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Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations

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Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of curcumin both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform:methanol (9.25:0.75 v/v). This system was found to give compact spots for curcumin (R_f value of 0.48 ± 0.02). Densitometric analysis of curcumin was carried out in the absorbance mode at 430 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r = 0.996$ and 0.994 with respect to peak height and peak area, respectively, in the concentration range 50–300 ng per spot. The mean value ± S.D. of slope and intercept were 1.08 ± 0.01, 51.93 ± 0.54 and 8.39 ± 0.21, 311.55 ± 3.23 with respect to peak height and area, respectively. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 8 and 25 ng per spot, respectively. Curcumin was subjected to acid and alkali hydrolysis, oxidation and photodegradation. The drug undergoes degradation under acidic, basic, light and oxidation conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and photo oxidation. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Curcumin analysis; HPTLC method; Validation; Stability indicating

1. Introduction

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione (Fig. 1) is a yellow colored phenolic pigment obtained from powdered rhizome of *Curcuma longa* Linn. (Family: Zinziberaceae) [1]. It has been used to relieve the pain and inflammation since ancient times in traditional medicine. Extensive researches have revealed potent anti-inflammatory effects of curcumin [2–4]. It appears to block synthesis of certain prostaglandins [5], reduces pro-inflammatory cytokine synthesis [6,7], inhibit pro-inflammatory arachidonic acid as well as neutrophils aggregation [8,9] during inflammatory states. However, the oxygen radical scavenging activity [10,11] of curcumin has also been implicated in its anti-inflammatory effects [12]. Curcumin is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Hydrolytic decomposition is reported even in in vitro physiological condition (isotonic phosphate buffer, pH 7.2) [13–15]. It undergoes photodegradation when exposed to light in solution as well as in solid form [13]. Various methods are available for the analysis of curcumin in the literature like UV [16], HPLC [17–19], TLC [20,21] and HPTLC [22], but there are very few reports on analytical methods for the estimation of curcumin in bulk and its dosage form. Moreover, none of them is stability-indicating method. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out

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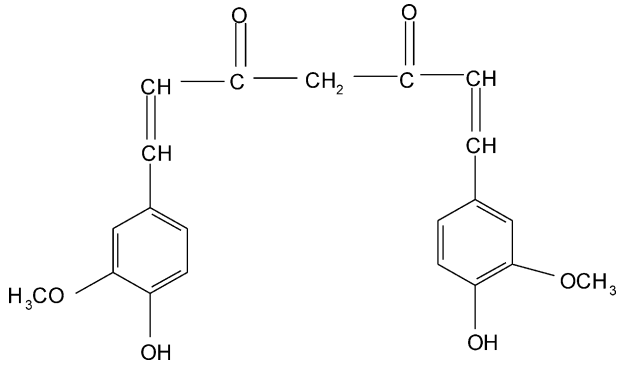

Fig. 1. Structure of curcumin.

to elucidate the inherent stability characteristics of the active substance [23]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. Nowadays, HPTLC is becoming a routine analytical technique due to its advantages [24–26]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Mobile phases having pH 8 and above can be employed. Suspensions, dirty or turbid samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. Simultaneous assay of several components in a multicomponent formulation is possible. The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of curcumin in the presence of its degradation products and related impurities as per ICH guidelines.

2. Experimental

2.1. Materials

Curcumin was purchased from Loba Chemicals Bangalore, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 3 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 150 nl/s was employed and space between two bands was 5.5 mm. The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. The mobile phase consisted of chloroform:methanol (9.25:0.75 v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was 65 mm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner IV in the absorbance mode at 430 nm. The source of radiation utilized was deuterium and tungsten lamp.

2.3. Calibration curves of curcumin

A stock solution of curcumin (100 μg/ml) was prepared in methanol. Different volumes of stock solution, 0.5, 1, 1.5, 2, 2.5 and 3 μl were spotted in duplicate on TLC plate to obtain concentrations of 50, 100, 150, 200, 250 and 300 ng per spot of curcumin, respectively. The data of peak height/area versus drug concentration were treated by linear least-square regression.

2.3.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (100 ng per spot of curcumin). The intra- and inter-day variation for the determination of curcumin was carried out at two different concentration levels of 100 and 200 ng per spot.

2.3.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of chloroform:methanol (9.5:0.5 and 9:1 v/v) was tried at two different concentration levels of 100 and 200 ng per spot.

