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

Iram Rakhshi[1], Pawar R K[2*], and Singh K C[3]

[1]Research Scholar, Shri Venkateshwara University Gajraula, Amroha, Uttar Pradesh, India.
[2*]Pharmacopoeias Laboratory for Indian Medicine, Ghaziabad 201- 002, Uttar Pradesh, India.
[3]R.S.S. (P.G.), College, Pilkhuwa, Hapur, Uttar Pradesh, India.

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 l60 GF254 and developed using Toluene Ethyl acetate (9:1) V/Vas mobile phase. The plate was sprayed (derivatized) with Anisaldehyde Sulphuric Acid reagent followed by heating at 110^0C 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.

Author for Correspondence:

R. K. Pawar,
Pharmacopoeia Laboratory for Indian Medicine,
Ghaziabad 201 002 (U.P.) Address- C-27,
First Floor, Swarn Jayanti Puram,
Ghaziabad-201 002, Uttar Pradesh, India.

Email: pawarplim@gmail.com

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 Avaleha, Chitraka Haritakietc. 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 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 officinal is, 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 gand 384 grams of honey. *Plumbagozeylanica* Linn Syn. *Plumbagorosea* 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 contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanolone[5]. Elliptinone, isozeylinone, catechol tannin, [8]isoshinanolone, dihydrosterone and β- sit sterol also islated from plant[3-8]. Plumbagin shows as anticancer and antitumor activity [5-9]. Aspartic acid, tryptophan, tyrosine, heroine, alanine, histidine, glycine, methionine, hydroxyproline, were isolated from the aerial parts[9,15]. Lupeol and lupenyl acetate have been isolated from the root[10-18]. Literature survey reveals that the TLC, HPLC and HPTLC methods are reported but no method as yet is reported for the determination of Gallic acid in Haritaki fruit and Amalaki fruit. A simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of Gallic acid in Haritaki fruit and Amalaki fruit and its compound formulations.

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 cm^2) 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 GF_{254} (20x10 cm^2; 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 gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene Ethyl acetate (9:1) V/Vas 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 110^0C 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 (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 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 & 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 co efficient 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 sampleCH1DB 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 is3.228mg 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 (±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.

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

S.No	Detection/ Visualization	Citrak Haritaki (Track T1, T2 and T3)		Standard- Piperine (Track S1, S2 and S3)		Pippali Fruit (Track SD1)		Kalimirch Fruit (Track SD2)	
		R_f. Values	Colour of band	R_f. Values	Colour of band	R_f. values	Colour of band	R_f. values	Colour of band
1	Under UV 254 nm	0.06 0.15 0.24 0.30	Grey dark grey grey grey	0.15	dark grey	0.06 0.15 0.24 0.30 0.68	dark grey ,, ,, ,, grey	0.06 0.15 0.24 0.30 0.82	dark grey ,, ,, ,, grey
2	Under UV 366 nm	0.06 0.15 0.38 0.42 0.48 0.52 0.55 0.68	sky blue sky blue red red sky blue red red sky blue	0.15	sky blue	0.06 0.15 0.36 0.48 0.55 0.68	sky blue sky blue sky blue sky blue sky blue sky blue	0.06 0.15 0.24 0.38 0.48 0.68	sky blue sky blue green red sky blue sky blue
3	After derivatization	0.06 0.15 0.30 0.32 0.42 0.72 0.84 0.68	greenish grey ,, violet violet violet violet violet violet	0.15	dark greenish grey	0.06 0.15 0.30 0.36 0.42 0.65 0.68	greenish grey ,, violet violet violet red dark sky blue	0.15 0.30 0.36 0.65 0.82	dark greenish grey violet violet red violet

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 Piperine via graph	Linearity and Regression Coefficient and Standard deviation via graph
1	T1	9µl	4500µg	2.779µg	Y = 17245.694 + 3305.754 * X r = 0.99768 sdv = 2.09%
2	T2	9µl	4500µg	3.204µg	
3	S1	2µl	2µg	2.000µg	
4	S2	4µl	4µg	4.000µg	
5	S3	6µl	6µg	6.000µg	
6	T3	9µl	4500µg	3.517µg	
7	SD1	3µl	1500µg	4.471µg	
8	SD2	3µl	1500µg	4.842µg	

