AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

COMPARISON & QUANTIFICATION OF MARKER COMPOUND OF TRIPHALA GUGGULU BY USING HPTLC METHOD

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ABSTRACT

Triphalaguggulu contains amala, baheda, harde ,pipali, and guggulu in powder form which contains many phytoconstituents other than phenols(gallicacid, ellagic acid) and alkaloids(piperine) and *Z*-guggulusterone. phenols are present in good amounts, and several as important anti-oxidant. Piperine is a bioavailability enhancer and *Z*-guggulusterone as anticholesterimic. Considering the current importance of these ingredients, an attempt has been made for simultaneous estimation of piperine, gallic acid, ellagic acid by using HPTLC methods A simple HPTLC method has been developed for the estimation of galic acid,ellegic acid, piperine and *Z*-guggggulusterone from methanolic extract of marketed and laboratory preparation of triphala guggulu. These compounds in the extracts have been estimated by using HPTLC method. The separation was performed on TLC aluminium Plates precoated with silica gel $60F_{254}$, good separation was achived in the mobile phase of toluene:ethyl acetate:formic acid:methanol(3:3:0.8:0.5v/v/v/v)and densiometric determination of galic acid, ellegic acid was carried out at 280 nm and piperine was carried out at 337nm and *Z*-guggulusterone was carried out at 248 nm & compare the these phytoconstituents with other marketed product

Key words: Triphala guggulu, HPTLC, Gallic acid, Piperine, *Z*-guggulusterone

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Received 9 June 2012, Accepted 20 June 2012

Please cite this article in press as: Patel SG *et al*., Comparison & Quantification of Marker Compound of Triphala Guggulu by using HPTLC method. American Journal of PharmTech Research 2012.

INTRODUCTION

Triphalaguggulu contain amala, baheda, harde ,pipali, and guggulu in powder form which contains many phytoconstituents other than phenols(gallic acid, ellagic acid) and alkaloids(piperine). And Z-guggulusterone. phenols are present in good amounts, and these phenols give good anti-oxidant effect. Piperine is a bioavailability enhancer and and *Z*-guggulusterone is anticholesterimic. Considering the current importance of these ingredients, an attempt has been made for simultaneous estimation of piperine, gallic acid, ellagic acid by HPTLC methods and determination of other phytoconstituents by different analytical methods.

MATERIALS AND METHODS:

Material:

The crude drugs i.e Amla, baheda, harde, pippali and guggulu were procured from reputed ayurvedic drug supplier, M/s Sanjivani,aushdhalay, Bhavanagar. Gallic acid, ellagic acid and piperine marker compounds were procured from Yucca Enterprises. Z-guggulusterone marker compound was procured from Sigma-Aldrich.ltd

TLC study:[1,2,3]

Thin layer chromatography(TLC) study gives a information about number of compounds present in sample TLC was carried out in following conditions:

Stationary phase: - Aluminum-backed silica gel 60 F254 pre -coated TLC Plate(E.merck)(0.2 mm thickness)

Mobile phase: - Toluene: ethyl acetate: formic acid: methanol **(**3 : 3 : 0.8 :0.5/v/v/v/v)

Developing Chamber:- CAMAG glass twin through chamber (20x10) cm

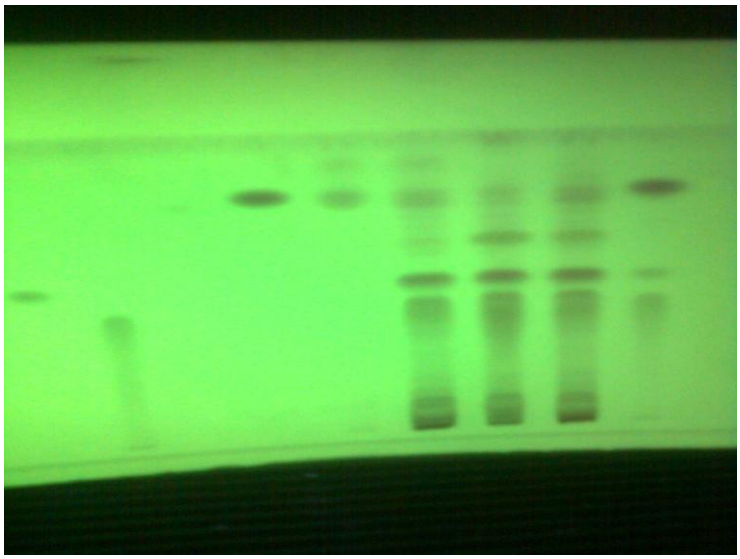
Chamber saturation: - 30 minutes

Standard solution: - E-ellagic acid, G-gallic acid, P=Piperine dissolved in methanol& Z-guggulusterone dissolved in chloroform

Test solution :- 1g of each sample were extracted under reflux condition for 15 min with methanol (4X25) extract were filtered, pooled, concentrated to 25 ml in volumetric flasks

Detection : - After developing the plates were dried at 105°C temperature. The TLC plate is shown in Figure-1.

G = gallic acid, E = ellagic acid, P = piperine, Z = *Z*- guggulusterone, CU1, CU2 , CU3, CU4, S= Mix of std



G , E, P, Z , CU2 ,CU3 ,CU1,CU4,S

Figure:-1 TLC plate

G = gallic acid, E = ellagic acid, P = piperine, Z = *Z*-guggulusterone, CU1=sample no. 1, CU2=sample no. 2, CU3=sample no.3, CU4=sample no. 4, S = mix of Standard solution

Preparation of sample & standard solution:[6]

1gm powder of amala, baheda, harde, pipali and guggul and laboratory and three various marketed each sample was extracted with methanol(4x25ml). The extract was filtered and filtrates were combined and concentrated to 25ml

Estimation of gallic acid[4,5]

Standard solution of gallic acid

A stock solution of Gallic acid (100µg/ml) was prepared by dissolving 1mg of accurately weighed Gallic acid in methanol and making up the volume to 10 ml with methanol.

