IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Simultaneous Quantitative Analysis of β- sitosterol and 18β- glcyrrhetinic acid in marketed herbal formulation by HPTLC method.

Shifa Aslam Shikalgar

Assistant Pofessor

M.C.E Society's Allana College of Pharmacy

ABSTRACT

Introduction: In recent years, herbal drugs are used popularly all over the world. The herbal drugs and medicines are used in the treatment and as well as in the prevention of various diseases. Herbal health supplements and tonics has been consumed by the customers as natural, safe, harmless and free from adverse side effects.

Aim and objective: The present studies aims in the development and validation of herbal markers and formulation using densitometric technique.

Material and methods: Separation and detection of two herbal markers was achieved by using Toluene: Ethyl Acetate: Methanol (2: 6: 2 v/v/v). This method was validated as per the norms of ICH guidelines.

Result and Conclusion: The β- sitosterol and 18β- glcyrrhetinic acid was found to be 0.21 ± 0.008 and 0.92 ± 0.007 respectively. The developed method was found to be simple, sensitive and selective, accurate, precise, and repeatable for analysis of β-sitosterol and 18β-glycyrrhetinic acid in market formulation without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation.

Keywords: β- sitosterol and 18β- glcyrrhetinic acid.

INTRODUCTION:

β- sitosterol is responsible for the reduction of cholesterol level in plasma and it also improves liver function and activity. 18β- glcyrrhetinic acid possesses antihyperglycemic action on streptozocin. In literature survey the data reveals that the few HPLC and HPTLC method had been reported for β- sitosterol and 18β- glcyrrhetinic acid individually or in combination with other drugs. HPTLC method requires small quantity of samples and mobile phase .therefore it reduces the time and cost of analysis. So, we are studying β- sitosterol and 18β- glcyrrhetinic acid by HPTLC method. Hence, this present study involves method development and validation for the simultaneous estimation of this two herbal drugs in the formulation by HPTLC.

MATERIALS AND METHOD:

Solvent and chemicals:

Standard β- sitosterol and 18β- glcyrrhetinic acid were procured from natural remedies private limited, banglore, india. Herbal drug formulation used in the study was procured from Immucor Tablets, Herbazen, pune. Toluene AR Grade, Methanol AR Grade, Ethyl Acetate AR Grade.

2 Instrument:

1. Camag HPTLC system:
    - Linomat- V applicator
    - Camag TLC Scanner 3
    - win CATS software V- 1.4.2
    - Merck TLC plates precoated with silica gel 60 F_{254}
    - Hamilton microlitre syringe
2. Jasco Model V-550 UV-Visible Double beam spectrophotometer with single Monochromator
3. Shimadzu Model AY-120 balance
4. Calibrated glasswares were used for the study

Selection of mobile phase and chromatographic conditions:

Chromatographic separation studies were carried out on the working standard solution of β-sitosterol (100 ng/band) and 18β-glycyrrhetinic acid (100 ng/band). Initially, trials were carried out using various solvents in various proportions on normal TLC plates, to obtain the desired system suitability parameters. After few trials, Toluene: Ethyl Acetate: Methanol (2: 6:2 v/v/v), was chosen as the mobile phase and TLC plates, which gave good resolution and acceptable peak parameters. Other chromatographic conditions like chamber saturation time, run length, sample application volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible R_f values and symmetrical peak shape for the drug peak.

Preparation of Standard stock solution:

Standard stock solution of B-sitosterol and 18β-glycyrrhetinic acid were prepared separately by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of β -sitosterol and 18β-glycyrrhetinic acid separately in methanol.

Selection of Detection Wavelength:

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 257 nm (Fig.)