2.3.3. Limit of detection and limit of quantification

In order to determine detection and quantification limits, curcumin concentrations in the lower part of the linear range of the calibration curve were used. Curcumin solutions of 50, 100 and 150 ng/μl were prepared and applied in triplicate (1 μl each). The amount of curcumin by spot versus average response (peak area) was graphed and the equation for this curve was determined, thereby obtaining an estimate of the target response: ybl. The ybl value corresponds to the intersection of the curve. Subsequently, a second curve was graphed showing the amount of curcumin by spot versus standard deviation of the responses. From the equation of this curve, we obtained an estimate of the standard deviation for target: sbl, which corresponds to the intersection of this curve. Detection and quantification limits were calculated by means of the equations [27]: detection limit = (ybl + 3 sbl)/*b*; quantification limit = (ybl + 10 sbl)/*b*, where "*b*" corresponds to the slope obtained in the linearity study of the method.

2.3.4. Recovery studies

The analyzed samples were spiked with extra 50, 100 and 150% of the standard curcumin and the mixtures were re-analyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

2.4. Analysis of the curcumin in prepared formulation

To determine the content of curcumin in tablets (labeled claim: 20 mg per tablet), the tablets were powdered and powder equivalent to 10 mg of curcumin was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. The 2 μl of the filtered solution (200 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

2.5. Forced degradation of curcumin

2.5.1. Acid and base induced degradation

The 50 mg of curcumin and complex equivalent to 50 mg of curcumin were separately dissolved in 50 ml of methanolic solution of 1 M HCl and 1 M NaOH. These mixtures were refluxed for 6 h at 90 °C in the dark in order to exclude the possible degradative effect of light. The resultant solutions were diluted 10 times, and applied on the TLC plate in triplicate (2 μl each, i.e. 200 ng per spot). The chromatograms were run as described in Section 2.2.

2.5.2. Hydrogen peroxide-induced degradation

To 25 ml of methanolic solutions of curcumin and complex (curcumin equivalent to 2 mg/ml), 25 ml of hydrogen peroxide (30.0%, v/v) were added separately. The solutions were heated in boiling water bath for 6 h to remove completely the excess of hydrogen peroxide. The resultant solutions were diluted appropriately and applied (2 μl each) on TLC plate in triplicate (200 ng per spot). The chromatograms were run as described in Section 2.2.

2.5.3. Photochemical degradation product

The 50 mg of curcumin and complex equivalent to 50 mg of curcumin were separately dissolved in 50 ml of methanol and exposed to direct sunlight and UV chamber at 254 nm for 24 h. The resultant solutions were diluted appropriately and applied on TLC plate (200 ng per spot) and then chromatograms were run as described in Section 2.2.

In all degradation studies, the average peak area of curcumin after application (200 ng per spot) of three replicates was obtained.

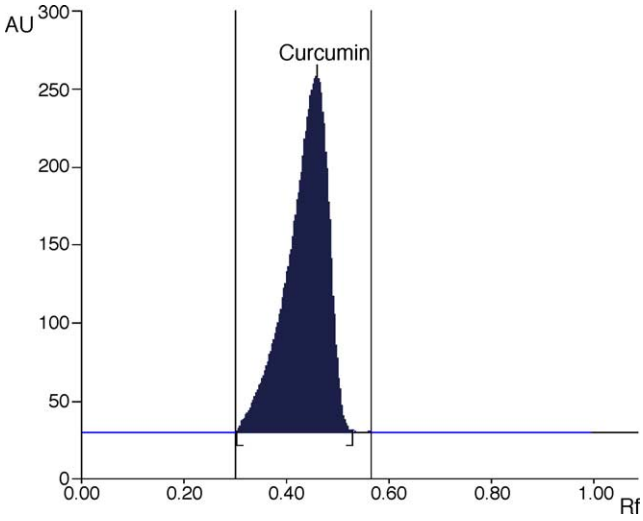

Fig. 2. A typical HPTLC chromatogram of curcumin (R_f = 0.48).

3. Results and discussion

3.1. Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method. Initially, chloroform:methanol in varying ratios was tried. The mobile phase chloroform:methanol (9.5:0.5 v/v) gave good resolution with R_f value of 0.48 for curcumin but typical peak nature was missing. Finally, the mobile phase consisting of chloroform:methanol (9.25:0.75 v/v) gave a sharp and well-defined peak at R_f value of 0.48 (Fig. 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

3.2. Calibration curves

The linear regression data for the calibration curves (n = 3) as shown in Table 1 showed a good linear relationship over the concentration range 50–300 ng per spot with respect to peak area.

