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Research Article

Chemical fingerprint analysis for quality assessment and control of *Curcuma longa* L. rhizomes from Vietnam using a high-performance liquid chromatography-diode array detector (HPLC-DAD)

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Abstract

Turmeric, extensively cultivated across Southeast Asia, especially in Vietnam, harbors active polyphenols, primarily curcumin (2–5%), renowned for its diverse health benefits. Pharmacopoeias recognize turmeric, yet it lacks standardized quality assessments and encounters challenges in extraction and identification due to natural variations and adulteration. This analytical method is vital for verifying the authenticity, purity, and quality of turmeric products in both the pharmaceutical and nutraceutical industries. This study successfully developed an efficient extraction process for curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) from *Curcuma longa* L. rhizomes. The herbal powder was extracted with methanol (1:30, w/v) by the ultrasound-assisted method for 10 minutes, and this process was repeated three times. A high-performance liquid chromatography-diode array detector (HPLC-DAD) method was validated for the simultaneous quantification of three analytes, following the AOAC guideline and achieving a correlation coefficient (R^2) value greater than 0.9950. Utilizing the HPLC-DAD method, the study developed a chemical fingerprint analysis for three analytes to identify the characteristic chemical components distinguishing turmeric from each region. Nineteen samples collected from various provinces across Vietnam were subjected to analysis. In all analyzed samples, the concentrations of CUR, DMC, and BDMC ranged from 0.77–10.30%, 0.33–6.92%, and 0.03–3.23%, respectively. CUR was determined to be the dominant compound in most samples, while BDMC consistently exhibited the lowest levels of content. Utilizing the findings derived from the analysis of RRT and RPA metrics, the research assessed variances across sample batches. It is suggested that this newly established approach can be applied to construct and develop raw material areas to serve the needs of each field.

Keywords

chemical fingerprint, curcuminoid, raw material, turmeric, ultrasound-assisted method

Introduction

Curcuma longa L. (Zingiberaceae), commonly known as turmeric, is a perennial herb native to India and widely cultivated throughout Southeast Asia, mostly in China and Indonesia. Since ancient times, Vietnamese people have known to use the powder of the rhizomes as a coloring and spice in many cuisines to increase appetite and also for food supplements as well as herbal healthy drinks. Current Vietnamese traditional medicines claim the use of its powder might be effective in treating jaundice, menstrual disorders, constipation, obesity, liver disorders, and stomach disease (Loi 2013; Chi 2021).

Turmeric contains highly active polyphenols called curcuminoids (Navekar et al. 2017). The major curcuminoids present are curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), and the most bioactive ingredient is curcumin (approximately 2–5%) (Navekar et al. 2017; Jin 2018). Many studies showed its various beneficial pharmacological effects, such as anti-inflammatory and antioxidant activities (Nishiyama et al. 2005; Tranchida et al. 2017), anti-cancer (Aggarwal et al. 2007; Wilken et al. 2011; Huynh et al. 2023), anti-bacterial (Parvathy et al. 2009; Bhawana et al. 2011), hepatoprotective activity (Noorafshan et al. 2013), and estrogenic activity (Bachmeier et al. 2010; Mohajeri et al. 2020). It is also official in the Pharmacopoeia of Vietnam and China as well as in other Asian countries such as Japan and Korea; its usage covers a wide range of health indications.

On one hand, as the value of the biological activities of curcuminoids from *Curcuma longa* L. (CL) has been inexorably authenticated in recent years, the extraction and use of these substances are being studied in many countries. However, the majority of studies were no longer feasible to apply effectively to various raw material areas. In addition, after an appropriate process has been found, it is also essential to evaluate its suitability for purposes and needs.

On the other hand, chemical fingerprints (CF) are chemical information about medicinal herbs expressed in the form of chromatograms, spectra, and graphs made by analytical techniques (Kamboj 2012). Many organizations, such as WHO, FDA, MFDS, and EMA, have mentioned and accepted chemical fingerprint chromatography as an official and reliable method for quality control of medicinal herbs (Liang et al. 2004).

