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Development and Validation of RP-LC Method for Curcumin in Pharmaceutical Formulations

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Curcumin in tablet formulations. The separation was achieved by using column Hypersil BDS C18, 150x4.6 mm, 5µ (Make: Thermo), in mobile phase consisted of tetrahydrofuran and citric acid buffer in the ratio of (550:450, v/v). The flow rate was 1.0 mL.min-1 and the separated curcumin was detected using UV detector at the wavelength of 425 nm. The retention time of curcumin, was noted to be 8.05 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography; Curcumin, Validation

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INTRODUCTION

Curcumin is the principal curcuminoid of popular Indian spice Turmeric, which is a member of Ginger family (Zingiberaceae). The other two curcuminoids are Demethoxy and Bis-demethxy Curcumin. The curcuminoids are natural phenols and responsible for the yellow colour of turmeric. Curcumin can exist in several tautomeric forms. The enol form is more energetically stable in the solid phase and in solution[1].

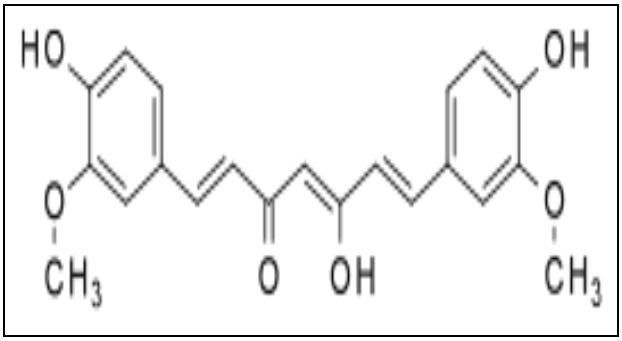

Figure 1: The Structure of Curcumin

IUPAC name is (1*E*, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl) -1, 6-heptadiene-3, 5-Dione. Curcumin is Anti microbial, Anti inflammatory, Hepato protective, Anti carcinogenic, Anti bacterial, Anti oxidant, Anti mutagenic, lower blood cholesterol level. Its molecular formula and molecular weight is $C_{21}H_{20}O_6$ and 368.38 g/mol. Curcumin is Bright yellow-orange powder. It is insoluble in water and soluble in THF, acetonitrile and methanol. Literature review revealed that some methods for the determination of curcumin in spectrophotometric[2], thin layer chromatography[3], HPTLC[4-9], RP-HPLC[10-16] there were no methods for the estimation of curcumin. Hence in the present chapter new sensitive, economical, stability indicating RP-HPLC method was developed and validated in accordance with ICH guidance.

MATERIALS AND METHOD

Chemicals and Reagents

Analytical-grade Citric acid, Tetrahydrofuran, Methanol, Acetonitrile and Water HPLC-grade, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, India.

Instrumentation and Chromatographic Conditions

Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Buffer preparation

Accurately weighed 5.5 g of Citric acid was dissolved in 1000 ml milli-Q water, the solution was filtered through 0.45µ filter paper and degassed.

Mobile phase preparation

550 volumes of buffer and 450 volumes of filtered and degassed Tetrahydrofuran were mixed and sonicated.

Diluent preparation

Mobile phase was used as blank.

Standard preparation

About 25 mg of Curcumin was accurately weighed and transferred into a 100 ml volumetric flask and added 25 ml of THF was added and sonicated for 15 min and volume was made up with Mobile phase. From the above stock solution 5ml was transferred to a 50 ml volumetric flask and the volume was made up with mobile phase. Filtered through the 0.45 µ membrane filter. (The final concentration of resulting was 25 µg/ml).

Sample preparation

20 tablets were weighed and average weight was found out. The tablets were powdered and about 5 mg equivalent of Curcumin (about 8.066 g of diazen tablet powder) was weighed and transferred into 200 ml volumetric flask. 40 ml THF was added and sonicated for 20 min and finally the volume was made up with mobile phase. Filtered through the 0.45 µ membrane filter. (The final concentration of resulting was 25µg/ml).

