Quality Assurance of Turmeric Formulations: UPLC Method Development and Validation for the Simultaneous Analysis of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin

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ABSTRACT

Background: Standardization of turmeric formulations requires a rapid and reliable analytical method for simultaneous estimation of major curcuminoids. **Objective:** The present study aimed to develop and validate a rapid RP-UPLC method for the simultaneous quantification of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in marketed turmeric tablets. **Methods:** Chromatographic separation was achieved on a Hypersil GOLD™ C18 column using a gradient mobile phase of 0.1% formic acid in water and acetonitrile at a flow rate of 0.30 mL/min with UV detection at 425 nm. The method was validated according to ICH Q2(R2) guidelines. **Results:** The analytes were well resolved within 10 min with excellent linearity (10–60 µg/mL, $r^2 = 0.9999$). Accuracy (99.49–99.93% recovery), precision (%RSD < 2.0%), ruggedness, robustness, and sensitivity (LOD 0.40–0.48 µg/mL; LOQ 1.22–1.46 µg/mL) met validation criteria. Assay of the marketed formulation showed 99.10% of the labeled curcuminoid content. **Conclusion:** The validated RP-UPLC method is rapid, accurate, precise, sensitive, and suitable for routine quality control and standardization of turmeric-based pharmaceutical and nutraceutical formulations.

Keywords: RP-UPLC, Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, Turmeric formulation

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INTRODUCTION

Turmeric, derived from the rhizomes of *Curcuma longa* L. (Family: Zingiberaceae), is one of the most extensively used medicinal plants in traditional systems of medicine, including Ayurveda, Traditional Chinese Medicine, and Unani medicine.[1] It has gained significant scientific attention owing to its diverse pharmacological properties, such as anti-inflammatory, antioxidant, antimicrobial, anticancer, hepatoprotective, and neuroprotective activities.[2] The therapeutic efficacy of turmeric is primarily attributed to a group of polyphenolic compounds collectively known as curcuminoids, among which curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are the principal bioactive constituents.[3]

The increasing global demand for turmeric-based dietary supplements, herbal medicines, functional foods, and nutraceutical products has highlighted the need for stringent quality assurance measures. Variations in geographical origin, cultivation practices, harvesting conditions, extraction methods, and formulation processes can significantly influence the curcuminoid content of turmeric products.[4] Consequently, ensuring batch-to-batch consistency and maintaining product quality have become major challenges for manufacturers and regulatory agencies. Accurate quantification of curcuminoids is therefore essential for standardization, quality control, efficacy evaluation, and regulatory compliance of turmeric formulations.[5]

Curcumin is generally considered the major curcuminoid, accounting for approximately 70–80% of total curcuminoids, while DMC and BDMC constitute smaller proportions .[6] Although curcumin has been widely investigated, growing evidence suggests that DMC and BDMC also contribute substantially to the overall biological activity and stability of turmeric extracts. Therefore, simultaneous determination of all three curcuminoids is necessary to provide a comprehensive assessment of turmeric quality and therapeutic potential. [7] Analytical methods focusing solely on curcumin may not adequately reflect the

complete phytochemical profile of turmeric-based products.

Various analytical techniques, including spectrophotometry, thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and capillary electrophoresis, have been employed for the analysis of curcuminoids [8]. Among these methods, HPLC has been widely accepted due to its reliability and reproducibility. However, conventional HPLC methods often require longer run times, higher solvent consumption, and lower analytical throughput, making them less suitable for routine quality control applications involving large sample numbers.[9]

Ultra-performance liquid chromatography (UPLC) has emerged as a powerful analytical tool offering enhanced chromatographic resolution, improved sensitivity, reduced solvent consumption, and shorter analysis times compared with conventional HPLC systems [10]. The use of smaller particle-size columns and higher operating pressures in UPLC enables rapid and efficient separation of structurally similar compounds, making it particularly suitable for the simultaneous analysis of curcuminoids in complex herbal matrices. Furthermore, UPLC facilitates high-throughput analysis, which is advantageous for industrial quality control laboratories and regulatory testing environments.[11]

Method development and validation are critical steps in establishing reliable analytical procedures for pharmaceutical and herbal products. According to guidelines established by the International Council for Harmonisation (ICH Q2(R2)), analytical methods must be validated for parameters such as specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability before routine application.[12] A validated UPLC method ensures that analytical results are accurate, reproducible, and scientifically acceptable for quality assurance purposes. Such validation is especially important for herbal formulations, where matrix complexity and natural variability may affect analytical performance.