Table No.3: Summary of results

S.No	Sample from	CH1DB	CH2BY	CH3ZB	Pippali Fruit	Kalimirch Fruit
1	Quantity of Piperine in 1g	0.617mg	0.712mg	0.781mg	2.980mg	3.228mg
2	% Piperine	0.0617% w/w	0.0712%w/w	0.0781%w/w	0.2980%w/w	0.3228%w/w

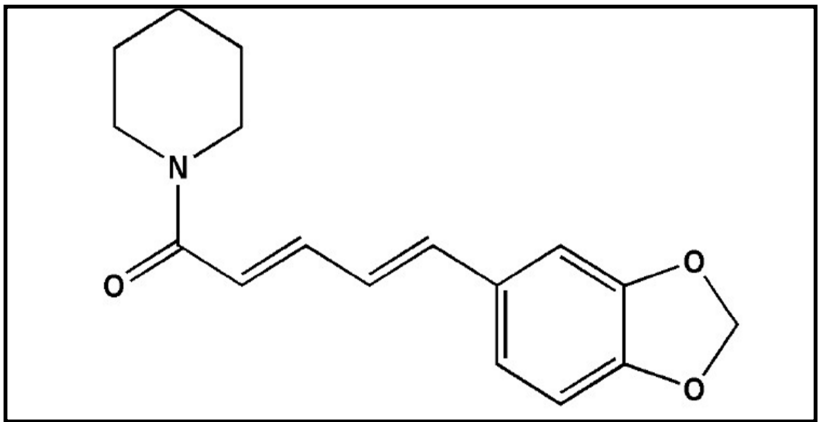
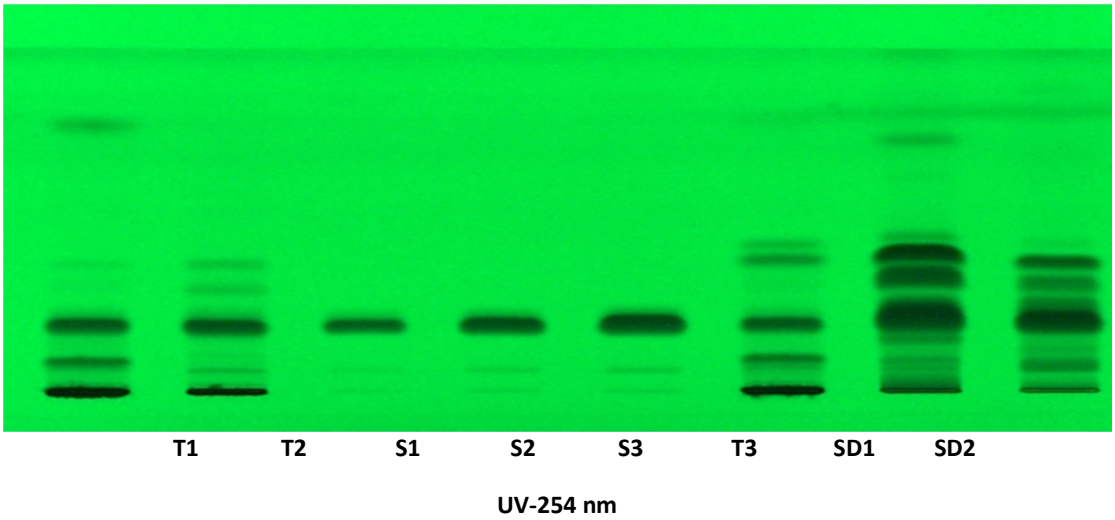
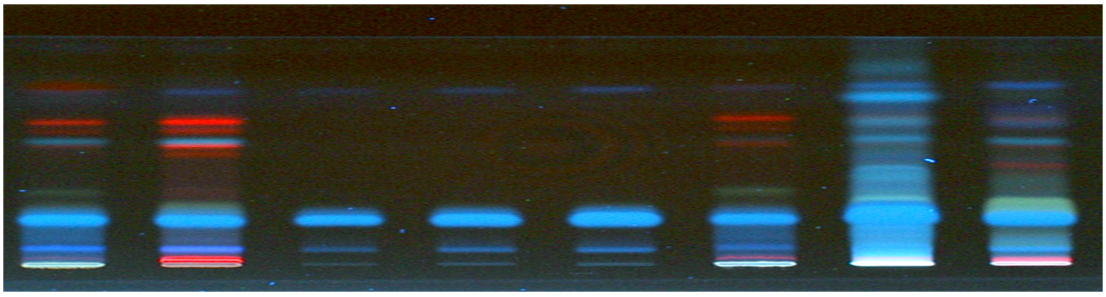


