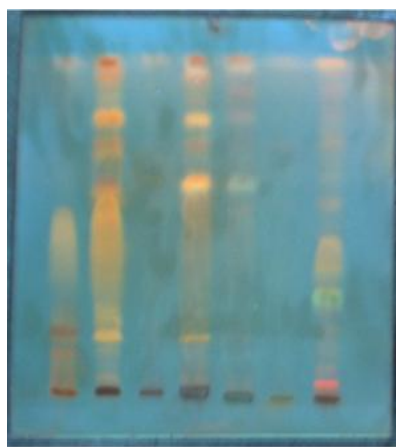
After development White R



After Derivatization At 254

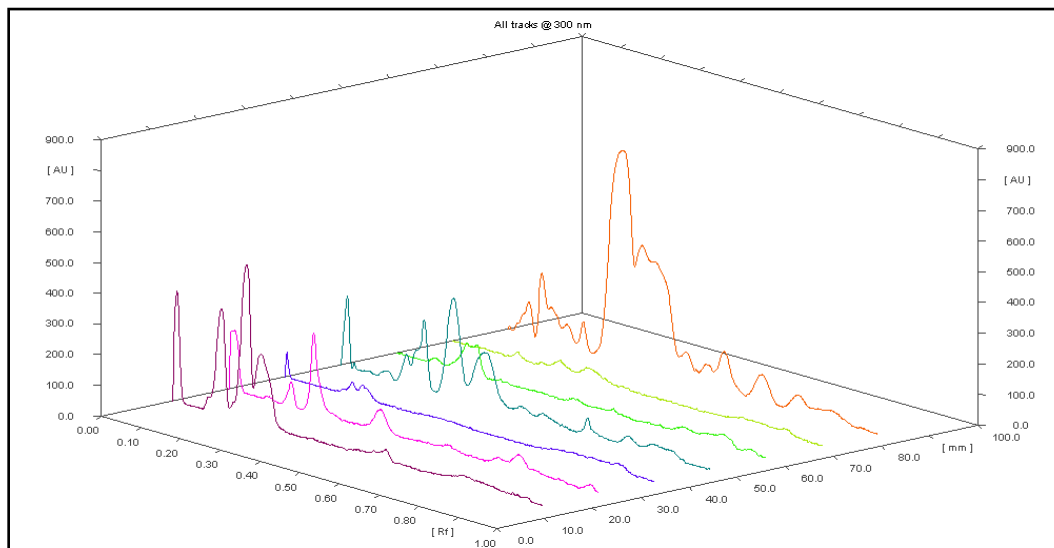


After Derivatization At 366

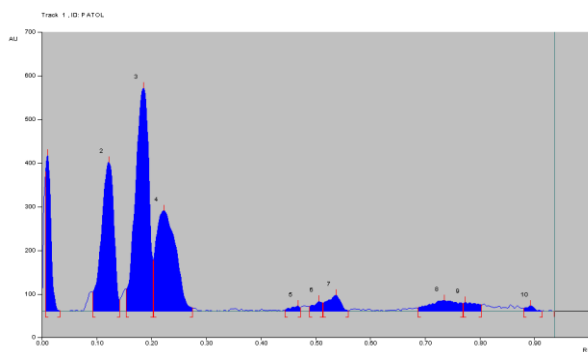


After Derivatization White R

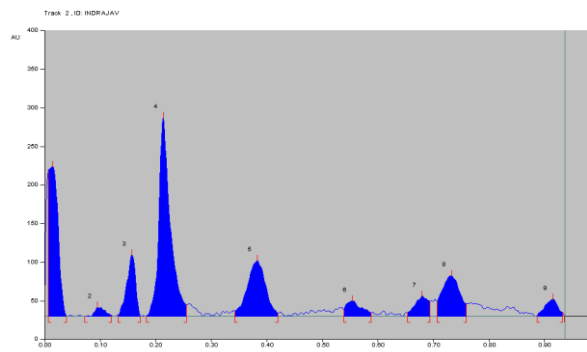




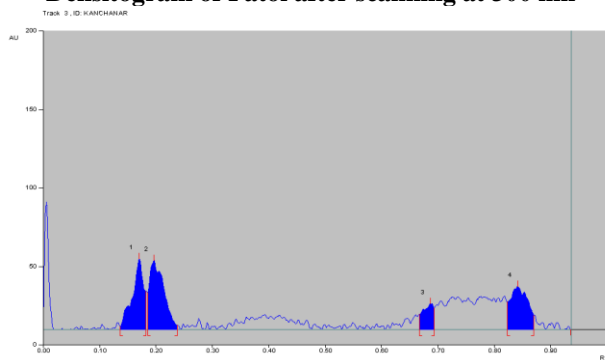
Chromatogram of Hypothyroidism tablet comparison with raw herbs



