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ESTIMATION OF CURCUMINOIDS IN HERBAL FORMULATIONS USING VALIDATED RP-HPLC METHOD

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ABSTRACT

Turmeric (Curcuma longa L. Family: Zingiberaceae) is a widely cultivated and used spice in India. Curcumin is the active constituent present in Curcuma longa along with two related compounds, Demethoxycurcumin and Bisdemethoxycurcumin are altogether known as Curcuminoids. Turmeric is rich in Curcuminoids and is recognized for its broad spectrum of biological activities such as Antioxidant, Antifungal, Antiseptic, Anti Inflammatory, and Anti-cancer. The present study involves the estimation of Curcuminoids content of in-house developed herbal formulations using simple, accurate, precise, and reproducible reverse-phase High-Performance liquid chromatography (RP-HPLC). Analysis was performed on an Agilent 1200, HiQ sil C18HS column (4.6 mm X 250mm) column with the mobile phase consisting of Acetonitrile: 0.02% Orthophosphoric acid in water (55:45) at a flow rate of 1.0 mL/min. UV detection was performed at 425 nm and 20µL sample was injected. The retention time for Bisdemethoxycurcumin, Demethoxycurcumin, and Curcumin was about 11.00, 12.03, and 13.18 minutes. The calibration curve was found linear (correlation coefficient = 0.9927) in the selected range. The method was validated for various parameters such as system suitability, specificity, linearity, and recovery, precision, ruggedness, robustness as per ICH guidelines. The system suitability parameters, such as theoretical plate, resolution, and relative standard deviation (RSD) for five standard replicates, were within the limit. The validated RP-HPLC method was found to be suitable, specific, linear, which was successfully used for the estimation of Curcuminoids content of marketed products as well as in-house developed formulations. This method can be used for the estimation of curcuminoids content in the formulation/ products containing turmeric / curcuminoids.

Keywords *– Turmeric, Curcuminoids, Curcuma longa, RP-HPLC, Tablet, Emulgel, Validation.*

1. INTRODUCTION

Turmeric (*Curcuma longa* of Family Zingiberaceae) is one of the widely used spices in India and is known as Haldi. Indian ancient medicine system Ayurveda talks about the medicinal properties of Turmeric. [1] Turmeric (*Curcuma longa)* has been reported to possess Antioxidant, Antifungal, Antiseptic, Anti Inflammatory, and Anti-cancer properties. [2] These effects are due to the presence of polyphenolic compounds known as Curcuminoids. Turmeric contains three Curcuminoids namely Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin. [3]