In this research, an HPLC method, integrated with a DAD, was formulated to analyze curcuminoids simultaneously in the rhizomes of CL. The developed method is also applied to establish CF because it has advantages such as convenience, high selectivity, sensitivity, resolution, and a short analysis time. In addition, this study will also provide an overview of the process of establishing chemical fingerprints to identify and evaluate the quality of turmeric species in some provinces of southern Vietnam.

Materials and methods

Chemicals and reagents

Curcumin, demethoxycurcumin, and bisdemethoxycurcumin were purchased from the Institute of Drug Quality Control in Ho Chi Minh City (Vietnam) with curcuminoid content ≥ 98%, and the structure is given in Fig. 1.

Ethanol (96%) was obtained by Kha Doanh Company, Vietnam. Acetic acid, formic acid, dichloromethane, and n-hexane were acquired from Sigma-Aldrich, Steinheim, Germany. Methanol, acetonitrile, and water used for HPLC were of chromatographic grade and purchased from Merck (Darmstadt, Germany). Membrane filters (0.45 μm pore size; PTFE; P/N E252) were obtained from Alain Laboratory Instruments (Zhejiang, China).

Plant materials

The fresh rhizomes of CL were collected from Kien Giang province, Vietnam, in March 2023. The specimen was identified by polymerase chain reaction (PCR) and gene sequencing techniques at the molecular biology laboratory, Biotechnology Research and Development Institute (Can Tho University, Can Tho, Vietnam). These were utilized in the process of developing and validating curcuminoids in turmeric.

All plant materials in this study were conducted as follows: The fresh rhizome was washed with water, sliced, and then dried using a conventional oven at 70 °C for 48 hours, until it reached constant mass. The dried slices were ground into a fine powder using an electric blender. All samples were stored in sealed plastic bags and reached the appropriate humidity according to Vietnamese Pharmacopoeia V (Ministry of Health 2018).

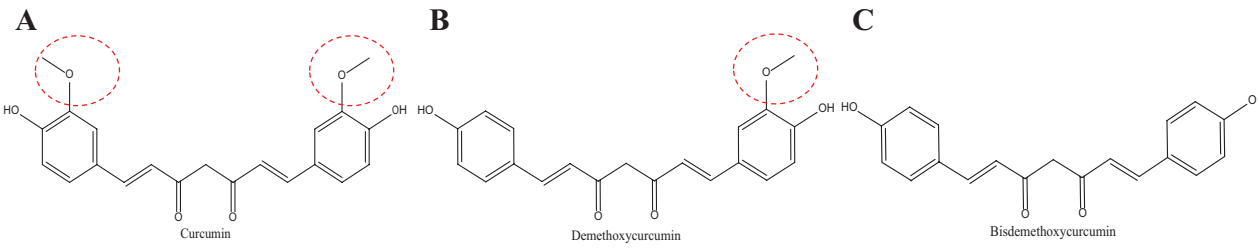

Figure 1. The chemical structure of curcumin (**A**); demethoxycurcumin (**B**); and bisdemethoxycurcumin (**C**).

Preparation of stock and working standard solutions

The stock standard solutions of CUR, DMC, and BDMC were precisely weighed and separately dissolved in methanol at a concentration of 1000 µg/mL for each analyte. An appropriate amount of each stock solution was mixed and diluted with methanol to achieve eight various working standard solutions in the range of 7.5–90 µg/mL for CUR and 2.5–30 µg/mL for DMC and BDMC for constructing the relevant calibration curves.

All solutions were kept at -20 °C and must be left at room temperature before being filtered through a 0.45 µm millipore filter into a vial for analysis on the HPLC system.

Preparation of a sample solution

About 150 mg of powdered rhizomes of CL were extracted with 4.5 mL of methanol, ultrasonicated for 10 min, and centrifuged at 10,000 rpm for 7 min. The residues were re-extracted an additional two times with methanol following the same procedure. The combined filtrates of each extraction were transferred to a 25-mL volumetric flask and adjusted to the mark with methanol. Then we accurately pipetted 1 mL from the 25-mL volumetric flask and diluted it to a final volume of 10 mL with the same solvent. This solution was filtered through a 0.45 µm membrane filter into a vial before injection. The extraction procedure flowchart is illustrated in Fig. 2.

