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DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF PIPERINE IN TRIKATU CHURNA – 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 Trikatu Churna of different manufactures. The alcoholic extract of Trikatu Churna and Pippali fruit samples were applied on TLC Aluminium plate pre coated with Silicagel60 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 110C for 10 minutes and detection and quantification were carried out densitometrically using anUV detector at wavelength of 254 nm. Content of marker compound in the samples were found similar.

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Keywords- *Trikatu Churna, Pippali Fruit, Piperine, HPTLC etc.*

1. INTRODUCTION

The Trikatu churna is one of the classical Ayurvedic dosage formused in Ayurvedic system of medicine. It is official in ayurvedic formulary of India is combination of three reputed herbs, comprised of the fruits Piper longum L (Pippali), Piper nigrumL (Marica) and rhizomes of Zingiber officianalis Roscoe (Saunth). Trikatu churna is an Ayurvedic proprietary medicine containing Pipalli as an important constituent,which is used for bronchitis and asthma. All these plant materials are used world wide as spices. They are also used as important ingredients in folklore medicine in many Asian countries [1-3]

Trikatu churna is also known as trikatu choornam, Trikatu choorna, katutraya churna, katutraya choorna etc. Trikatu means three herbs which are having Katu Rasa (Bitter taste). This combination streamlines the metabolism of the body this is the reason it is indicated in a wide range of health

problems. Pippali is well known for its immuno modulator action and rejuvenating effect on digestive and respiratory system. Shunthi is one of the best herbs which are rejuvenating for the whole body this is the reason it is also called as Vishvabhaishjya means the medicines of the world. Maricha is said to have Pramathi Guna i.e. it forcefully expels the toxins out of the body. [2-4]

Ayurvedic practitioners consider Trikatu a 'warming formula', used to awaken Agni According to Ayurvedic theory, poor quality food and inconsistent eating habits can create a dullness to the upper GI, which, if uncorrected, will result in further deviations from overall health. Specifically, it can lead to unhealthy food cravings. This creates a feedback loop, since the poor food choices driven by the cravings reinforce the digestive dysfunction.

Trikatu is a unqiue herbal combination. When used along with other herbs, Trikatu enhances the bio-availability of active constituents of the co-herbs in the target are of disease. Trikatu is used as ingredient in many Ayurvedic medicines such as Kanchnar Guggul, Navayasa Lahua, Dashamoolakatutraya kashaya, Ashwagandharishta etc.

Trikatu Churna ingredients -
Shunthi rhizome (Zingiber officinale)- 1 Part
Maricha Fruit (Piper nigrum)- 1 Part
Pippali Fruit (Piper longum)- 1 Part

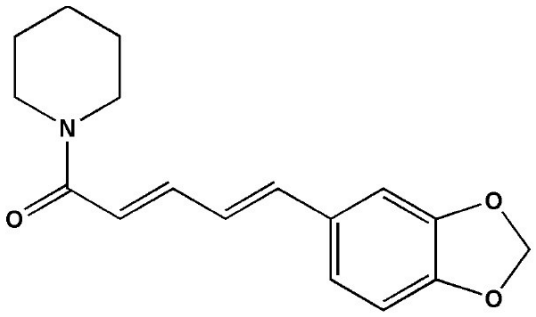
Trikatu churna– method of preparation: The Trikatu churna is a fine powder of drugs. It is prepared by mixing equal quantities of the powder of the dried fruits of Pipernigrum, Piper longum and rhizomes of Zingiber officinale and then sieved through muslin cloth. This churna is stored in airtight container. [5-8]

Trikatu is the remedy for stimulating a sluggish *agni*. It is indicated whenever there is low digestive activity with sluggishness, bloating, abdominal pain and flatulence due to high *kapha* or *vata*. It also helps in conditions of poor nutritional assimilation due to parasites, a leaky gut or low enzyme

secretions. May help in IBS, *Candida albicans*, diarrhoea from cold or food intolerances to damp, wet and heavy foods. It is a specific remedy to help burn *ama* and undigested toxins in the digestive tract and bloodstream. When there is nausea due to excess phlegm this can help. *As* trikatu rejuvenates the lungs it is used whenever there is a cough, wheezing or breathing difficulties with clear, sticky and white phlegm. Used in asthma, bronchitis, pneumonia, cough and colds. A superb remedy for hayfever and acute allergic rhinitis as an immediate way of drying up the copious nasal secretions; it has antiallergenic effects. Also used in sinus congestion and chronic nasal blockage. It has an affinity for all the orifices of the head and clears a muzzy head, clears blocked ears and treats sore throats. [8]

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 *Trikatu Churna* samples. [9-14]

Piperine is reported to have as an antidepressant, hepatoprotective, anti-metastatic, antithyroid, immunomodulatory, antitumor,antiplatelet, antioxidant,and antiamoebic activities.



