Journal of Advanced Pharmacy Education & Research
1:70-80 (2011)

Simultaneous estimation of Gallic acid, Curcumin and Quercetin by HPTLC method

Vinitkumar Y. Thakker*[1],Vaishali N. Shah[1], Uravashi D. Shah[1], Manish P. Suthar[2]

1. Department of Pharmacognosy, A.P.M.C. College of Pharmaceutical Education & Research, Himatnagar, India.
2. A.R College & G. H. Patel Institute of Pharmacy, V.V. Nagar, India.

*Corresponding author: vinit.thakker@gmail.com

ABSTRACT:

There are number of Polyherbal formulation available for psoriasis but standardization is essential for ensuring quality control of Polyherbal formulation. The aim of this study was to formulate psoriasis tablets and to standardize it by HPTLC using gallic acid, curcumin and quercetin as a biomarker. Chromatography was performed on silica gel $60F_{254}$ percolated TLC plate using toluene: ethyl acetate: forminc acid (4.5:3.0:0.2, *υ/υ/υ*) as solvent system. Densitometry scanning was performed under reflectance-absorbance mode at 366 nm to quantify the spots. The R_f values of gallic acid, curcumin and quercetin are 0.40, 0.73 and 0.55 respectively. The total peak areas of the standards (gallic acid, curcumin and quercetin) and the corresponding peak areas of extracts were compared and the Gallic acid, curcumin and quercetin contents were estimated to be 1.860, 1.682 and 0.4693 % w/w. The HPTLC method was validated as per the ICH guideline and to be simple, precise, specific, sensitive and accurate and can be used for routine quality control of raw material as well as formulation containing any of these compounds.

Keywords: Gallic acid, Curcumin, Quercetin, HPTLC, Standardization.

INTRODUCTION:

Psoriasis is a common chronic inflammatory dermatosis. Person of all ages may develop the disease [1]. In Ayurveda, there are three kinds of psoriasis called “Kitibha”, “Ekakushtha” and “Charmakhya” [2].

The effect of psoriasis on a patient is multidimensional, encompassing the physical, social, and psychological health of the person and is based largely on the patient’s view of condition. Indeed, the effect on the quality of life of the patient is far greater than what is suggested by the overall clinical severity of psoriasis, as assessed by the Psoriasis Area Severity Index (PASI), and duration of psoriasis [3].

In modern treatment topical medications are used but they produce disadvantages like skin irritation, dilated blood vessels, increased risk of skin aging, skin cancer with long-term treatment etc., Because of these disadvantages with the available treatments, there is a need for more safe and more effective approaches. The present study polyherbal formulation, psoriasis tablet whose major ingredients include *Azadirachta indica* Linn., *Curcuma longa* Linn., *Rubia cordifolia* Linn., *Tinospora cordifolia* Willd., *Acacia catechu* Wild and others.

Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition thus posing problems for quality control. There may be batch to batch variation, quality control of raw material, process control and quality control of finished products. Several external factors like environmental, genetic, methods of cultivation, collection, harvest time, preparation, storage etc., also affects quality of herbal drugs. Hence standardization is essential for ensuring quality control of Herbal drugs [4].

So, in the present study deals with Development of Polyherbal formulation for psoriasis this was developed in our institute and its standardization. Formulation was standardized by using gallic acid, curcumin and quercetin as a biomarker by HPTLC analysis.

MATERIALS AND METHODS:

Preparation of extract

The medicinal plants which were used for the preparation of the polyherbal formulation, the plant was identified and authenticated by the botanist Mr. M. M. Prajapati, H.N.S.B Science College, Himatnagar, and voucher specimen was retained in our laboratory (APMC 1101-1108). Methanolic extract of polyherbal formulation was prepared by distillation method. The extract was filtered using whatman filter paper and then concentrated in vacuum and dried.

Reagents and other materials

Gallic acid, curcumin and quercetin [Natural remedies, Bangaluru], toluene, acetone, ethyl acetate, dichloromethane, formic acid, glacial acetic acid and methanol [all Reagents of analytical grade, E-Merck] and silica gel 60F254 precoated TLC aluminium plates [E-Merck].