The development of a rapid, sensitive, and validated UPLC method for the simultaneous quantification of CUR, DMC, and BDMC can significantly strengthen the quality assurance framework for turmeric formulations. By enabling precise characterization of the principal bioactive markers, the method can support standardization efforts, detect adulteration or degradation, and ensure compliance with pharmacopeial and regulatory requirements. Moreover, comprehensive curcuminoid profiling contributes to product consistency and helps establish correlations between phytochemical composition and therapeutic efficacy.

Therefore, the present study aims to develop and validate a robust UPLC method for the simultaneous determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric formulations. The validated method is expected to provide a reliable analytical platform for routine quality control, standardization, and quality assurance of turmeric-based pharmaceutical and nutraceutical products.

MATERIAL AND METHOD

The curcumin, demethoxycurcumin, and bisdemethoxycurcumin were procured from Sigma-Aldrich, USA. UPLC-grade acetonitrile ,methanol, along with analytical-grade formic acid and orthophosphoric acid, were obtained from Finar Pvt. Ltd. Ultrapure. The marketed formulation selected for analysis was Carbamide Forte Curcumin Tablets was procured from Amazon India.

Preparation of Standard Stock Solution

Standard stock solutions of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were prepared by accurately weighing 10 mg of each reference standard into separate 10 mL volumetric flasks and dissolving in methanol. The volume was adjusted to obtain a concentration of 1000 µg/mL. The solutions were stored in amber-colored flasks at 2–8°C and protected from light. Working standard solutions were prepared by appropriate dilution with the mobile phase before analysis.

Optimized Chromatographic Conditions

Chromatographic conditions were optimized by evaluating different mobile phase compositions, flow rates, and column temperatures to achieve efficient separation of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The final separation was achieved using a Hypersil GOLD™ C18 column (100 mm × 2.1 mm, 1.9 µm) with a gradient mobile phase consisting of 0.1% formic acid in water (Mobile Phase A) and acetonitrile (Mobile Phase B). The flow rate was maintained at 0.30 mL/min, column temperature at 35°C, and detection wavelength at 425 nm. An injection volume of 2 µL and run time of 10 min were employed.

Assay preparation of Tablet formulation

Twenty Carbamide Forte Curcumin Tablets were weighed, powdered, and mixed uniformly. A quantity of powder equivalent to approximately 10 mg of total curcuminoids was transferred to a 50 mL volumetric flask and extracted with methanol using sonication for 30 min. The volume was adjusted with methanol, centrifuged at 4000 rpm for 10 min, and filtered through a 0.22 µm nylon syringe filter. The filtrate was suitably diluted with mobile phase to obtain concentrations within the

linearity range and injected into the UPLC system for analysis.

Validation Parameters

The developed RP-UPLC method was validated according to ICH Q2(R2) guidelines for system suitability, accuracy, precision, ruggedness, robustness, and sensitivity.

Specificity and selectivity

Specificity and selectivity were evaluated to confirm that the developed RP-UPLC method could accurately identify and quantify curcumin, demethoxycurcumin, and bisdemethoxycurcumin without interference from blank, solvent system, tablet excipients, piperine, or other co-extracted matrix components present in the selected marketed turmeric formulation. Blank solution, mixed standard solution, and sample solution were injected under the optimized chromatographic conditions, and the chromatograms were examined for interference at the retention times of the target analytes

Linerity

Linearity of the developed RP-UPLC method was evaluated for curcumin, demethoxycurcumin, and bisdemethoxycurcumin over a concentration range of 10–60 µg/mL. Calibration standards were prepared by appropriate dilution of the stock solutions with the mobile phase. Each concentration level was analyzed in triplicate under optimized chromatographic conditions. Calibration curves were constructed by plotting mean peak area against corresponding concentration, and linear regression analysis was performed using the least-squares method. The slope, intercept, and correlation coefficient (R^2) were determined to assess the linear relationship between analyte concentration and detector response.

Accuracy

Accuracy was assessed by recovery studies at 80%, 100%, and 120% levels using the standard addition method. Each level was analyzed in triplicate, and percentage recovery and %RSD were calculated. Recoveries within 98–102% with %RSD ≤ 2.0% were considered acceptable.

Precision

Precision was evaluated as repeatability, intra-day precision, and inter-day precision. Three concentration levels (20, 30, and 40 µg/mL) were analyzed in triplicate. Repeatability was assessed by six replicate injections of a 60 µg/mL mixed standard solution. Results were expressed as %RSD, with values ≤ 2.0% considered acceptable.

