International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Suppl 4, 2010

Research Article

HPTLC METHOD FOR THE DETERMINATION OF PLUMBAGIN FROM *PLUMBAGO ZEYLANICA* LINN. (ROOT)

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Received: 01 September 2010, Revised and Accepted: 30 September 2010

ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Plumbagin in different samples of *Plumbago zeylanica* Linn. root. The chloroform extract of *Plumbago zeylanica* Linn. root samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF_{254} and developed using Toluene : Ethyl acetate (3:1) v/v as a mobile phase. The plate was sprayed (derivatized) with Anisaldehyde-Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and detection and quantification were carried out densitometrically using an UV detector at wavelength of 270 nm. Content of marker compound in the samples were found similar.

Keywords: Plumbagin, *Plumbago zeylanica* Linn. Root., Chitrak root, Chitrakmul, HPTLC.

INTRODUCTION

Plumbago zeylanica Linn Syn. *Plumbago rosea* Linn (Family-Plumbaginaceae) known vernacularly as Chitrak, Chitra, Chitraka, Chitrakmul, Agni, Pathi, Ushana, Chita, Chitramulam, Ceylong Leadwort or white Leadwort is found wild in the tropics, subtropics and throughout India including West Bengal, Bihar and peninsular India. It is also widely cultivated as an ornamental plant. It is a much branched shrub with long tuberous root and a striate stem[1-4].

The root and root bark are bitter, stomachic carminative, astringent to bowels, anthelmintic, piles bronchitis, itching, diseases of lever, consumption, ascetics. The root is bitter, laxative, expectorant, tonic, abortifacient, alexipharmic, good appetizer, useful in laryngitis, rheumatism, diseases of spleen, ringworm, scabies. Paste of root with milk, vinegar or salt and water is applied in leprosy and other skin diseases externally. Tincture of root bark is used as an antipatriotic . it acts as a powerful sudorific. Leaves are caustic, vesicant aphrodisiac and good for scabies[5].

Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanolone[5]. Fructose, glucose, invertase and protease isolated from root bark. 3,3'-bisplumbagin, chitranone (binaphthaquinone), droserone, elliptinone, isozeylinone, catechol tannin 8]. Amino acids; β-(2, 3 dihydroxybenzoyl)-butyric acid (plumbagic acid), vanillic acid, 1,2(3)-tetra hydro-3,3'-bisplumbagin, isoshinanolone, dihydrosterone and β- sitosterol also islated from plant [3,8]. Plumbagin shows as anticancer and antitumor activity[5,9]. Aspartic acid, tryptophan, tyrosine, threonine, alanine, histidine, glycine, methionine, hydroxyproline, were isolatedfrom the aerial parts [9, 15].Lupeol and lupenyl acetate have been isolated from the root.

Literature survey reveals that the TLC, HPLC and HPTLC methods are reported but no method as yet is reported for the determination of Plumbagin in *Plumbago zeylanica* Linn., root. A simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of plumbagin in *Plumbago zeylanica* Linn., root powder. This method can be used for phytochemical profiling of *Plumbago zeylanica* Linn., root and quantification of Plumbagin.

MATERIAL AND METHOD

Plant material

The Chitrak root was procured from the Local Market, Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad. One genuine sample also taken from the Museum of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.

Equipments- Cammag Linomat V applicator, Cammag Twin Trough Chamber (size 20x10 cm) with SS lid, Cammag Dipping Chamber, TLC Aluminium pre-coated plate with Silica gel 60 GF_{254} (size 10X10 cm; 0.2 mm thick) E. Merck.

Chemicals- Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid and n-Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (10X10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard Plumbagin procured from Aldrich Chem. Co. Milw, WI 33201 (414-273-3850/19,064-0481-42-5).

Experimental

Sample preparation- 1g of coarsely powdered drug samples were extracted with 10 ml Chloroform for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 10 ml in a volumetric flask.

Standard Preparation- 5mg of standard Plumbagin dissolved in 5ml of Chloroform and made up to 5ml in standard volumetric flask.