Methodology for Simultaneous estimation of Gallic acid, Curcumin and quercetin in Polyherbal formulation for psoriasis

Apparatus

Spotting device: Linomat V Automatic Sample Spotter; CAMAG (Muttenz, Switzerland)
Syringe: 100µL Hamilton (Bonadzu, Switzerland)
Thin layer chromatography (TLC) Chamber: Glass with trough chamber (20×10 ×4 cm) (CAMAG)
Densitometer: TLC scanner 3 linked to WinCats Software (CAMAG)
HPTLC plates: 10×10 cm, 0.2 mm thickness precoated with silica gel 60 F_{254} (E. Merck, Mumbai, India)
Experimental conditions: Temperature 25 ± 2 ºC, relative humidity 40 %

Preparation of standard and sample solutions

Standard solution of gallic acid

A stock solution of gallic acid was prepared by dissolving 10 mg of accurately weighed gallic acid in methanol and making up the volume to 10 ml with methanol to get the final concentration of 1mg/ml.

Standard solution of curcumin

A stock solution of curcumin was prepared by dissolving 10 mg of accurately weighed curcumin in methanol and making up the volume to 10 ml with methanol to get the final concentration of 1 mg/ml.

Standard solution of quercetin

A stock solution of quercetin was prepared by dissolving 10 mg of accurately weighed quercetin in methanol and making up the volume to 10 ml with methanol to get the final concentration of 1 mg/ml.

Preparation of sample solutions

Sample solution was prepared by dissolving 50 mg of the methanolic extract of formulation in methanol and making up the volume to 5 ml to get the concentration of 10 mg/ml.

Calibration curve for gallic acid, curcumin and quercetin

Exactly 10 µl of each of the standard solution of gallic acid, curcumin and quercetin were applied in triplicate on TLC plates. The plates were developed in a solvent system of toluene: ethyl acetate: formic acid (4.5: 3: 0.2) at 25 ± 20C temperature and 40% relative humidity and allowed to travel up to a distance of 8 cm. After development the plates were dried in air and scanned densitometrically at 366 nm for gallic acid, curcumin and quercetin. The peak areas were recorded. Calibration curves of gallic acid, curcumin and quercetin were prepared by plotting peak areas versus concentration.

Quantification of gallic acid, curcumin and quercetin

Exactly 10 µl of sample solution was applied in triplicate on a pre-coated silica gel 60 F_{254} TLC plate (0.2 mm thickness) with the Linomat V Automatic sample Spotter. The Plate was developed in the solvent system of toluene: ethyl acetate: formic acid (4.5: 3: 0.2) and scanned at 366 nm for gallic acid, curcumin and quercetin. The amount of gallic acid, curcumin and quercetin in the sample was calculated using the respective calibration curves.

Validation of the method

ICH guidelines were (CPMP/ICH/381/95; CPMP/ICH/281/95) followed for the validation of the analytical procedure. The method was validated for precision, repeatability and accuracy. The repeatability of the method was checked by repeated scanning of the same spot of gallic acid (800 ng), curcumin (600 ng) and quercetin (200 ng) , seven times and was expressed as coefficient of variance (% CV). Variability of the method was studied by analyzing aliquots of the standard solution of gallic acid (800, 1000, 1200 ng), curcumin (600, 800, 1000 ng) and quercetin (200, 600, 1000 ng) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % CV. Accuracy of the method was tested by performing recovery studies at three levels (50%, 100% and 150% addition). The percent recovery as well as average percent recovery was calculated. For the determination of limit of detection and limit of quantification, different dilutions of the standard solutions of gallic acid, curcumin and quercetin were applied along with methanol as well as the blank and determined on the basis of signal to noise ratio.

RESULTS AND DISCUSSION:

Different concentration of standard solution of gallic acid, curcumin and quercetin were applied in triplicate on HPTLC plates and methanolic extract of formulation was applied on HPTLC plate for estimation of gallic acid, curcumin and quercetin. The HPTLC plates were developed in a solvent system toluene: ethyl acetate: formic acid (4.5: 3: 0.2, v/v/v) and dried in air and scanned densitometrically at 366 nm for gallic acid, curcumin and quercetin.

