Application of HPTLC Technique to Identify the Active Ingredients in a Polyherbal Formulation

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ABSTRACT:

Background: Benign prostatic hyperplasia is a condition that affects the prostate gland in men. A poly herbal formulation was formulated and standardized with the help of HPTLC instrument.

Objective: The present study shows the use of High Performance Thin Layer Chromatography (HPTLC) technique as a tool for the identification of active ingredients used in polyherbal formulated capsule used in the treatment of Benign Prostatic Hyperplasia. The formulated polyherbal product called Prostakare capsules is marketed by Piramal Life Sciences. This product is a brand formulation of Piramal Life Sciences. The formulation consists of five active ingredients namely Saw palmetto, Tribulus terrestris, Boerhaavia diffusa, Curcuma longa and Lycopene extracts. The active ingredients present in the formulation are pharmacologically proved for the treatment of Benign Prostatic Hyperplasia.

Methods: A HPTLC method was developed by applying the five active extracts individually along with the different extraction of samples on a TLC plate, developing with a proper solvent system, scanned and photodocumented.

Results: The HPTLC technique could successfully determine the respective ingredients from the polyherbal formulated capsule.

Keywords: High Performance Thin Layer Chromatography, Polyherbal Formulation, Prostate care, Analytical method.
1. INTRODUCTION

Benign prostatic hyperplasia is a condition that affects the prostate gland in men. The prostate is a gland found between the bladder (where urine is stored) and the urethra (the tube urine passes through). Enlargement of prostate takes place in men over 50 and is rarely found in younger men. Benign Prostatic Hyperplasia is characterized by enlargement of prostatic nodules due to proliferative process involving both stromal and epithelial elements of the prostate. As the prostate enlarges, the surrounding tissue offers resistance, causing the prostate to press against the urethra and it might lead to prostate cancer in later stages. Nowadays people more often opt for Ayurvedic or herbal drugs then allopathic drugs due to their erroneous side-effects.

Ayurvedic formulations are more demanding in the present scenario as they are natural and safe comparatively. As formulating the polyherbal drug is important likewise development of appropriate analytical methods to ensure the drug efficacy is also equally potential. There are different ways and sophisticated instruments by which we can develop numerous methods for a different drug molecule.

Likewise HPTLC is easy and best technique for polyherbal formulations and hence used as a tool to identify the ingredients present in the formulation. High performance Thin Layer Chromatography, is a simple separation technique, as name refers it is advance Thin Layer Chromatography widely and popularly used in the herbal drug field. It definitely differs from conventional Thin Layer Chromatography as it can be scanned and can be reported and photo documented. A non-enzymatic polyherbal drug plays a vital role in the treatment of Benign Prostatic Hyperplasia disorder. The formulated capsule actually helps to reduce excessive cell growth by inhibiting conversion of testosterone into DHT and preventing attachment of estrogen to its receptors in prostate tissue. The formulated capsule constitute of extracts of Saw Palmetto, Tribulus terrestris, Boerhaavia diffusa, Curcuma longa and Lycopene. As per the literature, these extracts have activity to prevent Benign Prostatic Hyperplasia disorder.

Table 1: Shows active ingredients of a formulated capsule

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Botanical Name</th>
<th>Morphological Part Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saw Palmetto extract</td>
<td>Fruit</td>
</tr>
<tr>
<td>2.</td>
<td>Tribulus terrestris extract</td>
<td>Whole plant</td>
</tr>
<tr>
<td>3.</td>
<td>Boerhaavia diffusa extract</td>
<td>Whole plant</td>
</tr>
<tr>
<td>4.</td>
<td>Curcuma longa extract</td>
<td>Rhizome</td>
</tr>
<tr>
<td>5.</td>
<td>Lycopene extract</td>
<td>Fruit</td>
</tr>
</tbody>
</table>

*Saw palmetto extract*: It is effective for the symptoms of benign prostatic hyperplasia. It is also used orally as a mild diuretic, a sedative, an anti-inflammatory, and as an antiseptic. *Saw palmetto* is also used to prevent complications during transurethral resection of the prostate. In combination with other herbs, *Saw palmetto* is used to treat prostate cancer also.

*Curcuma longa extract*: Curcumin a major constituent of the yellow spice turmeric derived from the rhizomes of *Curcuma spp.*, has been reported to be potential against prostate cancer. Besides it also helps in enhancing the apoptosis inducing potential of trial in prostate cancer cells. It is also having anti-inflammatory properties.

*Tribulus terrestris extract*: The extract of the fruits shows anti-urolithiatic activity. *Tribulus terrestris* is a testosterone enhancer.