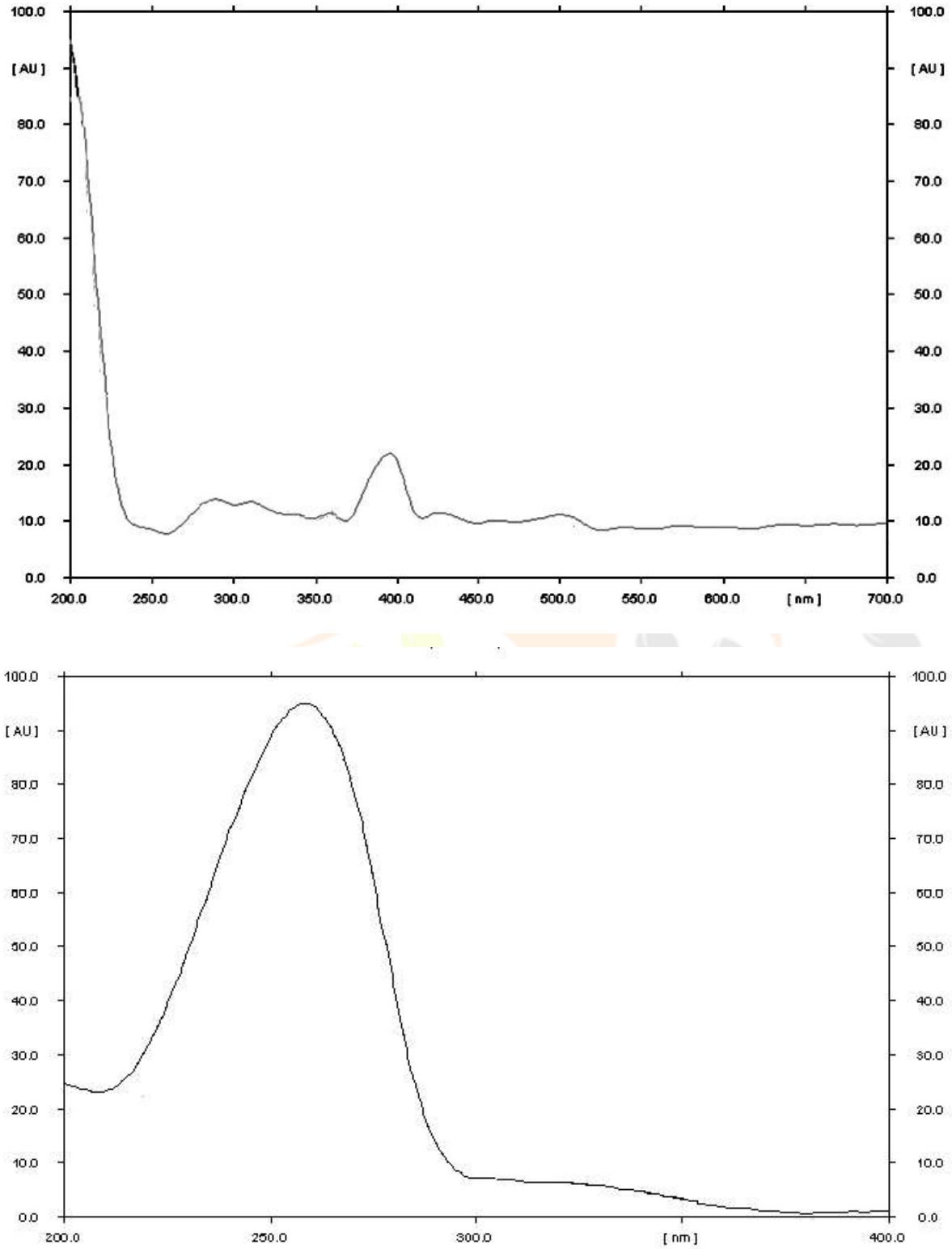

Fig. : In-situ UV-VIS Spectra of β-sitosterol and 18β-glycyrrhetinic acid

Preparation of sample solution (Herbal Tablet Formulation Analysis):

Ten tablets were weighed and powdered. 10 mg of tablet powder was transferred to 10 ml volumetric flask and was diluted with methanol and volume made to 10 ml with methanol. Solution was filtered and 10 µl of filtrate was applied on TLC plate.

Densitogram of the Standard drugs:

Solution of 50 µg/ml of β-sitosterol and 50 µg/ml of 18β-glycyrrhetinic acid was prepared. 2 µl (100 ng/band) of β-sitosterol and 2 µl (100 ng/band) of 18β-glycyrrhetinic acid solution was applied on TLC plate with the help of Hamilton syringe (100 µl), using Linomat IV sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned over 80 mm distance at 257 nm. The retention factor (Fig. 2) were found to be:

B-sitosterol = 0.21 ± 0.008

18β-glycyrrhetinic acid = 0.92 ± 0.007

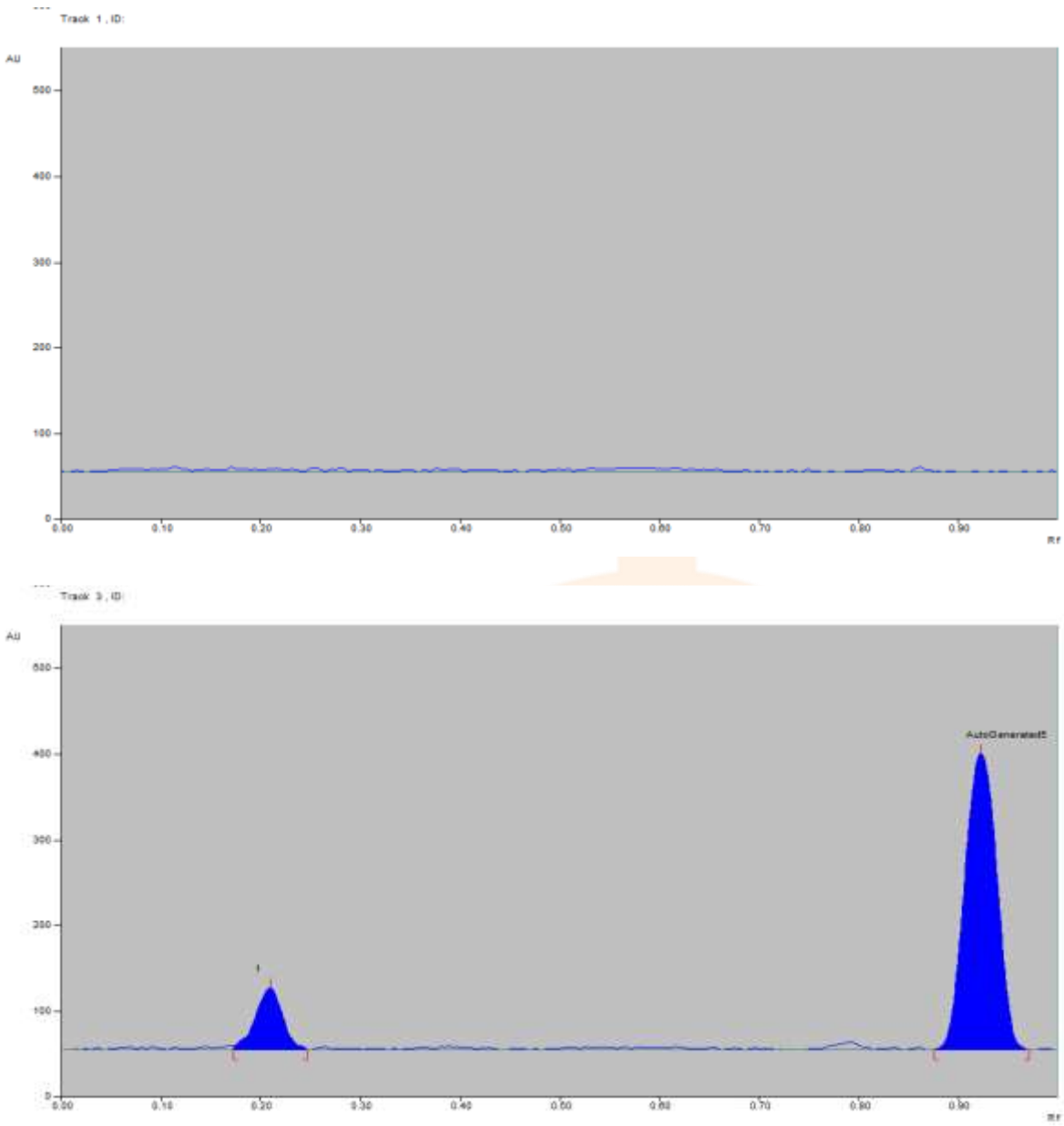

Fig. : Densitogram of blank and mixed standard solution of β-sitosterol and 18β-glycyrrhetinic acid

Summary of chromatographic parameters selected:

Chromatographic parameters are summarized in Table

Table: Chromatographic parameters

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminum plate precoated with silica gel 60 F_{254}
2.	Mobile phase	Toluene: Ethyl Acetate: Methanol (2: 6:2 v/v/v)
3.	Detection Wavelength	257 nm
4.	Saturation time	15 mins

Densitogram of Formulation:

10 µl volume of sample (Tablet) solution was applied on TLC plate with the help of Hamilton syringe (100 µl), using Linomat V sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance from start line. The plate was dried and was scanned over 80 mm distance at 257 nm. (Fig)