Calibration curve for gallic acid

10µl of each of the standard solution(150ng to 750 ng per respective spot) were applied on a TLC plate. The plate was developed in a solvent system of toluene: ethyla cetate : formic acid :methanol(3:3:0.8:0.5v/v/v/v) in a chamber upto a distance of 8 cm. after development, the plates were dried in air and scanned at 280 nm. The peak areas were recorded. Calibration curve of Gallic acid was prepared by plotting peak areas vs concentration. The calibration curve and 3D curve are shown in figure 2 & 3.

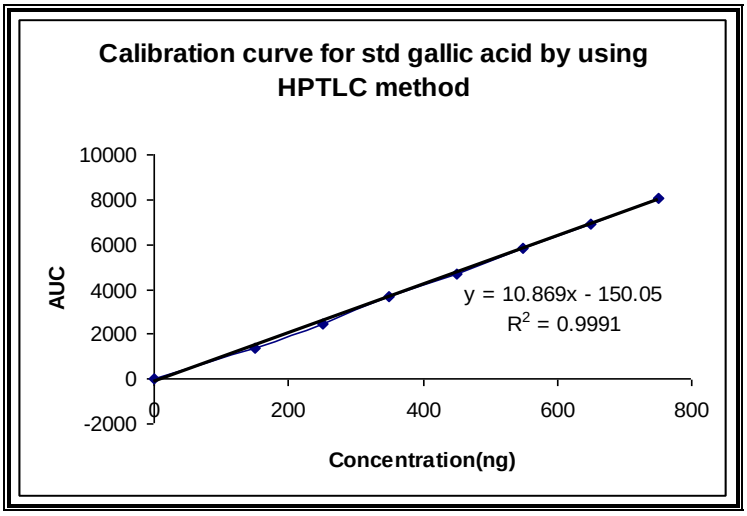


Figure:-2 Linearity curve for std gallic acid by using HPTLC method

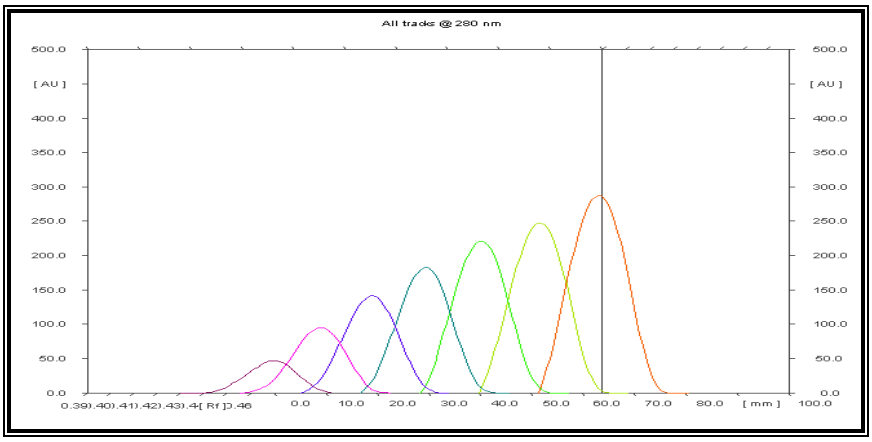


Figure :- 3 3D curve of gallic acid

Estimation of gallic acid in test solution:

10µl of each of the test solution were applied on a TLC plate.The plate was developed in a solvent system of toluene: ethyl acetate: formic acid: methanol(3:3:0.8:0.5v/v/v/v) in a twin-through chamber up to a distance of 8 cm. After development , the plates were dried in air and scanned at 280nm.The peak area was recorded. The amount of gallic acid in different samples was calculated using the respective calibration curve. HPTLC chromatogram of std gallic acid &sample of CU1,CU2,CU3,CU4 are shown in figure 6,8,9,10&11.

Table: 1 Data of HPTLC method for Gallic acid estimation

Test solution	Sample spot(µl/spot)	Peak area	% Gallic acid
CU1	5 µl	4348.05±1.53	0. 20
CU2	5 µl	5482.95±1.99	0.25
CU3	5 µl	4952.4±2.23	0. 23
CU4	5 µl	3822.33±1.55	0. 18

Mean(n=3)

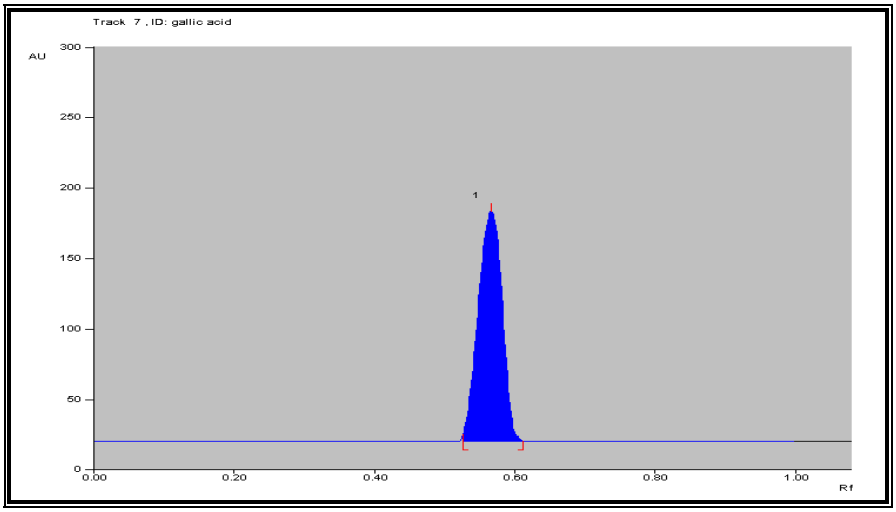


Figure:-6 HPTLC chromatogram of std gallic acid

Estimation of ellagic acid:[4,5]

Standard solution of ellagic Acid

A stock solution of ellagic acid(100µg/ml) was prepared by dissolving 1mg of accurately weighed ellagic acid in methanol and making up the volume to 10ml with methanol

Calibration curve for ellagic acid:

10µl of each of the standard solution(150ng to1300ng per respective spot) were applied on a TLC plate. The plate was developed in a solvent system of toluene:ethyl acetate:formica acid:methanol(3:3:0.8:0.5 v/v/v/v) in a chamber upto a distance of 8 cm. after development, the plates were dried in air and scanned at 280 nm.The peak areas were recorded. Calibration curve of ellagic acid was prepared by plotting peak areas were vs concentration. The calibration curve and 3D curve are shown in figure 4 & 5.