The peak areas were recorded. The method was validated in terms of precision, repeatability and accuracy (Table II and III). The relationship between the concentration and peak response was linear within the range of 600 to 1500 ng/spot for gallic acid, 200 to 1000 ng/spot for curcumin and 200 to 1500 ng/spot for quercetin with correlation coefficient of 0.9918, 0.9969 and 0.9969 respectively.

The % recovery of gallic acid was found to be 98.70%, 99.34%, 99.51% , for curcumin was found to be 98.95%, 99.00%, 99.28% and for quercetin was found to be 98.69%, 99.09%, 99.17%. Average recovery was found to be 99.18% for gallic acid, 99.07% for curcumin and 98.98% for quercetin.

The HPTLC method was developed for simultaneous quantification of gallic acid, curcumin and quercetin in presence of other plant constituents. The proposed method was found to be precise, simple, specific and sensitive.

Table 1. Method of Validation parameters for estimation of biomarker compound by HPTLC

Parameters	Gallic Acid	Curcumin	Quercetin
Linearity (correlation-coefficient)	0.991	0.996	0.996
Repeatability (% CV) (n=7)	0.6738	0.6636	0.6174
Limit of detection (ng/spot)	78.87	16.00	20.62
Limit of quantification (ng/spot)	262.91	53.34	68.73
Range (ng/spot)	600 - 1500	200 - 1000	200-1500
Specificity	specific	specific	specific

Table 2: Data for Validation and precision of Intraday and Interday precision for Gallic acid, Curcumin and Quercetin

Biomarker	Concentration (ng/spot)	Intraday		Interday	
		peak area ± SD (n=3)	%CV	peak area ± SD (n=3)	%CV
Gallic acid	800	4446.73 ± 30.03	0.67	4450 ± 29.85	0.67
	1000	5779.4 ± 41.44	0.71	5774.06 ± 40.77	0.7
	1200	6561.3 ± 50.17	0.76	6566.6 ± 49.20	0.75
Curcumin	600	4858.8 ± 32.84	0.67	4862.7 ± 32.26	0.66
	800	6424.13 ± 45.25	0.7	6445.7 ± 45.06	0.69
	1000	8435.36 ± 62.53	0.74	8438.86 ± 62.75	0.74
Quercetin	200	2811.13 ± 17.43	0.62	2810.9 ± 17.48	0.62
	600	9933.9 ± 69.21	0.69	9941.03 ± 68.06	0.68
	1000	16503.6± 129.11	0.78	16508.93± 128.41	0.77

Values are expressed as mean ± SD (n=3)

Table 3. Recovery study of marker compound by proposed HPTLC method

Biomarker	Amount in sample (ng)	Amount added (ng)	Peak area	Amount found	Recovery	Average Recovery
Gallic acid	300	150	1985.08 ± 14.89	444.15	98.70%	
	300	300	2869.56 ± 22.13	596.05	99.34%	99.18%
	300	450	3575.19 ± 26.90	746.35	99.51%	
Curcumin	80	40	914.16 ± 7.122	118.74	98.95%	99.07%
	80	80	1299.53 ± 9.78	158.4	99.00%	
	80	120	1535.32 ± 11.67	198.56	99.28%	
Quercetin	100	50	1786.52 ± 13.62	148.04	98.69%	
	100	100	2537.51 ± 19.56	198.18	99.09%	98.98%
	100	150	3267.26 ± 25.26	247.94	99.17%	

Values are expressed as Mean ± SD (n = 3)

