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An improved reversed-phase HPLC method for simultaneous estimation of curcuminoids in a dietary supplement formulation

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ABSTRACT

A reversed phase High Performance Liquid Chromatography method was developed for simultaneous estimation of Curcumin (C), Demethoxy curcumin (DMC) and Bis-demethoxy curcumin (BDMC) in a dietary supplement formulation. The formulation analysed also contained piperine along with curcuminoids. Separation of curcuminoids was performed on an HQsil HS C_{18} column using 50 mmol Potassium dihydrogen phosphate (PH-4.4): acetonitrile in a ratio 45:55 (v/v) as the mobile phase at the flow rate of 1.0 ml/min. UV detection for curcuminoids was done at 421nm. The retention time of BDMC, DMC, C were found to be 11.5 and 12.6 and 13.7 respectively. Linearity for curcuminoids was established at concentrations ranging from 12.8 µg/ml - 38.34 µg/ml for (C), 3.15 µg/ml - 9.49 µg/ml for (DMC) and 0.61 µg/ml - 1.95 µg/ml for (BDMC). The LOD and LOQ were found to be 0.101 µg and 0.305 µg for (BDMC), 0.63 µg and 1.89 µg for (DMC) and 2.37 µg and 7.11 µg for (C). This method was further validated in terms of precision, recovery and robustness as per ICH guidelines giving satisfactory results.

KEYWORDS

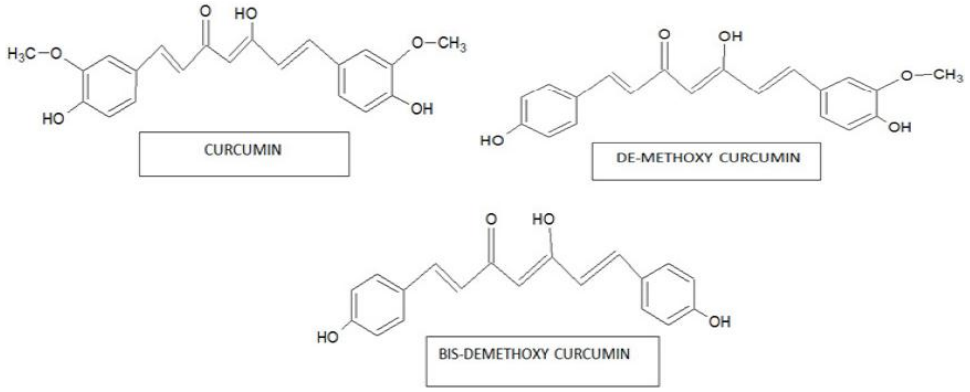
High performance liquid chromatography;
Reversed phase chromatography;
Curcuminoids;
Curcumin;
Demethoxy curcumin;
Bis demethoxy curcumin.

INTRODUCTION

The origin of the plant *Curcuma longa* L., which belongs to Zingiberaceae family is India. The plant is distributed throughout tropical and subtropical regions of the world and widely cultivated in Southeast Asian countries. Curcumin (C) along with Demethoxycurcumin (DMC) and Bis-demethoxycurcumin (BDMC) are the three major pharmacologically important curcuminoids that have been isolated from *C. longa* (turmeric) and have been shown to possess antioxidant, anti-inflammatory, anti-carcinogenic, antimutagenic, anti-fungal, anti-viral and anti-cancer activities[1]. Looking at its health benefits many herbal based manufacturing companies are manufacturing curcuminoids based dietary supplements (tabs, caps) containing all three curcuminoids along with piperine to act as a bioavailability enhancer of curcuminoids in human body.

However such companies face problems of standardizing such herbal preparations due to lack of analytical methods to analyse contents of such herbal formulations. Literature survey has revealed various analytical methods aiming to analyse curcuminoids like HPLC-UV in commercial food products[2], UPLC UV-MS[3], TLC[4], HPTLC[5], capillary electrophoresis[6],

LC-ESI-MS/MS[7]. But no HPLC-UV method was reported till date for simultaneous estimation of curcuminoids in a dietary supplement. Another utility of this developed method was that the estimation of Demethoxy curcumin (DMC), Bisdemethoxy curcumin (BDMC) and curcumin (C) was done using extracted curcuminoids in combination as a standard and not individual curcuminoid standards.



