ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.7826]

Journal Homepage: www.eijppr.com

Research Article

Development of HPTLC Method for the Determination of Piperine in Sitopladi Churna - An Ayurvedic Formulation

Shivani[1], Iram Rakhshi[2], Pawar R.K.[3], Sharma Shivani[4], Sharma Rajiv KR[5], Singh K.C.[6]
[1, 2, 4, 6] Department of chemistry, R.S.S. (P.G.) College, Pilkhuwa, Hapur (U.P.), India
[3, 5] Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad 201 002 (U.P.), India

Article info

Article History:
Received 14 Aug 2013
Accepted 24 Aug 2013

Keywords:
Sitopladi Churna, Pippali Fruit, Piperine, Standardisation, HPTLC.

Abstract

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Piperine in Ayurvedic formulations of Sitopladi Churna of different manufactures. The alcoholic extract of Sitopladi Churna and Pippali fruit samples were applied on TLC Aluminium plate pre coated with Silicagel 60 GF254 and developed using Toluene: Ethyl acetate (9:1) 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.

1. INTRODUCTION

Sitopaladi Churna is one of the very easy-to-make Ayurvedic herbal powder mix. It is one of the best and widely used Ayurvedic medicines for cough, even for kids. Sitopaladi Churna is also known as Sitopaladi Choorna, Sitopaladi Choornam, Sitopaladi Chooran etc.

1.1 Composition of Sitopaladi Churna

Sitopaladi Churna [1-3] ingredients include-
Sitopala – Sugar candy powder – 16 parts
Vamshalochana (*Bambusa arundinacea*- Inner white light part of bamboo tree) – 8 parts
Pippali (*Piper longum*) – 4 parts
Ela (*Elettaria cardamomum*) – 2 parts
Twak (*Cinnamomum zeylanicum*) – 1 part.

1.2 Method of Preparation of Sitopaladi Churna

Fine herbal powders of the above herbs are taken in the said proportions, mixed thoroughly and kept in air tight container.

1.3 Benefits of Sitopaladi Churna

Traditionally, Sitopaladi Churna is used in the treatment of respiratory diseases, cough, burning sensation in palms and feet, low digestion power, loss of sensation in tongue, pain in abdomen, flanks, anorexia, fever, bleeding from nose. It gets absorbed into the body and provides nutrition and energy to digest the mucous conditions. Sitopaladi is a botanical alternative medicine formulation which aids in the relief of respiratory tract disorders.
It is helpful to increase appetite, improves digestion with strength the body. It acts as a good expectorant hence recommended for seasonal coughs. It is a best supportive medicine in allergic and viral respiratory infection. It Reduces pharyngeal and chest congestion by soothing dry or wet cough and clearing mucous in the airways. Sugar candy has soothing property which controls throat irritation.

Corresponding Author:
R. K. Pawar,
C-27, I Floor, Swarn Jayanti Puram,
Ghaziabad-201 002 (U.P.), India
Tel. No. +91-9868998025
Email: pawarplim@gmail.com

1.4 Dose of Sitopaladi Choorna

Usually given in varied dose of 1 – 4 grams two or three times a day with honey, before or after food. Half a teaspoon of Sitopaladi Churna is given along with half a teaspoon of ghee and a teaspoon of honey. It is even given with anyone of honey and ghee.

1.5 Side Effetcs of Sitopaladi Churna

It is generally devoid of any side effects. But it may worsen gastritis in a few patients, especially if given before food. This herbal powder mix is not ideal for people with diabetes.
Standardization of a compound Ayurvedic formulation is a critical and essential issue to be considered in assuring the therapeutic efficacy and safety and to rationalize their use in the health care. *Sitopaladi churna* is a reputed polyherbal formulation of Ayurveda. It is prescribed for the treatment of pleurodynia, intercostal neuralgia, cold, cough associated with bronchitis, pneumonia, tuberculosis, viral respiratory infection, and in pharyngeal and chest congestion[3-6].
The investigation was carried out to develop standardization parameters. The objectives include physico-chemical analysis, and thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) fingerprint profile for the quantification of piperine in *Sitopaladi churna* samples.
Piperine (Fig.1) is reported to have as an antidepressant, hepatoprotective, anti-metastatic, antithyroid, immunomodulatory, antitumor, antiplatelet, antioxidant, and antiamoebic activities. Cinnamaldehyde is reported to show antidiabetic, antifungal, antibacterial, anticancer, antimutagenic, and anti-inflammatory activities[7-12].

