IJPSR (2018), Volume 9, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 October, 2017; received in revised form, 31 March, 2018; accepted, 12 June, 2018; published 01 July, 2018

HPTLC FINGERPRINTING FOR SIMULTANEOUS QUANTIFICATION OF HARMINE, KAEMPFEROL, DIOSGENIN AND OLEIC ACID IN THE FRUIT EXTRACT OF *TRIBULUS TERRESTRIS* L. AND ITS FORMULATION

Vikas V. Vaidya [1], Prakash L. Kondalkar [*1], Manjiri A. Shinde [1] and Sulekha Gotmare [2]

Department of Chemistry [1], Ramnarain Ruia College, Matunga, Mumbai - 400019, Maharashtra, India.
Department of Analytical Chemistry [2], S. N. D. T. Women's University, Santacruz, Mumbai - 400049, Maharashtra, India.

Keywords:

Tribulus terrestris L., Harmine, Kaempferol, Oleic acid, Diosgenin, CAMAG

Correspondence to Author:
Mr. Prakash L. Kondalkar

Student,
Department of Chemistry,
Ramnarain Ruia College, Matunga,
Mumbai - 400019, Maharashtra,
India.

E-mail: prakash.kondalkar@gmail.com

ABSTRACT: A simple, accurate and reproducible HPTLC method has been developed for the simultaneous quantification of diosgenin, oleic acid, harmine and kaempferol from the methanolic extract of fruits of plant *Tribulus terrestris* L. and its marketed formulation. The CAMAG HPTLC systems with winCATS software used for the analysis. Double development with mobile phase Toluene: Ethyl acetate: Formic acid: Glacial acetic acid (2:1:1:0.75 v/v/v/v) on TLC plate silica gel 60 F 254. Detection and quantification of harmine at 366 nm, kaempferol at 270 nm, diosgenin at 201 nm and oleic acid at 580 nm after derivatization with anisaldehyde sulphuric acid reagent. The quantity of harmine was found to be 0.014% and 0.006%, kaempferol was 0.018% and 0.006%, oleic acid was 0.259% and 0.108% and diosgenin was 0.086% and 0.022% in plant and formulation respectively. The developed method was then validated in terms of specificity, linearity, LOD, LOQ, precision and recovery. The validated method was successfully applied for quantification of four components in a formulation containing *Tribulus terrestris* L. extract.

INTRODUCTION: Many countries (including developed ones) suffer a big problem in standardizing and quality control of the herbal plants. This is due to many factors among which are the complex form of these products and the inability of the traditional methods to precisely estimate the quality of the herbs [1]. The quality of herbal medicines is defined in terms of the content of its bioactive compounds.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(7).3066-74

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(7).3066-74

Modern high-performance TLC (HPTLC) is an efficient instrumental analysis, and optimised quantitative HPTLC using a densitometric evaluation can produce results analogous to those obtained with gas chromatography (GC) and high performance liquid chromatography (HPLC) [2, 3].

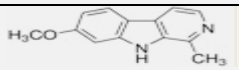
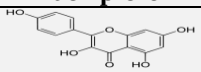
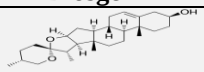
Thus, HPTLC 'fingerprint analysis' may be a powerful tool for the quality control of raw plant material and may be an alternative technique, particularly in the analysis of crude plant extracts [4]. An improvement over conventional TLC, HPTLC is an instrumental technique where by special plates and instrumental resources for sampling are used and the quantitative evaluation of separations is aided by densitometry [5, 6].

Tribulus terrestris L. is a valuable herb known for its application in the folk medicine in various parts of the world. In different regions the common names of this plant are Puncture vine, Caltrop, Yellow vine, Goathead, Burra Gokharu and bindii [6, 7]. This plant is extremely rich in substances having potential biological significance, including: saponins, flavonoids, alkaloids, and other nutrients [8, 9, 10]. Gokshura is extremely efficacious in most of the urinary tract disorders because it promotes the flow of urine, cools and soothes the membranes of the urinary tract, and aids in the expulsion of urinary stones and gout. It also stops bleeding from the tract and rejuvenates the urogenital system, both in males as well as females. Gokshura effectively controls the bleeding, in large doses, it imparts the laxative action, hence is used as an adjunct in the treatment of piles. It is commonly used in treating diabetes [11], urinary calculi, dysuria, gout and sexual debility [12].

Many forms of raw plant material and herbal drugs derived from *Tribulus terrestris* L. are distributed in herbal market; however, the content of bioactive components in these products have not necessarily been quality-controlled [13, 14, 15]. Therefore, a simple, low-cost, and rapid method for screening and quantitating bioactive components is strongly desired [17, 18].

Literature survey revealed that no method has been reported for simultaneous quantitation of diosgenin, oleic acid, harmine and kaempferol from methanolic extract of fruits of *Tribulus terrestris* L. Therefore, the aim of the study was to develop a rapid, precise and reproducible HPTLC method for quantification of diosgenin, oleic acid, harmine and kaempferol from *Tribulus terrestris* L. plant materials that can be used to determine their content in commercial herbal drugs.