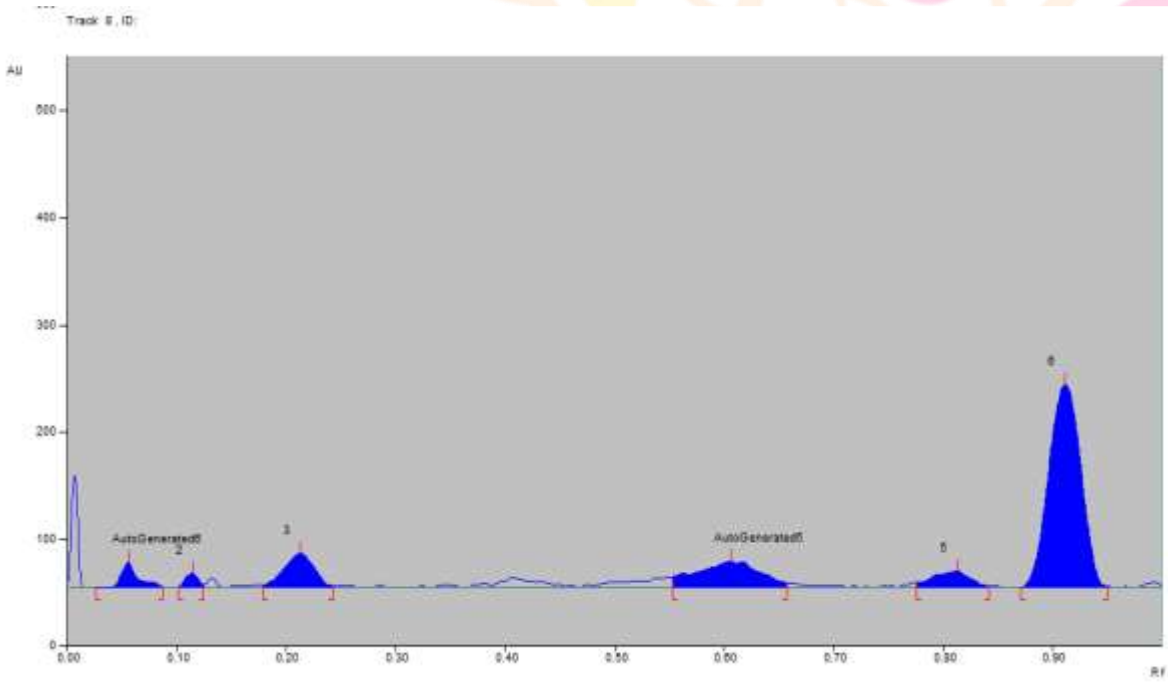

Fig. : Densitogram of Test Solution

RESULT AND DISCUSSION:

Validation of Analytical Method

Linearity

From the standard stock solution (1000 µg/ml) of β-sitosterol and 18β-glycyrrhetinic acid, solution was prepared containing 50 µg/ml of β-sitosterol and 50 µg/ml of 18β-glycyrrhetinic acid, separately. Different volumes were applied on TLC plate to obtain linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 50-500 ng/band for β-sitosterol and 50-300 ng/band for 18β-glycyrrhetinic acid.

The results obtained are shown in Table for β-sitosterol and in Table 18β-glycyrrhetinic acid

Table : Linearity study of β-sitosterol

Replicates	Concentrations of β-sitosterol (ng/ band)					
	50	100	200	300	400	500
	Peak Area					
1	1365.8	2327.4	4456.6	6035.1	7619.8	8976.9
2	1398.6	2339	4261	5825.5	7401.3	8682.7
3	1388.4	2367	4295.6	6070.7	7805.1	9101.6
4	1321.22	2373	4342.3	6079.9	7550.5	8923.8
5	1373	2403.5	4284.3	6098	7645	9284.9
6	1381.9	2278.9	4287.3	6091.4	7549.2	9014.1
Mean	1371.49	2348.13	4319.52	6033.44	7595.15	8997.34
Std.dev.	27.17	43.23	72.57	104.23	133.31	199.33
%RSD	1.98	1.84	1.67	1.72	1.76	2.21