Chromatographic conditions

Chromatographic analysis was performed on Hypersil BDS C18, 150x4.6 mm, 5µ (Make: Thermo) column. The mobile phase consisted of Tetrahydrofuran and citric acid buffer in the ratio of (550:450, v/v). The flow rate was 1.0 mL/min, column oven temperature ambient temperature, the injection volume was 20 µL, and detection was performed at 425 nm using a photodiode array detector (PDA).

RESULTS AND DISCUSSION

Method development

Spectroscopic analysis of compound curcumin showed that maximum UV absorbance (λmax) at 425 nm respectively. To develop a suitable and robust LC method for the determination of curcumin, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Hypersil BDS 250mm×4.6mm, 5µ with the following different mobile phase compositions like that Buffer and Acetonitrile in the ratio of 80:20 v/v 65:35 v/v & 50:50. It was observed that when curcumin was injected, Peak Tailing, not satisfactory. For next trial the mobile phase composition was changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 550:450 v/v. respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 425nm. The retention time of curcumin is 8.05 minutes and the peak shape was good. The chromatogram of curcumin standard using the proposed method is shown in **(Figure 2)** system suitability results of the method are presented in Table 1

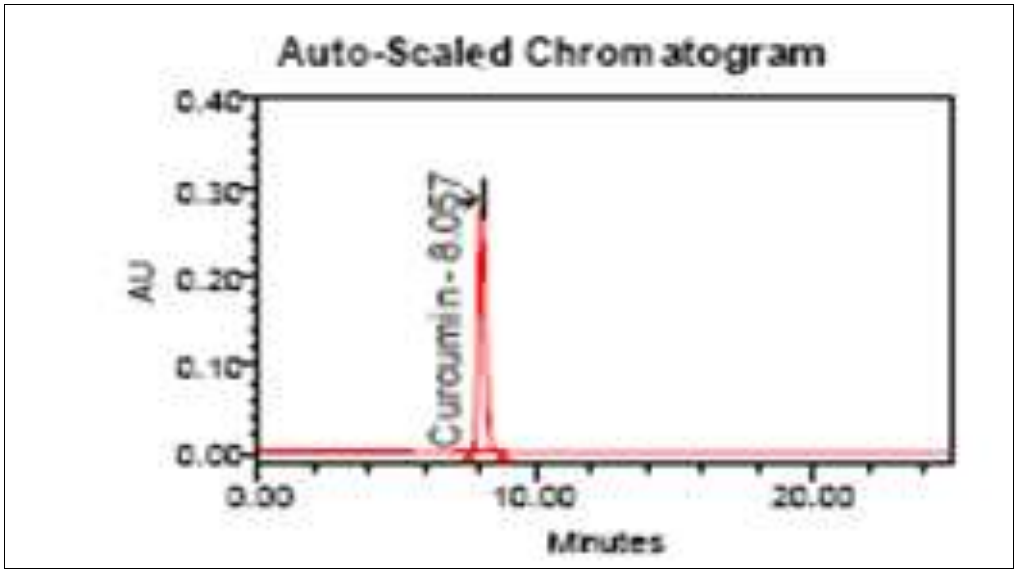

Figure 2: Chromatogram Showing the Peak of Curcumin

Method validation

The developed RP-LC method extensively validated for assay of curcumin using the following parameters.

Specificity

Preparation of blank solution:

Citric acid buffer and Tetrahydrofuran were mixed in the ratio of 55:45 and degassed.

Preparation of Placebo solution

Placebo solution was prepared in duplicate by weighing the equivalent amount of excipients present in the finished drug product and analyzed as per proposed method. Interference due to placebo was evaluated for each of the placebo preparations.

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Figure 3)** showed no peak at the retention time of curcumin peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of curcumin in curcumin tablets. Similarly chromatogram of placebo solution (Figure 4) showed no peaks at the retention time of curcumin peak. This indicates that the placebo used in sample preparation do not interfere in estimation of curcumin in curcumin tablets.

