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 110°C 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.

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 officinalis*, 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, fairy thick, shriveled, smooth or irregularly fissured bark. The roots have a short fracture an acid and biting taste and disagreeable odour.1-13. 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 scabies.5 Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3’-biplumbagin, elliptinone, chitrane, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanolone.5. Elliptinone, isozeylinone, catechol tannin, isoshin anolone, dihydrosterone and β- sitosterol also isolated 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.8,15. Lupeol and lupenyl acetate (Figure No.1) have been isolated from the root.10-18.

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 Pharmacopeial 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 Pharmacopeial 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

**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 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 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 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 GF$_{254}$,(20X10 cm$^2$; 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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Detection/ visualization</th>
<th>Citrak Haritaki (Track T1, T2 and T3)</th>
<th>Standard- Plumbagin (Track S1, S2 and S3)</th>
<th>Chitrak Root (Track SD1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( R_f ) values</td>
<td>Colour of band</td>
<td>( R_f ) values</td>
</tr>
<tr>
<td>1</td>
<td>Under UV 254 nm</td>
<td>0.22 0.38 0.51 0.60 0.88</td>
<td>grey grey grey dark grey</td>
<td>0.88</td>
</tr>
<tr>
<td>2</td>
<td>Under UV 366 nm</td>
<td>0.07 0.15 0.22 0.38 0.48 0.71 0.76 0.88</td>
<td>sky blue red sky blue sky blue green sky blue red bright red</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>After derivatization</td>
<td>0.21 0.38 0.51 0.71 0.88</td>
<td>greenish grey violet violet grey</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Track No.</th>
<th>Volume applied on plate</th>
<th>Quantity applied on plate</th>
<th>Quantity of Plumbagin via graph</th>
<th>Linearity and Regression Coefficient and Standard deviation via graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1</td>
<td>12µl</td>
<td>6000µg</td>
<td>3.567µg</td>
<td>[ Y = 27430.924 + 5323.825 * X -0.3331 * X^2 ] ( r = 0.99086 ) ( sdx = 3.14% )</td>
</tr>
<tr>
<td>2</td>
<td>T2</td>
<td>12µl</td>
<td>6000µg</td>
<td>3.642µg</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>S1</td>
<td>2µl</td>
<td>2µg</td>
<td>2.000µg</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S2</td>
<td>4µl</td>
<td>4µg</td>
<td>4.000µg</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S3</td>
<td>6µl</td>
<td>6µg</td>
<td>6.000µg</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SD1</td>
<td>3µl</td>
<td>1500µg</td>
<td>5.217µg</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>T3</td>
<td>12µl</td>
<td>6000µg</td>
<td>3.689µg</td>
<td></td>
</tr>
</tbody>
</table>

- 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 3: Summary of results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample from</th>
<th>CH1DB</th>
<th>CH2BY</th>
<th>CH3ZB</th>
<th>Citrak Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quantity of Plumbagin in 1g</td>
<td>0.595mg</td>
<td>0.607mg</td>
<td>0.615mg</td>
<td>3.478mg</td>
</tr>
<tr>
<td>2</td>
<td>% Plumbagin</td>
<td>0.0595%w/w</td>
<td>0.0607%w/w</td>
<td>0.0615%w/w</td>
<td>0.3478%w/w</td>
</tr>
</tbody>
</table>

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
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 Rf 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 (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of Rf 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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