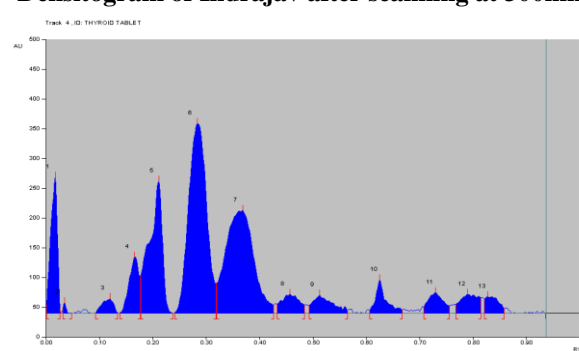
Densitogram of Patol after scanning at 300 nm



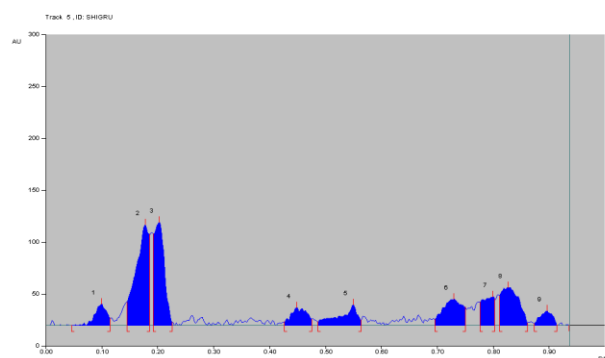
Densitogram of Indrajav after scanning at 300nm



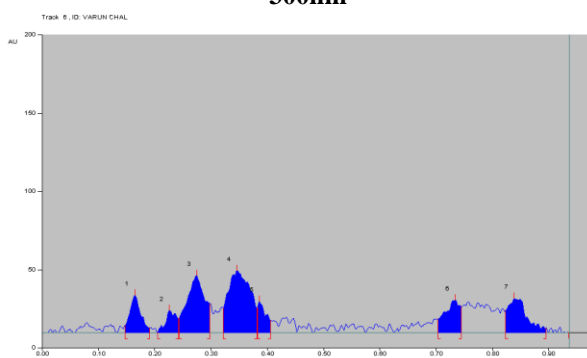
Densitogram of Kanchnar after scanning at 300nm



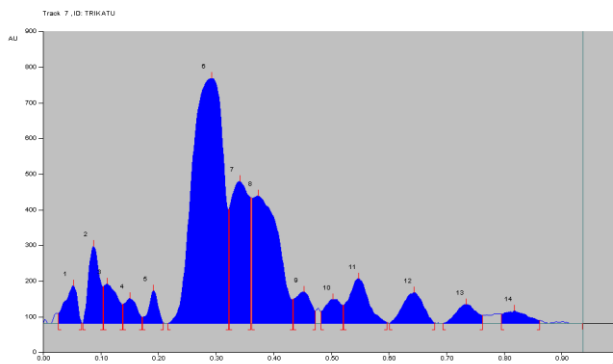
Densitogram of Hypothyroidism Tablet after scanning at 300nm



Densitogram of Shigru after scanning at 300nm



Densitogram of Varun Chal after scanning at 300nm



Densitogram of Trifala after scanning at 300nm

3. Conclusion

The developed HPTLC method for the qualitative evaluation of Patol (*Trichosanthes dioica*), Indrajav (*Holarrhena antidysenterica*), Kanchnar (*Bauhinia variegata*), Shigru (*Moringa oleifera*), Varun Chal (*Crataeva nurvala*) and Trikatu (*Piper longum*, *Piper nigrum* and *Zingiber officinalis*) was found to be specific, accurate and precise and can be used for the qualitative simultaneous evaluation of raw herbs of Patol (*Trichosanthes dioica*), Indrajav (*Holarrhena antidysenterica*), Kanchnar (*Bauhinia variegata*), Shigru (*Moringa oleifera*), Varun Chal (*Crataeva nurvala*) and Trikatu (*Piper longum*, *Piper nigrum* and *Zingiber officinalis*); also in its poly herbal formulations and in quality control labs.

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