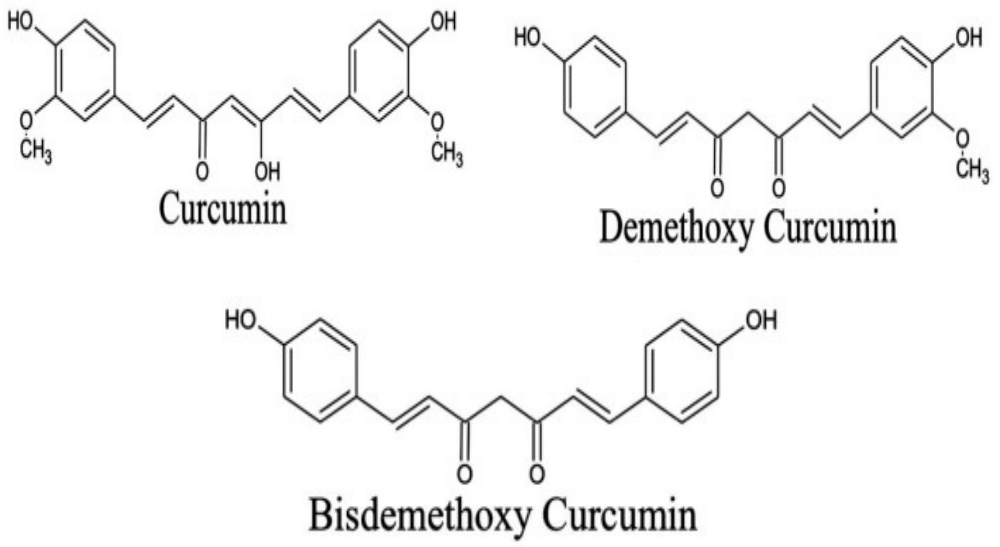

Fig. 1. Chemical structures of different Curcuminoids present in turmeric

The herbal formulation is at high risk due to changes in herbal raw material by various factors such as the method of extraction, moisture content, and the geographical condition that will lead to a change in the quality of the final product also the chances of the batch to batch variation are high. [4] To ensure the Quality and Safety of the products, quantitative estimation of active phytoconstituents is important, various methods such as Spectroscopic analysis, Thin Layer Chromatography (TLC), and High-Performance Thin Layer Chromatography (HPTLC), capillary electrophoresis, liquid chromatography-mass spectroscopy (LC/MS) are available. While taking into account sensitivity, specificity, and efficacy High-Performance Liquid chromatography (HPLC) is considered to be the most widely accepted method of estimation of all curcuminoids is possible by this method. Hence Present work involves Quantitative estimation of Curcuminoids content in-house developed herbal Tablet and Emulgel formulation. [5-7]

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Curcuminoids were procured as a gift sample from Sahyadri Phytoconstituent Pune, Standard Curcumin (Purity = 97%, Batch No. K085/0241110IX30) was purchased from High Purity Laboratory Chemicals Pvt. Ltd., Mumbai, India. All the chemicals and reagents were used of HPLC grade.

2.2 Instrumentation

A double-beam UV-Vis spectrophotometer (Shimadzu, Japan) with 1.0 cm quartz cells was used for all absorbance measurements. An Agilent 1200 high-performance liquid chromatography (HPLC) equipped with a Quaternary pump and UV detector was used for analysis. Detection was made at 425 nm using Chemstation software. The chromatographic analysis was performed on a HiQ sil C18HS column (4.6 mm X 250mm) column. Degassing of the mobile phase was done by using an Oscar ultrasonic bath sonicator (Mumbai, India).

2.3 Preparation of Standard Solution

The standard solution was prepared by adding about 100 mg of Pure Curcumin to 50 ml of Methanol in a 100 ml volumetric flask. This solution was sonicated for about 10 minutes and then the volume was made up to 100 ml with Methanol. The solution was filtered, and 5 ml of this solution was diluted up to 25 ml by methanol and kept for sonication for 20 minutes and used as Standard.

2.4 Preparation of Test Solution

The test solution was prepared by adding Tablet powder and Emulgel respectively equivalent to 200 ppm of Curcuminoids in methanol. This solution was sonicated for about 20 minutes.

2.5 Chromatographic Conditions

The method was developed using reverse phase, HiQ sil C18HS column (4.6 mm X 250mm) column. The run time was 20 min. The Injection volume was 20 µL. The mobile phase used was Acetonitrile and 0.02% orthophosphoric acid in water the ratio (55:45) at a flow rate of 1.0 ml/min, column temperature maintained at 27 °C and a detection wavelength of 425 nm using a UV-visible detector [8-12]

2.6 Validation of HPLC Method

The HPLC method was validated for the evaluation of Curcuminoids as per ICH guidelines. The selected method was validated for Specificity, Linearity, Precision, Accuracy, and Robustness. [13-15]

2.6.1 System Suitability

System suitability testing is used to verify that the Precision/Reproducibility of the system is adequate for the analysis to be performed. Parameters such as theoretical plates, resolution, and reproducibility (% RSD for the area of replicates) were determined. Five replicate injections of the standard solution were made into the HPLC system. Mean, Standard Deviation, and % RSD were calculated.

2.6.2 Specificity

A Blank and Placebo solution was injected to check the specificity of the method. The specificity of the method was evaluated by comparing the Chromatograms of Blank, Placebo, Standard, and Samples.

2.6.3 Linearity

Linearity was performed by preparing various concentrations of a standard solution in the range from 25-300□g/mL 20 µL of each concentration was injected in duplicate into the HPLC system. The response was read at 425 nm, and the corresponding chromatograms were recorded. Regressions of the plots were computed by the least square regression method.

2.6.4 Method Precision and Ruggedness (Intermediate Precision).

The precision of the method was performed as intraday precision and interday precision. To study the intraday precision, analysis was performed on 6 doses and the samples were analyzed by a test method. Ruggedness was carried out by changing the person and conducting precision experiments on different days. The percent relative standard deviation (% RSD) was calculated

2.6.5 Recovery

Accuracy of the method was evaluated in triplicate at three concentration levels (80,100, and 120 %), and the percentage recoveries were calculated. The study was carried out in triplicate at 160, 200, and 240 µg/mL respectively.