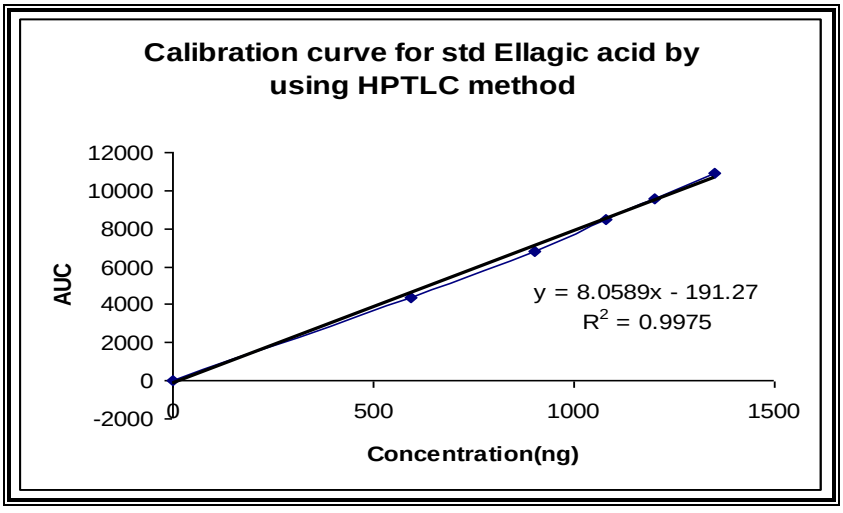


Figure:-4 Linearity curve for std ellagic acid by using HPTLC method

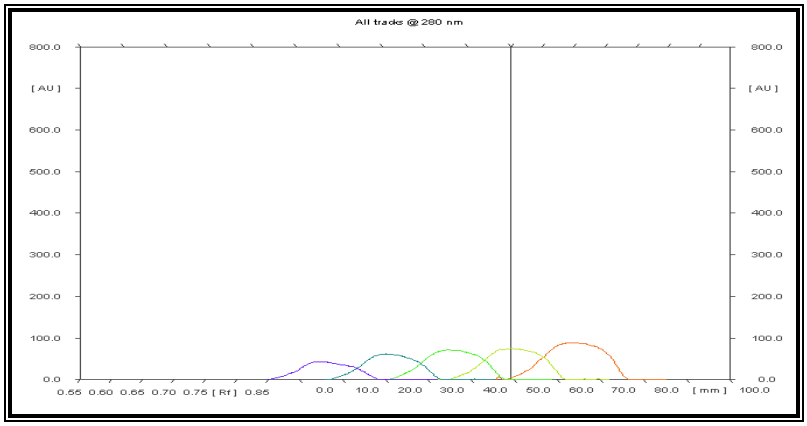


Figure:-5 3D Curve of ellagic acid

Estimation of ellagic acid in test solution:

10µl each of the test solution were applied on a TLC plate. The plate was developed in a solvent system of toluene:ethyl acetate:formic acid:methanol(3:3:0.8:0.5v/v) in a twin-through chamber upto a distance of 8cm.After development, the plates were dried in air and scanned at 280 nm. The peak area was recorded. The amount of ellagic acid in different samples was calculated using the respective calibration curve. HPTLC chromatogram of std ellagic acid &sample of CU1,CU2,CU3,CU4 are shown in figure 7,8,9,10&11.