Molecular 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 Trikatu churna.

2. EXPERIMENTAL & METHOD:

2.1 Material:

(1) The *Trikatu 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) TC1TH (ii) TC2VP (iii) TC3DV

(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 for study.

2.2 Phytochemical evaluation:

Tests for the following major groups of organic constituents for both samples has been done and results given in the Table No. 1

Table No. 1: Phytochemical evaluation of *Trikatu churnas*

S. No.	Name of Organic Groups	Result
1.	Alkaloids	Positive
2.	Carbohydrates	Positive
3.	Flavonoids	Positive
4.	Glycosides	Positive
5.	Proteins	Positive
6.	Saponins	Negative
7.	Steroids	Positive
8.	Tannins	Positive
9.	Resins	Positive

2.3 Determination of physico-chemical constants:

The following Physico-chemical constants has been analysed for both samples and results are given in Table No. 2.

Table No. 2: Physico-chemical constants of *Trikatu churnas*

S. No.	Name of Physico-chemical constants	TC1TH	TC2VP	TC3DV
1.	Moisture content	4.01% w/w	3.87% w/w	4.27% w/w
2.	pH (of 5% aq. Solution)			
3.	Total ash	2.34% w/w	2.67% w/w	2.93% w/w
4.	Acid in-soluble ash	0.75% w/w	0.96% w/w	0.83% w/w
5.	Water soluble ash	1.05% w/w	1.12% w/w	1.17% w/w
6.	Water soluble extractives	31.36% w/w	32.03% w/w	31.78% w/w
7.	Ethanol soluble extractives	15.17% w/w	14.94% w/w	15.04% w/w
8.	Chloroform soluble extractives	6.23% w/w	7.02% w/w	6.743% w/w
9.	Hexane soluble extractives	6.22% w/w	6.91% w/w	6.23% w/w

3. Material and Method:

3.1 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 Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

Chemical & Reagents-

Analytical grade; Toluene, ethyl acetate, 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} (20x10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard- Piperine procured from Aldrich (Lot No.08214 PE-027).

Sample & Standard preparation-

Sample preparation: 1g of drug samples were extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extracts were filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask and used for H.P.T.L.C. work.

Standard Preparation: 5mg of standard Piperine dissolved in 3ml of absolute alcohol and made up to 5ml in standard volumetric flask.

3.2 Chromatography:

TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethylacetate (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 110^0C 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 (Fig. 1). 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.

3.3 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 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 (Fig. 1). 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. 0.15(dark grey). The peaks, graph and spectra obtained were given in Fig.4.3 and 4.4 and R_f. values, colour of bands (Table No.3), quantity of Piperine, linearity, standard deviation & regression coefficient found via graph (Table No. 4) and calculated quantity of Piperine was given in Table No. 5.

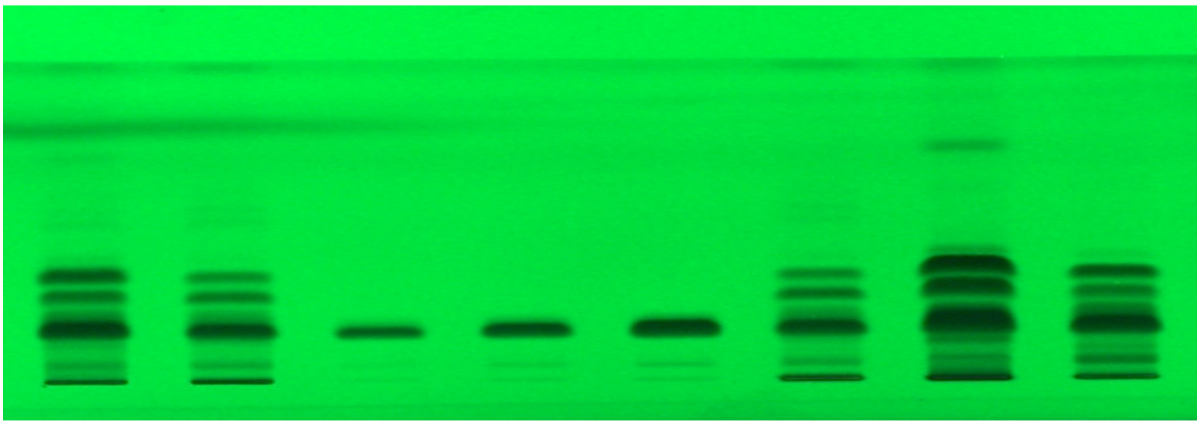
Table No. 3: HPTLC details of alcoholic extract of Trikatu churna