EXPERIMENTAL

Materials

Standard curcuminoids were extracted from a good quality turmeric powder using a reported method and authenticated for presence of all three curcuminoids by TLC test. HPLC grade water, methanol, acetonitrile were purchased from E-Merck India. Curcuminoid based dietary supplement formulation named Doctors best curcumin C3 complex with bioperine was procured from the U.S market for analysis.

Preparation of mobile phase

Mobile phase constituted of 50 mmol potassium dihydrogen phosphate buffer (pH-4.4): Acetonitrile in a ratio (45:55). Buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 400 ml of HPLC grade water. The mobile phase was filtered through 0.22 µm filter and sonicated before each analysis.

Instrumentation and Chromatographic conditions

Analysis was performed with HPLC system comprising of two JASCO 2080 plus pumps, a UV-2075 plus (UV-VIS detector), a Rheodyne 7725i manual injector valve with a 20 µl sample loop and a Jasco 2080-31 mixing module. Curcuminoids were separated on HQsil HS C-18 column with (250 mm× 4.6 mm i.d, 5.0 µm particle size) under reverse phase chromatographic conditions. Mobile phase used was 50 mM Potassium dihydrogen phosphate: Acetonitrile in a ratio of (45:55). The flow rate was 1.0 ml/min and wavelength chosen for analysis was 421 nm. Software used was Jasco Borwin. Room temperature was maintained at (25±1°C) using air conditioning system. The mobile phase was filtered through 0.22 µm filter paper and then degassed before use. The system was equilibrated before each injection.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 2.5 mg of curcuminoids standard in 25 ml of methanol.

Assay of dietary supplement formulation

Formulation analysed by this method claimed to have 950 mg of curcuminoids per serving (in each tablet). Label claim also stated that within this 950mg curcuminoids, 712.5 mg was Curcumin, 190 mg was Demethoxy curcumin and 42.75 mg was Bis-demethoxy curcumin. For analysis of this formulation, twenty tablets were taken and ground to coarse powder and average weight (1.205g) was calculated. Tablet powder equivalent to the average weight was transferred to a 100 ml volumetric flask, to it 50 ml of methanol was added and sonicated for 10 min. Volume was made up to 100 ml with methanol and then resonicated for 5 min. The flask was shaken for 2 min and the resultant solution was then centrifuged at 1800 rpm for 5 min. This extraction process yielded a sample solution of concentration 9.5 mg/ml. From this solution 0.105 ml (105 µl) was pipetted out and transferred to 10 ml volu-

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metric flask and volume was made up to the mark. This made the concentration of sample solution to be 100µg/ml. Finally, from this solution 332 µl was pipette out and transferred to a microcentrifuge tube of capacity (1.5ml) and volume was made up to 1ml with methanol. This gave the total concentration of curcuminoids to be 33.2µg/ml. This dilution was calculated theoretically to contain 24.83 µg/ml of (C), 6.60 µg/ml of (DMC) and 1.46 µg/ml of (BDMC). (TABLE 1) (Figure 1)