TABLE 1: STRUCTURES AND PROPERTIES OF BIOACTIVE COMPONENTS

Component	Harmine	Kaempferol	Oleic acid	Diosgenin
Structure				
Molecular formula	$C_{15}H_{12}N_2O$	$C_{15}H_{10}O_6$	$C_{18}H_{34}O_2$	$C_{27}H_{42}O_3$
Molecular weight	212.25	286.24	282.46	414.6
PKa	6.44	6.44	4.99	Neutral
Group	Alkaloid	Flavonoid	Essential fatty acid	Saponin

MATERIALS AND METHOD:

Collection of Plant: *Tribulus terrestris* L. were collected from Padadhari, around 30 km away from Rajkot, Gujarat, India in the month of December and it was authenticated with specimen no. 10291(2) of H. Santapau at 'Blatter Herbarium' in St. Xavier's college, Mumbai - 400001.

Preparation of Plant Material: The fruits were washed thoroughly with tap water. The fruits were dried initially using tissue paper to remove excess of water and later were air dried thoroughly under shade at room temperature to avoid direct loss of phytoconstituents from sunlight. The shade dried material was powdered using grinder and sieved through an ASTM 80 mesh. It was then homogenized to fine powder and stored in an air-tight container for further analysis [19].

Preparation of the Fruit Extracts: About 5 gm of dried fruit powder of *Tribulus terrestris* L. was weighed into a round bottom flask. 150 ml of mixture of 2N hydrochloric acid and methanol in the ratio of 20:80 was added to the flask and the mixture was refluxed at controlled 80 °C on a boiling water bath for about 6 h. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India) and extracted with chloroform (50 ml × 3). The three chloroform extracts were combined and rinsed thrice times with 2N NaOH and then rinsed thrice with distilled water. The extract was then passed through a filter bed of Na_2SO_4 to eliminate any remaining water.

The samples were concentrated to dryness by evaporating the solvent at reduced pressure on Rotavapor buchi at 60 °C and reconstituted the residue to final 50 ml volume in volumetric flask. This solution was further used for assay.

Preparation of the formulation extracts: About 15 gm of formulation Gokhshuradi Guggul containing *Tribulus terrestris* L. was weighed into a round bottom flask. 150 ml of mixture of 2N hydrochloric acid and methanol in the ratio of 20:80 was added to the flask and the mixture was

refluxed at controlled 80 °C on a boiling water bath for about 6 h. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India) and extracted with chloroform (50ml × 3). The three chloroform extracts were combined and rinsed thrice times with 2N NaOH and then rinsed thrice with distilled water. The extract was then passed through a filter bed of Na_2SO_4 to eliminate any remaining water. The samples were concentrated to dryness by evaporating the solvent at reduced pressure on Rotavapor buchi at 60 °C and reconstituted the residue to final 50 ml volume in volumetric flask. This solution was further used for assay.

Reagents and Standards: All chemicals and solvents used were of analytical grade and purchased from Merck (Darmstadt, Germany). Analytical standards diosgenin, oleic acid, harmine and kaempferol were procured from Sigma-Aldrich (Bengaluru, India).

Preparation of Standard Solutions: Stock solutions of standards were prepared in methanol just before use. 25.78 mg of diosgenin, 60.54 mg of oleic acid and 24.58 mg of harmine and 23.78 mg kaempferol were dissolved separately in 25 ml of methanol.

Chromatographic Conditions:

TABLE 2: CAMAG SYSTEM

Applicator	ATS 4 (Automatic TLC sampler 4)
Chamber	ADC 2
Scanner	TLC Scanner 4
Visualizer	TLC Visualizer 2
HPTLC software	win CATS

TABLE 3: CHROMATOGRAPHIC CONDITION

Stationary phase	Merck, TLC plate silica gel 60 F 254 (200 × 100 mm)
Method	Double development 2 chambers (20 × 10 cm)
Mobile phase 1	Toluene : Ethyl acetate : Formic acid: Glacial acetic acid (2 : 1 : 1: 0.75 v/v/v/v)
Saturation time	20 min
Development	Development till 40 %
Drying time & temperature	5 min & room temperature
Mobile phase 2	Toluene : Ethyl acetate : Formic acid: Glacial acetic acid (2 : 1 : 1: 0.75 v/v/v/v)
Saturation time	20 min
Development	Development till 80%
Drying time & temperature	5 min & room temperature
Derivatization	Dip method with Anisaldehyde Sulphuric Acid Reagent
Heating	At 110 °C for 10 min

TABLE 4: DETECTION PARAMETERS

Component	Lamp	Scanning	Wavelength	Colour
Harmine	Mercury	Pre derivatization	366 nm	Blue
Kaempferol	Deuterium	Pre derivatization	270 nm	Blackish green
Diosgenin	Deuterium	Pre derivatization	202 nm	No colour/ green after derivatization
Oleic acid	Tungsten	Post derivatization	580 nm	Purple

Validation of the Method: The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice [20].

Specificity: During the experiments scan ranging from 200 to 800 nm in the time window of the analytes using TLC Scanner 4 was performed with the aim of revealing eventual interfering compounds and evaluating the selectivity of the method. Specificity of the intended method was established by comparing the HPTLC retention factor (R_f) and of target peaks from the analysed samples with those of the reference compounds.

Specificity test was carried out by applying 4 µL of each of Tribulus *terrestris* L. fruits methanolic extract, formulation extract, 2 µL of 50 µg/mL diosgenin standard solution, 0.5 µL of standard solution of 100 µg/mL of harmine, 1 µl of 100 µg/ml kaempferol, 1 µl of 800 µg/ml of oleic acid, 4 µL of diluent and mobile phase.