*Boerhaavia diffusa extract*: It is an anti-inflammatory and diuretic. It is also hepatoprotective.

*Lycopene*: Although primarily considered as an antioxidant, lycopene appears to have an influence on cellular proliferation and differentiation as well as immune response. Epidemiological studies have shown an inverse association between dietary intake of lycopene and prostate cancer risk.
2. MATERIALS AND METHODS

2.1 Chemicals and Reagents:
All the chemicals used in the experiment were of analytical grade. Extracts of *Saw Palmetto*, *Tribulus terrestris*, *Boerhaavia diffusa*, *Curcuma longa* and Lycopene were procured from approved vendors. All the solvents used in the experiment were procured from Merck Pvt. Ltd, Mumbai, India.

2.2 Apparatus:
Applicator Device: Linomat V Automatic sampler applicator; CAMAG, (Muttenz, Switzerland).
Syringe: 100µl Hamilton (Switzerland)
Thin Layer Chromatography Chamber: Glass twin trough chamber (20x20)
Densitometer: Thin Layer Chromatography Scanner 3 with WinCATS software; CAMAG, Switzerland.
TLC Plate: 20x20 cm, 0.2mm precoated with silica gel 60F\textsubscript{254}; Merck.
Spraying Reagents: Vanillin Sulphuric acid reagent.
Sonicator: Model no. 3.7L100H

2.3 High Performance Thin Layer Chromatography Profile
HPTLC study consists of three different types of methanolic extracts of the formulation to check the separation and presence of active ingredients in the drug.

2.4 Extraction procedures of the sample:
2.4.1 Methanolic extract: Blend of one capsule taken in a conical flask, 10 ml of methanol added, sonicated for 10 minutes, heated for another 1 minute, cooled and filtered through Whatman Filter paper no.1. Sample is used for the application. 10µl of sample is applied with 100µl Hamilton syringe on 20x20 cm, 0.2mm precoated with silica gel 60F\textsubscript{254} TLC plate. Stability sample was also prepared just to confirm the HPTLC pattern and was prepared by methanolic extraction only.

2.4.2 Dichloromethane : Methanol extract (1:1): Blend of one capsule taken in a conical flask, 10 ml mixture of Dichloromethane : methanol (1:1) added, sonicated for 10 minutes and heated for another 1 minute and filtered through Whatman Filter paper No.1. Sample is used for the application. 10µl of sample is applied with 100µl Hamilton syringe on 20x20 cm, 0.2mm precoated with silica gel 60F\textsubscript{254} TLC plate.

2.4.3 Alkaline methanolic extract: Blend of one capsule taken in a conical flask, 1 ml of 30% Ammonia solution is added to it, then 9 ml of methanol added to it, sonicated for 10 minutes and heated for another 1 minute and filtered through Whatman Filter paper No.1. Sample is used for the application. 10µl of sample is applied with 100µl Hamilton syringe on 20x20 cm, 0.2mm precoated with silica gel 60F\textsubscript{254} TLC plate.

The HPTLC analysis was performed by applying bands of 8.0 mm of each of extracts and samples on a TLC (20x20 cm with 250µm thickness) plate using Linomat V Applicator and 100µl Hamilton syringe. The TLC plate consists of total of 11 bands comprising of 4 bands of *Saw Palmetto*, *Tribulus terrestris*, *Curcuma longa* and Lycopene extracts respectively, 2 bands of *Boerhaavia diffusa* extract out of which one was with increased volume(for band identification), 1 extra band of *Saw palmetto* extract in dichloromethane but the pattern obtained from dichloromethane solution was same as that from methanol solution, 3 bands each of three different methanolic extractions and one extra band of stability sample applied to confirm the HPTLC pattern. The bands were applied with the help of nitrogen spray. The TLC plate was developed with a particular solvent system and was dried with a dryer. Densitometric scanning was performed on CAMAG, HPTLC scanner III in the absorbance / reflectance mode.

2.5 Extraction procedure for Extracts:
Weighed each active extract as per the quantity added in the capsule i.e. 160 mg of *Saw palmetto* extract in 10 ml of methanol, sonicated for 15 minutes, filtered through Whatman Filter paper No.1. Filtrate obtained is used as a reference standard for the application.
(a) 50 mg of *Tribulus terrestris* extract in 10 ml of methanol, sonicated for 15 minutes, filtered through Whatman Filter paper No.1. Filtrate obtained is used as a reference standard for the application.

(b) 50 mg of *Curcuma longa* extract in 10 ml of methanol, sonicated for 15 minutes, filtered through Whatman Filter paper No.1. Filtrate obtained is used as a reference standard for the application.