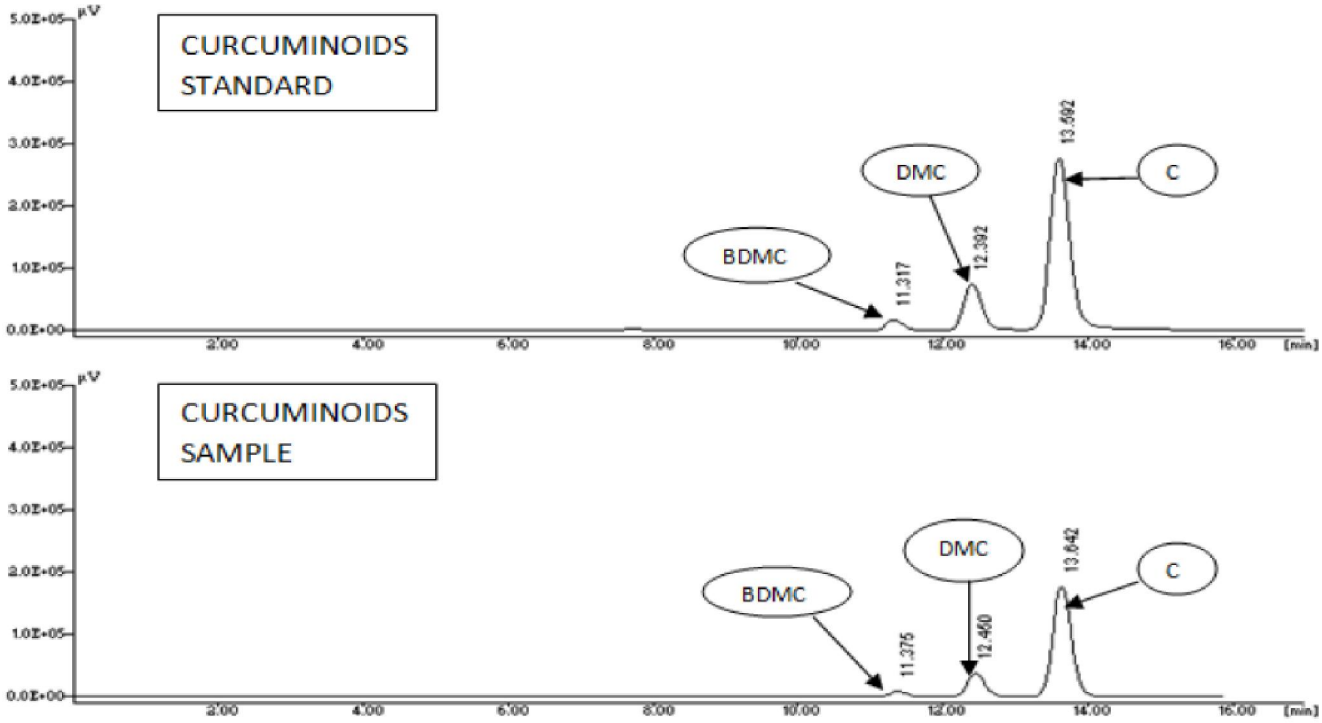

Figure 1 : Chromatogram correlating standard and sample curcuminoids.

TABLE 1 : Assay of dietary supplement formulation.

Sr. No.	Name of drug	Theoretical Concentration (µg/ml)	Practical Concentration (µg/ml)	%Drug Content ±SD
1.	Bisdemethoxy curcumin	1.46	1.289	88.83±0.012
2.	Demethoxy curcumin	6.60	5.96	90.3 ±0.09
3.	Curcumin	24.83	26.11	105.15 ±0.21

Validation parameters

Method has been validated as per ICH guidelines[8]

Linearity

For establishing linearity of all the three curcuminoids simultaneously different volumes 166 µl, 249 µl, 332 µl, 415 µl and 498 µl of standard curcuminoids solution was transferred into Micro centrifuge tubes of capacity (1.5 ml) and volume was made up to 1 ml with methanol. These dilutions were then analysed for areas it yielded for individual curcuminoids (C, DMC and BDMC) simultaneously, through HPLC system. Linearity ranges of three curcuminoids were found to be 12.82 µg- 38.34 µg for (C), 3.15 µg-9.49 µg for (DMC) and 617 ng-1.95 µg for (BDMC). (TABLE 2), (Figure 2, 3)

TABLE 2 : Linearity of curcumin (C), demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC).