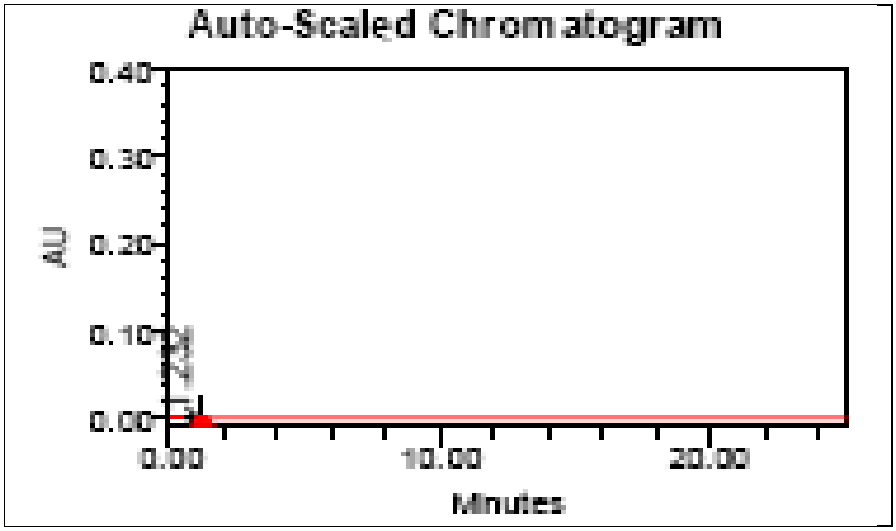

Figure 3: Chromatogram showing the no interference of diluent for curcumin

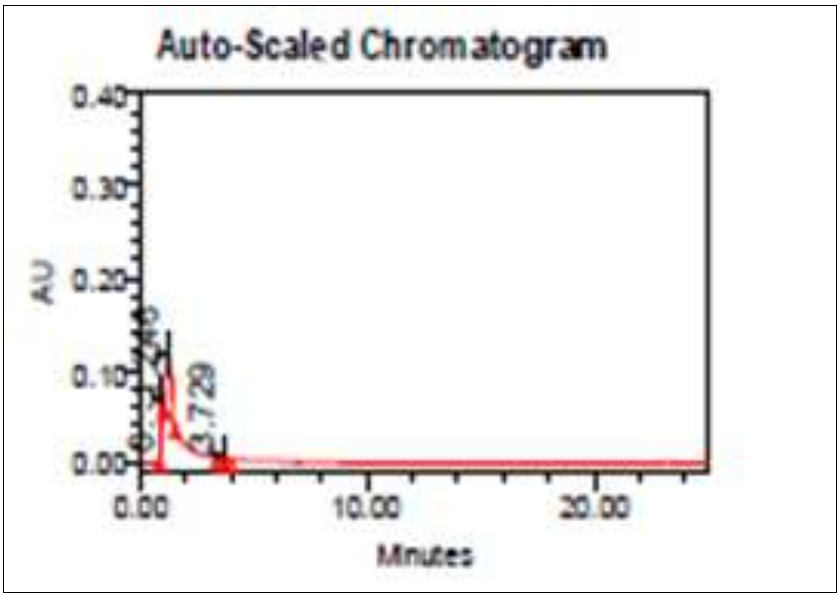

Figure 4: Chromatogram showing the no interference of placebo for curcumin

Table 1: System Suitability Parameters for Curcumin by Proposed Method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Curcumin	8.057	8566	1.1

System precision

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The chromatogram was shown in Figure 5 and data were shown in Table 2

