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DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF PLUMBAGIN IN CHITRAK HARITAK – AN AYURVEDIC FORMULATION

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Plumbagin in Ayurvedic formulations of Chitrak haritaki of different manufactures. The alcoholic extract of Chitrak Haritaki and Chitrak Root samples were applied on TLC Aluminum plate pre coated with Silicagel60 GF254 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 110⁰C for 10 minutes and detection and quantification were carried out densitometrically using an UV detector at wavelength of 254 nm. Content of marker compound in the samples were found similar.

KEYWORDS

Chitrak Haritaki, Chitrak Root, Plumbagin, Standardization and HPTLC.

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INTRODUCTION

Chitrak haritaki is a very famous Ayurvedic medicine used in treating chronic respiratory conditions. It is in herbal jam form. It is also known as Chitrak haritaki Avaleha, Chitraka Haritaki etc. Avaleha suggests that it is a herbal jam. Chitraka and haritaki are two herbs, which are the main ingredients of this product.

Chitrak Haritaki Uses

It is used in the treatment of chronic respiratory conditions, Asthma, bronchitis, rhinitis and tuberculosis. It is also used to improve digestion power and to treat bloating and intestinal worm.

Chitrak Haritaki Dose

3 - 6 grams once or two times a day after food with milk. This medicine is quite hot in nature. Hence it is advised to be taken along with milk, which is a coolant and has a calming effect over stomach.

Chitrak Haritaki Ingredients

4.8 liters of decoction is prepared with each of - Chitraka - *Plumbago zeylanica*, Amalaki-*Embellica officinallis*, Guduchi - *Tinospora cordifolias* and Dashamoola. It is added with 4.8 kg of jaggery and 3.072 kg of Haritaki - *Terminalia chebula*. This mixture is heated till semi solid consistency. It is added with Trikatu - pepper, long pepper and ginger - 96 g each, Cinnamon - 96 g, Tejpatra - *Cinnamomum tamala* - 96 g, Yavakshara - 24 g and 384 grams of honey.

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 one of the main ingredient of this formulations and is found wild in the tropics, subtropics and throughout India including West Bengal, Bihar and peninsular India. The dried roots occur as cylindrical pieces of varying length, less than 1.25 cm in width, reddish brown in colour with a brittle, fairly thick, shriveled, smooth or irregularly fissured bark. The roots have a short fracture an acrid and biting taste and disagreeable odour¹⁻¹³.

The root and root bark are bitter, stomachic carminative, astringent to bowels, anthelmintic, piles bronchitis, itching, diseases of liver, consumption and ascetics. It acts as a powerful sudorific. Leaves are caustic, versicant aphrodisiac and good for scabies⁵. Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanone⁵. Elliptinone, isozeylinone, catechol tannin⁸, isoshin anolone, 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 isolated from the aerial parts^{9,15}. Lupeol and lupenyl acetate (Figure No.1) have been isolated from the root¹⁰⁻¹⁸.

STRUCTURE OF PLUMBAGIN

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 and its compound formulations.

EXPERIMENTAL

MATERIALS AND METHODS

(1) The Chitrak haritaki of three different manufactures was procured from the Local Market Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded for further study.

(i) CH1DB (ii) CH2BY (iii) CH3ZB

(2) The Chitrak root was procured from the Local Market, Ghaziabad and also identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded as SD1 for study.

H.P.T.L.C. (High Performance Thin Layer Chromatography)

Equipment

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm²) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

Chemicals and Reagents

Analytical grade; Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Alcohol, 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 GF₂₅₄ (20x10 cm²; 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).

Sample and Standard preparation

Sample preparation

1g of coarsely powdered crude drug and Citrak Haritaki 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. Filtrate was concentrated to 2 ml and used for H.P.T.L.C.

Standard Preparation

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

Chromatography

Procedure

TLC Aluminium pre coated plate with Silica gel60 GF₂₅₄ (20x10 cm²; 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 cm²) 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 110⁰C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under

UV 254 nm, 366 nm and after derivatization (Figure No.2). 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.

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 GF₂₅₄, (20X10 cm²; 0.2 mm thick), 2µl, 4µl, 6µl on Track No.S1, S2 and S3 respectively and for assay, 12µl of Chloroform extract of samples applied on Track No.T1 T2 and T3 and 3µl Chitrak root on Track No.SD1 on the same plate. TLC plate 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 (Figure No.3). The plate was scanned immediately 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. It was observed that Plumbagin appeared at R_f 0.88 (dark grey colour). The peaks, graph and spectra obtained were given in Figure No.3 and 4 and R_f values, colour of bands (Table No.1), quantity of Plumbagin, linearity, standard deviation and regression coefficient found via graph (Table No.2) and calculated quantity of Plumbagin were given in (Table No.3).