Sr. No.	Area			Concentration in (µg/ml)		
	C	DMC	BDMC	C	DMC	BDMC
1.	1840326	453495	88557	12.82	3.15	0.61
2.	2882277	713645.8	138839.8	19.21	4.75	0.92
3.	3792440	947857	182162	25.57	6.39	1.22
4.	4460222	1115183	215735.5	31.96	7.99	1.54
5.	5389604	1334838	275146	38.34	9.49	1.95

Precision studies

Precision of analytical method was expressed in terms of % RSD. Repeatability studies were performed twice by injecting and measuring peak areas of six replicates of same concentrations 24.9 µg/ml of curcuminoids on same day (repeatability study-1) and on a different day (repeatability study-2). (TABLE 3).

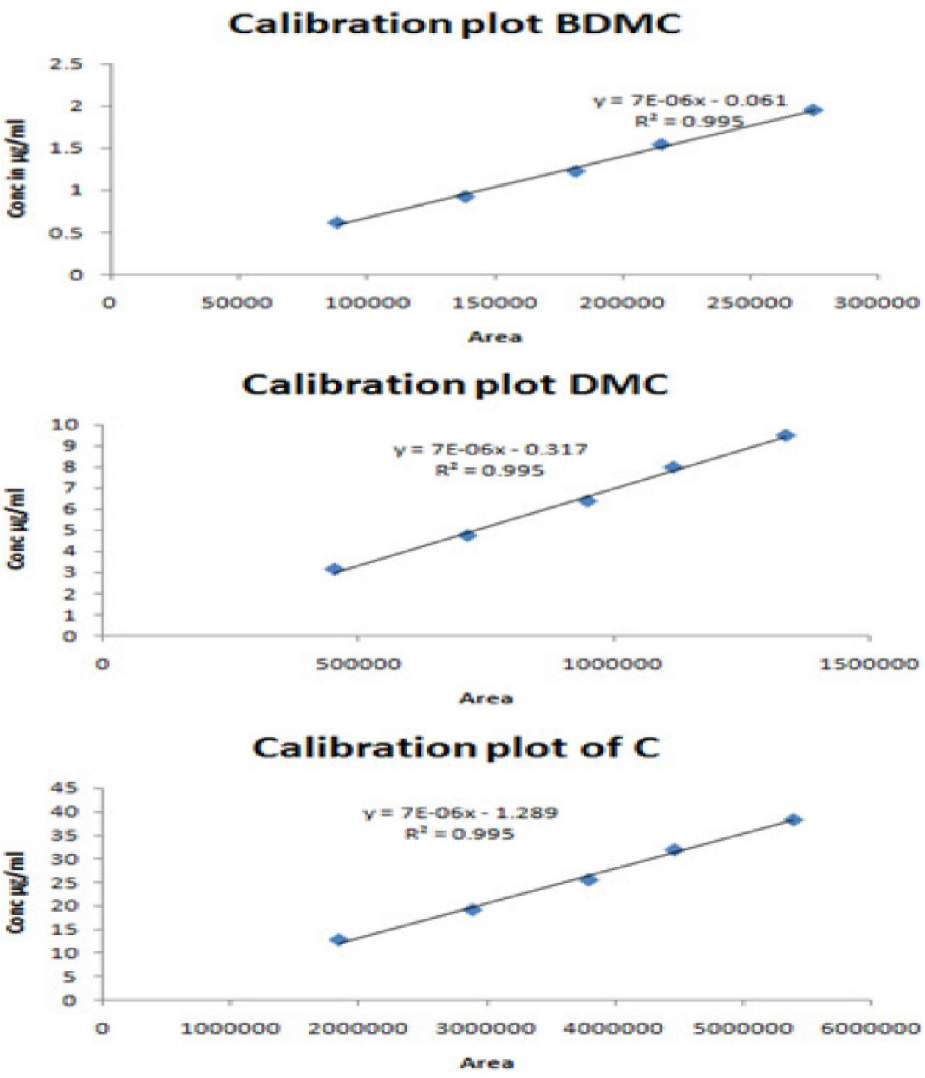

Figure 2 : View of calibration plots of Bisdemethoxy curcumin (BDMC), Demethoxy curcumin (DMC) and Curcumin (C).