Precision: The variability of the method was studied by carrying out repeatability and intermediate precision. Repeatability was carried out in same laboratory, on same day, by analysing quality control samples containing the mixture of diosgenin, oleic acid, harmine and kaempferol using optimized chromatographic conditions. The experiment for inter-day precision was carried out using quality control samples of diosgenin, oleic

acid, harmine and kaempferol on different days. The developed method was found precise with % CV<2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ): ICH defines the limit of detection (LOD) is the lowest concentration of an analyte that can be detected under the operational conditions of the method but not necessarily quantitated as an exact value. The limit of quantification (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy, under the operational conditions of the method.

Linearity: The Linearity of a method is the measure of how well a calibration plot of detector response against concentration approximates to a straight line. Seven concentration levels of each marker were selected for linear dynamic range for experiment. For harmine, concentration levels of 24.58 µg/mL to 196.64 µg/mL were selected. Concentrations of kaempferol were 47.56µg/mL to 190.24 µg/mL, Concentrations of oleic acid were 484.32 to 1937.28 µg/mL, for diosgenin, concentrations of 50.56µg/mL to 206.24µg/mL, were selected for linear dynamic range for experiment. The correlation coefficient was found to be ≥0.995.

Assay: 4 µL of sample solution *i.e. Tribulus terrestris* L. fruits extract and 4 µL of formulation were injected six times separately and analysed using the optimized chromatographic conditions. Peak areas were recorded for each analyte of interest and the amount of all the four analytes (harmine, kaempferol, oleic acid and diosgenin) was calculated by use of the calibration plot.

Recovery: The recovery experiment was carried out to check if there is any interference of other constituents with the peaks of diosgenin, oleic acid, harmine and kaempferol present in fruits of *Tribulus terrestris* L. and formulation Gokhshuradi Guggul containing extract of *Tribulus terrestris* L. Accuracy of the method was established by carrying out recovery experiment at three different levels, using standard addition method. To 4 µL fruits extract and 4 µL of formulation, known amounts of pure standards of diosgenin, oleic acid, harmine and kaempferol were added at different levels. The sample was then analysed by HPTLC method using the developed optimized chromatographic conditions. Each sample was analysed in three replicates and the amounts of diosgenin, oleic acid, harmine and kaempferol recovered for each level, were determined. The value of percentage recovery for the four components was then calculated.

$$\text{Recovery (\%)} = [(\text{amount found} - \text{original amount}) / \text{amount added}] \times 100.$$

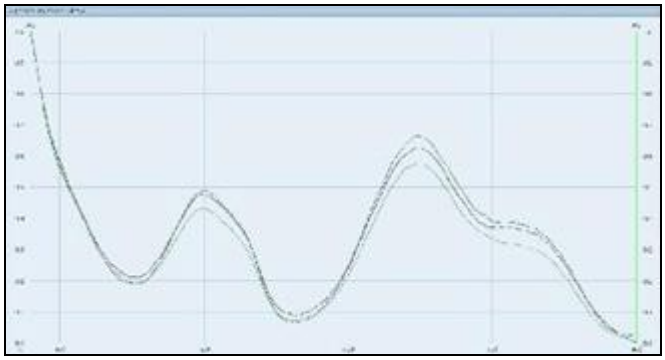
Values within the range of 85 - 115% were accepted.

Robustness: Robustness of the method was studied by determining the effects of small variations of mobile phase saturation time (20 ± 5 min), TLC plate drying time (5 ± 2 min) and mobile phase composition of Ethyl acetate (1 ± 0.2). Effect of these deliberate changes on the response (area) and retention factor of QC samples of harmine, kaempferol, oleic acid and diosgenin was observed during the analysis. The results were expressed in terms of % mean difference. Values within a difference range of ± 5% were accepted.

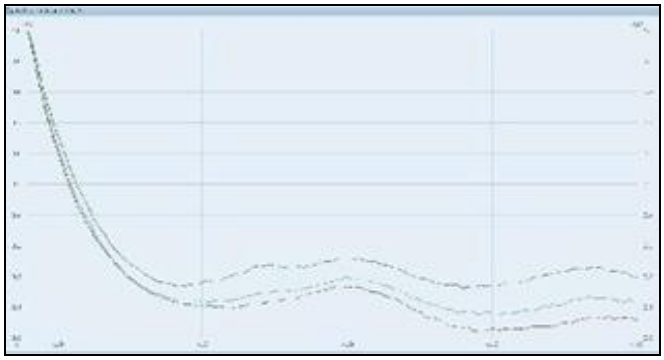
Solution Stability: The stability of the stock solutions of all the three standards was evaluated by storing the solutions in refrigerator at 2 - 8 ºC for 72 h and then comparing the results against freshly prepared stocks for each standard. Samples in triplicate were also subjected to bench top stability at 0 h, 24 h, 48 h and 72 h respectively. Values within a difference range of ±5% were accepted.