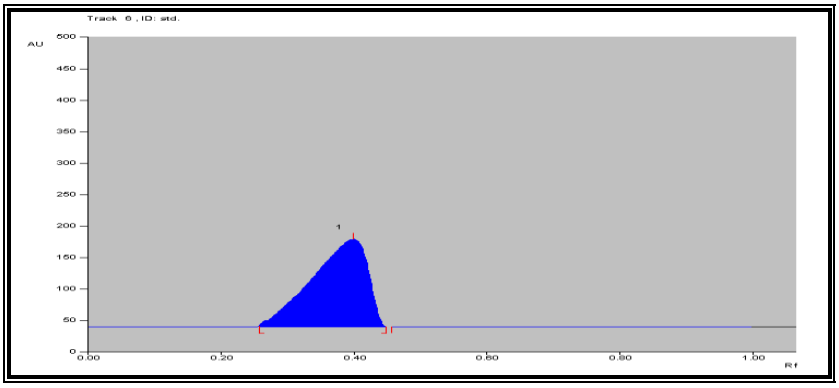


Figure :-7 HPTLC chromatogram of std ellagic acid

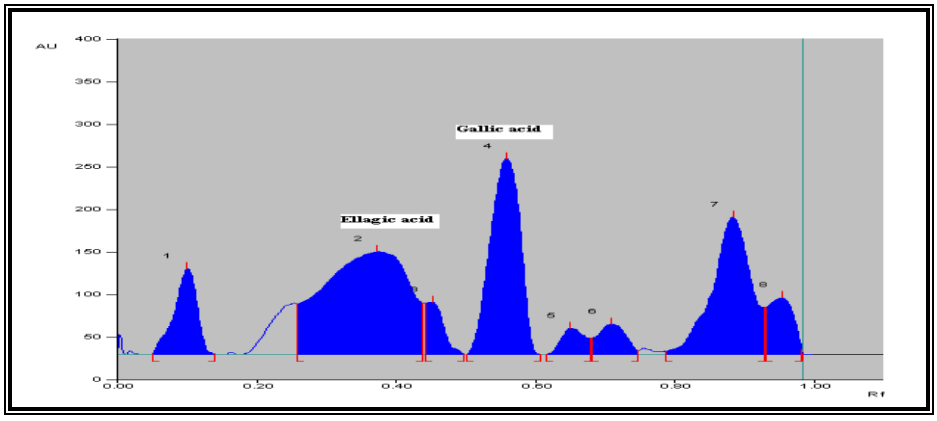


Figure :-8 HPTLC chromatogram of gallic and ellagic acid of CU1 sample

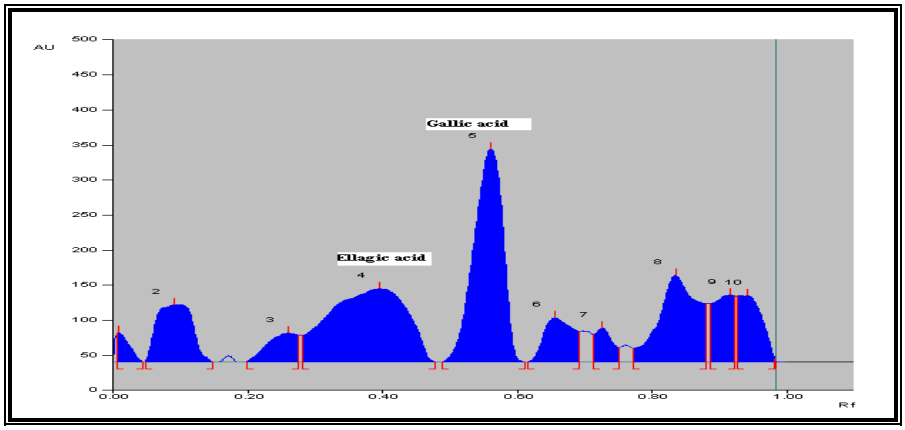


Figure :-9 HPTLC chromatogram of gallic and ellagic acid of CU2 sample

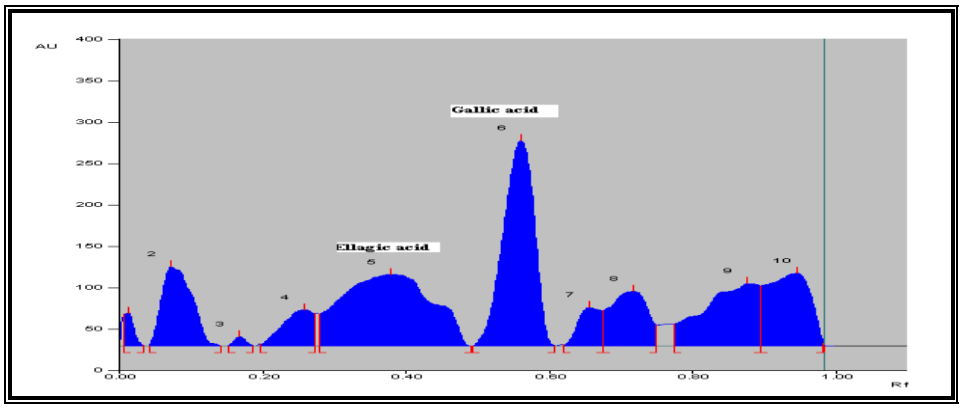


Figure :10 HPTLC chromatogram of gallic acid and ellagic acid of CU3 sample

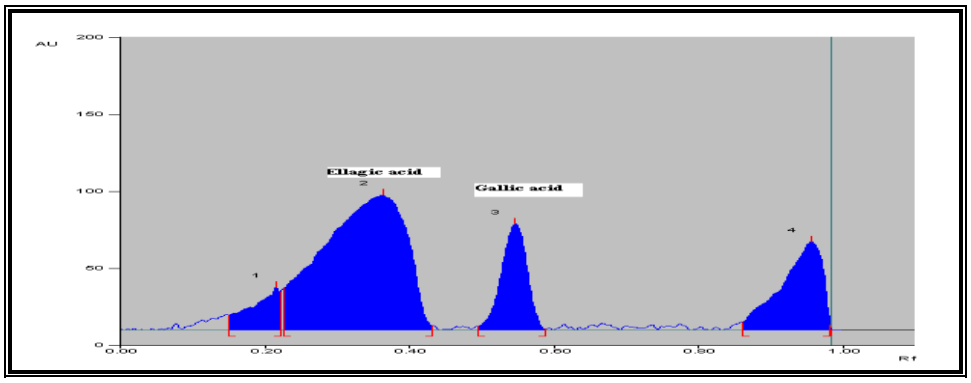


Figure :-11 HPTLC chromatogram of gallic acid and ellegic acid CU4 sample [INSERT TABLE-1 HERE]

Table: 2 Data of HPTLC method for Ellgic acid estimation

Test solution	Sample spot(µl/spot)	Peak area	% Ellagic acid
CU1	5 µl	5040.9±2.10	0.32
CU2	5 µl	4700±1.04	0.30
CU3	5 µl	4750±2.43	0.30
CU4	5 µl	5700±2.42	0.36

Mean(n=3)

Estimation of Piperine:[6,]

Standard solution of piperine

A stock solution of piperine(100µg/ml) was prepared by dissolving 1mg of accurately weighed piperine in methanol and making up the volume to 10ml with methanol.

Calibration curve for piperine

10µl each of the standard solution (10ng to 130ng per respective spot) were applied in triplicate on a TLC plate. The plate was developed in a solvent system toluene: ethyl acetate :formic acid: methanol(3:3:0.8:0.5v/v/v/v) in a chamber upto a distance of 8cm. After development, the plates were dried in air and scanned at 337 nm. The peak areas were recorded. Calibration curve of piperine was obtained by plotting peak areas vs concentration of piperine applied. The calibration curve and 3D curve are shown in figure 12 &13.