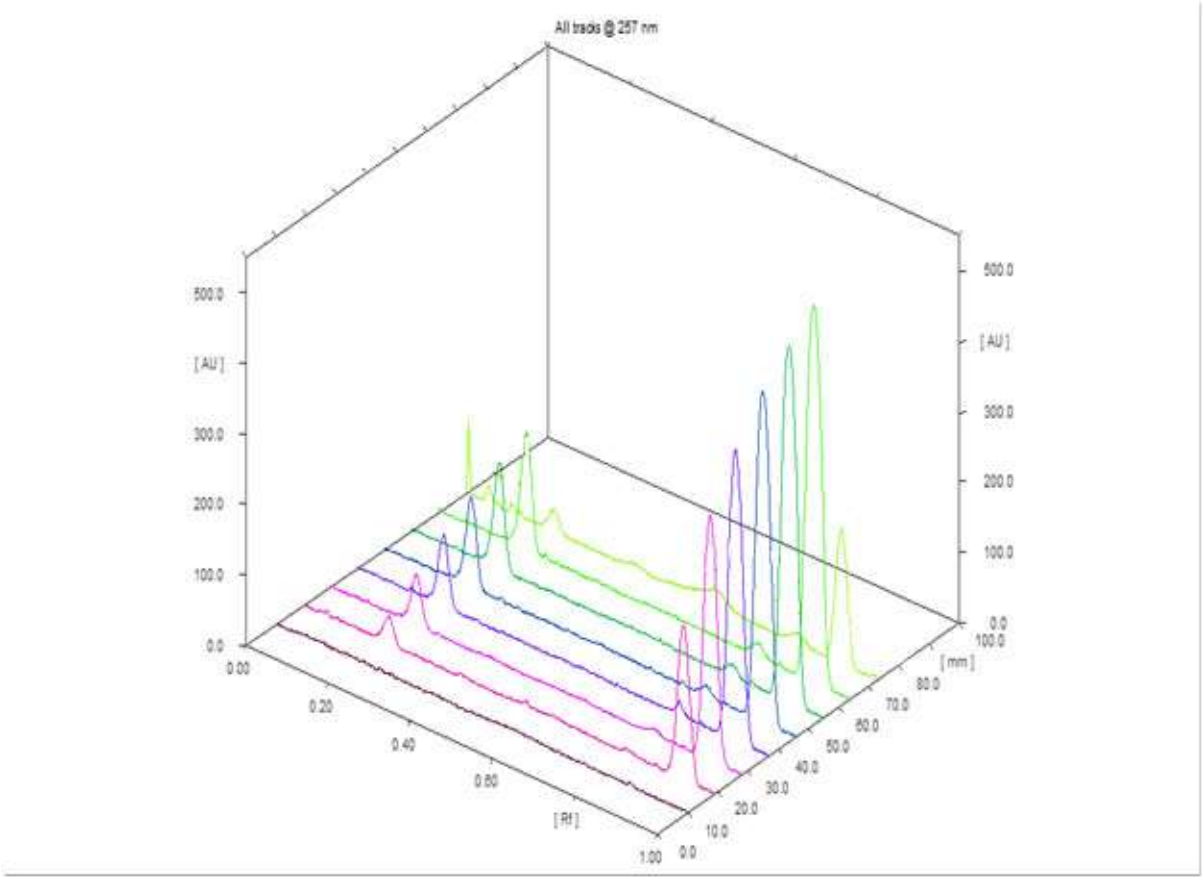

Fig. : Densitogram of linearity of β-sitosterol and 18β-glycyrrhetinic acid

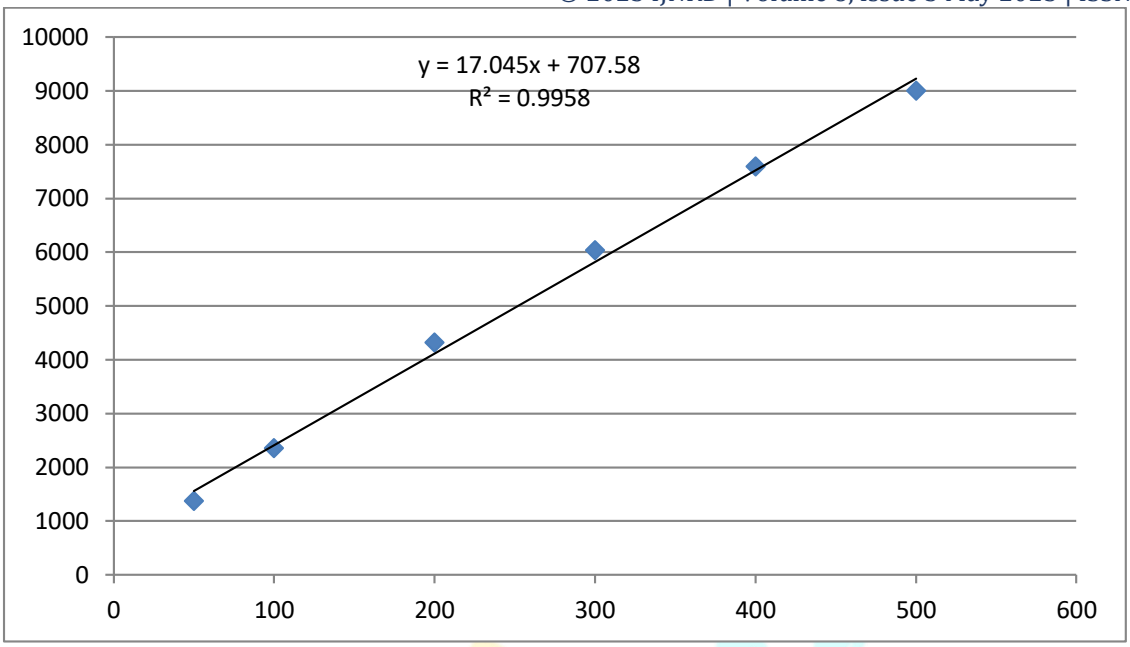

Fig.: Calibration curve for β-sitosterol

Table: Linearity study of 18β-glycyrrhetinic acid

Replicates	**Concentrations of 18β-glycyrrhetinic acid (ng/band)**					
	50	**100**	**150**	**200**	**250**	**300**
	Peak Area					
1	2352	3989.6	5527	7680.7	9521.11	11595.3
2	2383.6	4041.6	5622	7681.11	9539.6	11510.6
3	2379.4	4192	5697	7802.9	9601.8	11353.9
4	2419.5	4157.8	5689	7643.4	9627	11718.3
5	2355.1	4059	5691.4	7749	9618	11714
6	2412.7	4143.3	5679.9	7650.5	9623	11229.8
Mean	2383.72	4097.22	5651.05	7701.27	9588.42	11520.32
Std.dev.	28.16	78.63	66.70	62.23	46.16	197.24
%RSD	1.18	1.91	1.18	0.81	0.48	1.71