Table No.1: HPTLC details of chloroform extract of Citrak Haritaki

S.No	Detection/ visualization	Citrak Haritaki (Track T1, T2 and T3)		Standard- Plumbagin (Track S1, S2 and S3)		Chitrak Root (Track SD1)	
		R _f values	Colour of band	R _f Values	Colour of band	R _f values	Colour of band
1	Under UV 254 nm	0.22 0.38 0.51 0.60 0.88	grey grey grey grey dark grey	0.88	dark grey	0.22 0.38 0.51 0.88	grey grey grey dark grey
2	Under UV 366 nm	0.07 0.15 0.22 0.38 0.48 0.71 0.76 0.88	sky blue red sky blue sky blue green sky blue red bright red	0.88	bright red	0.07 0.32 0.41 0.48 0.71 0.88	sky blue sky blue sky blue green sky blue bright red
3	After derivatization	0.21 0.38 0.51 0.71 0.88	greenish grey violet violet violet grey	0.88	grey	0.38 0.51 0.58 0.88	violet violet violet grey

Table No.2: Quantity applied on plate and values found via graph

S.No	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of Plumbagin via graph	Linearity and Regression Coefficient and Standard deviation via graph
1	T1	12µl	6000µg	3.567µg	$Y = 27430.924 + 3323.826 * X - 63.331 * X^2$ $r = 0.99086 \quad s_{dv} = 3.14\%$
2	T2	12µl	6000µg	3.642µg	
3	S1	2µl	2µg	2.000µg	
4	S2	4µl	4µg	4.000µg	
5	S3	6µl	6µg	6.000µg	
6	SD1	3µl	1500µg	5.217µg	
7	T3	12µl	6000µg	3.689µg	

- T1** - Chloroform extract of CH1DB
T2 - Chloroform extract of CH2BY
S1 - Plumbagin Std. Chloroform solution (1mg/ml)
S2 - Plumbagin Std. Chloroform solution (1mg/ml)
S3 - Plumbagin Std. Chloroform solution (1mg/ml)
SD1 - Chloroform extract of Chitrak Root
T3 - Chloroform extract of CH3ZB

Table No.3: Summary of results

S.No	Sample from	CH1DB	CH2BY	CH3ZB	Citrak Root
1	Quantity of Plumbagin in 1g	0.595mg	0.607mg	0.615mg	3.478mg
2	% Plumbagin	0.0595% w/w	0.0607% w/w	0.0615% w/w	0.3478% w/w