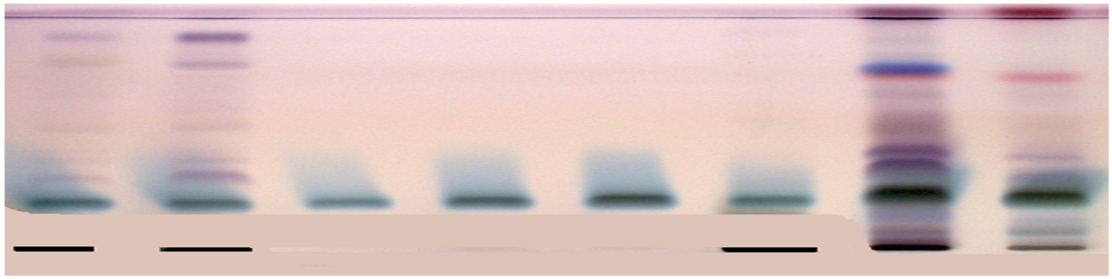
Figure No.1: Molecular structure of Piperine

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

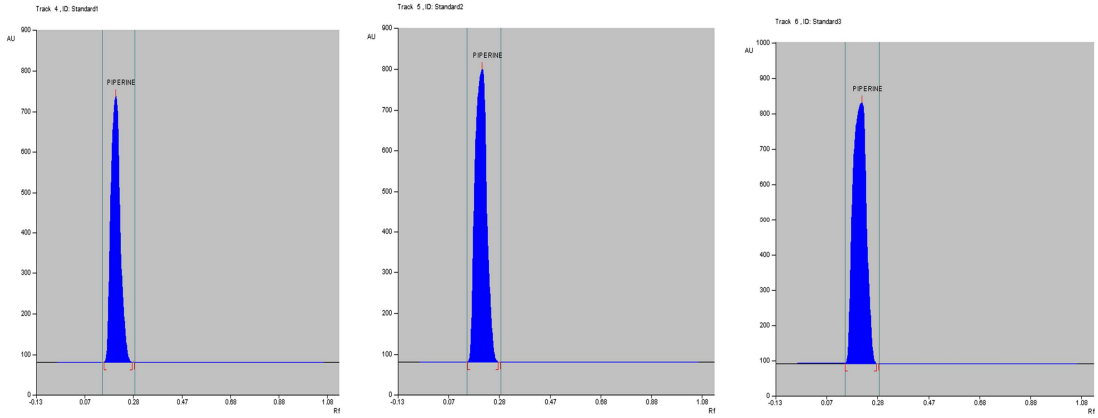




UV-366 nm



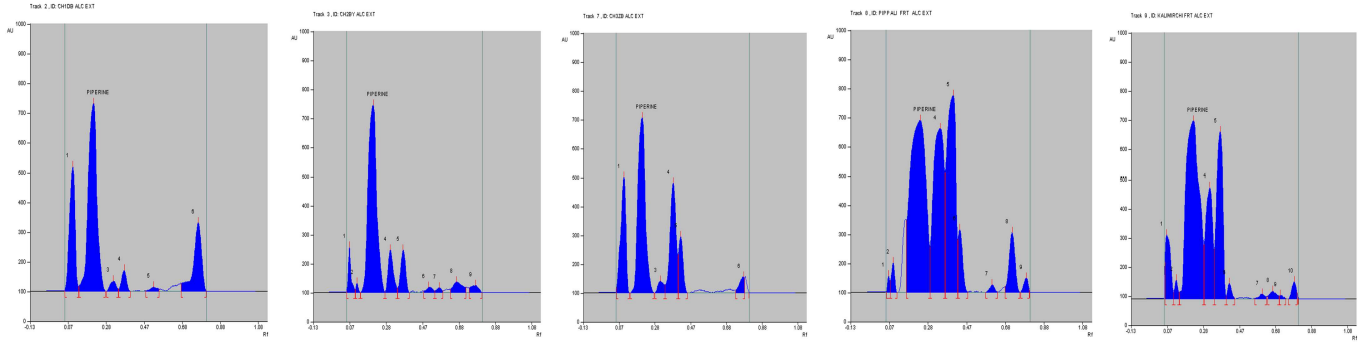
After derivatization



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

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