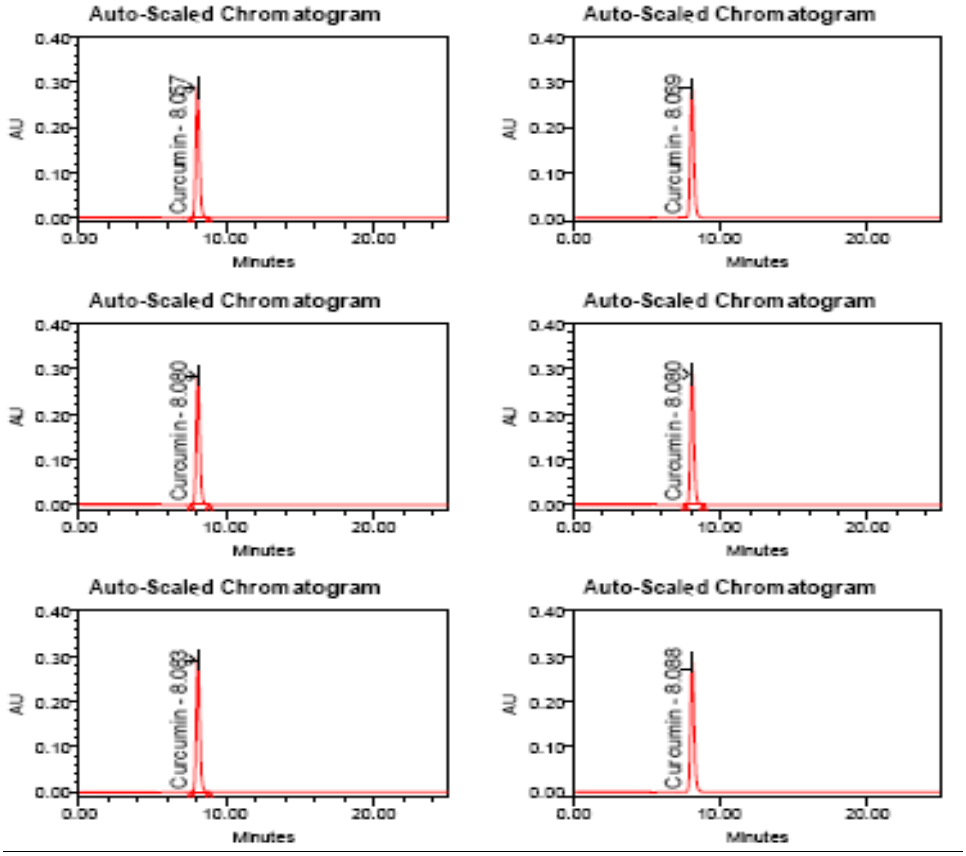

Figure 5: System precision standard chromatogram

Table 2: System Precision Data for Curcumin

No. of injections	Area
1	4765464
2	4769515
3	4789437
4	4629991
5	4600278
6	4600598
Average	4692546
SD	91115.256
% RSD	1.9

Method precision

The precision of test method was evaluated by doing assay for six samples of curcumin tablet as per test method. The content in mg and % label claim for curcumin for each of the test preparation were calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in **Figure 6** and data were shown in **Table: 3**