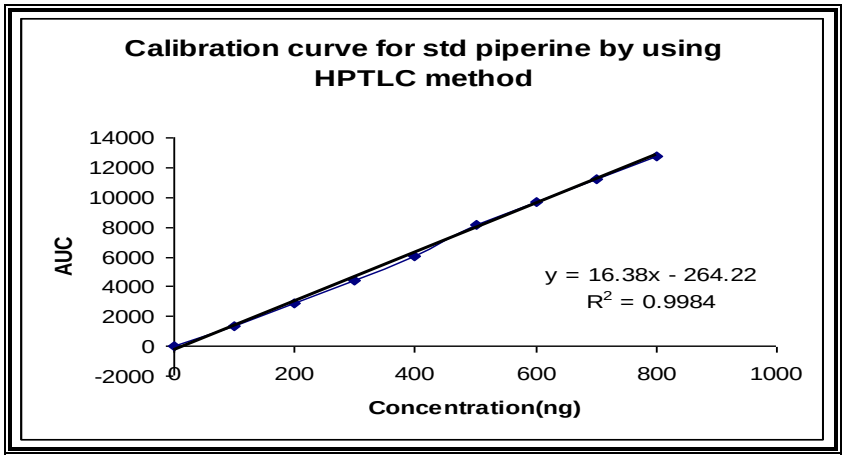


Figure:-12 Linearity curve for std piperine by using HPTLC method

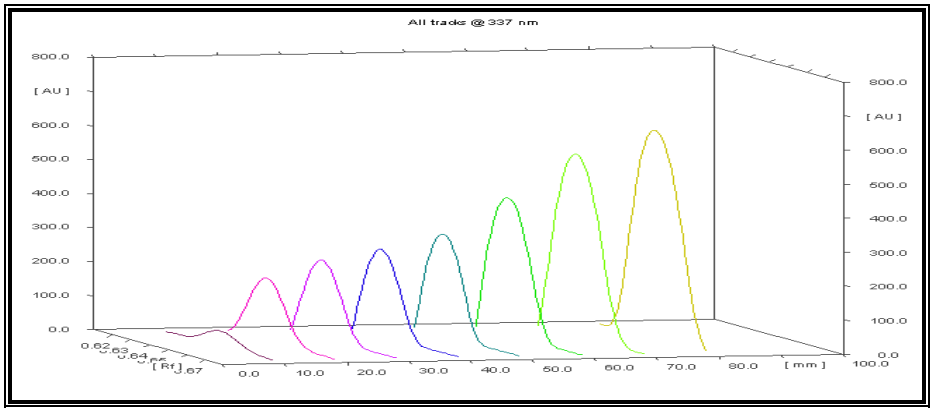


Figure:-13 3D curve of piperine

Estimation of piperine in test solution

10µl of each of the test solution were applied on a TLC plate. The plate was developed in a solvent system toluene: ethyl acetate: formic acid: methanol(3:3:0.8:0.5v/v/v/v) in a chamber up

to a distance of 8 cm. After development, the plates were dried in air and scanned at 337 nm. The peak area was recorded. The amount of piperine in different samples was calculated using the respective calibration curve. HPTLC chromatogram of std piperine &sample of CU1,CU2,CU3,CU4 are shown in figure 14,15,16,17&18.