2.6.6 Robustness

Robustness was evaluated by changing the flow rate by ±0.2 mL, wavelength by ±2 nm, and temperature by ±2□ C and analyzing the samples by making the above changes in the HPLC method. The factors of the robustness study are summarized in Table No. 1.

2.6.7 Solution Stability

Solution stability was performed over a period of 24 hours, verifying the response of the standard and sample solution stored at room temperature.

Table 1. Summary of Robustness Parameters used in the study

Parameter	Low	Nominal	High
Wavelength (nm)	423	425	427
Flow rate (ml/min)	0.8	1.0	1.2
Temperature (℃)	25	27	29

3. RESULTS and DISCUSSIONS

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and choice of stationary and mobile phase. The HPLC parameters were optimized on trial and error basis. The selection of stationary phase depends upon the chemical nature of the sample, solubility, and the molecular weight. Curcuminoids are water insoluble compound and hence reverse phase columns was selected.Various combinations of methanol, acetonitrile, orthophosphoric acid, ammonium dihydrogen phosphate, and triethylamine were tested.Composition of mobile phase on the retention time of Curcuminoids was thoroughly investigated. At this optimized concentration, Curcuminoids gave symmetric peak with short runtime.

The representative chromatogram of placebo standard and tests (Emulgel and Tablet) are represented in Figures 2a to 2d

Method validation was performed to ensure that the developed method is reliable and adequate for its intended purpose. The optimized chromatographic conditions were validated as per ICH guidelines.