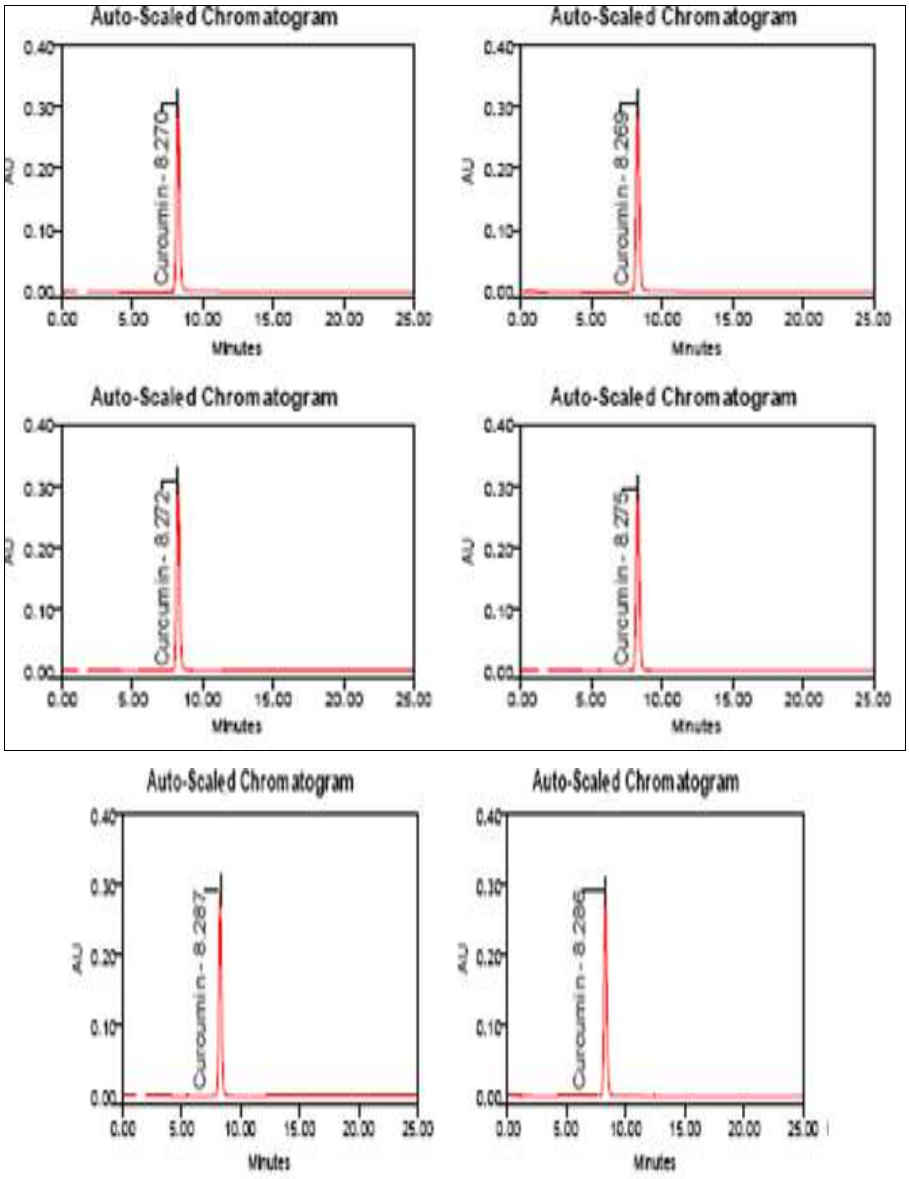

Figure 6: Method precision sample chromatogram

Table 3: Method precision data for Curcumin

No. of injections	Curcumin % Assay
1	109.6
2	109.4
3	108.8
4	109.8

5	109.8
6	108.8
Average	109.4
SD	0.01
%RSD	0.42

Linearity of detector response

The standard curve was obtained in the concentration range of 20.0-30.0µg/ml for curcumin. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure 7 to demonstrate the linearity of the proposed method. From the data obtained which given in Table 4 the method was found to be linear within the proposed range.

Table 4: Linearity studies for curcumin by proposed method

Level no.	Curcumin Linearity concentration	Concentration (µg / ml)	Average area response
1	80	19.9564	3817757
2	90	22.5963	4349852
3	100	24.8896	4812110
4	110	27.6058	5359588
5	120	29.9517	5832441
Correlation coefficient:			0.9980
Slope (m):			19551
Intercept (y):			-12054