Chromatographic conditions

Chromatographic analysis was performed by an HPLC system (Hitachi L-2000, Tokyo, Japan) equipped with an L-2455 diode array detector (DAD), an autosampler, a quaternary pump, a degasser, a column thermostat, and connected to OpenLAB Control Panel software.

The separation was performed using a Thermo Scientific – C18 column (5 µm, 4.6 × 250 mm, I.D. Thermo Corporation) with an isocratic mobile phase consisting of acetonitrile – 1% acetic acid (50:50, v/v) at a flow rate of 1 mL/min. The column temperature was maintained at 25 °C. The injection volume was 20 µL. The resolution of each curcuminoid was greater than 1.5, and the total analysis time was 15 min. The detection wavelength of 425 nm was selected as the maximum wavelength of curcuminoids for simultaneous quantitative analysis.

Method validation

According to the Association of Official Analytical Chemists guidelines (AOAC 2023), the validation process for the method assessing the simultaneous quantitative analysis of curcuminoids encompassed evaluating system suitability, selectivity, and the linearity of calibration curves, as well as determining the limits of detection (LOD) and quantification (LOQ), alongside precision and accuracy assessments.

System suitability

A system suitability test is the process of evaluating and ensuring the suitability and consistency of the chromatographic system, including the analytical method, instruments, and conditions, for the intended analysis. The testing was conducted by injecting six times the sample containing CUR, DMC, and BDMC at 20 µg/mL. The relative standard deviation (RSD) values of the retention time, peak area, capacity factor, resolution, asymmetry, and theoretical plate number were calculated to evaluate the system's suitability.

Specificity

Specificity is described as the ability of a method to discriminate the analyte from all potentially interfering substances. Specificity was evaluated by obtaining the spectra of mobile phase solvent, sample solvent, standard, sample, and spiking (standard addition sample).

Linearity of calibration curves, LOD, and LOQ

The calibration curve was drawn with eight standard solutions at concentrations ranging from 2.5–90 µg/mL. The methods were evaluated by determining the coefficient

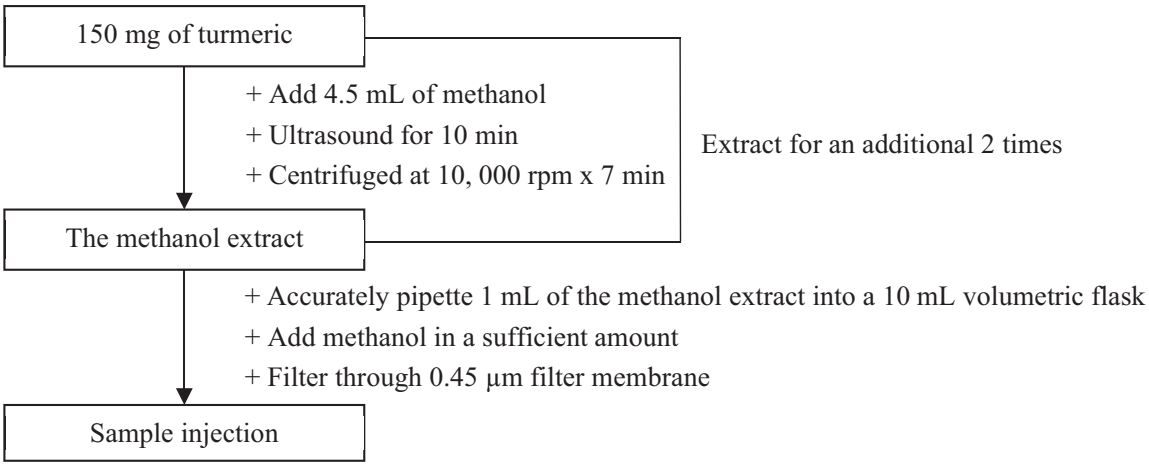

Figure 2. Extraction procedure.

of determination (R^2). The sensitivity of the present study was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ). LOD and LOQ of each standard were determined based on the signal-to-noise (S/N) ratio by injecting the diluted solutions until S/N > 3 for LOD and S/N > 10 for LOQ, respectively.