Sr. No.	Detection/ visualization	Trikatu churna (Track T1, T2 & T3)		Standard-Piperine (Track S1, S2 & 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 0.36 0.68	grey dark grey grey dark grey grey	0.15	dark grey	0.06 0.15 0.24 0.30 0.68	dark grey dark grey dark grey dark grey	0.06 0.15 0.24 0.30 0.82	grey dark grey grey dark grey grey
2.	**Under UV 366 nm**	0.06 0.15 0.24 0.36 0.42 0.48 0.55 0.76	sky blue sky blue green red red sky blue red sky blue	0.15	sky blue	0.06 0.15 0.24 0.36 0.42 0.48 0.55 0.68	sky blue sky blue green red red sky blue red sky blue	0.06 0.15 0.24 0.36 0.48 0.76	sky blue sky blue green red sky blue
3.	**After derivatization**	0.06 0.15 0.24 0.35 0.48 0.52 0.65 0.68 0.84	violet greenish grey violet violet brown brown red sky blue violet	0.15	Dark greenish grey	0.15 0.30 0.35 0.42 0.65 0.68	dark greenish grey violet violet brown red sky blue	0.15 0.30 0.35 0.65 0.84	Dark greenish grey violet violet red violet

Table No. 4: Quantity applied on plate and values found via graph

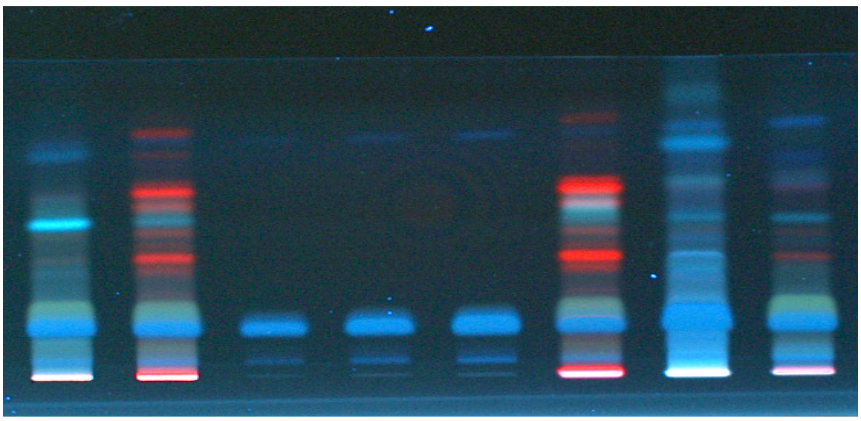
Sr. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of Piperine via graph	Linearity & Regression Coefficient and Standard deviation via graph
1.	T1	5µl	500µg	3.747µg	Y = 14247.956 + 3221.956 * X r = 0.99907 sdv = 1.45%
2.	T2	5µl	500µg	3.971µg	
3.	S1	2µl	2.000µg	2.000µg	
4.	S2	4µl	4.000µg	4.000µg	
5.	S3	6µl	6.000µg	6.000µg	
6.	SD1	9µl	900µg	3.346µg	
7.	T3	5µl	500µg	3.894µg	
8.	SD2	9µl	900µg	3.589µg	