No significant difference was observed in the slopes of standard curves (ANOVA, $P > 0.05$).

Table 1
Linear regression data for the calibration curves (n = 3)

Linearity range (ng)	50–300
Correlation coefficient	0.994
Slope ± S.D.	51.93 ± 0.54
Confidence limit of slope[a]	51.32–52.54
Intercept[b] ± S.D.	311.55 ± 3.23
Confidence limit of intercept[a]	307.90–315.21

[a] 95% confidence limit.
[b] Percentage of bias of intercept = −0.018.

Table 2
Intra- and inter-day precision of HPTLC method ($n = 6$)

Amount (ng/spot)	Intra-day precision				Inter-day precision			
	Mean area	S.D.	%RSD	S.E.	Mean area	S.D.	%RSD	S.E.
100	5420.67	32.06	0.62	13.09	5398.56	36.53	0.75	15.26
200	11044.23	40.52	0.36	16.54	10956.28	42.35	0.58	16.84

Table 3
Robustness of the method ($n = 3$)

Amount (ng/spot)	Mobile phase composition	
	Chloroform:methanol (9.5:0.5 v/v) (%RSD)	Chloroform:methanol (9:1 v/v) (%RSD)
100	1.35	0.89
200	0.65	1.12

Table 4
Recovery studies ($n = 6$)

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	%RSD	S.E.
0	100	101.83	0.73	0.31
50	150	101.21	0.53	0.22
100	200	98.85	0.66	0.54
150	250	99.55	0.56	0.57

3.3. Validation of the method

3.3.1. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of %RSD and results are depicted in Table 2, which revealed intra- and inter-day variation of curcumin at two different concentration levels of 100 and 200 ng per spot.

3.3.2. Robustness of the method

The low values of %RSD obtained after introducing small changes in mobile phase composition indicated robustness of the method as indicated in Table 3. There was no significant variation in the slope values (ANOVA, $P > 0.05$).

3.3.3. LOD and LOQ

The calibration curve in this study was plotted between amount of analyte versus average response (peak area) and the regression equation was obtained ($Y = 59.49X - 428.89$) with a regression coefficient of 0.999. Detection limit and quantification limit was calculated by the method as described

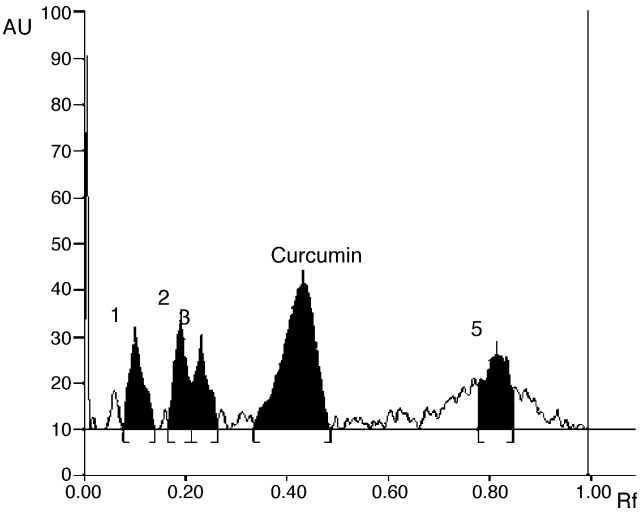

Fig. 4. HPTLC chromatogram of acid degradation products of curcumin complex.

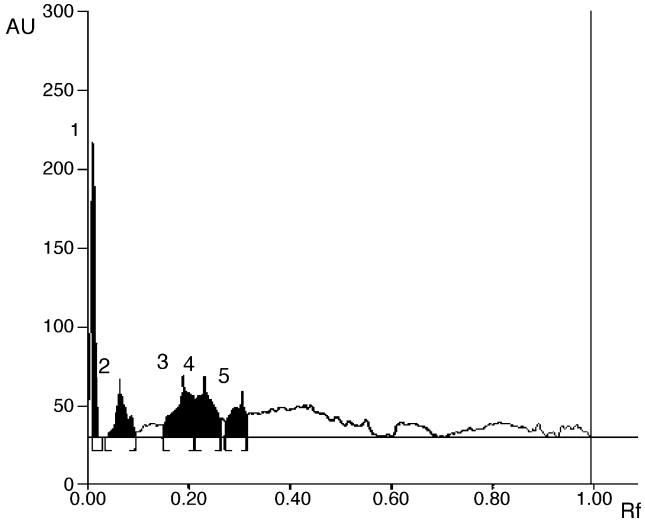

Fig. 3. HPTLC chromatogram of acid degradation products of curcumin.