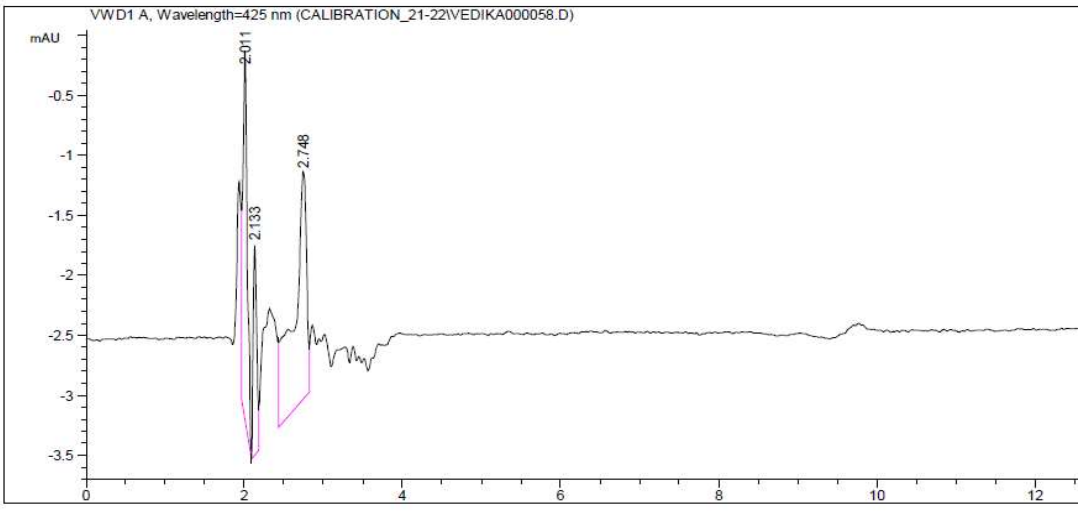

Fig. 2a. A Typical Chromatogram of Placebo

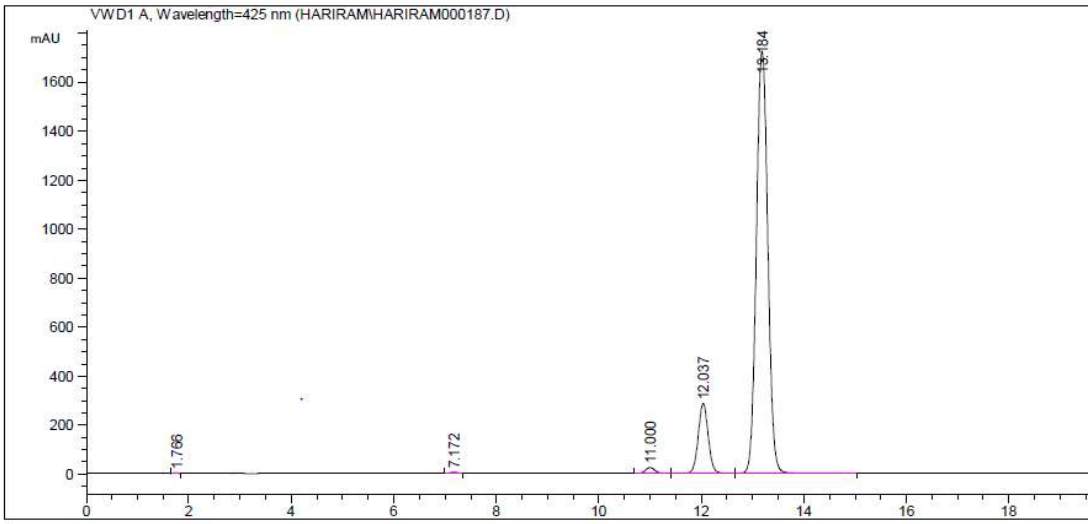

Fig. 2b. A Typical Chromatogram of Standard

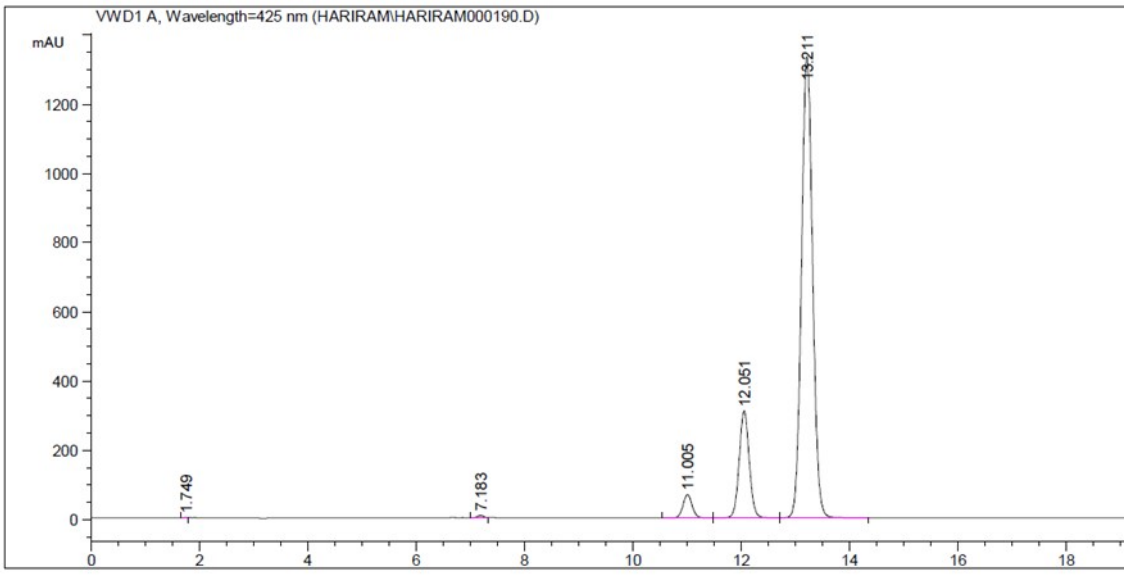

Fig. 2c. A Typical Chromatogram of Test - 1 (Emulgel)