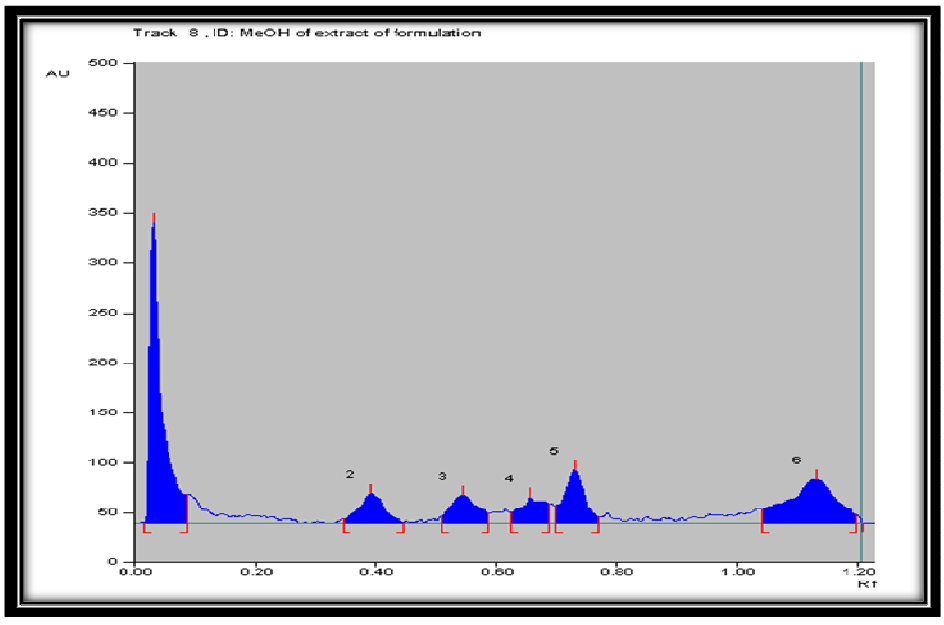

Fig. 1. HPTLC Chromatogram for formulation

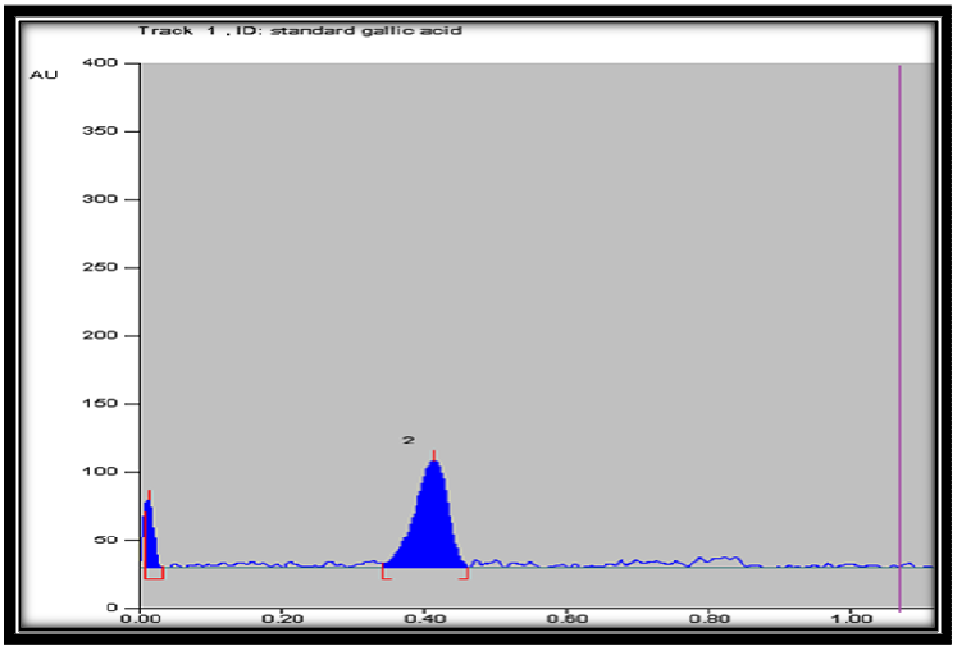

Fig. 2. HPTLC Chromatogram of Gallic acid