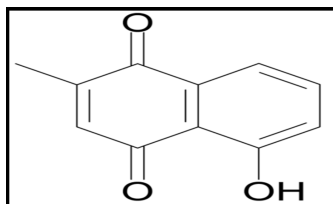


Figure No.1: Lupeol and lupenyl acetate

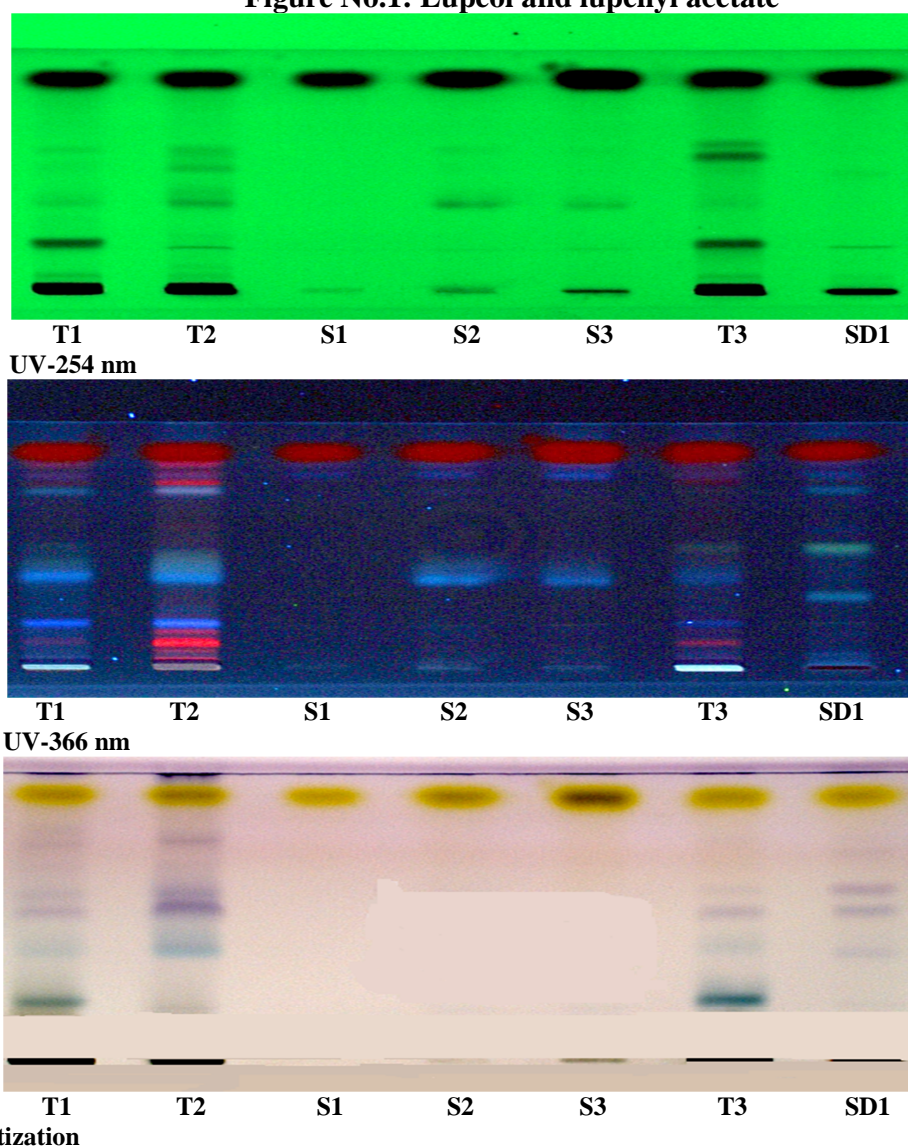
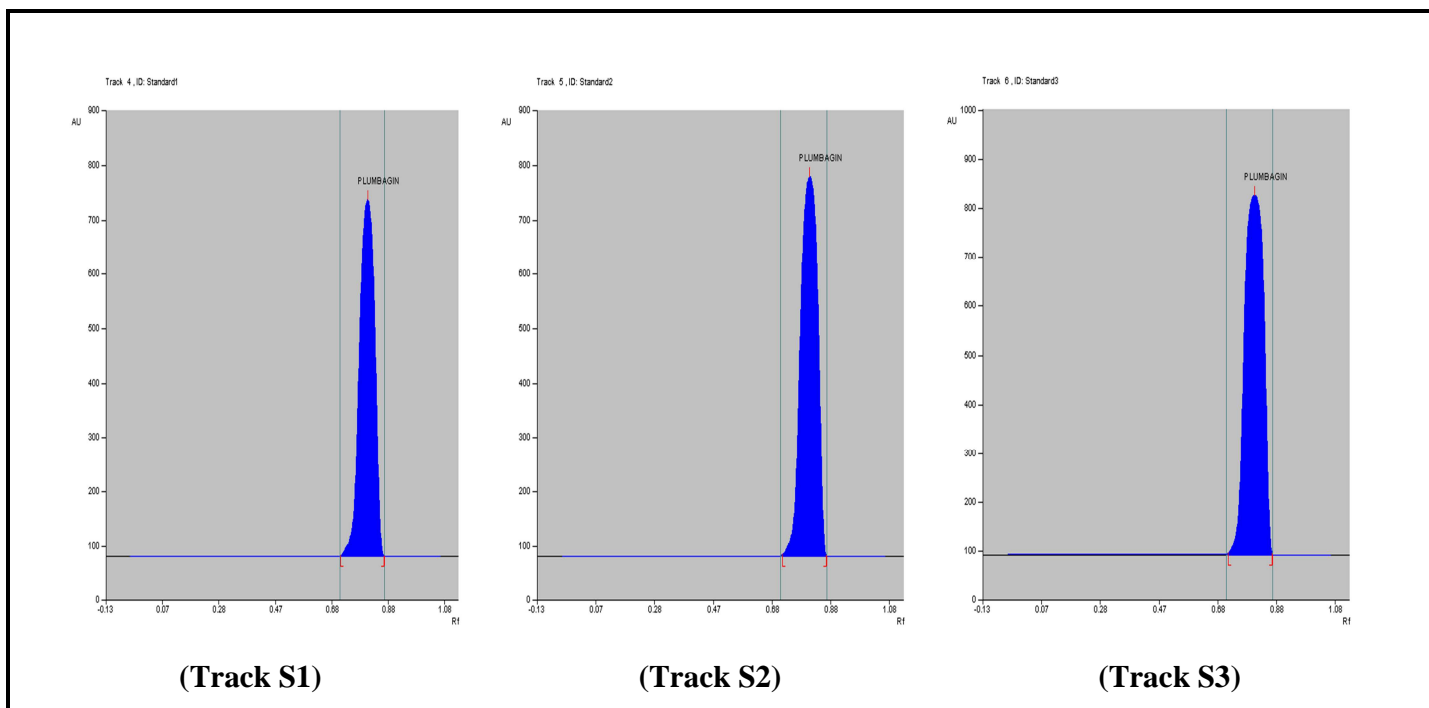
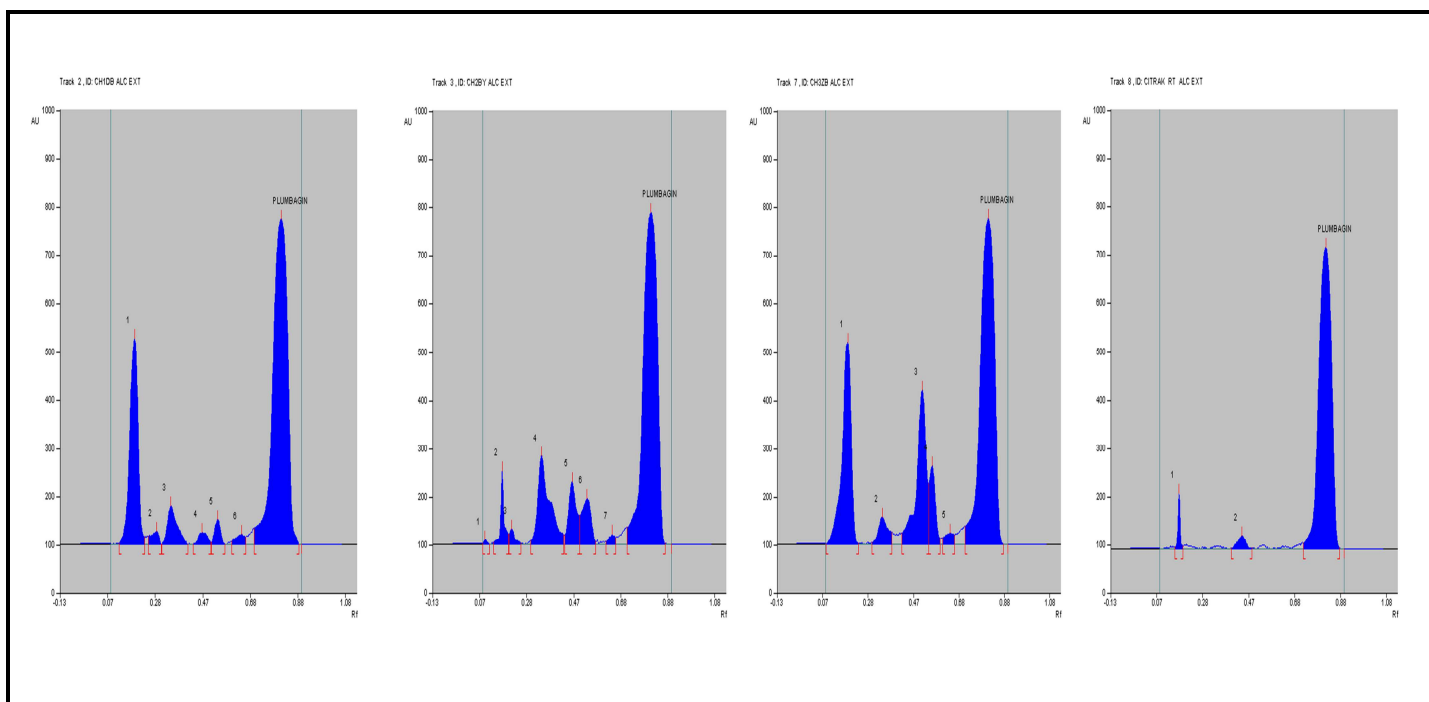