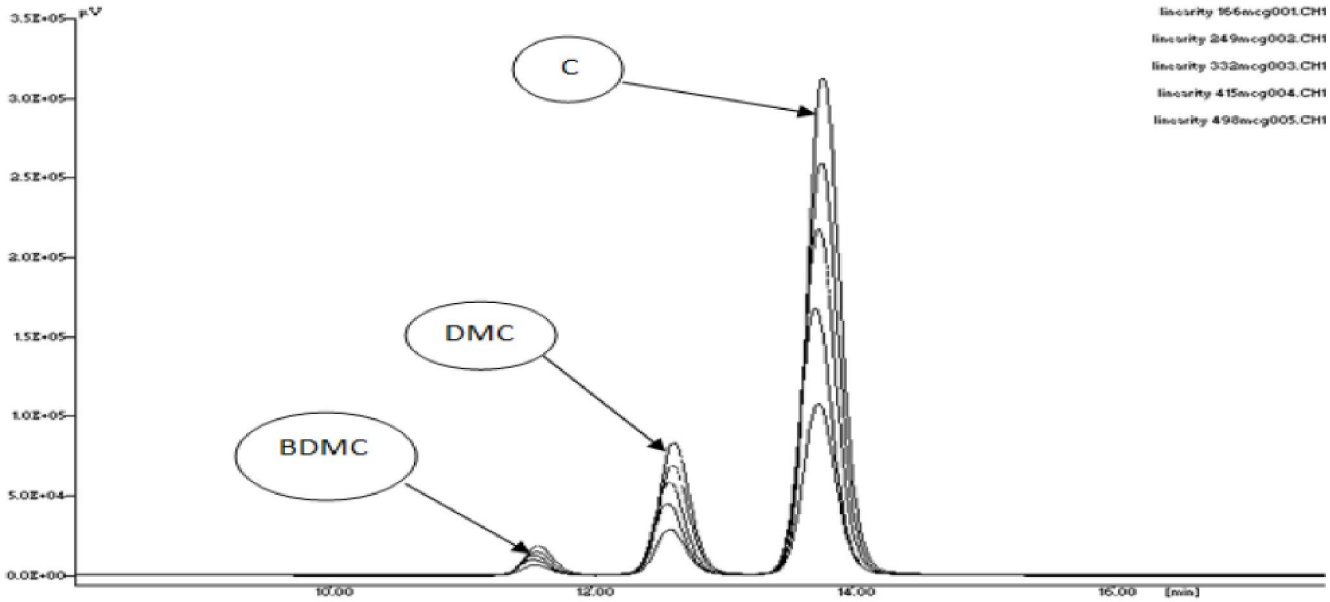

Figure 3 : Linearity overlay view of (C), (DMC) and (BDMC).

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TABLE 3 : Data of repeatability studies 1 and 2.

Sr. No.	Concentration of curcuminoids in (µg/ml)	Area			Concentration in (µg/ml)			%RSD
		C	DMC	BDMC	C	DMC	BDMC	
Repeatability study-1 (n=6)								
1.	24.9	2920125	727470	145096	22.6	5.63	1.12	C 1.82
2.	24.9	3017250	746129	147747	23.3	5.77	1.14	
3.	24.9	2941978	749952	146444	22.8	5.80	1.13	DMC 1.33
4.	24.9	2845138	749990	147789	22.1	5.80	1.14	
5.	24.9	2941517	758980	143503	22.8	5.86	1.11	BDMC 1.27
6.	24.9	2984593	743556	143587	23.1	5.75	1.11	
Repeatability study-2 (n=6)								
1.	24.9	2998385	744503.5	145358.9	25.6	6.35	1.24	C 1.75
2.	24.9	2920125	727470.3	145096.7	25.5	6.36	1.27	
3.	24.9	2941978	749952	146444.7	25.4	6.48	1.26	DMC 2.00
4.	24.9	2844485	709782	143873.5	25.5	6.37	1.29	
5.	24.9	2988593	743576.5	143567	23.1	5.75	1.11	BDMC 0.77
6.	24.9	2941517	758980.5	143503	25.4	6.55	1.23	

LOD and LOQ

Limit of detection and limit of quantitation were determined based on the standard deviation of the response and the slope as per the ICH guidelines. They were calculated based on the following formulas:

LOD = 3.3 sigma/slope

LOQ = 10 sigma/ slope

Sigma = Standard deviation of the response.