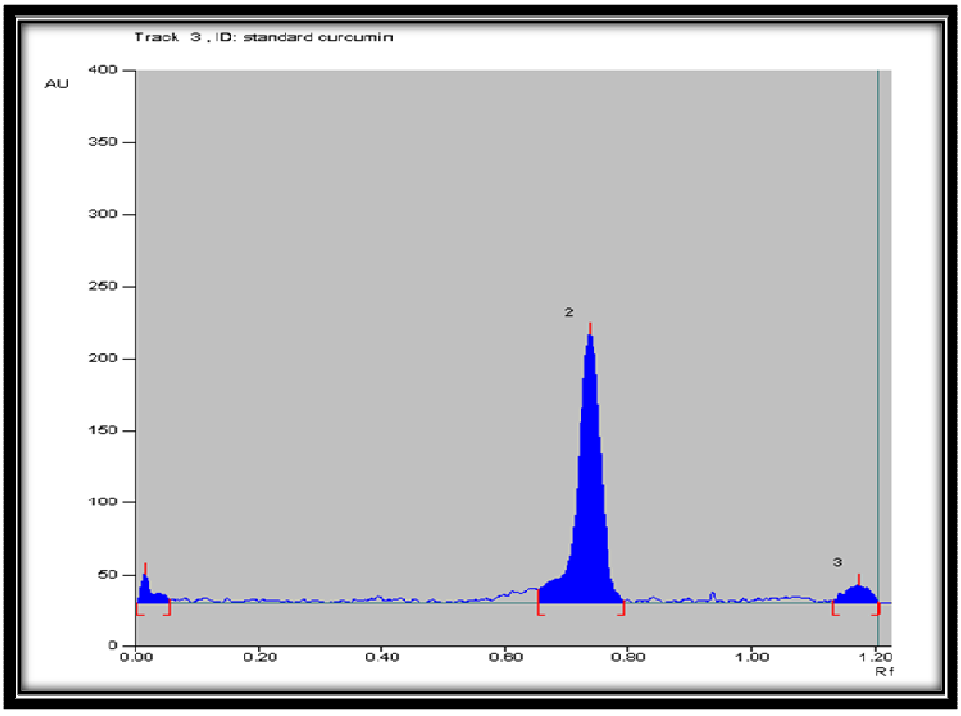

Fig. 3. HPTLC Chromatogram of Curcumin

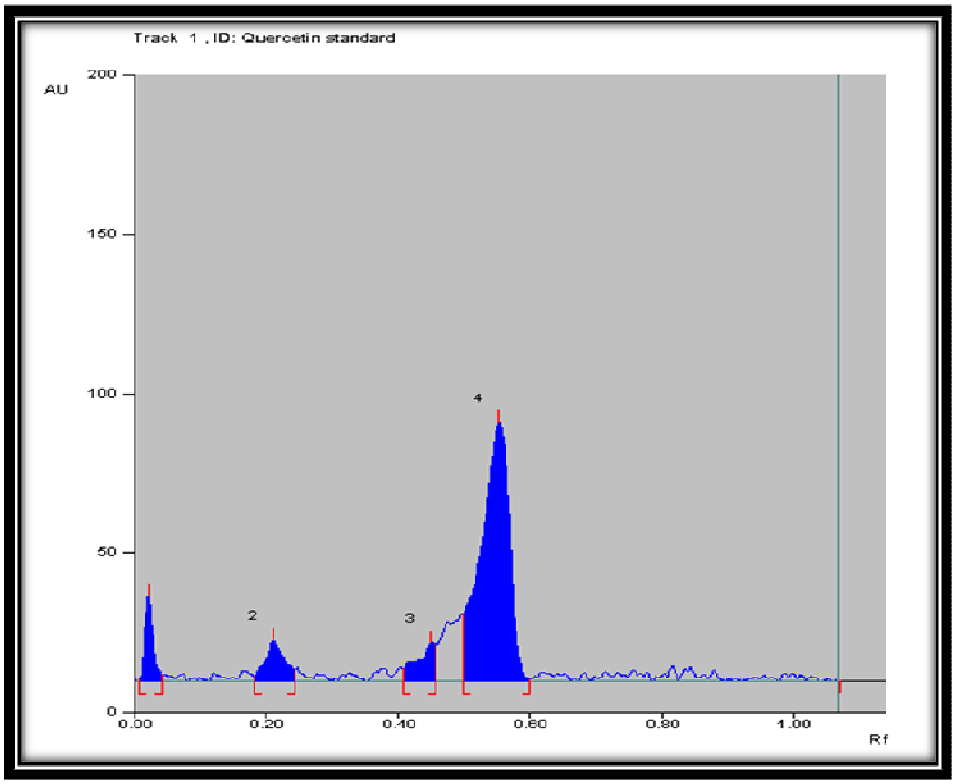

Fig. 4. HPTLC Chromatogram of Quercetin