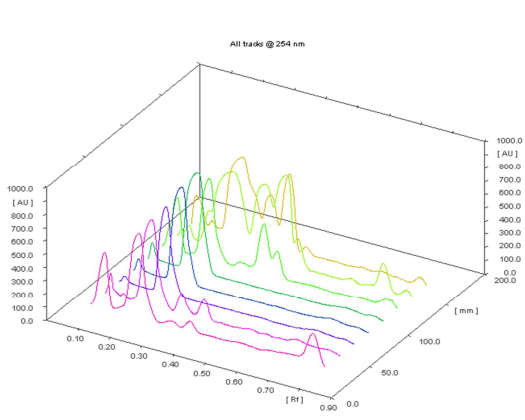
Peaks of Plumb gin @ 270nm



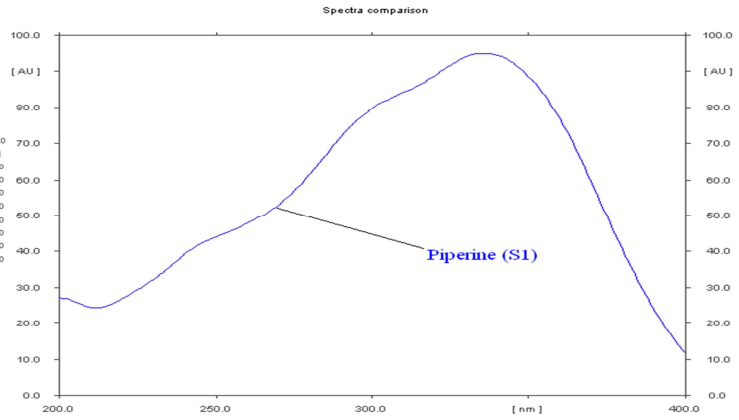
(Track T1) (Track T2) (Track T3) (SD1) (SD2)

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

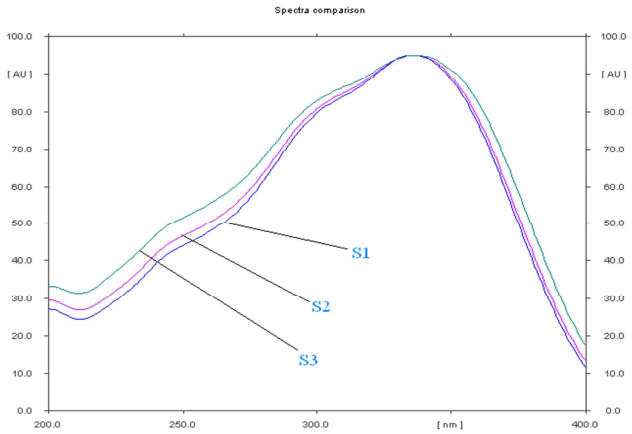
Peaks of Citrak Haritaki and Pippali and Kalimirch CHCl3 Extract @ 270nm