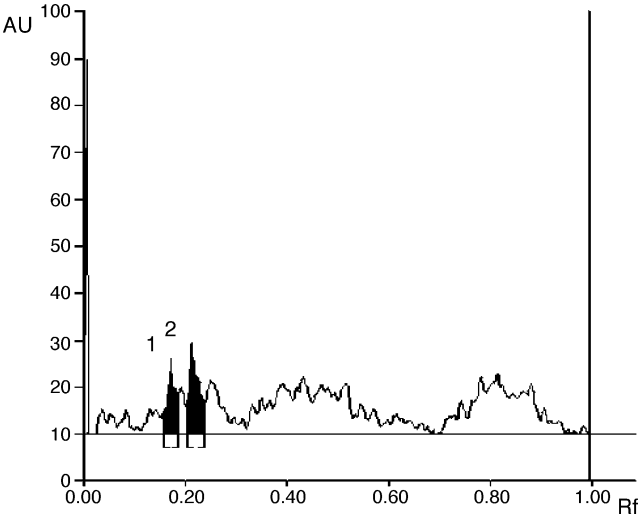

Fig. 5. HPTLC chromatogram of base degradation products of curcumin.

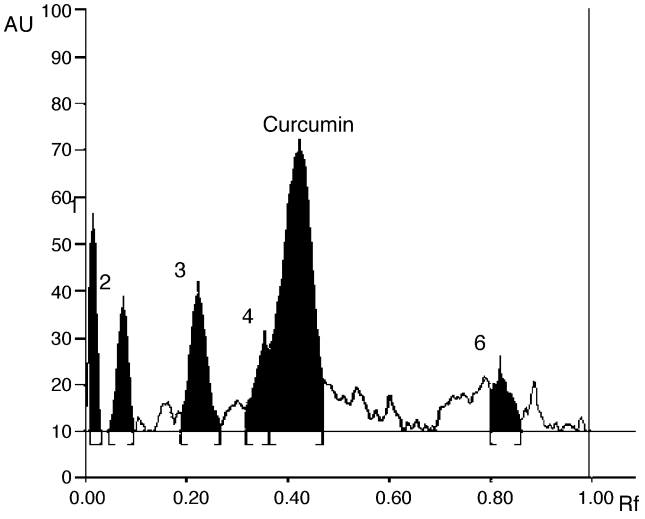

Fig. 6. HPTLC chromatogram of base degradation products of curcumin complex.

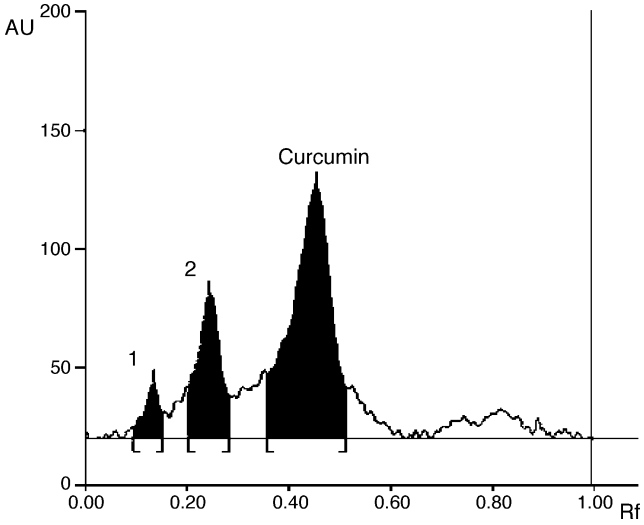

Fig. 8. HPTLC chromatogram of oxidation degradation products of curcumin complex.

in Section 2.3.3 and found 8 and 25 ng, respectively, which indicates the adequate sensitivity of the method.

3.3.4. Recovery studies

The proposed method when used for extraction and subsequent estimation of curcumin from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug afforded recovery of 98–101% as listed in Table 4.