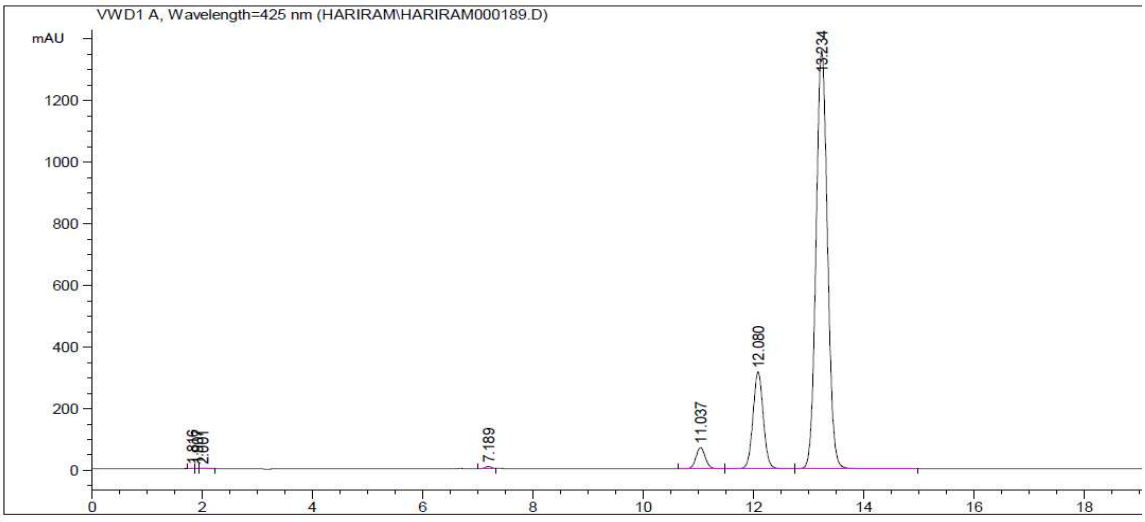

Fig. 2d. A Typical Chromatogram of Test - 2 Tablet

Fig 2: Chromatographic separation for specificity. (2a) Placebo; (2b) Standard; (2c) Emulgel; (2d) Tablet

3.1 System Suitability

The results of system suitability parameters are summarized in Table no.2.The theoretical plates obtained were more than 2000 and resolution between the peaks was more than 1.5 .The % RSD for five replicate injections was found to be less than 2.

3.2 Specificity

The method specificity is represented in Figure 2. There are no peaks observed at the retention time of Curcuminoids in the blank as well as placebo sample (Figure 2a). The retention time for Curcuminoids in the in-house developed herbal formulation samples (Figures 2c and 2d) is following the standard solution of Curcuminoids (Figure 2b). This indicates the specificity of the developed analytical method.

3.3 Linearity

The calibration curves created were linear in the specified ranges of 25-300 (µg/mL). The correlation coefficient (r2) was noted as 0.9926 with the linear regression equation Y = 107.98x+ 4808.7 The rationale for developing linearity at different ranges for Standard solution was based on their linearity. A good regression coefficient (r2) was observed within the range of 25-300 (µg/mL). Linearity is summarized in Table No. 3 (Refer Fig.3)

Table 2. Summary of System suitability parameters

Injections	Retention time			Area			Theoretical plate			Resolution			Selectivity Factor		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
1	8.8	9.5	10.2	1143.27	7139.26	28900	11405	11391	9137	7.84	1.95	1.87	1.39	1.08	1.08
2	9.3	10.09	10.87	1070.77	6773.14	28400	11698	11825	9549	8.04	2.01	1.93	1.4	1.08	1.08
3	9.3	10.11	10.9	1070.31	6767.9	28400	11759	11708	9584	8.27	2.01	1.92	1.4	1.08	1.08
4	8.93	9.61	10.34	1068.08	6652.04	27300	11735	11666	9769	7.99	1.97	1.9	1.39	1.08	1.08
5	8.91	10.02	10.43	1052.32	6812.41	28500	11541	11428	9678	8.13	2.01	1.94	1.4	1.08	1.08
Mean	9.04	9.86	10.54	1080.95	6828.95	28300	11627.6	11603.6	9543.4	8.05	1.99	1.91	1.39	1.08	1.08
SD	0.25	0.31	0.35	36.7	211.63	691.72									
% RSD	2.60	2.92	3.01	3.29	2.68	2.16									

Table 3. Results of Linearity

Concentration (µg/mL)	Area (mAU)
25	6742.74
50	9966.29
100	16195.2
200	27903.0
300	36102.5
Slope – 107.98	
Intercept – 4804.7	
Correlation Coefficient (r^2) - 0.9927	