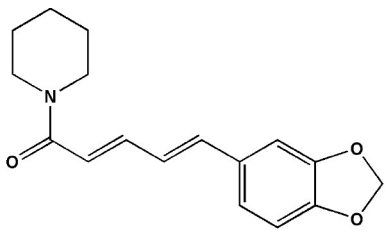


Fig.1: Chemical structure of piperine

With increasing demand for herbal products in medicines and cosmetics there is an urgent need for standardization. So the aim of the work is to develop a simple, rapid, selective and cost effective HPTLC method for the identification of Sitopladi churna.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 The *Sitopaladi churnas* 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) SC1DB (ii) SC2BY (iii) SC3DV

2.1.2 The Pippali fruit 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.

2.1.3 Chemicals : Analytical grade; Toluene, Ethyl acetate, Formic acid, Anisaldehyde, Sulphuric acid, Alcohol were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (10X10 cm; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard- Piperine procured from Aldrich (Lot No.08214 PE-027, CAS 94-62-2, P 459007).

2.2 Development of TLC Profile

2.2.1 Equipment's Used
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

2.2.2 Sample Preparation
1g of coarsely powdered crude drug and Shitopladi Churna samples were extracted with 10 ml absolute alcohol 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 HPTLC.

2.2.3 Standard Preparation
5mg of standard Piperine dissolved in 5ml of absolute alcohol and made up to 5ml in standard volumetric flask.

2.2.4 Chromatography
TLC Aluminium pre coated plate with Silica gel60 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. 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°C 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. 2). The derivatized plate was scanned immediately using Camag TLC Scanner III at wavelength 455nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

2.2.5 Linearity of Detector Response and Assay
In order to establish linearity, standard solution of Piperine (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 alcoholic extract of samples applied on Track No. T1, T2 and T3 on the same plate. TLC plates were 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. 2). 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 455nm. Wincats 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. 0.16 (dark grey). The peaks, graph and spectra obtained are given in Fig. 3 and 4 and R_f. values, colour of bands (Table No.1), quantity of Piperine, linearity, standard deviation & regression coefficient found via graph (Table No. 2) and calculated quantity of Piperine is given in Table No. 3.

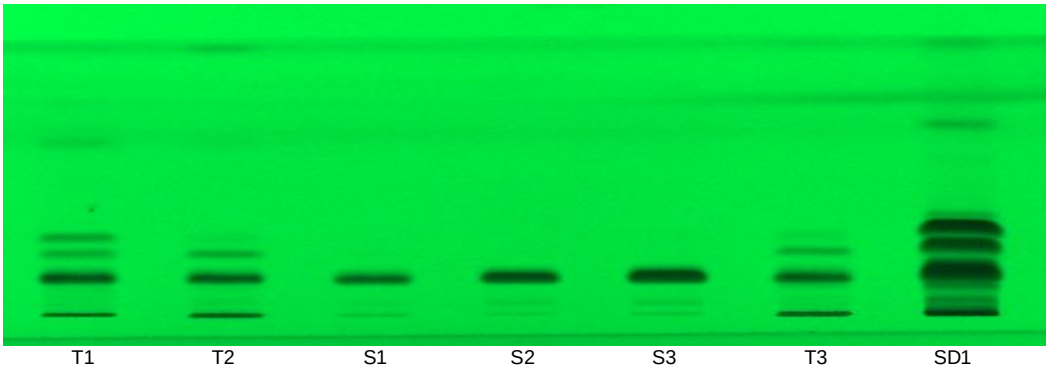
Table 1: HPTLC details of alcoholic extract of Sitopaladi churna