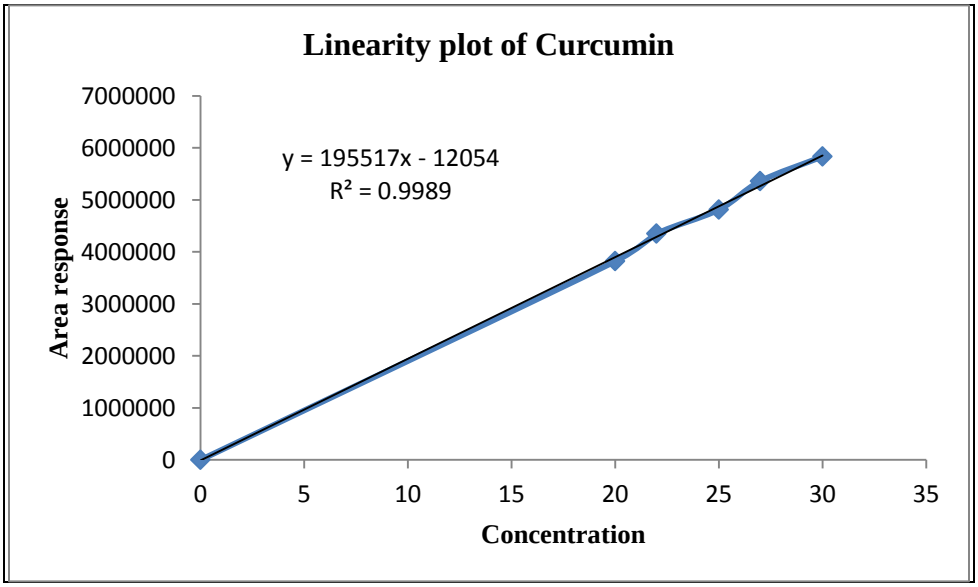

Figure 7: Calibration curve for Curcumin

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend

collected from 20 tablets of Curcumin, analyzed as per the proposed method. The percentage recoveries with found in the range of 98.9 to 100.4 for curcumin. The chromatogram was shown in Figure 7 to 9 the data obtained which given in Table 5 the method was found to be accurate.