RESULTS AND DISCUSSION:

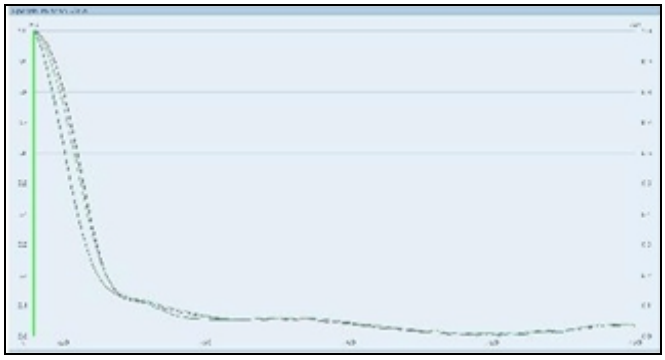
Optimization of the Chromatography: Initial trial experiments were conducted to select a suitable mobile phase for accurate analysis of the standard with the various mobile phases and compositions. UV-visible spectra using TLC Scanner 4 corresponding to the four standards, *T. terrestris* L. fruit extract and marketed formulation are represented in **Fig. 1**. Photo Scan of TLC plate at 366 nm for estimation of harmine, at 270 nm for kaempferol and at 580 nm after derivatization forestimation of oleic acid and visualization of diosgenin are represented in **Fig. 2** to **4**. There are 14 spots. Spot number 1 to 3 are of Formulation extract, spot number 4 to 11 of linearity levels (7 out of 8 are selected to plot calibration curve) and spot number 12 to 14 are of *T. terrestris* L. fruit extract.



SPECTRA OVERLAY OF HARMINE AND SAMPLE (FORMULATION AND FRUIT)



STANDARD SPECTRA OVERLAY OF KAEMPFEROL AND SAMPLE (FORMULATION AND FRUIT)



SPECTRA OVERLAY OF OLEIC ACID AND SAMPLE (FORMULATION AND FRUIT)

STANDARD SPECTRA OVERLAY OF DIOSGENIN AND SAMPLE (FORMULATION AND FRUIT)

FIG. 1: OVERLAY OF UV SPECTRA

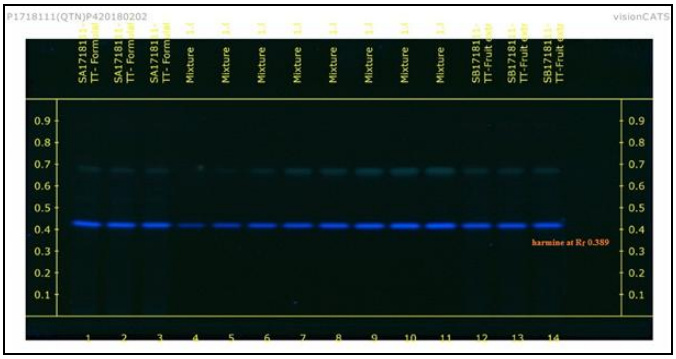


FIG. 2: TYPICAL SCAN OF TLC PLATE AT 366 nm FOR ESTIMATION OF HARMINE AT R_f 0.389

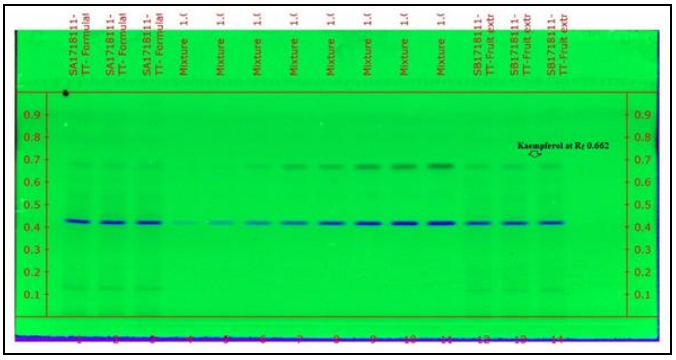


FIG. 3: TYPICAL SCAN OF TLC PLATE AT 366 nm FOR ESTIMATION OF KAEMPFEROL AT R_f 0.662

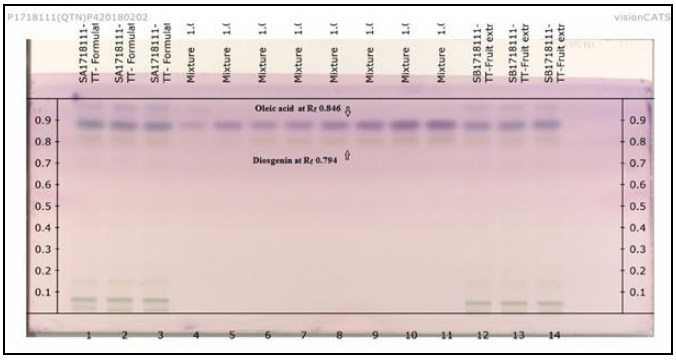
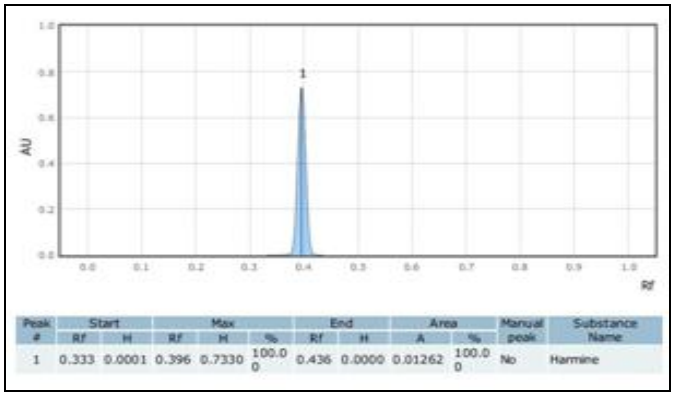
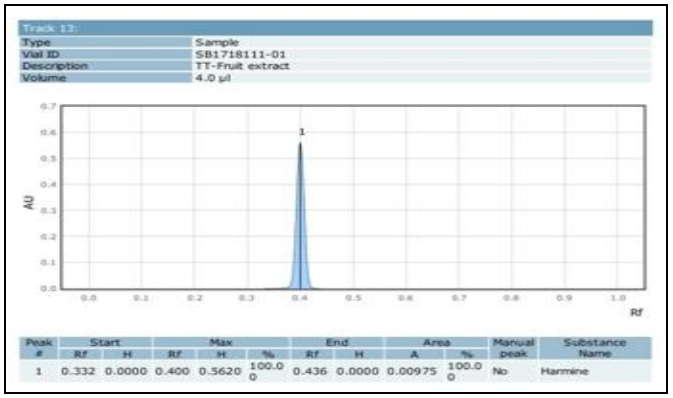