Sr. No.	Detection / visualization	Sitopaladi churna (Track No. T1, T2, and T3)		Standard- Piperine (Track No. S1, S2 and S3)		Pippali Fruit (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	0.16 0.27 0.35 0.66	Dark grey Grey Grey Grey	0.16	Dark grey	0.07 0.16 0.27 0.35 0.42 0.71	Grey Dark grey Grey Dark grey Grey Grey
2.	Under UV 366 nm	0.07 0.16 0.38 0.60 0.75	Sky blue Sky blue Red Red Sky blue	0.16	Sky blue	0.07 0.16 0.38 0.52 0.71	Sky blue Sky blue Sky blue Sky blue Sky blue
3.	After derivatization	0.07 0.16 0.32 0.53 0.73	Light Violet Greenish grey Violet Violet Sky blue	0.16	Greenish grey	0.07 0.16 0.32 0.53 0.73 0.81	Greenish grey Greenish grey Violet Violet Dark sky blue Violet

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

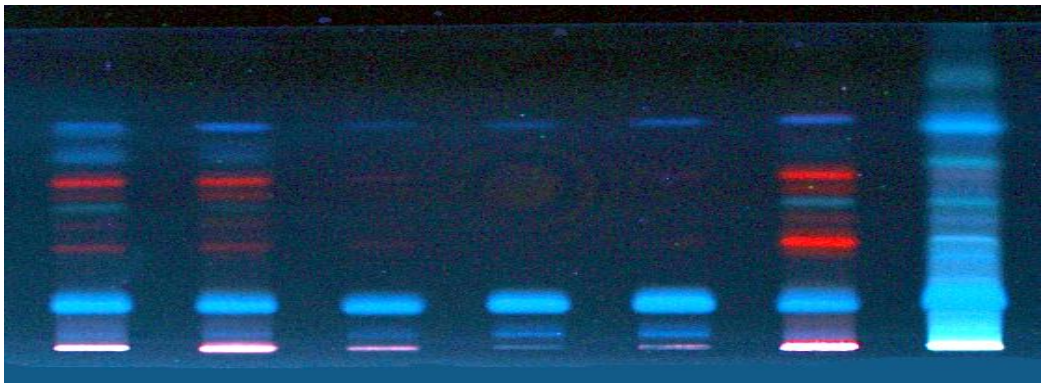
Sr. No.	Track No.	Volume applied on plate	Sample quantity applied on plate	Quantity of Piperine via graph	Linearity & Regression Coefficient and Standard deviation via graph
1.	T1	9µl	4500µg	4.095µg	Y = 13282.449 + 2806.067 * X r = 0.99520 sdv = 3.18%
2.	T2	9µl	4500µg	4.222µg	
3.	S1	2µl	2.000µg	2.000µg	
4.	S2	4µl	4.000µg	4.000µg	
5.	S3	6µl	6.000µg	6.000µg	
6.	T3	9µl	4500µg	5.036µg	
7.	SD1	4µl	2000µg	5.826µg	

T1- Alcoholic extract of SC1DB , T2- Alcoholic extract of SC2BY ,S1- Piperine standard solution (1mg/ml), S2- Piperine standard solution (1mg/ml), S3- Piperine standard solution (1mg/ml), T3- Alcoholic extract of SC3DV , SD1- Alcoholic extract of Pippali Fruit