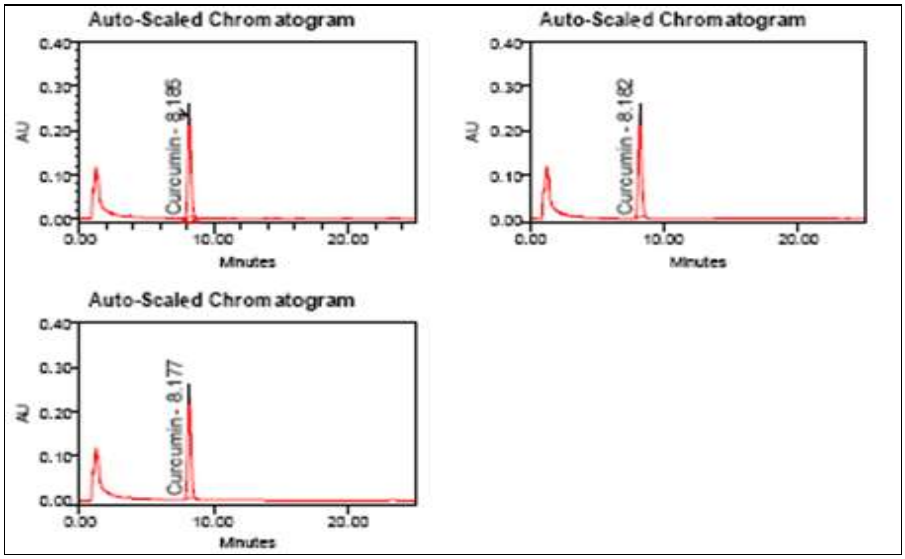

Figure 7: Accuracy (Spike level 80%) chromatogram

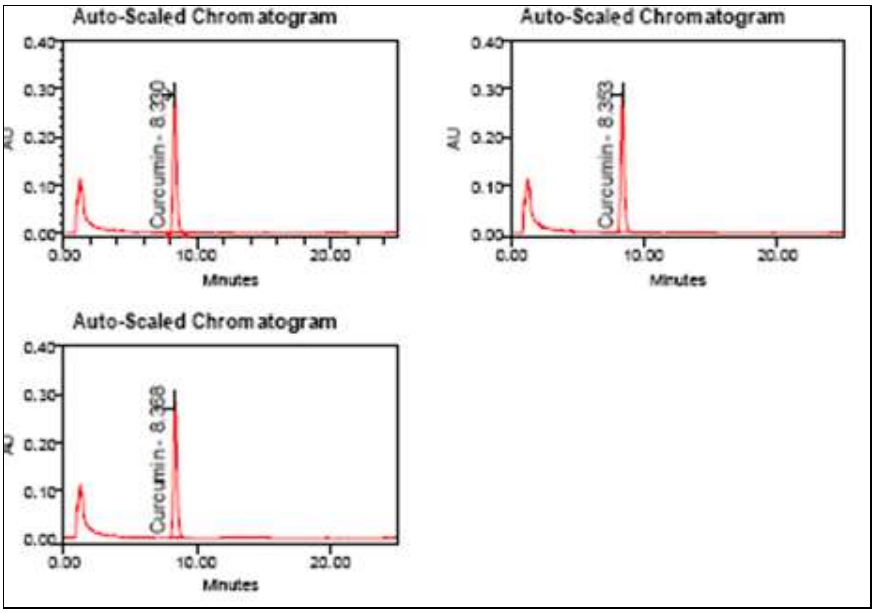

Figure 8: Accuracy (Spike level 100%) chromatogram

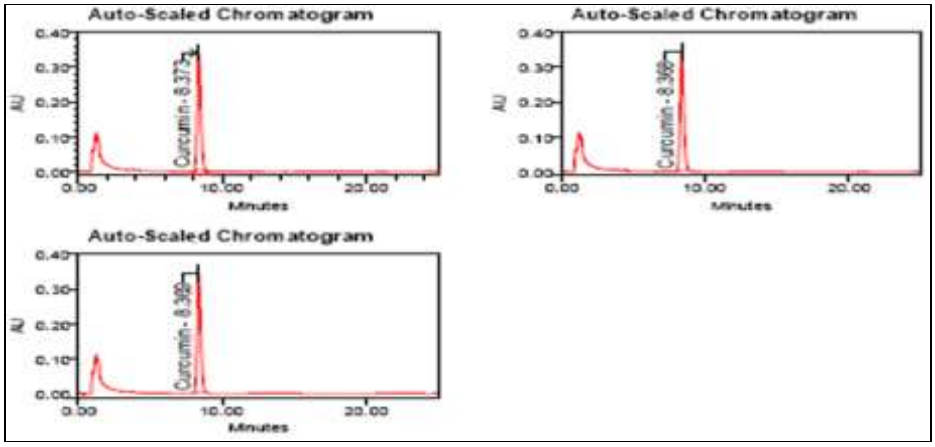

Figure 9: Accuracy (Spike level 120%) chromatogram

Table 5: Recovery studies for curcumin by proposed method

Recovery Level	Curcumin Amount Added (mg)	Amount recovered (mg)	Percentage recovery	Average Recovery (%)	% RSD
50%	4.02	3.98	99.0	98.9	0.4
	4.05	3.99	98.5		
	4.03	4.00	99.3		
100%	5.12	5.05	98.6	99.4	0.8
	5.07	5.05	99.6		
	5.05	5.06	100.1		
150%	6.13	6.17	100.7	100.4	0.9
	6.08	6.15	101.2		
	6.18	6.14	99.4		
Overall percentage recovery				99.6	
Overall percentage RSD for percentage recovery				0.8	

CONCLUSION

An RP-HPLC method for estimation of curcumin was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 20-30µg/ml. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the drug or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. We have developed a fast, simple and reliable analytical method for determination of curcumin in pharmaceutical preparation using RP-LC. As there is no interference of blank and

placebo at the retention time of curcumin. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of curcumin in its different pharmaceutical dosage forms.

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