Ruggedness

Ruggedness was determined by analyzing standard and sample solutions independently by two analysts under the same chromatographic conditions. Assay values and peak areas were compared, and %RSD ≤ 2.0% was considered acceptable.

Robustness

Robustness was evaluated by deliberately varying chromatographic conditions, including flow rate (±0.02 mL/min), column temperature (±2°C), and organic phase composition (±2%). The effects on chromatographic performance and system suitability parameters were monitored.

Sensitivity

The sensitivity of the method was expressed as limit of detection (LOD) and limit of quantification (LOQ), calculated using the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

RESULT AND DISCUSSION

Method Development and Optimized Chromatographic Conditions

An RP-UPLC method was developed and optimized for the simultaneous determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in a marketed turmeric formulation. Optimal separation was achieved using a Hypersil GOLD™ C18 column with a gradient mobile phase consisting of 0.1% formic acid in water and acetonitrile at a flow rate of 0.30 mL/min. Detection was carried out at 425 nm with a run time of 10 min. Gradient elution, acetonitrile as the organic modifier, and acidification with 0.1% formic acid provided improved peak shape, resolution, and baseline stability, resulting in efficient separation of all three curcuminoids. The compounds eluted at approximately 4.20, 4.79, and 5.39 min, respectively, with sharp, symmetrical, and well-resolved peaks. The gradient program provided efficient separation, stable baseline response, and suitable chromatographic performance for routine analysis.

Table 1: optimized chromatographic performance

Analyte	Retention time Trial 1 (min)	Trial 2 (min)	Trial 3 (min)	Mean RT ± SD (min)	Peak shape observation
Curcumin	4.18	4.21	4.20	4.20 ± 0.02	Sharp and symmetrical
Demethoxycurcumin	4.76	4.79	4.81	4.79 ± 0.03	Sharp and symmetrical
Bisdemethox	5.38	5.4	5.3	5.3	Sharp

ycurcumin		1	9	9 ± 0.02	and symmetrical

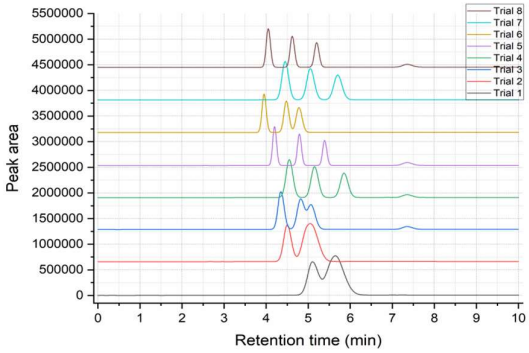

Figure 1: Overlay chromatogram of method optimization trials

The marketed Carbamide Forte Curcumin Tablets showed a total curcuminoid assay of 99.10 ± 0.39% of the labeled claim, with individual curcuminoids ranging from 98.40% to 99.20%**.** The low %RSD (<2.0%) indicates good precision and confirms that the formulation complies with acceptable assay specifications.

Table 2. Assay Results of Marketed Carbamide Forte Curcumin Tablets by RP-UPLC

Parameter	**Curcumin**	**Demethoxycurcumin**	**Bisdemethoxycurcumin**	**Total Curcuminoids**
Label claim (mg/tablet)	475.0	12.5*	12.5*	500.0
Amount found (mg/tablet)	470.8	12.3	12.4	495.5
Assay (% of label claim)	99.12	98.40	99.20	99.10
Mean assay (%)	99.12 ± 0.48	98.40 ± 0.55	99.20 ± 0.43	99.10 ± 0.39
(n = 6)				
% RSD	0.48	0.56	0.43	0.39

Method Development and Validation

Specificity and selectivity

Specificity and selectivity were evaluated by injecting blank, mixed standard, and sample solutions under optimized RP-UPLC conditions. No interference from the solvent, excipients, piperine, or other matrix components was observed at the retention times of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, confirming the method's specificity and selectivity.

Table 3: Specificity and selectivity evaluation of the developed RP-UPLC method

Injection type	**Curcumin RT (min)**	**DMC RT (min)**	**BDMC RT (min)**	**Observation**	**Inference**
Blank / diluent	Not detected	Not detected	Not detected	No peak at analyte RTs	No diluent interference
Mixed standard	4.20	4.79	5.39	Three well-resolved peaks observed	Standard peaks identified
Tablet sample 1	4.21	4.78	5.40	Peaks matched with standard RTs	Analytes present
Tablet sample 2	4.19	4.80	5.38	No interfering matrix peak observed	Method selective
Tablet sample 3	4.22	4.81	5.41	Clear baseline separation observed	Suitable for assay

Linearity

The developed RP-UPLC method exhibited excellent linearity for curcumin, demethoxycurcumin, and bisdemethoxycurcumin over the concentration range of 10–60 µg/mL, with correlation coefficients of 0.9999 for all analytes, demonstrating a strong linear relationship between concentration and detector response.