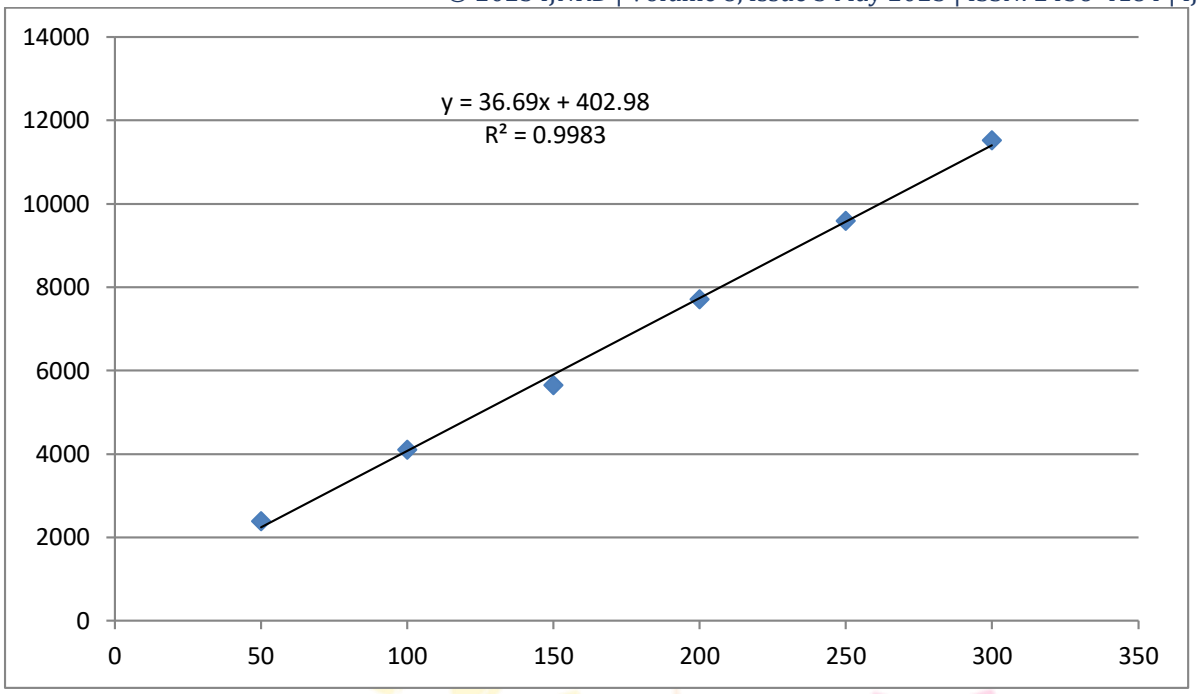

Fig.: Calibration curve for 18β-glycyrrhetinic acid

Range

β-sitosterol = 50 – 500 ng/band

18β-glycyrrhetinic acid = 50- 300 ng/band

Precision

The precision of the method was demonstrated by intra-day and inter-day studies. In the intra-day precision 2, 4, 6 µl of solution of concentration 50 µg/ml of β-sitosterol (100, 200, 300 ng/band) and 2, 3, 4 µl of solution of concentration 50 µg/ml of 18β-glycyrrhetinic acid (100, 150, 200 ng/band) were prepared and six replicates were analyzed, % RSD as calculated. The results obtain for intra-day variations are shown in Table

In the inter day variation studies, same procedure was repeated once a day for three consecutive days. The percentage RSD was calculated. The result obtained for inter day variations are shown in Table

Table: Intra-day precision study of β-sitosterol

Concentration (ng/spot)	Area	% Recovery	Mean % Recovery ± SD	% RSD
100	2396.3	98.08	99.46 ± 0.51	1.68
	2412.6	100.04		
	2399.4	99.26		
200	4172.4	101.64	100.55 ±1.08	
	4098.3	99.47		
	4134.5	100.53		
300	5915.3	101.85	102.86 ± 0.91	
	6003.9	103.58		
	5982.6	103.16		

Table: Intra-day precision study of 18β-glycyrrhetinic acid

Concentration (ng/spot)	Area	% Recovery	Mean % Recovery ± SD	% RSD
100	4117	101.23	100.36 ± 0.76	0.52
	4073.5	100.04		
	4065.1	99.82		
150	5885.2	99.62	100.05 ± 1.01	
	5869.3	99.33		
	5972.4	101.20		
200	7702.1	99.47	99.35 ± 0.11	
	7687.5	99.27		
	7690.2	99.31		

Table: Inter-day precision of β-sitosterol

Concentration (ng/spot)	Area	% Recovery	Mean % Recovery ± SD	% RSD
100	2373.9	97.77	98.86 ± 0.95	0.87
	2402.1	99.42		
	2401.5	99.39		
200	4119.3	100.09	100.47 ± 0.55	
	4124.5	100.24		
	4153.9	101.10		