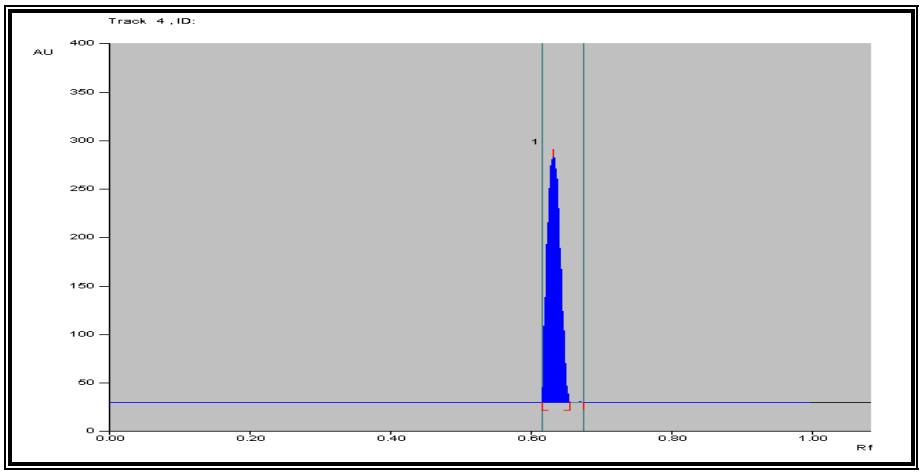


Figure:-14 HPTLC chromatograms of standard piperine

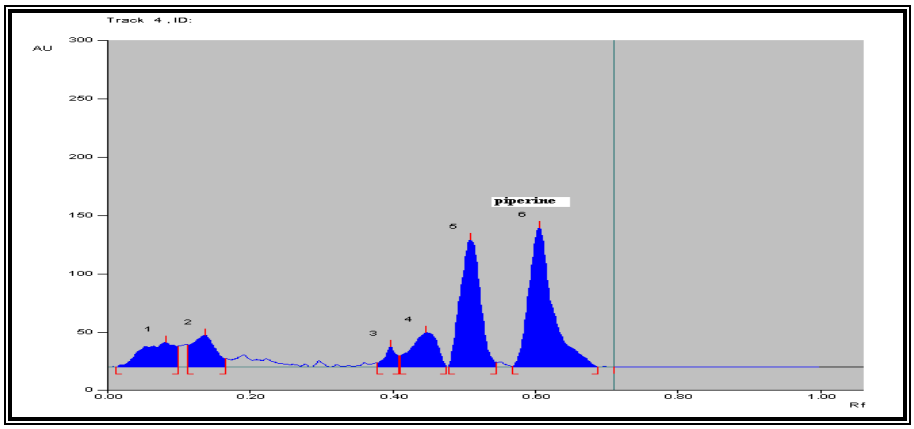


Figure:-15 HPTLC chromatograms of piperine of CU1 sample

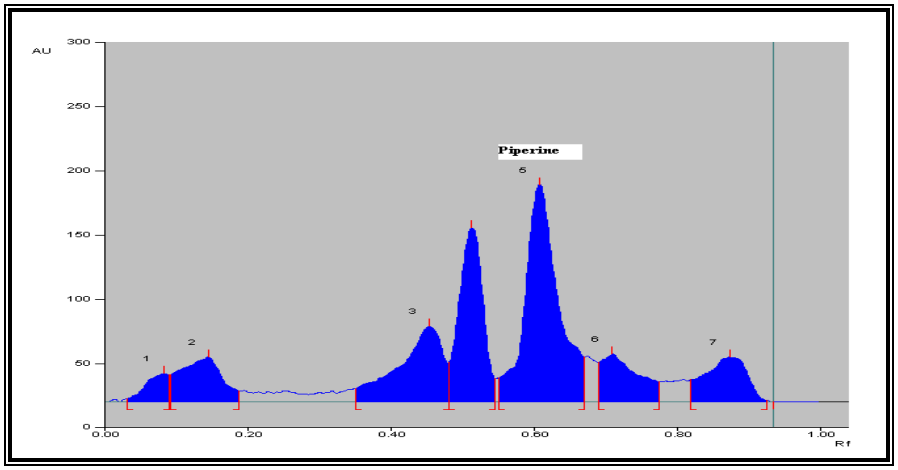


Figure:-16 HPTLC chromatograms of piperine of CU2 sample

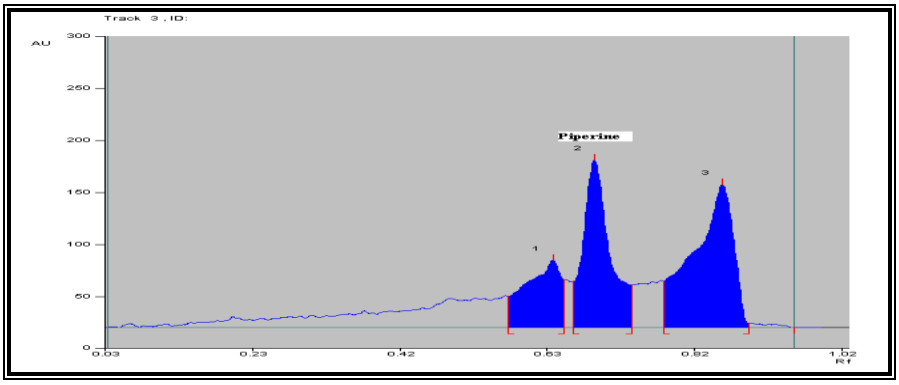


Figure:-17 HPTLC chromatograms of piperine of CU3 sample

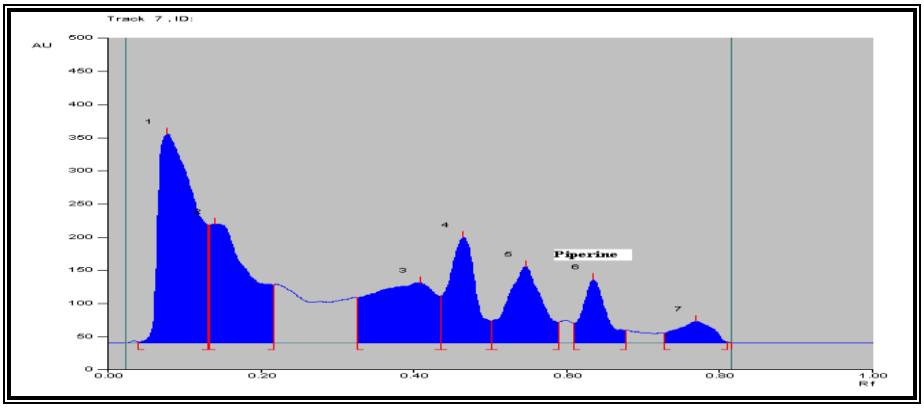


Figure :-18 HPTLC chromatogram of piperine of CU4 sample

Table: 3 Data of HPTLC method for Piperine estimation

Test solution	Sample spot(µl/spot)	Peak area	% Piperine
CU1	5 µl	4037.7±1.05	0.013
CU2	5 µl	5994.16±0.99	0.019
CU3	5 µl	5724.1±1.20	0.0182
CU4	5 µl	1964.1±1.08	0.0068

Mean(n=3)

Estimation of *Z*-guggulusterone[6]

Standard solution of *Z*-guggulusterone:

A stock solution of Z-guggulusterone was prepared by dissolving 5mg of accurately weighed Z-guggulusterone in chloroform and making up the volume to 5ml with chloroform

Calibration curve for Z-guggulusterone:

10 µl of each of the standard solution (100ng to 600ng per respective spot) were applied on a TLC plate . The plate was developed in a solvent system of toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.5v/v/v/v) in a chamber upto a distance of 8 cm. After development ,The plates were dried in air and scanned at 248 nm. The peak areas were recorded. calibration curve of *Z*-guggulusterone was prepared by plotting peak areas vs concentration. The calibration curve