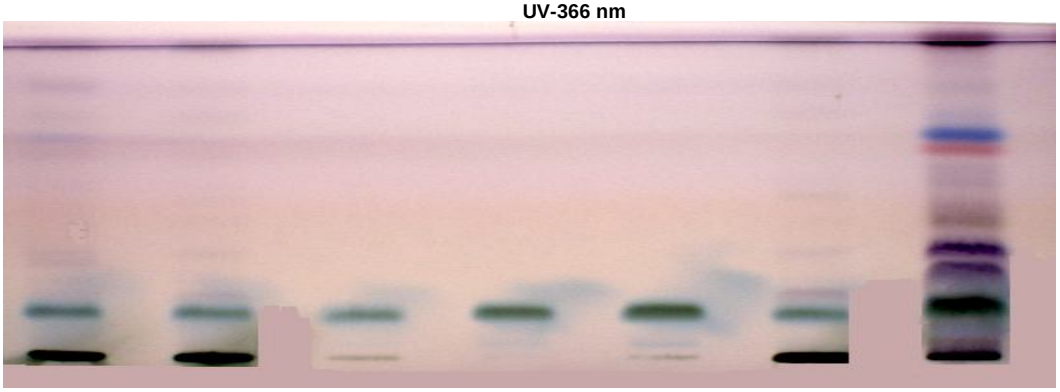
T1 T2 S1 S2 S3 T3 SD1

UV-254 nm



T1 T2 S1 S2 S3 T3 SD1

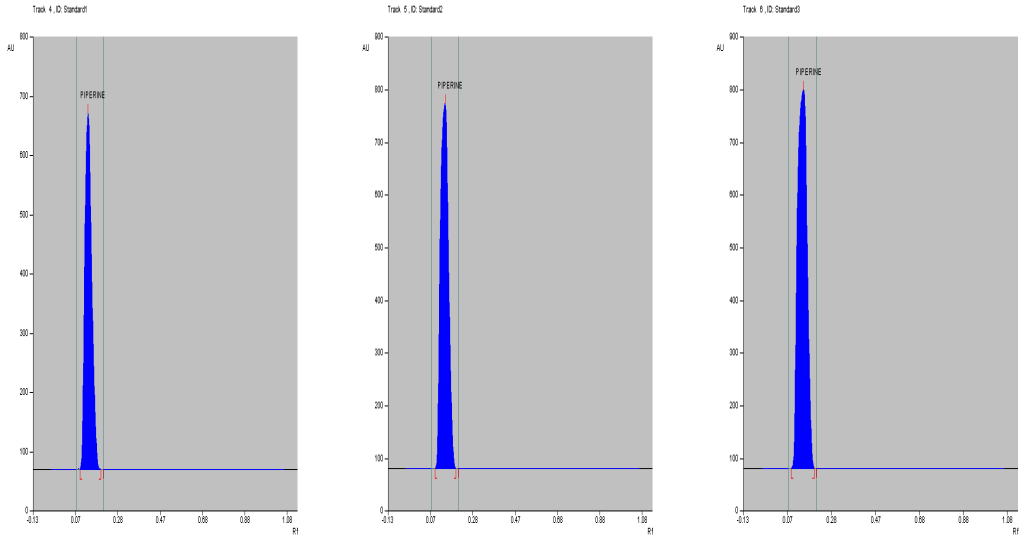
UV-366 nm



T1 T2 S1 S2 S3 T3 SD1

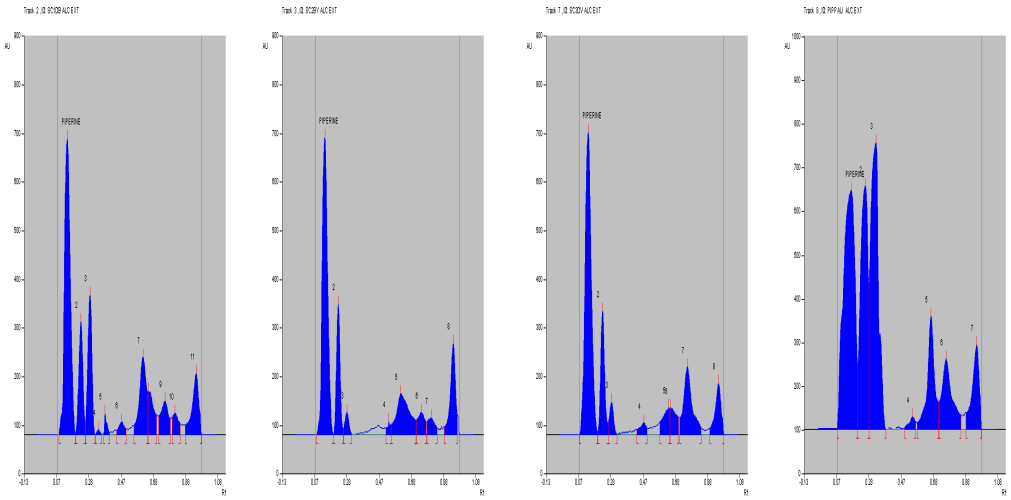
After Derivatization

Fig. 2: H.P.T.L.C. Finger print of Sitopaladi churna



(Track S1) (Track S2) (Track S3)