300	5785.2	99.14	99.13 ± 0.24	
	5762.3	98.69		
	5781.5	99.07		

Table: Inter-day precision of 18β-glycyrrhetinic acid

Concentration (ng/spot)	Area	% Recovery	Mean % Recovery ± SD	% RSD
100	4140.43	101.87	101.11 ± 0.71	1.36
	4089.2	100.47		
	4108.38	100.99		
150	5783.5	97.77	98.41 ± 0.58	
	5826.7	98.55		
	5846.1	98.91		
200	7724.1	99.77	99.59 ± 0.20	
	7694.46	99.37		
	7713.48	99.63		

Herbal tablet formulation analysis (Content analysis)

Herbal tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded for each drug. Concentration and % content was determined from linear equation.

	β-sitosterol		**18β-glycyrrhetinic acid**	
Sr. no.	**Peak area**	**Amount recovered (ng/band)**	**Peak area**	**Amount recovered (ng/band)**
1	2031.3	77.665	2567.3	58.991
2	2015.55	76.741	2541.6	58.290
3	2007.8	76.286	2596.8	59.795
4	1988.1	75.130	2589.5	59.596
5	1999.8	75.817	2577.75	59.276
6	2040.1	78.181	2591.3	59.645
Mean	2013.608	**76.627**	2574.590	**59.266**
% RSD	0.974	1.501	0.796	0.942

Calculation of content of β-sitosterol and 18β-glycyrrhetinic acid in herbal formulation

1. **β-sitosterol**

10 µl volume applied contains = 76.627 ng of β-sitosterol

So 1000 µl (1 ml) volume contains = 7662.7 ng (7.662 of µg) of β-sitosterol

therefore 10 ml (10 mg of sample) volume contains = 76.62 of µg of β-sitosterol

100 mg of sample contains = 766.2 µg of β-sitosterol

(ie 0.7662 mg/100 mg of sample or 0.7662 % w/w)

2. **18β-glycyrrhetinic acid**

10 µl volume applied contains = 59.266 ng of 18β-glycyrrhetinic acid

So 1000 µl (1 ml) volume contains = 5926.6 ng (5.926 of µg) of 18β-glycyrrhetinic acid

therefore 10 ml (10 mg of sample) volume contains = 59.26 of µg of 18β-glycyrrhetinic acid

