DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF PIPERINE IN CHITRAK HARIKAKI AN AYURVEDIC FORMULATION

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ABSTRACT
A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Piperine in Ayurvedic formulations of Chitrak Haritaki of different manufactures. The alcoholic extract of Chitrak Haritaki, Pippali fruit and Kalimirch fruit samples were applied on TLC Aluminium plate pre coated with Silicage 160 GF254 and developed using Toluene Ethyl acetate (9:1) V/V as 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 wave length of 254 nm. Content of marker compound in the samples were found similar.

KEYWORDS
Chitrak Haritaki, Haritaki fruit, Amalaki fruit, Piperine, Standardization and HPTLC.

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INTRODUCTION
Chitrakharitaki is a very famous Ayurvedic medicine used in treating chronic respiratory conditions. It is in herbal jam form. It is also known as Chitrakharitaki Avalahe, Chitraka Haritaki etc. Avalahe 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 tuber culosis. 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
A 4.8 liters of decoction is prepared with each of Chitraka - *Plumbagozeylanica*, Amalaki- *Embellica officinalis*, Guduchi - *Tinosporacordifoli* as and Dashamoola. It is added with 4.8 kg of jaggery and 3.072 kg of Haritaki - *Terminaliachebula*. This mixture is heated till semi solid consistency. It is added with *Trikatu* - pepper, long pepper and ginger - 96 g, Cinnamon - 96 g, Tejpatra – *Cinnamomumtamala* - 96 g, Yavakshara - 24 g and 384 grams of honey.

EXPERIMENTAL
Material and Method
(1) The Chitrak Heritage 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 Pippali fruit and Kalimirch fruit were 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 and SD2 respectively 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 Win cats an integrated Software 4.02 (Switzerland).

Chemical 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 Aluminum 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 Piperine procured from Aldrich Chem. Co. (Lot 08214 PE -027/CAS 94-62-2 P459007).

Sample and Standard preparation
Sample preparation
1g of coarsely powdered crude drug and Citrak Haritaki samples were extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extracts were filtered by Whitman 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 Piperine dissolved in 5ml of absolute alcohol and made up to 5ml in standard volumetric flask.
Chromatography

Procedure

TLC Aluminum precoated plate with Silica gel 60 GF$_{254}$ (20x10 cm$^2$; 0.2 mm thick) was used with Toluene Ethyl acetate (9:1) V/V as mobile phase. Alcoholic extract of samples and Piperine standard solution applied on plate by using Linomat V applicator. Camag 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$^\circ$C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (Figure No.5C.2). The plate was scanned before derivatization using Camag TLC Scanner III at wavelength 270nm. Win cats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

LINEARITY OF DETECTOR RESPONSE AND ASSAY

In order to establish linearity, standard solution of Pipelines (1mg/ml) applied on TLC Aluminum pre coated plate with Silica gel 60 GF$_{254}$ (20x10 cm$^2$; 0.2 mm thick), 2µl, 4µl, 6µl on Track No. S1, S2 & S3 respectively and for assay, 9µl of alcoholic extract of samples applied on Track No. T1 T2 & T3 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.1). The plate was scanned immediately before derivatization using Camag TLC Scanner III at wavelength 270nm. Win cats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Piperine appeared at R$_f$. 0.15 (dark grey colour). The peaks, graph and spectra obtained were given in Figure No.2 and Figure No.3 and R$_f$. values, colour of bands (Table No.1), quantity of Piperine linearity, standard deviation and regression coefficient found via graph (Table No. 2) and calculated quantity of Piperine given in Table No.3.