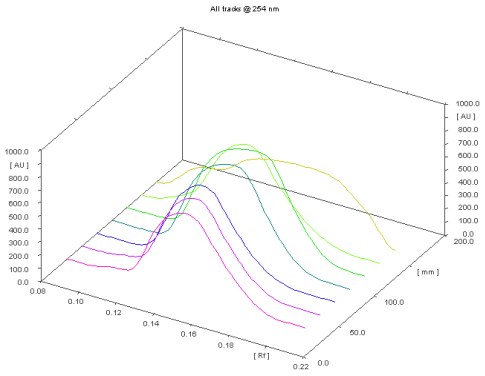
Peaks of Piperine @ 254nm



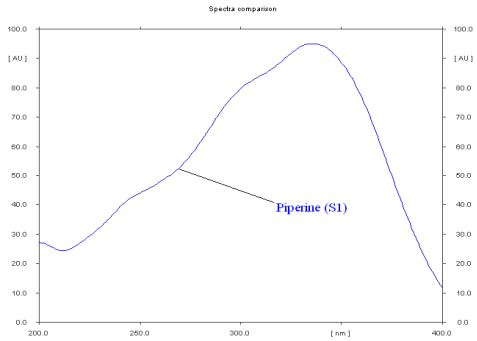
(Track T1) (Track T2) (Track T3) (Track SD1)

Peaks of Sitopaladi churna extract @ 254nm

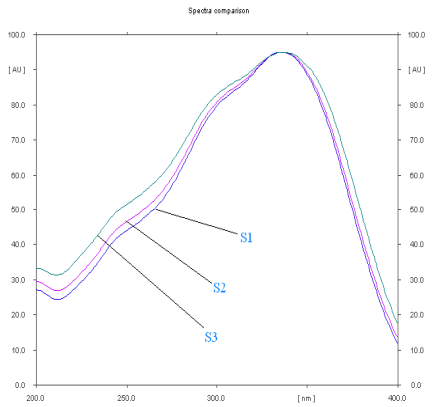
Fig. 3: Peaks of Sitopaladi churna in all Tracks