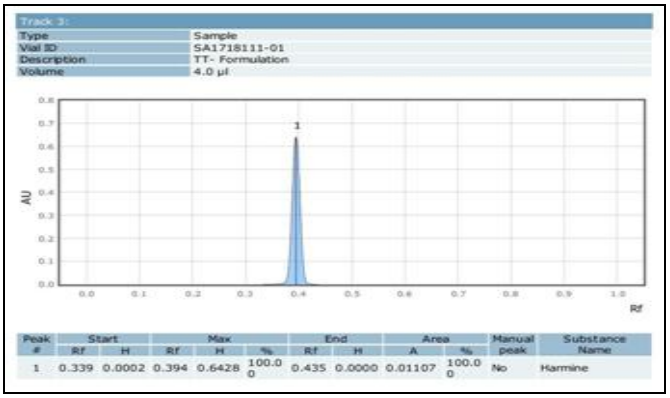
FIG. 4: TYPICAL SCAN OF TLC PLATE AT 580 nm AFTER DERIVATIZATION FOR ESTIMATION OF OLEIC ACID AT R_f 0.846 AND DIOSGENIN AT R_f 0.794



STANDARD HARMINE

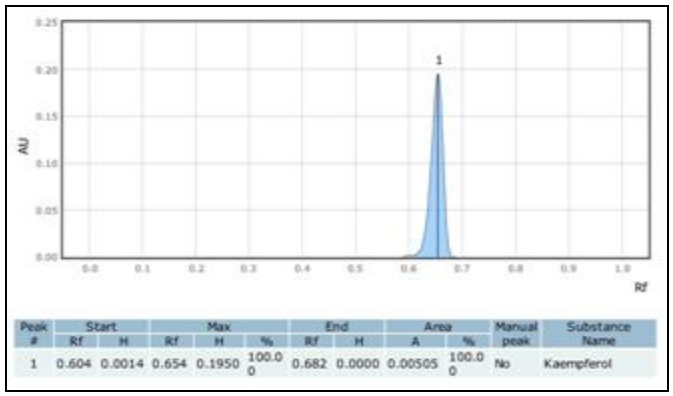


T.T. FRUIT SAMPLE

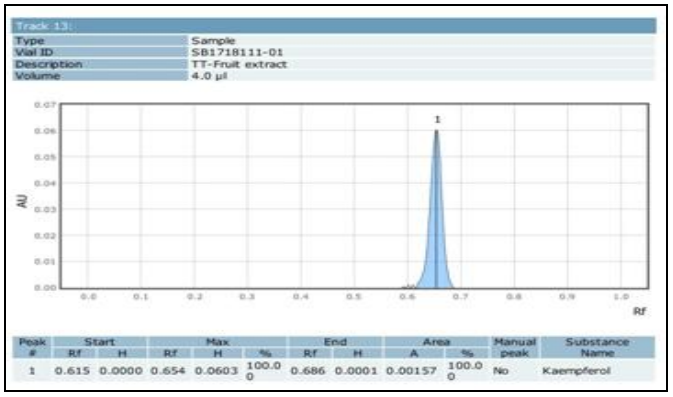


T.T. FORMULATION

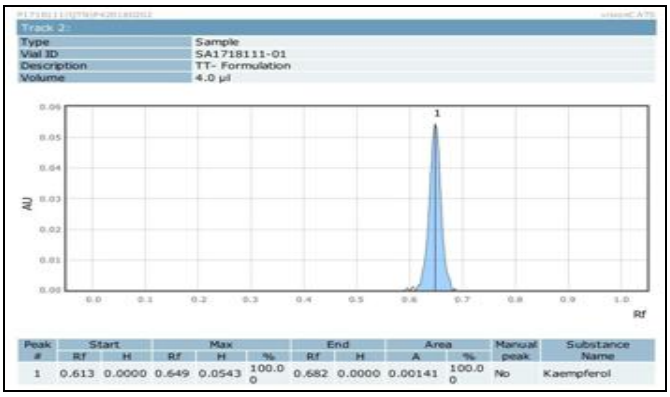
FIG. 5: DENSITOGRAMS OF STANDARD HARMINE, T.T. FRUIT SAMPLE AND T.T. FORMULATION AT 366 nm