RESULT AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene. Ethyl acetate (9:1) v/v and the active principle Piperine resolved as a dark grey colour band at R$_f$. 0.15 very efficiently from the other components in alcoholic extract of Pippali and Kalimirch (fruit) (Figure No.1). Sharp peaks of Piperine (Standard and samples) were obtained when the plate was scanned at wavelength 254nm (Figure No.2). Quantity of Piperine found in samples were obtained automatically (Table No. 2) via graph (Figure No.2) and % Piperine found in samples and % recovery were calculated (Table No.3). Quantity of Piperine found in sample CH1DB is 0.617mg in 1g drug sample (0.0617% w/w) in CH2BY is 0.712mg in 1g drug sample (0.0712% w/w) in CH3ZB is 0.781mg in 1g drug sample (0.0781% w/w) and quantity of Piperine found in Pippali Fruit is 2.980mg in 1g drug sample (0.2980% w/w) and in Kalimirch Fruit is 3.228mg in 1g drug sample (0.3228% w/w). 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 plumb again was observed, indicating the robustness of the method.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Detection/Visualization</th>
<th>Citrak Haritaki (Track T1, T2 and T3)</th>
<th>Standard- Piperine (Track S1, S2 and S3)</th>
<th>Pippali Fruit (Track SD1)</th>
<th>Kalimirch Fruit (Track SD2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R&lt;sub&gt;f&lt;/sub&gt; Values</td>
<td>Colour of band</td>
<td>R&lt;sub&gt;f&lt;/sub&gt; Values</td>
<td>Colour of band</td>
</tr>
<tr>
<td>1</td>
<td>Under UV 254 nm</td>
<td>0.06 0.15 0.24 0.30</td>
<td>Grey dark grey grey grey</td>
<td>0.06 0.15 0.24 0.30</td>
<td>dark grey</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Under UV 366 nm</td>
<td>0.06 0.15 0.38 0.42 0.48 0.52 0.55 0.68</td>
<td>sky blue sky blue red red sky blue red red sky blue</td>
<td>0.06 0.15 0.36 0.48 0.55 0.68</td>
<td>sky blue sky blue sky blue sky blue sky blue sky blue</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>After derivatization</td>
<td>0.06 0.15 0.30 0.32 0.42 0.72 0.84 0.68</td>
<td>greenish grey violet violet violet violet violet violet</td>
<td>0.06 0.15 0.36 0.42 0.65 0.68</td>
<td>greenish grey violet violet violet red dark sky blue</td>
</tr>
<tr>
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</table>

<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 Piperine 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>9µl</td>
<td>4500µg</td>
<td>2.779µg</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T2</td>
<td>9µl</td>
<td>4500µg</td>
<td>3.204µg</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>T3</td>
<td>9µl</td>
<td>4500µg</td>
<td>3.517µg</td>
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</tr>
<tr>
<td>7</td>
<td>SD1</td>
<td>3µl</td>
<td>1500µg</td>
<td>4.471µg</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>SD2</td>
<td>3µl</td>
<td>1500µg</td>
<td>4.842µg</td>
<td></td>
</tr>
</tbody>
</table>

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

\[
Y = 17245.694 + 3305.754 * X \\
r = 0.96768 \quad sdv = 2.09% 
\]
Table No.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>Pippali Fruit</th>
<th>Kalimirch Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quantity of Piperine in 1g</td>
<td>0.617mg</td>
<td>0.712mg</td>
<td>0.781mg</td>
<td>2.980mg</td>
<td>3.228mg</td>
</tr>
<tr>
<td>2</td>
<td>% Piperine</td>
<td>0.0617% w/w</td>
<td>0.0712% w/w</td>
<td>0.0781% w/w</td>
<td>0.2980% w/w</td>
<td>0.3228% w/w</td>
</tr>
</tbody>
</table>

Figure No.1: Molecular structure of Piperine

T1 - Alcoholic extract of CH1DB  
T2 - Alcoholic extract of CH2BY  
S1 - Piperine Std. alcoholic solution (1mg/ml)  
S2 - Piperine Std. alcoholic solution (1mg/ml)  
S3 - Piperine Std. alcoholic solution (1mg/ml)  
T3 - Alcoholic extract of CH3ZB  
SD1 - Alcoholic extract of Pippali Fruit  
SD2 - Alcoholic extract of Kalimirch Fruit

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After derivatization

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

Peaks of Plumb gin @ 270nm

Figure No.3: Peaks of Citrak Haritaki in all Tracks
Peaks of Citrak Haritaki and Pippali and Kalimirch CHCl3 Extract @ 270nm

3D Representation

Spectra of Piperine @ 254nm

Spectra of Piperine in all Std. @ 254nm

Super Imposable UV Spectra of Piperine in all tracks @ 254nm

Conc. in µg. Graph Area vs AU

Figure No.4: 3D representation, Spectra and Graph of Citrak Haritaki
CONCLUSION
The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of Pippali and Kalimirch (fruit) and quantitative determination of Plumbagin in Chitrak Heritage.

CONFLICT OF INTEREST
We declare that we have no conflict.

BIBLIOGRAPHY
1. Dhiman Anil Kumar, Ayurvedic Drug Plants, Daya Publishing House, Delhi, 2006, 105.

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