Figure No.2: H.P.T.L.C. Finger print of Citrak Haritaki

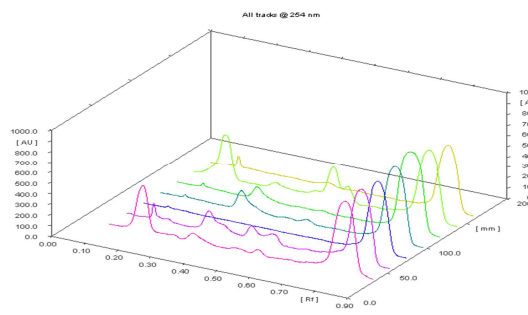


Peaks of Plumbagin @ 270nm

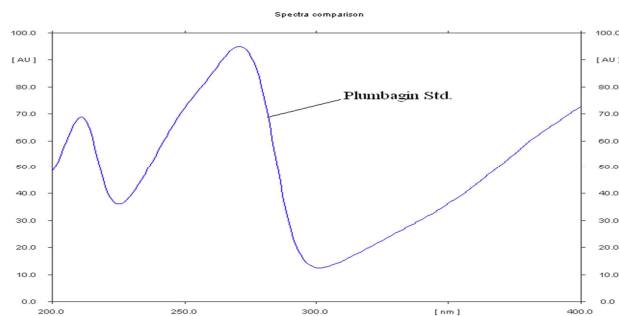


Peaks of Citrak Haritaki and Citrak Root CHCl3 Extract @ 270nm

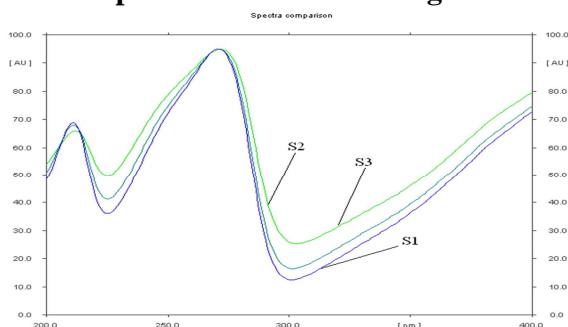
Figure No.3: Peaks of Citrak Haritaki in all Tracks



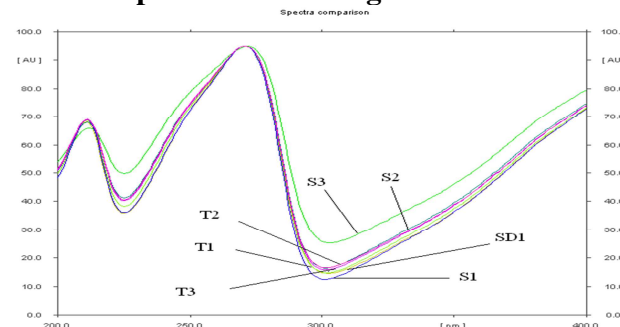
3D representation of Plumbagin



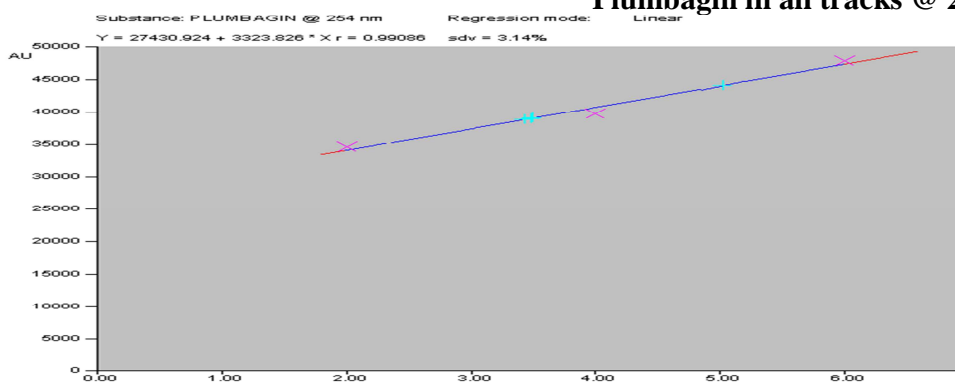
Spectra of Plumbagin @ 270nm



Spectra of Plumbagin in all Std. @ 270nm



Super Imposable UV Spectra of Plumbagin in all tracks @ 270nm



Conc. in µg, Graph Area vs AU

Figure No.4: 3D representation, Spectra and Graph of Citrak Haritaki

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 Citrak Haritaki (Figure No.2). Sharp peaks of Plumbagin (Standard and samples) were obtained when the plate was scanned at wavelength 270nm (Figure No.3). Quantities of Plumbagin found in

samples were obtained automatically (Table No.1) via graph (Figure No.4) and % Plumbagin found in samples was calculated (Table No.3). Quantity of Plumbagin found in CH1DB is 0.595mg in 1g drug sample (0.0595% w/w), in CH2BY is 0.607mg in 1g drug sample (0.0607% w/w), in CH3ZB is 0.615mg in 1g drug sample (0.0615% w/w) and Quantity of Plumbagin found in Citrak Root is 3.478mg in 1g drug sample (0.3478% w/w).

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 ($\pm 2\%$), 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.

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 Chitrak Haritaki.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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