STANDARD KAEMPFEROL

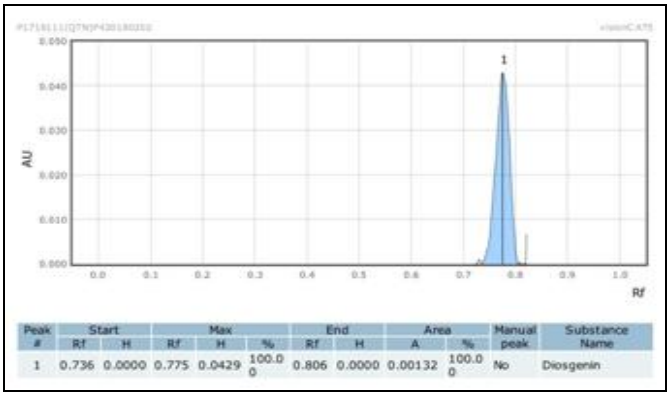


T.T. FRUIT SAMPLE



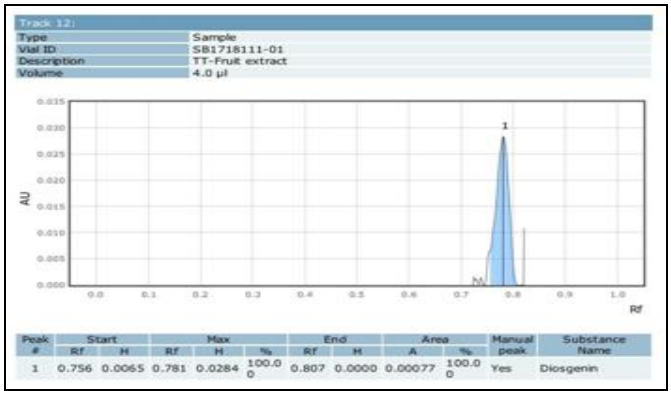
T.T. FORMULATION

FIG. 6: DENSITOGRAMS OF STANDARD KAEMPFEROL, T.T. FRUIT SAMPLE AND T.T. FORMULATION AT 270 nm



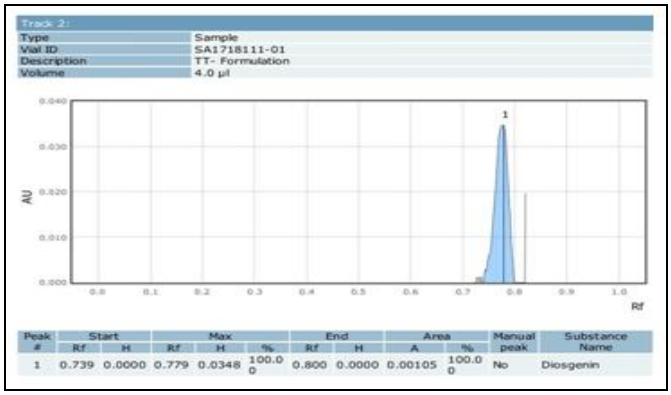
Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.736	0.0000	0.775	0.0429	100.00	0.806	0.0000	0.00132	100.00	No	Diosgenin

STANDARD DIOSGENIN



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.756	0.0065	0.781	0.0284	100.00	0.807	0.0000	0.00077	100.00	Yes	Diosgenin

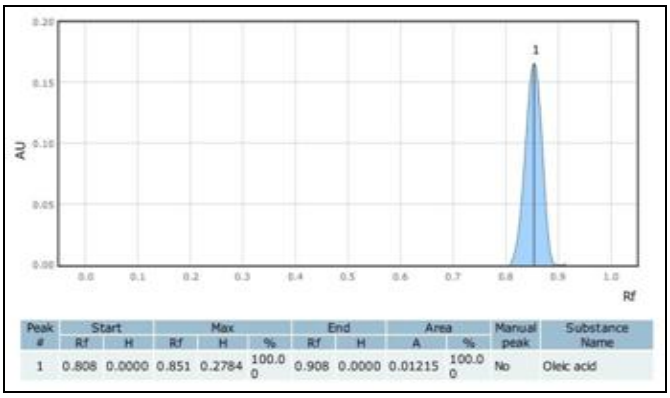
T.T. FRUIT SAMPLE



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.739	0.0000	0.779	0.0348	100.00	0.800	0.0000	0.00105	100.00	No	Diosgenin

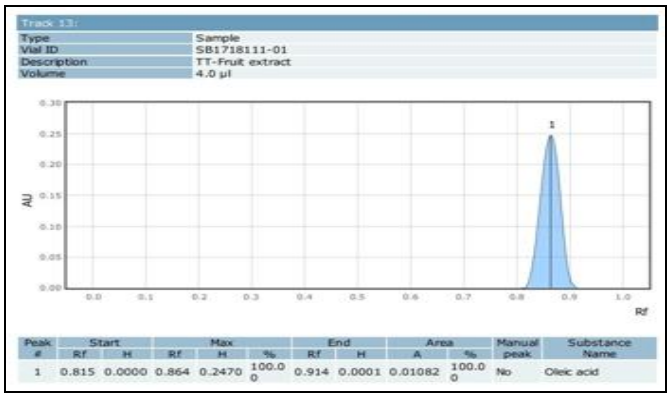
T.T. FORMULATION

FIG. 7: DENSITOGRAMS OF STANDARD DIOSGENIN, T.T. FRUIT SAMPLE AND T. T. FORMULATION AT 201 nm



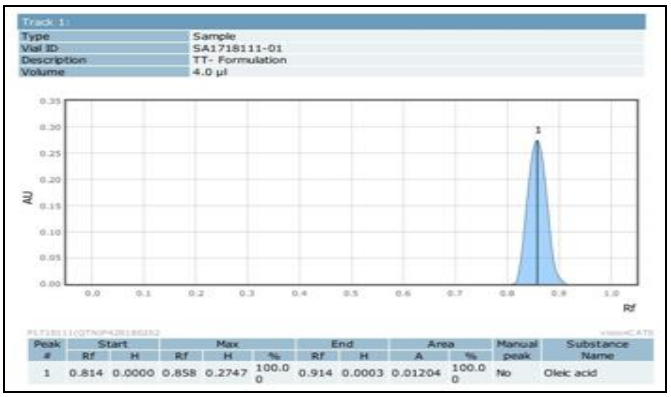
Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.808	0.0000	0.851	0.2784	100.00	0.908	0.0000	0.01215	100.00	No	Oleic acid