3.3.5. Analysis of the prepared formulation

A single spot at R_f 0.48 was observed in the chromatogram of the curcumin samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The curcumin content was found to be 99.55% with a %RSD of 0.98. It may therefore be inferred that degradation of curcumin had not occurred in the formulations that were analyzed by this method. The low %RSD value indicated the suitability of this method for routine analysis of curcumin in pharmaceutical dosage forms.

3.4. Stability-indicating property

The chromatograms of the samples degraded with acid, base, hydrogen peroxide and light showed well-separated spots of pure curcumin as well as some additional peaks at different R_f values. The spots of degraded product were well resolved from the drug spot as shown in Figs. 3–12. The number of degradation products with their R_f values, content of curcumin remained, and percentage recovery were calculated and listed in Table 5.

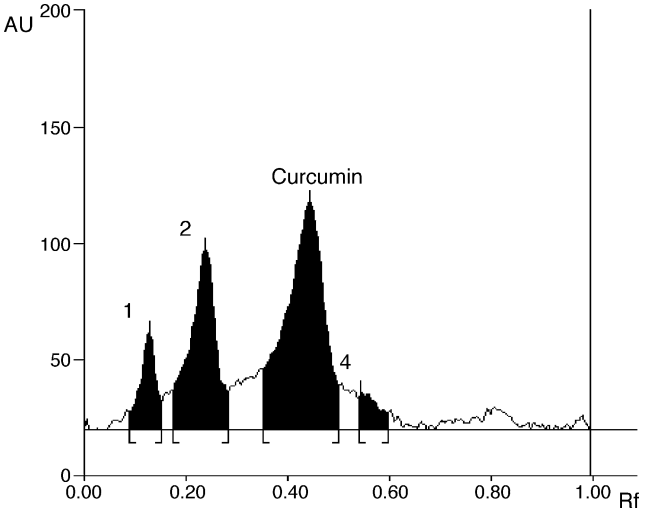

Fig. 7. HPTLC chromatogram of oxidation degradation products of curcumin.

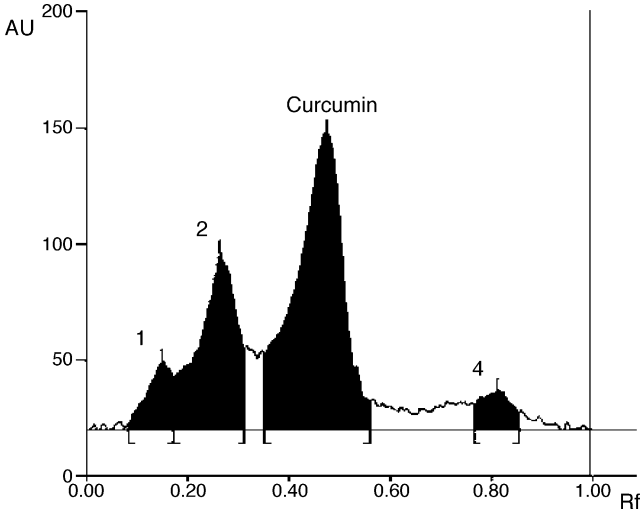

Fig. 9. HPTLC chromatogram of UV degradation products of curcumin.

Table 5
Forced degradation of curcumin and curcumin complex

Serial number	Sample exposure conditions	Number of degradation products (R_f value)	Figure	Curcumin remained (ng/200 ng) (±S.D., $n = 4$)	S.E.M.	Recovery (%)
1	Curcumin–acid, 1 M HCl	5 (0.01, 0.06, 0.19, 0.23, 0.31)	Fig. 3	Not detectable	–	0
2	Curcumin complex–acid, 1 M HCL	4 (0.10, 0.19, 0.23, 0.82)	Fig. 4	15.54 (±3.71)	2.62	7.8
3	Curcumin–base, 1 M NaOH	2 (0.17, 0.21)	Fig. 5	Not detectable	–	0
4	Curcumin complex–base, 1 M NaOH	5 (0.01, 0.07, 0.22, 0.35, 0.82)	Fig. 6	44.54 (±9.1)	6.43	22.3
5	Curcumin–H_2O_2, 30 vol.	3 (0.13, 0.24, 0.55)	Fig. 7	38.07 (±6.29)	4.45	19.0
6	Curcumin complex–H_2O_2, 30 vol.	2 (0.13, 0.24)	Fig. 8	79.16 (±5.00)	3.54	39.6
7	Curcumin–UV-254 nm	3 (0.15, 0.26, 0.81)	Fig. 9	97.98 (±2.82)	1.99	49.0
8	Curcumin complex–UV-254 nm	2 (0.14, 0.27)	Fig. 10	160.23 (±1.35)	0.96	80.0
9	Curcumin–day light	5 (0.17, 0.21, 0.30, 0.77, 0.82)	Fig. 11	107.07 (±0.92)	0.65	53.5
10	Curcumin complex–day light	2 (0.15, 0.28)	Fig. 12	121.06 (±3.86)	2.73	60.5