and 3D curve are shown in figure 19 &20

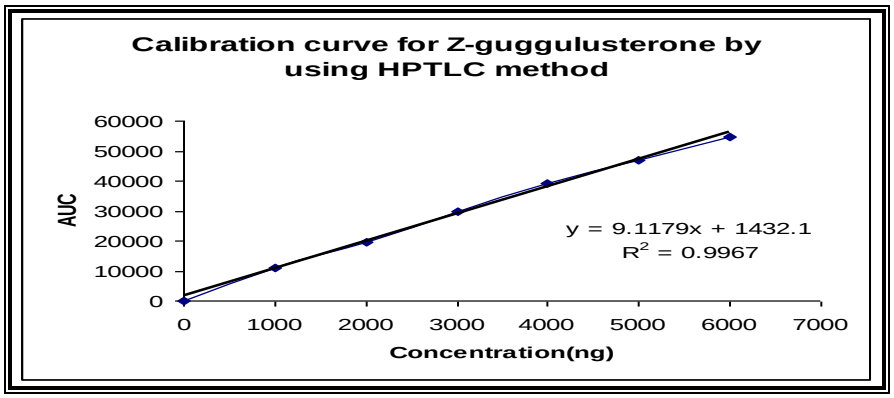


Figure:-19 Linearity curve for std *Z*-guggulusterone

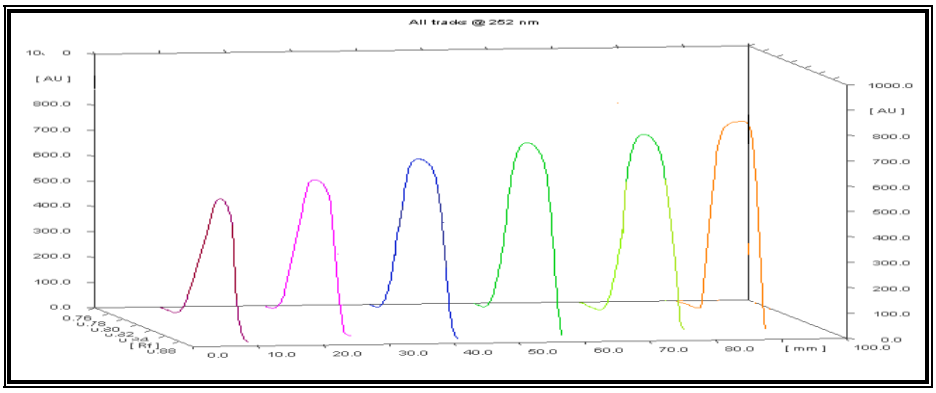


Figure:-20 3D curve of *Z*- guggulusterone

Estimation of Z-guggulusterone in test solution:

10µl each of the test solution were applied on a TLC plate. The plate was developed in a solvent system of toluene: ethyl acetate: formic acid: methanol(3:3:0.8:0.2v/v/v/v) in a twin –through chamber up to a distance of 8cm. after development, the plates were dried in air and scanned at 248 nm. The peak area was recorded. The amount of Z-guggulusterone in different samples was calculated using the respective calibration curve. HPTLC chromatogram of std Z-guggulusterone &sample of CU1,CU2,CU3,CU4 are shown in figure 21,22,23,24&25.