Chromatography

TLC Aluminum pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethyl acetate (3:1) V/V as mobile phase. Chloroform extract of samples and Plumbagin standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20x10 cm^2) with SS lid was used for development of TLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization (Fig.1). The plate was scanned before derivatization using Camag TLC Scanner III at wavelength 270nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Method validation and recovery study

To study the accuracy and precision of the proposed method, recovery experiment was carried out. To a fixed amount of chloroform extract of samples, the standard solution of Plumbagin

was added (ratio 9:1 v/v) and total amount of standard Plumbagin were determined. Percent recovery was calculated from the amount of Plumbagin found via graph (Table No. 3).

Linearity of detector response, assay and recovery

In order to establish linearity, standard solution of Plumbagin (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20X10 cm^2; 0.2 mm thick), 2µl, 4µl, 6µl on Track No. S1, S2 & S3 respectively and for assay, 9µl of Chloroform extract of both samples applied on Track No. T1 & T2 and for recovery study, the n-hexane extract of both samples were spiked with standard Plumbagin solution (ratio 9:1v/v) and applied 10µl on Track No. T3 & T4 on the same plate. TLC plates was developed to 8 cm distance above the position of the sample application and removed from the chamber and air dried at room temperature. This HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (Fig.1). The plate was derivatized with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and scanned immediately using Camag TLC Scanner III at wavelength 270nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Plumbagin appeared at R_f. 0.84 (dark grey colour). The peaks, graph and spectra obtained were given in Fig.2 and 3 and R_f. values, colour of bands (Table No.1), quantity of Plumbagin, linearity, standard deviation & regression coefficient found via graph (Table No. 2) and calculated quantity of Plumbagin & % recovery were given in Table No. 3.

RESULTS AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate (3:1) v/v and the active principle Plumbagin resolved as a dark grey colour band at R_f. 0.84 very efficiently from the other components in Chloroform extract of *Plumbago zeylanica* Linn. (root) (Fig.1). Sharp peaks of Plumbagin (Standard and samples) were obtained when the plate was scanned at wavelength 270nm (Fig.2). Quantity of Plumbagin found in samples were obtained automatically (Table No. 2) via graph (Fig.3) and % Plumbagin found in samples and % recovery were calculated (Table No.3). Quantity of Plumbagin found in Local Market Sample, Ghaziabad (U.P.) is 4.9589mg in 1g drug sample (0.49589% w/w) and quantity of Plumbagin found in Museum Sample of PLIM, Ghaziabad is 4.7922 mg in 1g drug sample (0.47922%w/w). The % recovery of Plumbagin in Local Market Sample, Ghaziabad (U.P.) is 99.84% w/w and 99.67%w/w in Museum Sample of PLIM, Ghaziabad (U.P.). The mean % recovery was 99.76%.

The accuracy and reproducibility of the method was established by means of recovery experiment. The mean recovery was close to 100% which indicates the accuracy of the method.

The robustness of the method was studied, during method development, by determining the effect of small variation, of mobile phase composition (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of R_f. or response to Plumbagin was observed, indicating the robustness of the method.

Table 1: TLC Details of CHCl3 Extract of *Plumbago zeylanica* Linn. (Root)

Sr. No.	Detection/ visualization	Citrak Root (Track No. T1, T2, T3 and T4)		Standard-Plumbagin (Track No. S1, S2 and S3)	
		R_f. values	Colour of band	R_f. values	Colour of band
1.	**Under UV**	0.05	grey		
	254 nm	0.10	grey		
		0.46	grey		
		0.84	dark grey	0.84	dark grey
2.	**Under UV**	0.05	light green		
	366 nm	0.10	brown		
		0.25	bright green		
		0.46	bright sky blue		
		0.70	sky blue		
		0.84	red	0.84	red
		0.96	blue		
3.	**After derivatization**	0.05	violet		
		0.10	violet		
		0.15	violet		
		0.46	dark violet		
		0.57	light violet		
		0.65	light violet		
		0.73	light violet		
		0.84	yello	0.84	yellow