Correlation coefficient: 0.991, Slope: 5.664 Intercept: 156.6

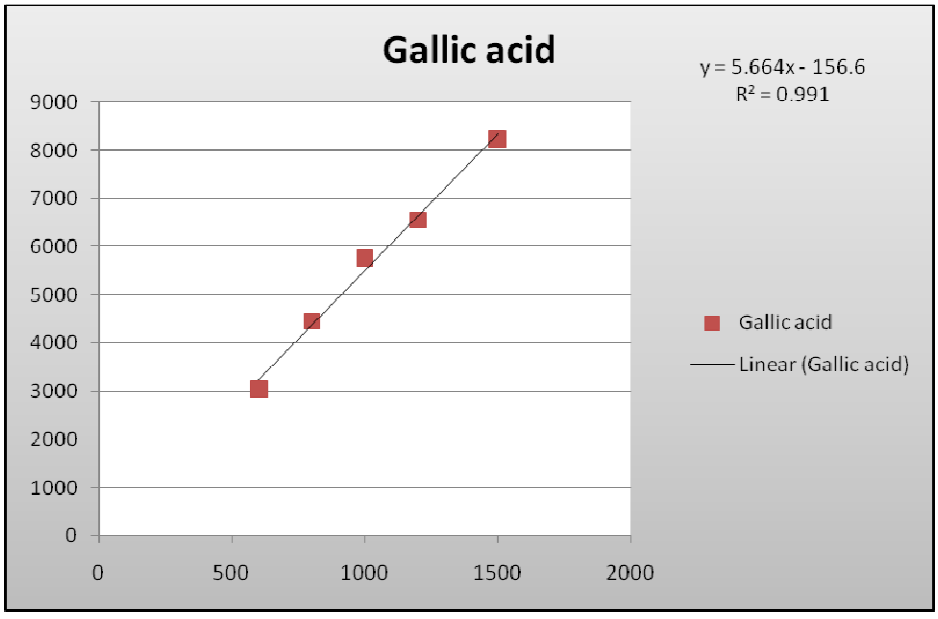

Fig. 5. Calibration curve for standard Gallic acid (Concentration Vs peak area)