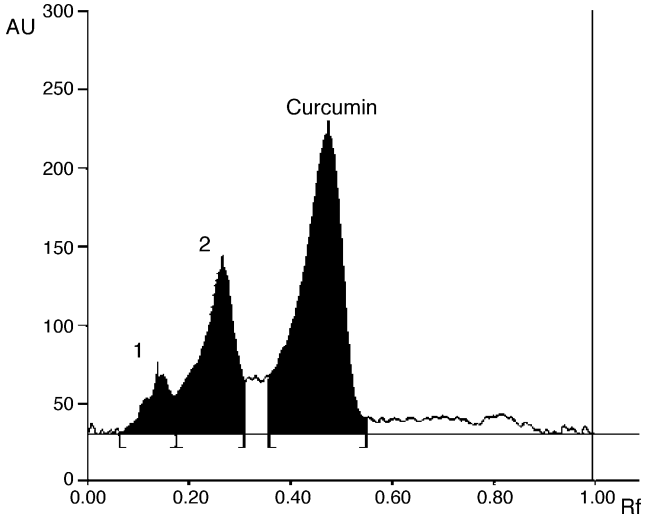

Fig. 10. HPTLC chromatogram of UV degradation products of curcumin complex.

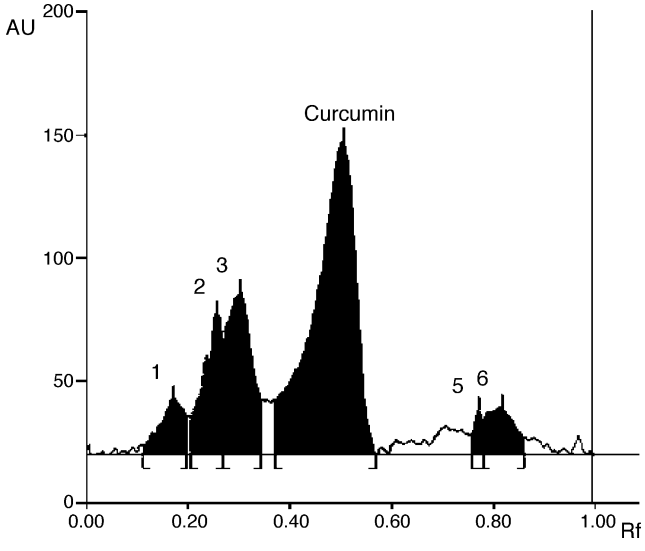

Fig. 11. HPTLC chromatogram of day light degradation products of curcumin.

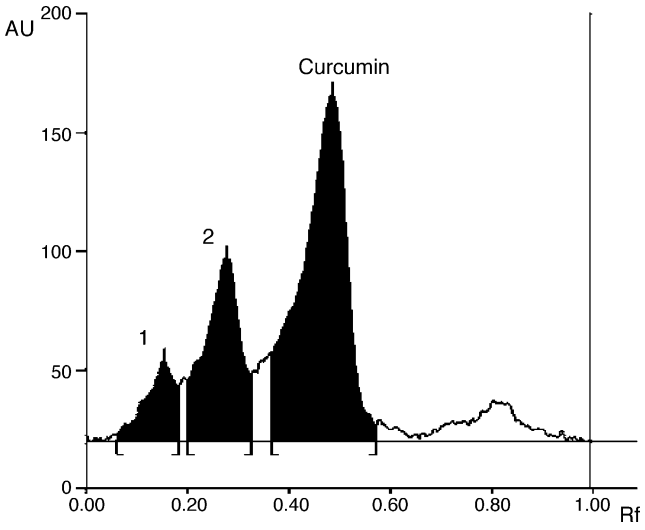

Fig. 12. HPTLC chromatogram of day light degradation products of curcumin complex.

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is repeatable and selective for the analysis of curcumin as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. It may be extended to study the degradation kinetics of curcumin and for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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