Table 4: Regression parameters for curcumin, demethoxycurcumin, and bisdemethoxycurcumin

Analyte	Linearity range (µg/mL)	Regression equation	Slope	Intercept	Correlation coefficient (r^2)
Curcumin	10–60	y = 151517.27x + 15.13	151 57.27	15.13	0.9999
Demethoxy curcumin	10–60	y = 12298.42x − 177.11	122 98.42	−17 7.11	0.9999
Bisdemeth oxycurcum in	10–60	y = 9758.93x − 41.49	975 8.93	−41. 49	0.9999

Accuracy

The developed RP-UPLC method demonstrated excellent accuracy, with mean recoveries ranging from 99.49% to 99.93% for curcumin, demethoxycurcumin, and bisdemethoxycurcumin at 80%, 100%, and 120% recovery levels. All results were within the acceptance criteria (98–102%), confirming the accuracy of the method.

Table 5:Accuracy (Recovery) Study of Curcuminoids by RP-UPLC

Analyte	Recovery Level (%)	Mean Recovery (%) ± SD	Overall Mean Recovery (%)	Acceptance Criteria	Inference
Curcumin	80	99.85 ± 0.25	99.85	98–102%	Pass
Curcumin	100	99.93 ± 0.23	99.85	98–102%	Pass
Curcumin	120	99.77 ± 0.27	99.85	98–102%	Pass
Demethoxy curcumin	80	99.66 ± 0.24	99.78	98–102%	Pass
Demethoxy curcumin	100	99.78 ± 0.24	99.78	98–102%	Pass
Demethoxy curcumin	120	99.89 ± 0.25	99.78	98–102%	Pass
Bisdemetho xycurcumin	80	99.49 ± 0.26	99.57	98–102%	Pass
Bisdemetho xycurcumin	100	99.64 ± 0.24	99.57	98–102%	Pass
Bisdemetho xycurcumin	120	99.59 ± 0.28	99.57	98–102%	Pass

Precision

The developed RP-UPLC method demonstrated excellent precision for curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The intra-day, inter-day, and repeatability studies showed %RSD values ranging from 0.07% to 0.30%**,** which were well within the acceptable limit of ≤2.0%**.** These findings confirm that the method is precise, reproducible, and suitable for routine quantitative analysis of curcuminoids in marketed turmeric formulations.

Table 6 Inter-day precision study

Analyte	Concentration (µg/mL)	Day 1 peak area	Day 2 peak area	Day 3 peak area	Mean peak area ± SD	%RSD
Curcumin	20	30 30 26	30 24 82	30 37 68	303 092.00 ± 645.69	0.21
Curcumin	30	45 50 82	45 42 36	45 59 18	455 078.67 ± 841.01	0.18
Curcumin	40	60 65 84	60 57 26	60 74 38	606 582.67 ±	0.14

					856.01	
Demethoxycurcumin	20	2456 18	2450 26	2463 14	245652.67 ± 644.61	0.26
Demethoxycurcumin	30	3687 84	3679 26	3696 02	368770.67 ± 838.14	0.23
Demethoxycurcumin	40	4917 92	4908 46	4926 74	491770.67 ± 914.13	0.19
Bisdemethoxycurcumin	20	1953 16	1947 64	1959 26	195335.33 ± 581.24	0.30
Bisdemethoxycurcumin	30	2930 48	2922 46	2938 12	293035.33 ± 783.09	0.27
Bisdemethoxycurcumin	40	3904 12	3895 86	3912 84	390427.33 ± 849.09	0.22

Table 7: Repeatability study by six replicate injections

Injection	Curcumin RT (min)	Curcumin peak area	DMC RT (min)	DMC peak area	BDMC RT (min)	BDMC peak area
1	4.19	909128	4.78	737286	5.39	585104
2	4.21	910642	4.80	738528	5.41	586216
3	4.20	909784	4.79	737842	5.40	585628
4	4.22	911036	4.81	738964	5.42	586784
5	4.18	908692	4.77	736954	5.38	584918
6	4.20	910216	4.79	738136	5.40	585906
Mean ± SD	4.20 ± 0.01	909916.33 ± 887.62	4.79 ± 0.01	737951.67 ± 731.53	5.40 ± 0.01	585759.33 ± 685.67
%RSD	0.33	0.10	0.31	0.10	0.27	0.12

Ruggedness

The developed RP-UPLC method exhibited excellent ruggedness, with mean assay values of 99.95–99.96% for Analyst I and 99.86–99.89% for Analyst II. The %RSD values were below 2.0% for all analytes, indicating negligible analyst-to-analyst variation and confirming that the method is rugged, reliable, and suitable for routine analysis.