100 mg of sample contains = 592.6 µg of 18β-glycyrrhetinic acid

(ie 0.5926 mg/100 mg of sample or 0.5926 % w/w)

Accuracy

To check accuracy of the method, recovery studies were carried out by overspotting standard drug solution to sample solution at three different levels. Basic volume of sample chosen was 10 µl to which 1, 2, 3 µl of β-sitosterol (50 µg/ml) and 1, 2, 3 µl of 18β-glycyrrhetinic acid (50 µg/ml) standard solutions were applied by overspotting. These solutions were applied on TLC plates in triplicate to obtain the densitogram. The drug concentrations of β-sitosterol and 18β-glycyrrhetinic acid were calculated by using linearity equations. The results obtained are shown in Table

Table: Recovery studies of β-sitosterol

Level	Conc. (ng/band)		Area		Mean % Recovery ± SD
	Sample	Std.			
1	76.63	50	2868.2	100.11	99.41 ± 0.64
			2849.9	99.26	
			2841.2	98.86	
2	76.63	100	3746.1	100.93	100.41 ± 0.46
			3726.5	100.28	
			3718.9	100.03	
3	76.63	150	4582.7	100.32	99.98 ± 0.97
			4598.7	100.74	
			4527.2	98.89	

Table: Recovery studies of 18β-glycyrrhetinic acid

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± SD
	Sample	Std.			
1	59.27	50	4471	101.47	100.22 ± 1.09
			4397.6	99.64	
			4393.3	99.53	
2	59.27	100	6301.7	100.95	100.56 ± 0.54
			6293.5	100.81	
			6242.6	99.93	
3	59.27	150	8032.9	99.37	100.13 ± 0.82
			8157.3	101.00	
			8081.6	100.01	

Limit of Detection (LOD)

LOD is calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

LOD of β-sitosterol = 15.99 ng/band

LOD of 18β-glycyrrhetinic acid = 11.02 ng/band

Limit of Quantification (LOQ)

The Quantitation limit is expressed as:

$$LOQ = \frac{10\,\sigma}{S}$$

LOQ of β-sitosterol = 48.47 ng/ band

LOQ of 18β-glycyrrhetinic acid = 33.40 ng/band

Specificity:

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.997, indicating the non interference of any other peak of degradation product or impurity.

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, chamber saturation time were, Time form application to development and Time form development to scanning are altered and the effects on the Rf values and area were noted. The results obtained are shown in Table

Table: Robustness study

Sr. No.	Prarmeters	Variation	% RSD	
			β-sitosterol	18β-glycyrrhetinic acid
1.	Chamber saturation period	± 1 mins	1.09	0.60
2.	Wavelength	± 1 nm	0.60	0.51
3	Time form application to development	0,30,60 mins	0.32	1.06
4	Time form development to scanning	0,30,60 mins	0.77	1.08

Summary of validation study

The summary of validation parameters are summarized in Table

Table: Summary of validation study

Sr. No.	Validation Parameter	Results	
		β-sitosterol	18β-glycyrrhetinic acid
1.	Linearity	y = 17.04 x + 707.5 R^2 = 0.995	y = 36.69 x + 402.9 R^2 = 0.998
2.	Range	50-500 ng/band	50-300 ng/band
3.	Assay (Formulation Content Analysis)		
4.	Precision	%RSD	%RSD
	A) Intraday precision	1.68 %	0.52 %
	B) Interday precision	0.87 %	1.36 %
5.	Accuracy	% recovery	% recovery
		99 .41 ± 0.64	100.22 ± 1.09
		100.41 ± 0.46	100.56 ± 0.54
		99.98 ± 0.97	100.13 ± 0.82
6.	LOD	15.99 ng/ band	11.02 ng/band
7.	LOQ	48.47 ng/band	33.40 ng/band
8.	Specificity	Specific	Specific
9.	Robustness	Robust	Robust

Conclusion:

The developed method was found to be simple, sensitive and selective, accurate, precise, and repeatable for analysis of β-sitosterol and 18β-glycyrrhetinic acid in market formulation without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. The results indicated the suitability of the method to study stability of B-sitosterol and 18β-glycyrrhetinic acid under various forced degradation conditions like acid, base, dry heat, neutral, oxidative and photolytic degradation. It can be concluded that as the method could separate the drug from its degradation products.