T1- Alcoholic extract of TC1TH
T2- Alcoholic extract of TC2VP
S1- Piperine standard solution (1mg/ml)
S2- Piperine standard solution (1mg/ml)
S3- Piperine standard solution (1mg/ml)
SD1- Alcoholic extract of Pippali Fruit
T3- Alcoholic extract of TC3DV
SD2- Alcoholic extract of Kalimirch Fruit



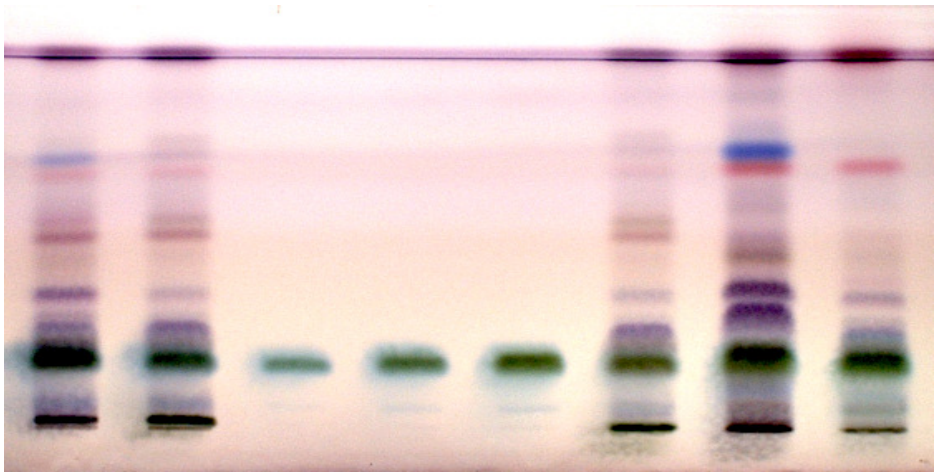