Table 2: Quantity applied on plate and values found via graph

Sr. No.	Track No.	Volume of extract and standard solution applied on plate	Quantity of β-Boswellic acid via graph	Linearity & Regression Coefficient and Standard deviation via graph
1.	Track T1	9µl	4.466µg	Y = 1788.136 + 3320.881 * X + -83.331 * X2 r = 0.99999 sdv = 0.00%
2.	Track T2	9µl	4.318µg	
3.	Track S1	2µl	2.000µg	
4.	Track S2	4µl	4.000µg	
5.	Track S3	6µl	6.000µg	
6.	Track T3	10µl	5.459µg -1µg = 4.459µg	
7.	Track T4	10µl	5.307µg -1µg = 4.307µg	

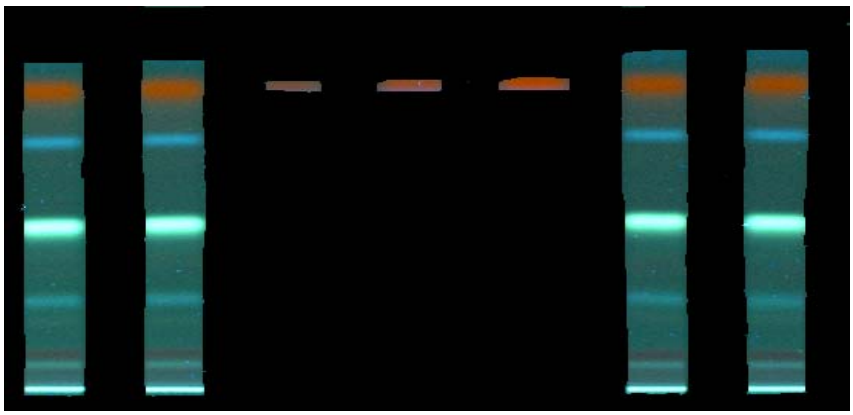
T1- Chloroform extract of Local Market sample, Ghaziabad, T2- Chloroform extract of Museum Sample of PLIM, Ghaziabad, S1- Plumbagin standard solution (1mg/ml), S2- Plumbagin standard solution (1mg/ml), S3- Plumbagin standard solution (1mg/ml), T3- Chloroform extract (spiked with std. solution) of Local Market Sample, Ghaziabad, T4- Chloroform extract (spiked with std. solution) of Museum Sample of PLIM, Ghaziabad

Table 3: Summary of results

Sr. No. ↓	Sample from →	Local Market Sample, Ghaziabad	Museum Sample of PLIM, Ghaziabad
1.	Quantity of Plumbagin in 1g	4.9589mg	4.7922 mg
2.	% Plumbagin	0.4959 % w/w	0.4792 % w/w
3.	% Recovery	99.84% w/w	99.67% w/w