STANDARD OLEIC ACID



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.815	0.0000	0.864	0.2470	100.00	0.914	0.0001	0.01082	100.00	No	Oleic acid

T.T. FRUIT SAMPLE



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.814	0.0000	0.858	0.2747	100.00	0.914	0.0003	0.01204	100.00	No	Oleic acid

T.T. FORMULATION

FIG. 8: DENSITOGRAMS OF STANDARD OLEIC ACID, T.T. FRUIT SAMPLE AND T.T. FORMULATION AT 580 nm AFTER DERIVATIZATION

Method Validation Parameters:

TABLE 5: LOD, LOQ AND LINEARITY

Compound	R_f*	Regression equation	r^2	Linear range (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)
Harmine	0.389 ±0.010	Y= 77.056 X +1267.6	0.9971	24.56-196.64	14.75	4.87
Kaempferol	0.662 ±0.010	Y=31.402 X -979.13	0.9983	47.56-190.24	19.02	9.51
Oleic acid	0.846 ±0.010	Y=8.013 X +2406.5	0.9965	484.32-1937.26	48.43	15.98
Diosgenin	0.794 ±0.010	Y=9.636 X-205.69	0.9966	51.56-206.24	30.94	15.47

*R_f Retention factor

Recovery: The recovery values summarized in **Table 6** for all the four components were within acceptable limits (85.0 to 115.0%). This indicated that the method was reliable and accurate.

TABLE 6: % RECOVERY IN FRUIT EXTRACT OF *TRIBULUS TERRESTRIS* L. FRUIT AND FORMULATION EXTRACT

Level		80%			100%			120%		
		i	ii	iii	i	Ii	iii	i	ii	iii
Harmine	Spiked conc. (µg/mL)	20.08	20.08	20.08	25.10	25.10	25.10	30.12	30.12	30.12
	% Recovery*	95.30	95.31	95.76	92.82	97.71	92.50	95.82	95.27	95.35
	% Recovery**	96.81	94.29	94.08	96.43	99.78	94.89	93.56	93.22	97.23
Kaempferol	Spiked conc. (µg /mL)	20.02	20.02	20.02	25.03	25.03	25.03	30.04	30.04	30.04
	% Recovery*	98.96	99.86	98.02	99.08	97.71	97.45	92.69	93.80	93.30
	% Recovery**	96.44	94.07	96.37	94.25	95.86	96.38	96.31	97.31	99.56
Oleic acid	Spiked conc. (µg/mL)	160.32	160.32	160.32	200.40	200.40	200.40	240.48	240.8	240.48
	% Recovery*	96.08	99.21	99.77	106.57	99.99	99.56	96.40	97.00	98.39
	% Recovery**	95.04	94.50	92.47	95.72	97.91	97.21	95.50	93.36	94.41
Diosgenin	Spiked conc. (µg/mL)	40.42	40.42	40.42	50.53	50.53	50.53	60.64	60.64	60.64
	% Recovery*	95.72	94.93	92.87	93.78	92.23	96.72	98.70	103.5	95.56
	% Recovery**	97.61	102.42	96.54	96.24	95.42	91.77	94.77	91.08	98.24

* % Recovery in fruit extract; ** % Recovery in formulation extract

TABLE 7: SUMMARY OF METHOD VALIDATION PARAMETERS

Parameter		Harmine	Kaempferol	Oleic acid	Diosgenin
Specificity		Specific	Specific	Specific	Specific
Precision*		0.17%	0.31%	0.14%	0.34%
Quantity in T.T fruit Extract		0.014%	0.018%	0.259%	0.086%
Quantity in T.T Formulation Extract		0.006%	0.006%	0.108%	0.022%
Robustness*	Saturation time (20 ± 5 min)	0.42%	0.55%	0.98%	1.22%
	Composition of Ethyl acetate	0.88%	0.42%	1.08%	1.38%
	Plate drying time (5 ± 2 min)	0.34%	0.48%	0.69%	0.18%
Recovery (Plant)**		95.09%	94.62%	99.22%	95.97%
Recovery (Formulation)**		95.59%	96.28%	95.12%	96.01%
Stability at RT***		6 h	6 h	6 h	6 h
Stability at 2-8 °C		72 h	72 h	72 h	72 h

*Values are average % CV; ** Values are average % Recoveries of all levels of concentrations; *** Room temperature

CONCLUSION: A precise, accurate and reproducible HPTLC method is validated for simultaneous quantification of four bioactive markers harmine, kaempferol, oleic acid and diosgenin. Proposed HPTLC method can be used as an analytical tool for quality evaluation of plants and formulations containing harmine, kaempferol, oleic acid and diosgenin as chemical markers. It is an efficient method to screen *Tribulus terrestris* L. fruit samples in order to assess its quality and authenticity. Hence, it can be demonstrated that HPTLC is a powerful practical tool for comprehensive quality control of plant raw materials and its formulations.

ACKNOWLEDGEMENT: Authors wish to acknowledge Anchrom Enterprises, Mumbai, India for providing excellent facilities to carry out HPTLC work.

CONFLICT OF INTEREST: The authors have declared no conflicts of interest.