T1 T2 S1 S2 S3 SD1 T3 SD2

UV-254 nm



T1 T2 S1 S2 S3 SD1 T3 SD2

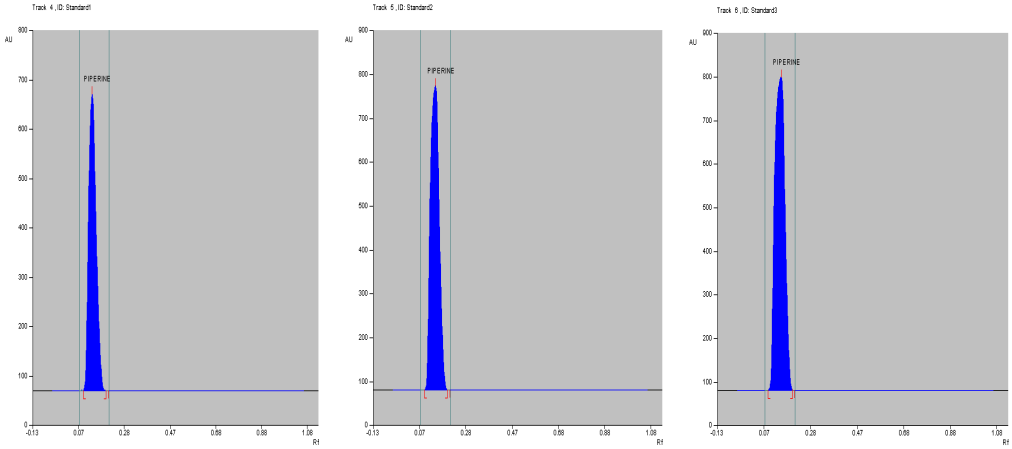
UV-366 nm



T1 T2 S1 S2 S3 SD1 T3 SD2

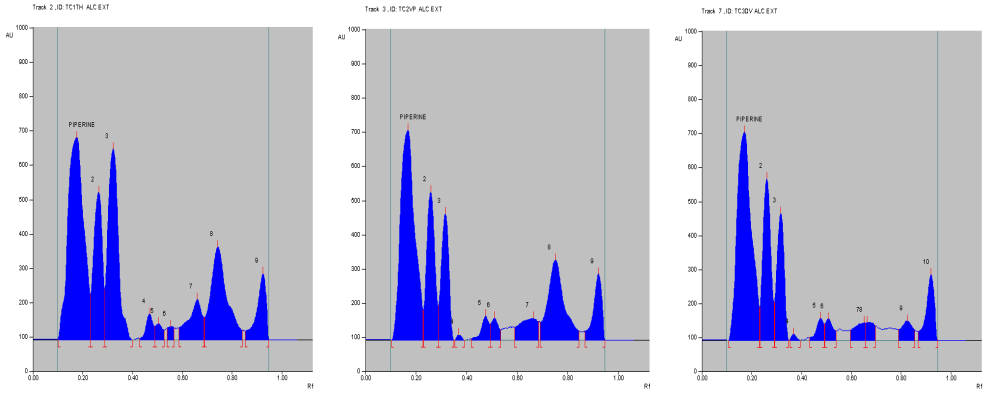
After Derivatization

Fig. 1: H.P.T.L.C. Finger print of Trikatu churna



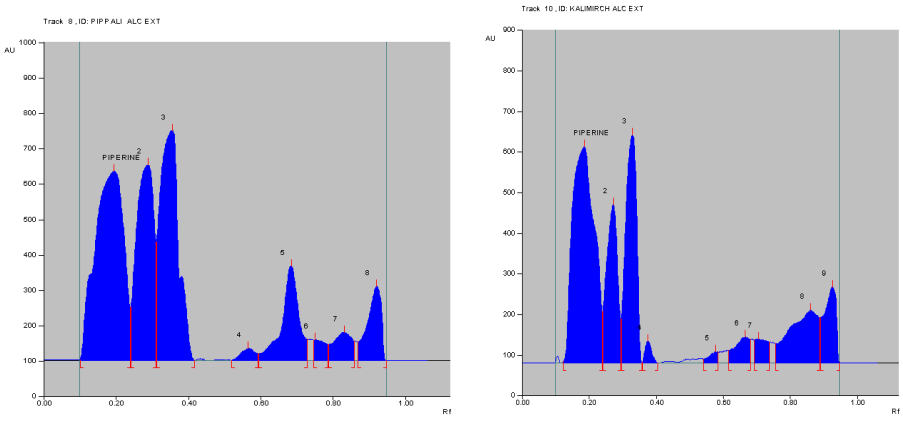
(Track S1) (Track S2) (Track S3)