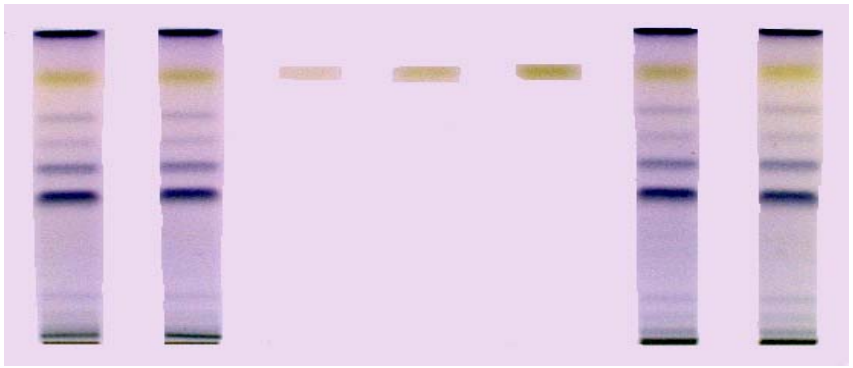
T1 T2 S1 S2 S3 T3 T4

UV-254 nm



T1 T2 S1 S2 S3 T3 T4

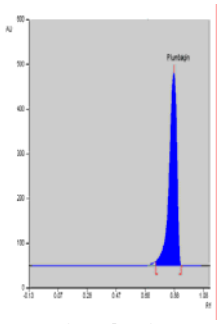
UV-366 nm



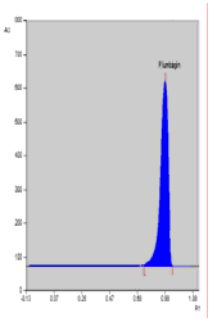
T1 T2 S1 S2 S3 T3 T4

After derivatization

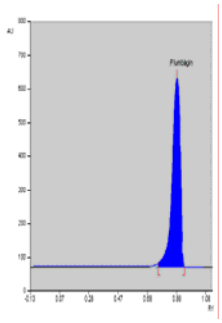
Fig. 1: H.P.T.L.C. Finger print of *Plumbago zeylanica* Linn. (Root)



(Track S1)

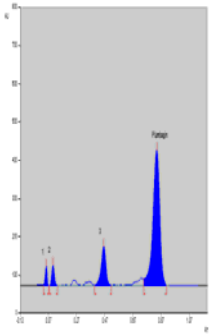


(Track S2)

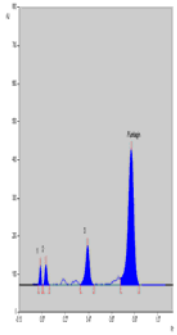


(Track S3)

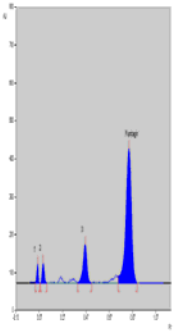
Peaks of Plumbagin @ 270nm



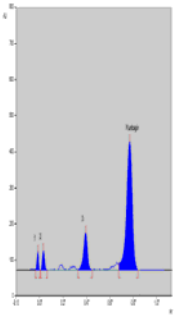
(Track T1)



(Track T2)



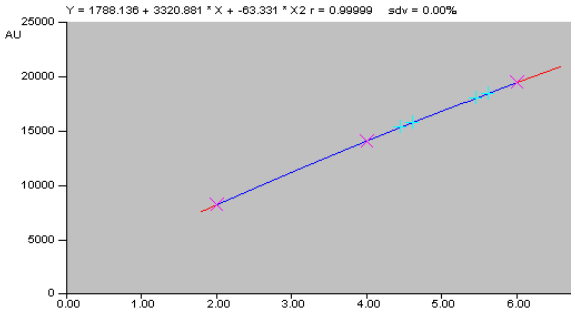
(Track T3)



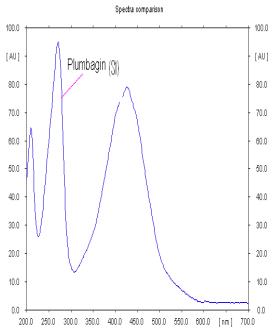
(Track T4)

Peaks of Plumbago zeylanica CHCl3 Extract @270nm

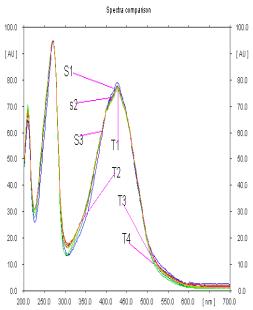
Fig.2: Peaks of *Plumbago zeylanica* Linn. (Root) in all Tracks



Graph Conc. vs AU



Spectra of Plumbagin @270nm



Spectra of Plumbagin in all trcks@270nm

Fig. 3: Graph and spectrra of *Plumbago zeylanica* Linn. (Root)

CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Plumbago zeylanica* Linn. (root) powder and quantitative determination of Plumbagin in root powder.

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