Slope = Slope of the calibration curve.

Robustness

The robustness of the analytical method was evaluated by producing variations in certain system suitability parameters like change of flow rates, change of room temperature during analysis. The method showed satisfying results in terms of peak retention time, peak asymmetry, Number of theoretical plates per peak with not much variation (TABLE 4).

Recovery studies

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method at different levels corresponding to 50%, 100% and 150% of the label claim of curcuminoids. A known amount of curcuminoids (C,DMC,BDMC) were added to preanalysed tablet solution which were then mixed, extracted and analysed at standard optimized chromatographic condition (TABLE 5).

TABLE 4 : Study of robustness.

Flow rate (ml/min)	Levels	Rt shifts in curcuminoids		
		C	DMC	BDMC
By changing the flow rate				
0.8	-1	15.7	14.4	13.3
1.0	0	13.7	12.5	11.4
1.2	+1	12.7	11.6	10.7
Mean ± S.D		14.03±1.34	12.8±1.42	11.8±1.52
By change in temperature				
Temperature	**Levels**	**Rt shifts in curcuminoids**		
		C	**DMC**	**BDMC**
24	-1	13.3	12.1	10.9
25	0	13.7	12.5	11.4
26	+1	13.5	11.9	11.1
Mean ± S.D		13.5±0.2	12.1±0.30	11.13±0.25

TABLE 5 : Recovery studies.

Sr. No.	% Levels	Amount found in (µg/ml)			Average % Recovery ± SD (n=3)			% C.V		
		C	DMC	BDMC	C	DMC	BDMC	C	DMC	BDMC
1.	50	40.7	7.85	1.65						
2.	50	39.3	7.93	1.59	100.55 ±0.96	88.84 ± 0.06	84.43 ± 0.04	2.79	1.13	1.90
3.	50	41.5	8.03	1.59						
4.	100	59.1	12.91	2.57						
5.	100	57.3	12.24	2.41	108.81 ± 0.15	106.42 ± 0.01	97.65 ± 0.01	0.26	0.12	0.60
6.	100	58.9	12.89	2.55						
7.	150	71.8	14.45	2.90						
8.	150	73.9	14.88	2.96	108.6 ± 1.07	98.74 ± 0.22	91.55 ± 0.03	1.47	1.56	1.33
9.	150	73.2	14.80	2.98						

RESULT AND DISCUSSION

A variety of mobile phases in different compositions were tried. A mobile phase containing 50 mmol potassium dihydrogen phosphate: acetonitrile in a ratio (45:55) was found to be most suitable for better resolution of curcumin (C), Demethoxy curcumin (DMC), Bisdemethoxy curcumin (BDMC). Linearity was established by least square linear regression analysis. Linear response were established at concentration ranges of 12.8 µg to 38.34 µg for (C), 3.15 µg to 9.49 µg for (DMC) and 617 ng to 1.95 µg for (BDMC). LOD and LOQ of the developed method was found to be 2.37 µg and 7.11 µg for (C), 632 ng and 1.89 µg for (DMC) and 0.101 µg and 0.305 µg for (BDMC). A %RSD <2 obtained by comparing peak area of curcumin for repeatability and intermediate precision studies suggested an excellent precision of developed method. The method was also found to be robust as it did not show wide variations in peak retention time, peak asymmetry on changing certain system suitability parameters. Recovery studies were done at three different levels of 50%, 100% and 150% of test concentration which gave satisfactory results for all three curcuminoids.

CONCLUSION

Simple, precise, accurate and a robust method has been developed and validated for the simultaneous estimation of Curcumin (C), Demethoxy curcumin (DMC) and Bis demethoxy curcumin (BDMC) in a dietary supplement formulation.

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