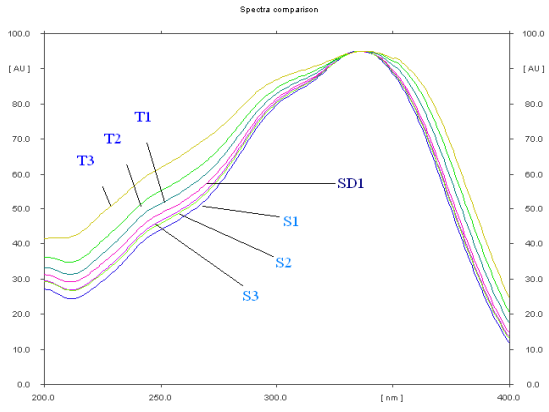
3D Representation



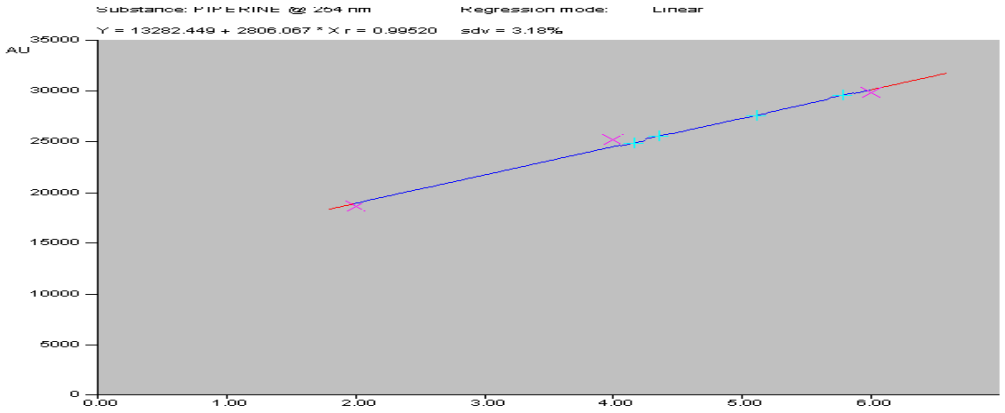
Spectra of Piperine @ 254nm



Spectra of Piperine in all Std.@ 254nm



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



Linearity graph of Conc. (µg) vs AU

Fig.4: 3D Representation, Spectra and Graph of Sitopaladi churna

Table 3: Summary of results

Sr. No.	Sample from	SC1DB	SC2BY	SC3DV	Pippali Fruit
1.	Quantity of Piperine in 1g	0.910mg	0.938mg	1.119mg	2.913mg
2.	% Piperine	0.0910% w/w	0.0938%w/w	0.1119%w/w	0.2913%w/w

3. RESULTS 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. 0.16 very efficiently from the other components in ethanolic extract of Sitopaladi churna (Fig. 2). Sharp peaks of Piperine (Standard and samples) were obtained when the plate was scanned at wavelength 254nm (Fig. 3). Quantities of Piperine found in samples were obtained automatically (Table No. 2) via graph (Fig. 4) and % Piperine found in samples was calculated (Table No. 3). Quantity of Piperine found in sample SC1DB is 0.910mg in 1g drug sample (0.0910% w/w); in SC2BY is 0.938mg in 1g drug sample (0.0938%w/w); in SC3DV is 1.119mg in 1g drug sample (0.1119%w/w) and quantity of Piperine found in Pippali Fruit is 2.913mg in 1g drug sample (0.2913%w/w).

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 Piperine was observed, indicating the robustness of the method.

4. CONCLUSION

The present work was carried out for standardization of Ayurvedic formulation- Sitopaladi churna. A TLC densitometric method has been developed for quantification of piperine using HPTLC. The developed and validated HPTLC methods are simple, precise, and accurate, and can be used for the quantification of piperine in herbal raw materials as well as in their formulations. Hence, these quality-control parameters and the developed HPTLC methods may be considered as a tool for assistance for scientific organizations and manufacturers in developing standards.

REFERENCES

1) Ayurvedic formulary of India, Central Council for Research for Ayurveda and Siddha. 2 nd ed. Part 1. Ministry of Health and Family Welfare, Government of India New Delhi; 2003. p. 116.
2) The Wealth of India, Raw Materials, Revised Edition, Vol IX, Rh-So, NISCAIR, CSIR, New Delhi, 1999,p-367-368.
3) Aswatharam HN, Kaushik U, Lachake P, Shreedhara CS. Standardization of Avipattikar churna- a polyherbal formulation. Pharmacognosy Res 2009; 4:224-7.
4) Sriwastava NK, Shreedhara CS, Aswatharam HN. Standardization of Ajmodadi churna, a polyherbal formulation. Pharamacognosy Res 2010; 2: 98-101.
5) Kenawy IM, Hafez M, Akl M, Lashien R. Determination by AAS of some trace heavy metal ions in some natural as biological samples after their preconcentration using newly chemical modified and chloromethylated polystyrene-PAN ion exchanger. Anal Sci 2000; 16:493.
6) Elamthuruthy AT, Shah CR, Khan TA, Tatke PA, Gabhe SY., J Pharm Biomed Anal 2005;37:937-41

7) Shailajan S, Yeragi M, Purohit A., Int J Pharm Sci Rev Res 2011;9:146-51.
8) Shailajan S, Singh A, Tiwari B, Int J Biomed Res Anal 2010; 1:78-81.
9) Shialajan S, Menon S., J Pharm Res 2011; 4:467-70.
10) Shailajan S, Menon S, Singh A. Int J Pharma Biosci 2009; 1:1-10.
11) Rai N., Pawar R.K., Kumar, Sharma S., Singh K.C. and Sharma R. K.; Hippocratic Journal of Unani Medicine, January- March 2012, Vol. 7 No.1, pg. 49-64.
12) Pawar R.K., Sharma S., Singh K.C. and Sharma R. K.; International Journal of Current Pharmaceutical Research, Vol. 4, Issue 1, 2012, p-43-48.