REFERENCES:

1. Agrawal S: Advances in Medical Plants. Oxford Book Company, Jaipur, India, First Edition 2009.
2. Hostettmann K: Handbook of Chemical and Biological Plant Analytical Methods. Wiley, First Edition 2014.
3. Alam P, Alqasoumi SI and Abdel-Kader MS: Simultaneous determination of menthol and eucalyptol by densitometric HPTLC method in some external analgesic

formulations. Journal of Chromatographic Science 2016; 54(1) 58- 63.
4. Nair JCV, Ahamad S, Khan W, Anjum V and Mathur R: Development and validation of high-performance thin-layer chromatography method for simultaneous determination of polyphenolic compounds in medicinal plants. Pharmacognosy Res. 2017; 9(S1): S67-S73.
5. Reich E and Schibli A: High-Performance Thin Layer Chromatography for Analysis of Medical Plants. Thieme Medical Publishers, Inc. First Edition 2007.
6. No author: Ayurvedic Pharmacopoeia of India. Publication of Govt. of India, Edition 1, Vol.126: 49-52.
7. Manda VK, Avula B and Ali Z: Characterization of *in-vitro* ADME properties of Diosgenin and Dioscin from *D. villosa*. Planta Medica 2013; 79(15): 1421-1428.
8. Louveaux A, Jay M, El-Hadi OTM and Roux G: Variability in flavonoid content of four *Tribulus terrestris*. Journal of Chemical Ecology 1998; 24(9): 1465-1481.
9. Shiquan X and Ruihai L: Content comparison of flavonoids in *Tribulus terrestris* from different habitats. China Pharmacist 2015; 18: 1671-1673.
10. Baba SA and Malik SA: Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii*. Blume. J Taibah Univ Med Sci. 2015; 9: 49-54.
11. Ali W, Shaikh H, Ansari A and Khanam S: Standardization of Unani antidiabetic tablet-Qurse Tabasheer. Pharmacognosy Res. 2016; 8: 147-52.
12. Do J, Choi S, Choi J and Hyun JS: Effects and mechanism of action of a *Tribulus terrestris* extract on penile erection. Korean Journal of Urology 2013; 54(3): 183-188.
13. Kalyani R, Komal K, Shukla VJ and Prajapati PK: A study to evaluate Diosgenin in Laghu gokshur (*T. terrestris*) and Brihat gokshur (*Pedalium murex*) by HPTLC method. Int J Pharm Biol Archive 2012; 3: 1117-1120.
14. Gupta PK, Nagore DH, Kuber VV and Purohit S: A validated RP-HPLC method for the estimation of Diosgenin from polyherbal formulation containing *Tribulus terrestris* Linn. Asian J Pharm Clin Res 2012; 5: 91-94.
15. Soni H, Patgiri B and Bhatt S: Quantitative determination of three constituents of Rasayana Churna (a classical Ayurvedic formulation) by a reversed phase HPLC. Int J Res Ayurveda Pharm 2014; 5: 17-22.
16. Fang HJ, Bi KS and Qian ZZ: HPLC-DAD-ELSD determination of five active components in *Tribulus terrestris* L. Chinese. Journal of Pharmaceutical Analysis 2012; 32: 6.
17. Mendhulkar VD and Kharat SN: HPTLC assay for quercetin and rutin flavonoids in *Elephantopus scaber* L. grown under induced heat stress condition. Int J Pharm Biol Sci 2015; 6(B): 36-52.
18. Laila O, Murtaza I, Abdin MZ, Ahmad S, Ganai NA and Jehangir M: Development and validation of HPTLC method for simultaneous estimation of diosgenin and quercetin in fenugreek seeds (*Trigonella foenum-graceum*). ISRN Chromatogr 2014; 583047: 1-8.
19. Kole PL, Venkatesh G, Kotecha J and Sheshala R: Recent advances in sample preparation techniques for effective bioanalytical methods. Biomed Chromatogr. 2011; 25: 199-217.
20. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
21. Loescher CM, Morton DW, Razic S and Agatonovic-Kustrin S: High performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) for the qualitative and quantitative analysis of Calendula officinalis-advantages and limitations. J Pharm Biomed Anal. 2014; 98: 52-59.
22. Mallick MN, Singh M, Parveen R, Khan W, Ahmad S and Najm ZM: HPTLC analysis of bioactivity guided anticancer enriched fraction of hydroalcoholic extract of *Picrorhiza kurroa*. Biomed Res Int. 2015; 513875.
23. Abdel-Kader MS, Al-Qutaym A, Saeedan ASB, Hamad AM and Alkharfy KM: Nephroprotective and hepatoprotective effects of *Tribulus terrestris* L. growing in Saudi Arabia. Journal of Pharmacy & Pharmacognosy Research 2016; 4(4): 144-152.
24. El-Sheikh TMY, Al-Fifi ZIA and Alabboud MA: Larvicidal and repellent effect of some *Tribulus terrestris* L. (Zygophyllaceae) extracts against the dengue fever mosquito, *Aedes aegypti* (Diptera: Culicidae). Journal of Saudi Chemical Society 2016; 20(1): 13-19.

How to cite this article:
Vaidya VV, Kondalkar PL, Shinde MA and Gotmare S: HPTLC fingerprinting for simultaneous quantification of harmine, kaempferol, diosgenin and oleic acid in the fruit extract of *Tribulus terrestris* L. and its formulation. Int J Pharm Sci & Res 2018; 9(7): 3066-74. doi: 10.13040/IJPSR.0975-8232.9(7).3066-74.