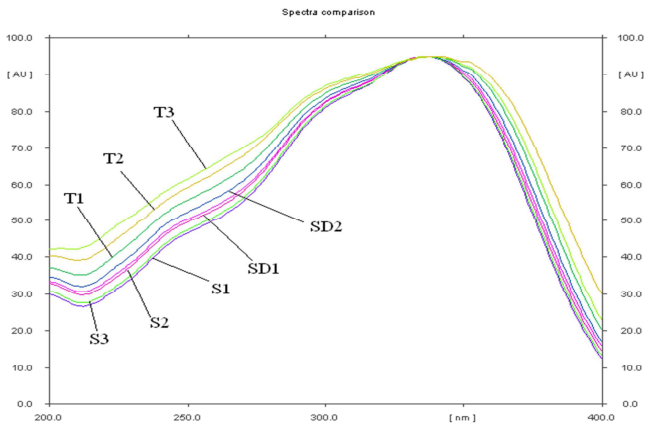
3D Representation



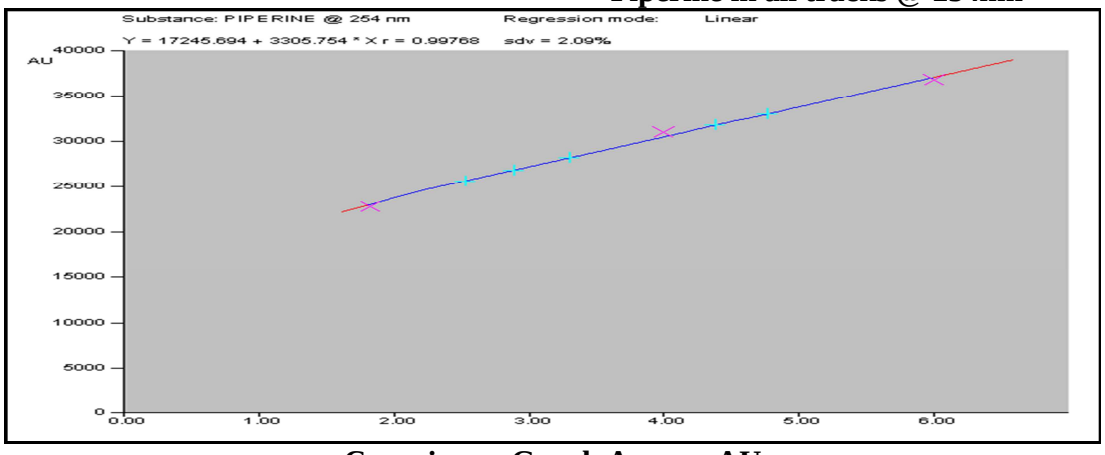
Spectra of Piperine @ 254nm



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.
2. Husain Akhtar and *et al*. Dictionary of Indian Medicinal Plant, *CIMAP, Lucknow*, 1992, 358.
3. The Wealth of India, Raw Materials, Revised Edition, Vol. VIII, *NISCAIR, CSIR, New Delhi*, 1992, 162.
4. Parapet Purohit and *et al*. A Handbook of Medicinal Plants, A Complete Source Book, *Agrobio, India*, 2004, 410.
5. Indian Herbal Pharmacopoeia, Vol. 2, 1999, 103.
6. Thakur R S, Puri H S, Husain Akhtar. Major Medicinal Plant of India, *CIMAP, Lucknow*, 1989, 415.
7. Recent progress in Medicinal plants, Vol.11, Drug Development from Molecules, 267.
8. The Treatise on Indian Medicinal Plants, Vol.-4, 55.
9. Elizabeth M. Williamson Major Herbs of Ayurveda, 2002, 239.
10. Rastogi Ram P, Mehrotra B N. Compendium of Indian Medicinal Plants, *CDRI, PID, New Delhi*, 1993, Vol.2, 547.
11. Rastogi Ram P, Mehrotra B N. Compendium of Indian Medicinal Plants, *CDRI, PID, New Delhi*, Vol.3, 512.
12. Rastogi Ram P, Mehrotra B N. Compendium of Indian Medicinal Plants, *CDRI, PID, New Delhi*, Vol.4, 579.
13. Rastogi Ram P, Mehrotra B N. Compendium of Indian Medicinal Plants, *CDRI, PID, New Delhi*, Vol.5, 673.
14. Madhava Chetty K, Sivaji K, Sudarsanam G, Hindu Sekar P. Pharmaceutical Studies and Therapeutic uses of *Plumbago Zeylanica* L. Roots (Chitraka, Chitramulamu), *Ethan botanical Leaflets*, 10, 2006, 294-304.
15. Dina B, Saha S. *Journal of Indian Chemical Society*, 64(4), 1987, 261.
16. Dina B, Saha S. *Journal of Indian Chemical Society*, 67-88, 1990.
17. Ashwani J Purkar, A R. Balap, Sathiyanarayanan L, Mahadik K R. Development and validation of HPTLC method for simultaneous determination of A Rosuvastatin Calcium and Aspirin pure and Pharmaceutical dosage forms, *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(5), 2014, 704-706.
18. Chawla S and Bansal Y K. Qualitative and quantitative estimation of beta -Sit sterol from *in vitro* regenerated stem bark and callus of Helicteres isora L, *Asian Journal of Pharmaceutical and Clinical Research*, 7(3), 2014, 178-180.

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