Peaks of Piperine @ 254nm



(Track T1) **(Track T2)** **(Track T3)**

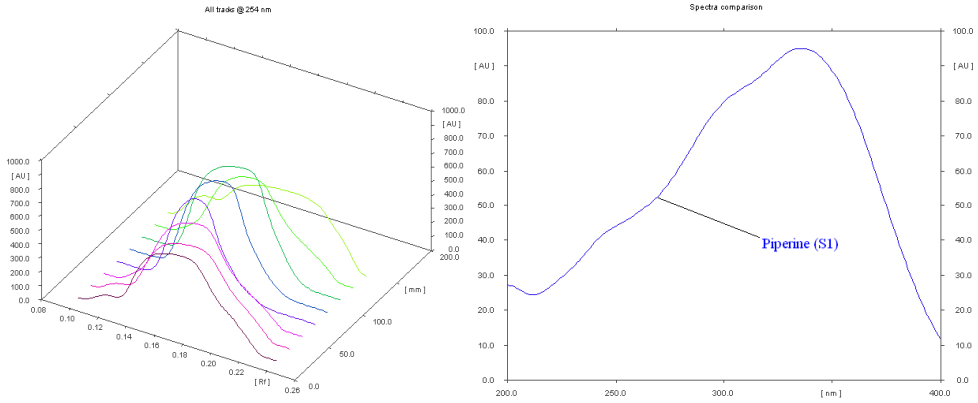
Peaks of Trikatu churna extract @ 254nm



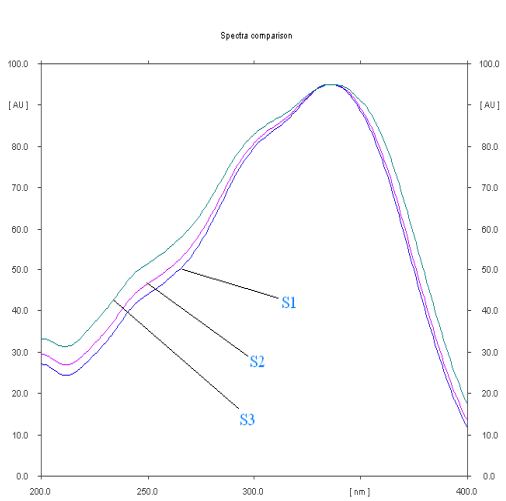
(Track SD1) **(Track SD2)**

Peaks of Pippali Fruit and Kalimirch Fruit extract @ 254nm

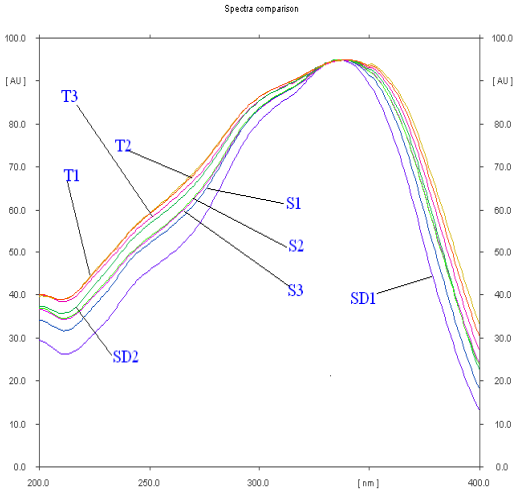
Fig. 2- Peaks of Trikatu churna in all Tracks



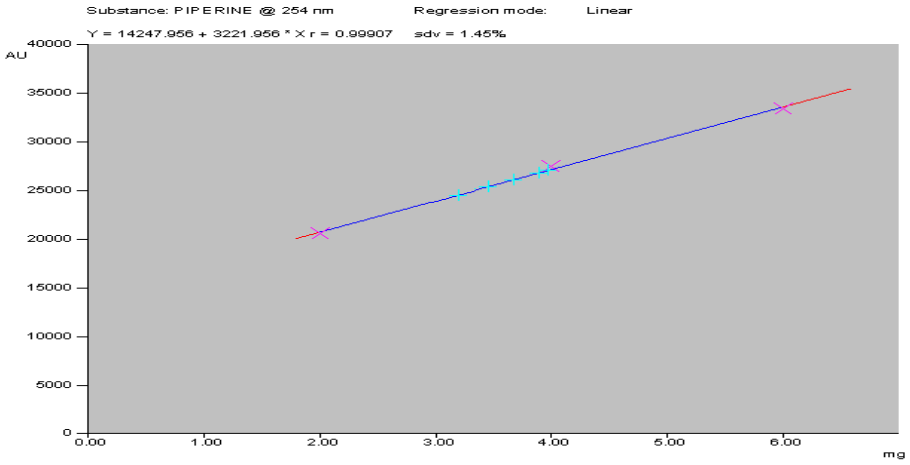
3D Representation **Spectra of Piperine @ 254nm**



Spectra of Piperine in all Std.@ 254nm



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



Conc. in µg

Graph- Conc. vs AU

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

Table No. 5: Summary of results

Sr. No. ↓	Sample from →	TC1TH	TC2VP	TC3DV	Pippali Fruit	Kalimirch Fruit
1.	**Quantity of Piperine in 1g**	7.494mg	7.942mg	7.788mg	3.717mg	3.987mg
2.	**% Piperine**	0.7494% w/w	0.7942%w/w	0.7788%w/w	0.3717%w/w	0.3987%w/w

4. 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 brown colour band at R_f. 0.33 very efficiently from the other components in ethanolic extract of Trikatu churna (Fig. 4.2). Sharp peaks of Piperine (Standard and samples) were obtained when the plate was scanned at wavelength 254nm (Fig. 1). Quantity of Piperine found in samples were obtained automatically (Table No. 4) via graph (Fig. 3) and % Piperine found in samples was calculated (Table No. 5). Quantity of Piperine found in sample TC1TH is 7.494mg in 1g drug sample (0.7494% w/w); in TC2VP is 7.942mg in 1g drug sample (0.7942%w/w); in TC3DV is 7.788mg in 1g drug sample (0.7788%w/w) and quantity of Piperine found in Pippali Fruit is 3.717mg in 1g drug sample (0.3717%w/w) and in Kalimirch Fruit is 3.987mg in 1g drug sample (0.3987%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.

5. CONCLUSION:

The present work was carried out for the formulation and standardization of Trikatu churna. 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.

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