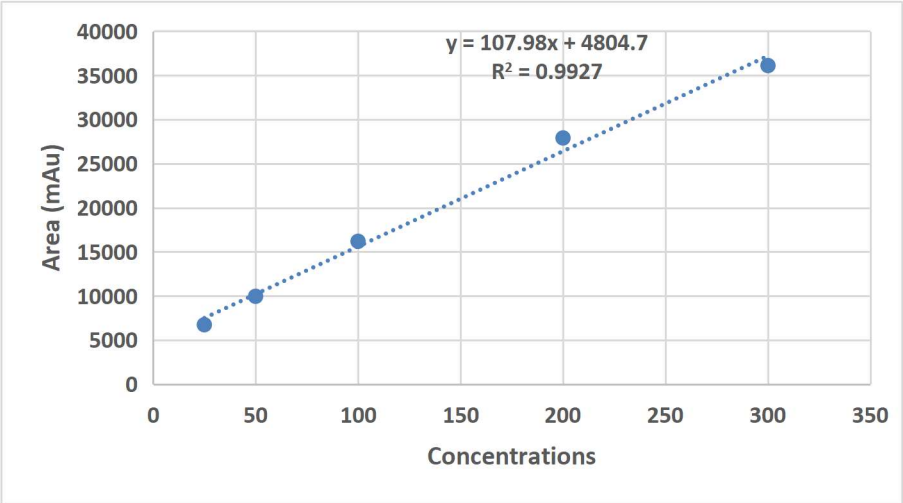

Fig. 3. Linearity curve of standard (Curcuminoids)

3.4 Precision

The results of Method precision and intermediate precision are summarized in Table no.4.The % RSD value was found to be less than 2 indicating that the method is precise and rugged.

Table 4. Results of intra-day and inter-day precision

Replicates	Method Precision Day-1 Analyst -1 (% Assay)	Intermediate Precision Day-1 Analyst -1 (% Assay)
Assay 1	99.93	101.41
Assay 2	101.14	100.28
Assay 3	99.8	98.98
Assay 4	101.73	101.98
Assay 5	101.97	98.87
Assay 6	98.50	101.18
Average	100.51	100.45
Standard deviation (SD)	1.33	1.30
% Relative Standard Deviation	1.327	1.296

3.6 Recovery Study

Recovery accuracy was expressed as the percentage of analysts recovered by the assay method.The mean percentage recoveries (Accuracy) obtained were found in between 95% to 105%. The results of the recovery study are summarized in Table 5

Table 5. Results of Recovery study

% Recovery of Curcuminoids				
Formulation	**Level (%)**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**
Emulgel	80	2000	1921	96.05
	100	2500	2431	97.24
	120	3000	2883	96.1
Tablet	80	60	57.50	95.83
	100	75	73.40	97.86
	120	90	84	95.11

3.6 Robustness

The results of the Robustness Study are summarized in Table no. 6. It was observed that there were no marked changes in chromatograms as well as % assay and the method was found to be reliable during normal usage due to small changes in some analytical parameters like change in wavelength, flow rate, and temperature, which demonstrated that the developed method was robust in nature.

Table 6. Robustness test of the developed method

Parameter	**Variation**	**% Assay**	**% Relative standard deviation**
Wavelength (nm) 425±2nm	423	102.12	1.32
	427	99.18	1.43
Flow rate (ml/min) 1±0.2 ml / min	0.8	101.15	1.27
	1.2	100.88	1.18
Temperature ℃ 27**±2℃**	25	100.99	0.95
	27	99.01	1.1

3.7 Solution Stability

Solution stability data indicate that the solution was stable for 24 hrs at room temperature.

3.8 Determination of Curcuminoids in herbal formulations

The in-house tablet and emulgel formulation were prepared using pure curcuminoids and suitable excipients. The Curcuminoid content in the marketed product and developed in-house formulations was determined using the above-validated method. The results of curcuminoids content are summarized in Table No. 7 and 8.

Table 7. Results of Curcuminoid content in the marketed product

Sr. No.	**Formulation**	**Label Claim**	**Amount found**	**% Recevory**
1	Marketed Tablets	73	71.7	98.35

Table 8. Results of Curcuminoid content in In-house developed herbal formulations

Formulation	% Curcuminoid Content
Emulgel	99.5
Tablet	99.7

4. CONCLUSION

The RP-HPLC method was successfully applied to quantify the amount of curcuminoids in marketed product as well as in-house developed herbal formulations. The obtained positive outcomes of this study indicated that the developed method can be further explored to quantify their phytoconstituents in biological samples during the preclinical or clinical studies. And in the Quality control laboratory for the determination of curcuminoids in the marketed Ayurvedic or herbal formulations.

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. ACKNOWLEDGMENT

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