Correlation coefficient: 0.996, Slope: 8.235 Intercept: 27.40

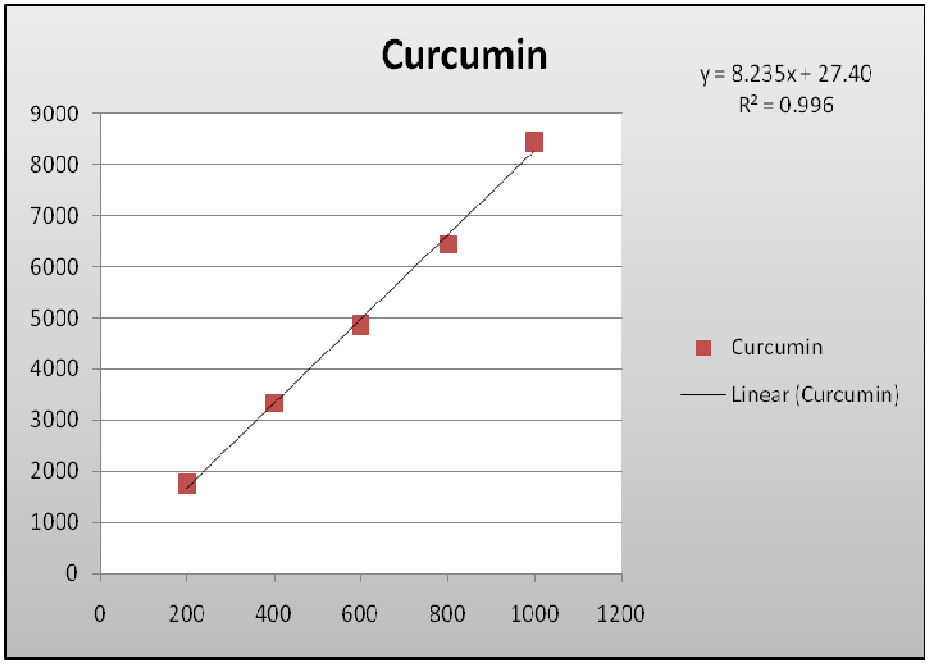

Fig. 6. Calibration curve for standard Curcumin (Concentration Vs peak area)

Correlation coefficient: 0.996, Slope: 16.47 Intercept: 141.5

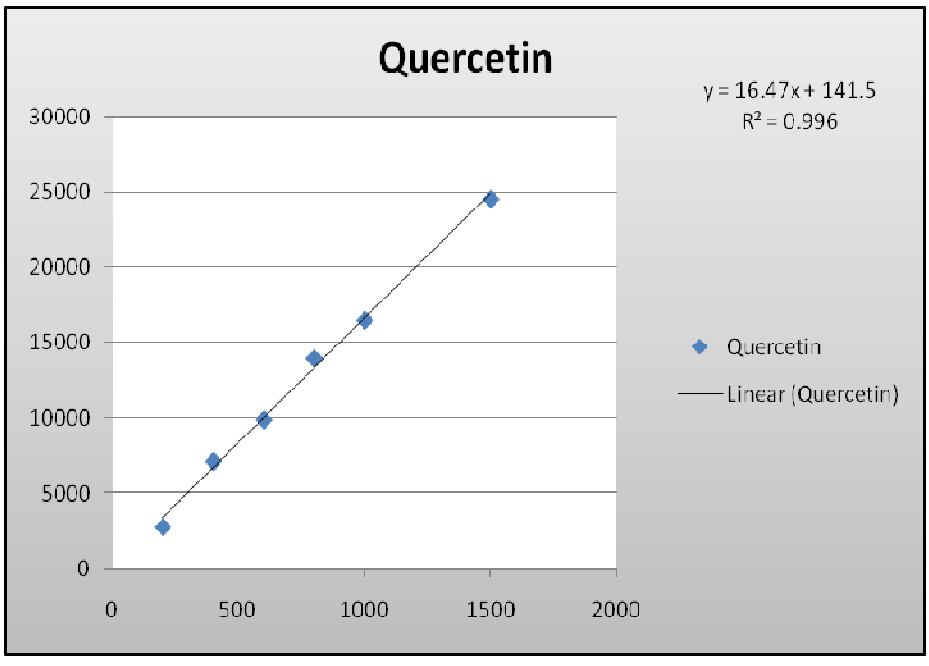

Fig. 7. Calibration curve for standard Quercetin (Concentration Vs peak area)

CONCLUSION:

A TLC densitometric method is established for the simultaneous quantification of gallic acid, curcumin and quercetin from polyherbal formulation Psoriasis using HPTLC fingerprinting. The methods were found to be simple, precise, specific, sensitive and accurate and can be used for their quantification in plant materials and also in routine quality control of the raw materials as well as formulations containing any of these compounds.

ACKNOWLEDGEMENTS:

The authors are thankful to the Department of Pharmacognosy, A.P.M.C. College of Pharmaceutical Education & Research, Himatnagar, for their help during this research work.

REFERENCES:

1. Walter L.F., Schaumburg G., Histopathology of the skin Boston, Massachusetts. 1981; 156 - 164.
2. Bajracharya, M.B. Ayurveda- The science of life series – vol.14, 2nd ed. Piyushvarshi Aushadhalaya Publishers: 2003.
3. Weiss S.C., Kimball A.B., Liewehr D.J., Blauvelt A., Turner M.L., Emanuel E.J. Quantifying the harmful effect of psoriasis on health-related quality of life. J Am Acad Dermatol. 2002; 47:512-518**.**
4. Agrawal, S.S. Paridhavi, M. Herbal Drug Technology, Universities Press (India) Private Limited, Hyderabad, 2007.