(c) 25 mg of *Boerhaavia diffusa* extract in 10 ml of methanol, sonicated for 15 minutes, filtered through Whatman Filter paper No.1. Filtrate obtained is used as a reference standard for the application.

(d) 10 mg of Lycopene extract, in 10 ml of methanol sonicated for 15 minutes filtered through Whatman Filter paper No.1. Filtrate obtained is used as a reference standard for the application.

3. RESULTS

The well resolved TLC plate pattern was observed. Formulation shows the separation which corresponds to four active ingredients.

![Figure 1: TLC Plate under UV lamp](image)

T1-Sample extraction 1, T2-Sample extraction 2, T3-Sample extraction 3, T4-Stability Sample, T5-Methanolic *Saw palmetto*, T6-Dichloromethane *Saw palmetto*, T7-*Tribulus terrestris*, T8-*Boerhaavia diffusa*, T9-*Curcuma longa*, T10-Lycopene, T11-*Boerhaavia diffusa* 30μl.

Solvent System: Ethyl acetate : Hexane : Methanol : Ammonia solution (30%) (7:1.5:1:0.5)

Saturation Time : 15 mins.

Length of Run : 15 cms

Above HPTLC Study shows the presence of *Curcuma longa* in all the extractions of the formulation. Under UV lamp (i.e. 254 nm) three prominent bands of the extracts at Rf about 0.34, 0.38 & 0.45 are seen which corresponds at the same Rf in the formulation. The three spectra of the curcuma matches to that obtained in the formulation (given in Fig.2,3,4).

![Figure 2](image)

![Figure 3](image)

![Figure 4](image)

Figure 2 shows overlapping spectra of first band of *Curcuma longa* extract and the formulated samples.

Figure 3 shows overlapping spectra of second band of *Curcuma longa* extract and the formulated samples.

Figure 4 shows overlapping spectra of third band of *Curcuma longa* extract and the formulated samples.
Visually, under fluorescence lamp (i.e. 366 nm) a fluorescent blue band in Tribulus terrestris extract at Rf 0.10 is present in the four extracted samples of formulation. Bright fluorescent blue band observed in Saw Palmetto extract at Rf 0.79 showed similar band at the same Rf in the formulation.

Under visible light, light orange colored band observed at Rf 0.87 in Lycopene extract showed the resemblance at the same Rf in the formulation. Similarly, three yellow prominent bands of curcumin are also observed in extract as well as in the formulation.

Boerhaavia diffusa extract not showed any separation in the above solvent system.

HPTLC Method for Boerhaavia diffusa extract

Extraction procedure for Boerhaavia diffusa extract:

Weighed accurately 25 mg of extract in a conical flask and added 10 ml of chloroform and sonicated for 10 minutes, then added 6-7 drops of Concentrated Hydrochloric acid and again sonicated for another 5 minutes, added 1 ml of methanol and heated to evaporate the solvent completely, then reconstituted with 10 ml of chloroform and filtered through Whatman filter paper No.1. Filtrate obtained is used as a reference standard for application. 30μl of sample is applied with 100μl Hamilton syringe on 10x10 cm, 0.2 precoated with silica gel 60F254 TLC plate.

Extraction procedure of the sample:

Blend of one capsule taken in a conical flask, 5 ml of Concentrated Hydrochloric acid was added, then evaporated to dryness, cooled and reconstituted with 10 ml of chloroform, finally filtered through Whatman filter paper No.1. Filtrate obtained is used for application. 30μl of sample is applied with 100μl Hamilton syringe on 10 x10 cm, 0.2 precoated with silica gel 60F254 TLC plate.
Figure 7: Shows TLC Plate under UV lamp

T1- *Boerhaavia diffusa* extract, T2-Sample
Solvent System: Toluene : Ethyl acetate : Glacial Acetic acid (8.5:2.0:0.5),
Saturation Time : 10 mins
Application : 30μl,
Length of Run : 8cm.

Figure 8: Shows overlapping spectra of *Boerhaavia diffusa* in both extract and sample

Under UV lamp (i.e.254 nm) a band at Rf 0.22 shows resemblance with the same Rf in the sample. Overlapping spectrum of the *Boerhaavia Diffusa* extract matches with the same Rf in the formulation (given in Fig.8).

4. DISCUSSIONS AND CONCLUSION

According to the results obtained we can conclude that the above HPTLC methods succeed in identifying the active ingredients that are added in the polyherbal formulated product. *Boerhaavia diffusa* is separately determined by different HPTLC method. Apart from *Boerhaavia diffusa* extract, rest of the four extracts can be identified easily by applying single HPTLC method. Above HPTLC methods used are reproducible and less time consuming and can be analysed with ease.
5. REFERENCES


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