Table 8: Summary of ruggedness study

Analyte	Mean assay by Analyst I (%)	Mean assay by Analyst II (%)	Acceptance criteria	Inference
Curcumin	99.95	99.88	%RSD ≤ 2.0%	Pass
Demethoxycurcumin	99.96	99.89	%RSD ≤ 2.0%	Pass
Bisdemethoxycurcumin	99.96	99.86	%RSD ≤ 2.0%	Pass

Robustness

The developed RP-UPLC method was found to be robust against small deliberate variations in flow rate (±0.02 mL/min), column temperature (±2°C), and mobile phase composition (±2% acetonitrile). Only minor changes in retention time and peak area were observed, while resolution (>2.0), tailing factor, and theoretical plate count remained within acceptable limits. These results confirm that the method is robust and reliable for routine quantitative analysis of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

Table 9: robustness study

Variation studied	Observed effect	System suitability status	Inference
Flow rate 0.28 mL/min	Slight increase in retention time	Passed	Method unaffected
Flow rate 0.32 mL/min	Slight decrease in retention time	Passed	Method unaffected
Column	Minor	Passed	Method

temperature 33°C	increase in retention time		unaffected
Column temperature 37°C	Minor decrease in retention time	Passed	Method unaffected
Acetonitrile +2%	Slightly faster elution	Passed	Method unaffected
Acetonitrile −2%	Slightly delayed elution	Passed	Method unaffected

Sensitivity Study: Limit of Detection and Limit of Quantification

The developed RP-UPLC method demonstrated high sensitivity, with LOD values of 0.40–0.48 µg/mL and LOQ values of 1.22–1.46 µg/mL for curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These values were well below the linearity range (10–60 µg/mL), confirming that the method is sufficiently sensitive for reliable routine quantification of curcuminoids in marketed turmeric formulations.

Table 10: Sensitivity data for curcumin, demethoxycurcumin, and bisdemethoxycurcumin

Analyte	Slope (S)	SD of response (σ)	LOD (µg/mL)	LOQ (µg/mL)
Curcumin	15157.27	1842.16	0.40	1.22
Demethoxycurcumin	12298.42	1636.74	0.44	1.33
Bisdemethoxycurcumin	9758.93	1428.62	0.48	1.46

CONCLUSION

The present study successfully developed and validated a rapid, simple, accurate, precise, and sensitive RP-UPLC method for the simultaneous determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in marketed turmeric tablet formulations. The optimized chromatographic conditions provided excellent separation of the three curcuminoids within a short run time of 10 minutes, with sharp, symmetrical peaks and satisfactory system suitability parameters. The use of a Hypersil GOLD™ C18 column with a gradient mobile phase of 0.1% formic acid in water and acetonitrile resulted in efficient resolution and reproducible chromatographic performance.

Method validation performed in accordance with ICH Q2(R2) guidelines demonstrated that the developed method met all predefined acceptance criteria. The method exhibited excellent specificity without interference from formulation excipients or matrix components and showed outstanding linearity over the concentration range of 10–60 µg/mL (r^2 = 0.9999). Accuracy studies produced recoveries between 99.49% and 99.93%, while precision, ruggedness, and robustness studies yielded %RSD values well below 2.0%, confirming the reliability and reproducibility of the analytical procedure. The low LOD and LOQ values further demonstrated the high sensitivity of the method for quantitative estimation of curcuminoids.

Application of the validated method to Carbamide Forte Curcumin Tablets showed an assay value of approximately 99.10% of the labeled curcuminoid content, indicating compliance with quality specifications and confirming the suitability of the formulation for therapeutic use. Overall, the developed RP-UPLC method provides a reliable, cost-effective, and high-throughput analytical approach for routine quality control, standardization, and batch-to-batch consistency evaluation of turmeric-based pharmaceutical and nutraceutical formulations. The method can also serve as a valuable analytical tool for regulatory compliance and future quality assurance studies involving curcuminoid-containing herbal products.

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