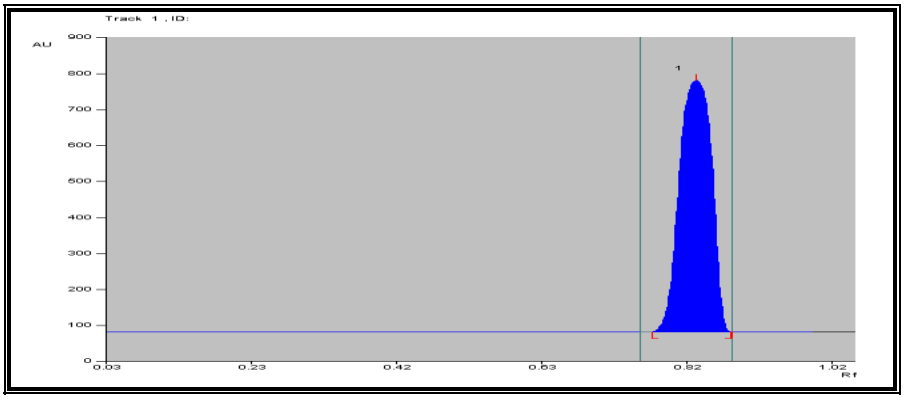


Figure:-21 HPTLC chromatogram of standard *Z*- guggulusterone

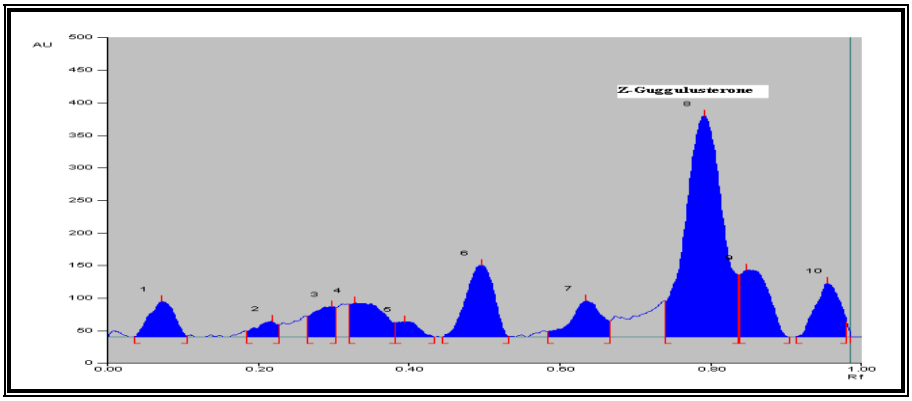


Figure:-22 HPTLC chromatogram of *Z*- guggulusterone of CU1sample

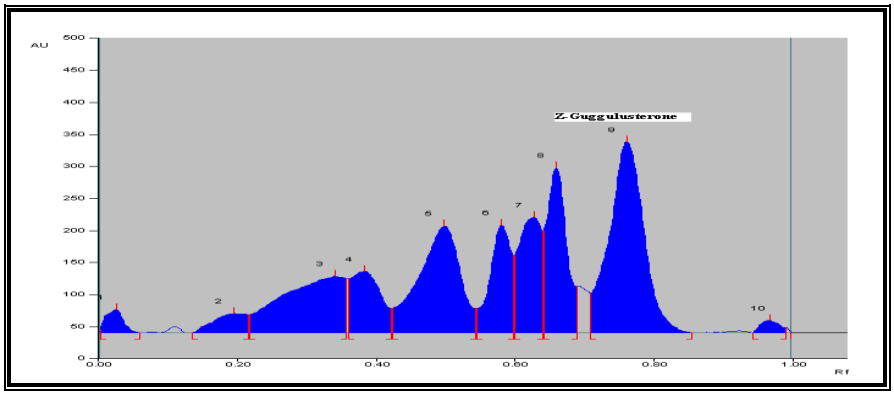


Figure:-23 HPTLC chromatogram of *Z* -guggulusterone of CU2 sample

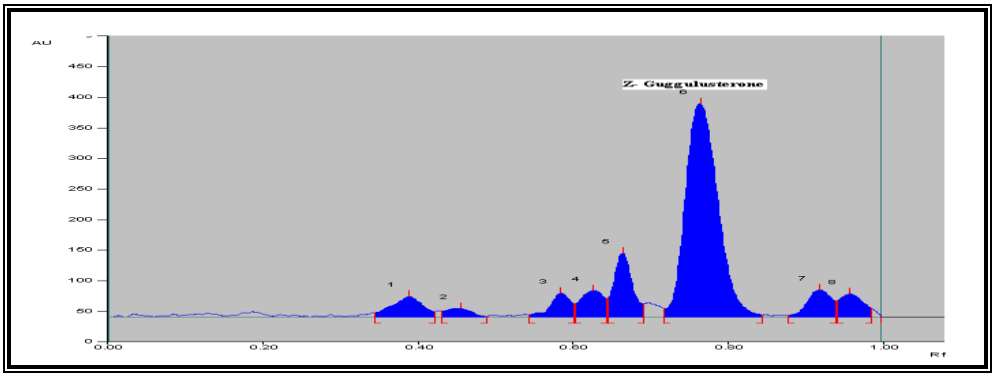


Figure:- 24 HPTLC chromatogram of *Z*- guggulusterone of CU3 sample

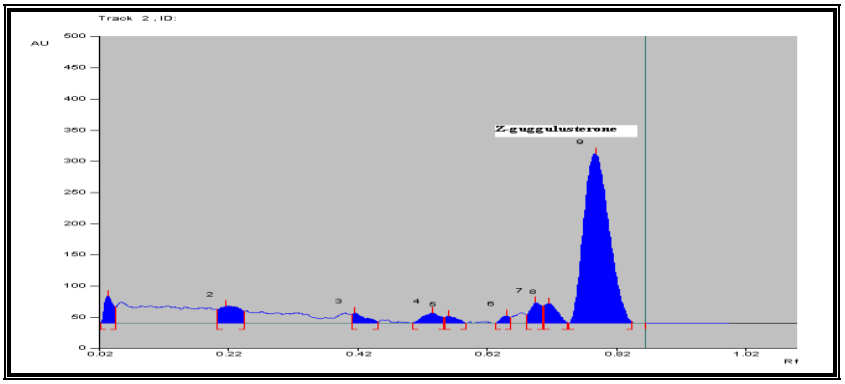


Figure:-25 HPTLC chromatogram of *Z*-guggulusterone of CU4 sample

Table: 4 Data of HPTLC method for Z-guggulusterone estimation

Test solution	Sample spot(µl/spot)	Peak area	%Z-guggulusterone
CU1	5 µl	12180.55±1.16	0.58
CU2	5 µl	13816.3±0.95	0.67
CU3	5 µl	13873.75±1.52	0.68
CU4	5 µl	11826.95±1.52	0.57

Mean(n=3)

RESULTS AND DISCUSSION:

TLC as confirmatory test

Further more important phytoconstituents was confirmed by the TLC Study . Test solution spotted along with mixture of standard solution On TLC plate showed 4 spots at R_f 0.48 & R^f 0.57 and R_f 0.75 and R_f 0.78 under day light. The thin layer chromatographic test showed that triphalaguggulu sample have the same R_f 0.48 & R_f 0.57and R_f 0.75 and R_f 0.78 value of ellagic acid and gallic acid and piperine and *Z*-guggulusterone respectively compare to standard which was used for comparision in this test in day light and UV-254

Estimation of Gallic acid by using HPTLC method:

Concentration of gallic acid in test sample was calculated from regression equation:

$Y= 10.869x-150.05$ ($R^2=0.9991$)

Where, X= concentration of the sample

Y= AUC(peak area)

Estimation of Ellagic acid by using HPTLC method :

Concentration of ellagic acid in test sample was calculated from regression equation:

$Y= 8.0589x-191.27$($R^2=0.9975$)

Where, X= concentration of the sample

Y= AUC (peak area)

Estimation of piperine by using HPTLC method

Concentration of Piperine in test sample was calculated from Regression equation:

$Y= 16.38x-264.22$($R^2=0.9984$)

Where, X= concentration of the sample

Y= AUC (peak area)

Estimation of *Z*-guggulusterone by using HPTLC method:

Concentration of *Z*-guggulusterone in test sample was calculated from regression equation:

Y= 911.79x+1432.1

Where, X= concentration of the sample

Y= AUC(peak area)

Table5 :- Data of HPTLC method for Quantification of marker Compounds

Phytoconstituents	CU1	CU2	CU3	CU4
Gallic acid	0.20	0.25	0.23	0.18
Ellagic acid	0.32	0.30	0.30	0.36
Piperine	0.013	0.019	0.0182	0.0068
Z-guggulusterone	0.58	0.67	0.68	0.57

Mean(n=3)

CONCLUSION:-

The quantitative determination of ellagic acid, gallic acid, piperine and *Z*-guggulusterone in triphala guggulu formulation was performed by HPTLC densiometry, the conents of of these marker are similar in CU2 and CU3 while CU1 and CU4 are also similar but in CU4 has very less amount of piperine

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