Accuracy and precision

A sample solution (80%, 100%, and 120% of the target concentration) was used to validate intra-day and inter-day precisions and accuracies. For the precision test, intermediate precision was determined by six identical sample solutions on three consecutive days. Percentage recovery was calculated for accuracy, while % RSD was calculated for precision.

Applications

The plant materials for the fingerprint analysis were obtained from various locations in Vietnam. The sampling part, collection source, and time of the 19 test samples are summarized in Table 1.

Table 1. Sample information for *Curcuma longa* L. used in the present study.

No.	Sample code	Source	Collection time
1	CLR-01	Ca Mau	Oct, 2023
2	CLR-02	Kien Giang	Oct, 2023
3	CLR-03	Bac Lieu	Nov, 2023
4	CLR-04	Ben Tre	Sep, 2023
5	CLR-05	An Giang	Nov, 2023
6	CLR-06	Can Tho	Sep, 2023
7	CLR-07	Hau Giang	Nov, 2023
8	CLR-08	Tra Vinh	Oct, 2023
9	CLR-09	Vinh Long	Oct, 2023
10	CLR-10	Dong Thap	Nov, 2023
11	CLR-11	Soc Trang	Nov, 2023
12	CLR-12	Tien Giang	Sep, 2023
13	CLR-13	Long An	Oct, 2023
14	CLR-14	Ho Chi Minh	Nov, 2023
15	CLR-15	Vung Tau	Sep, 2023
16	CLR-16	Binh Duong	Nov, 2023
17	CLR-17	Dong Nai	Oct, 2023
18	CLR-18	Tay Ninh	Oct, 2023
19	CLR-19	Binh Phuoc	Oct, 2023

CLR: *Curcuma longa* Rhizoma.

Results and discussion

Optimization of HPLC conditions

To achieve the requirements for quantitative analysis and chromatographic fingerprint analysis and have a good baseline separation of the desired analytes in the chromatogram. The stationary phase and mobile phase compositions were examined, and the wavelength for detection was optimized. For each analyte, the parameters of retention time, peak area, resolution, asymmetry coefficient, number of theoretical plates, and purity were used as the parameters for choosing the optimized chromatographic conditions.

A few different columns (Phenomenex Luna C8, Phenomenex Synergi Hydro-RP) were tested before Thermo Scientific C18 (Thermo Fisher Scientific, USA) was finally selected as the column of choice. Due to the medium polarity of CUR, DMC, and BDMC, whose log P values are 3.29, 3.15, and 3.16, respectively, employing a C18 column improves the separation of curcumin and its derivatives. This separation is facilitated by hydrophobic interactions (Jiang et al. 2021; Sayeli and Shenoy 2021). It was found to be more suitable, which gave good peak separation with stable baselines. To obtain sharp and symmetrical peaks, the mobile phases (acetonitrile, methanol, or water) with different modifiers (formic acid and acetic acid) were investigated under different isocratic elution modes. As a result, acetonitrile -1% acetic acid (50:50, v/v) gave a much better separation for CUR, DMC, and BDMC. The system's backpressure was lower than with pure methanol or water. The presence of acid in a mobile-phase system can eliminate the peak tailing of target compounds.

The effect of flow rate (0.5–1.5 mL/min) and injection volume (1–20 µL) on peak height was examined. Compromising between sensitivity and sampling frequency, the flow rate of 1 mL/min was selected as the optimum flow rate. The injection volume of 20 µL was chosen as a compromise between sensitivity and analysis time. The effect of column temperatures (20–50 °C) on the separation process was also tested. Most of the peaks in the HPLC chromatograms were well resolved at 25 °C (room temperature). The detection wavelength of 425 nm was selected as the maximum wavelength of curcuminoids for quantitative analysis and fingerprint analysis because it gave higher sensitivity compared to other wavelengths.

For this analysis, the parameters, such as flow rate and injection volume, were not much different from previous studies. While the analysis time for CUR exceeded that reported by Eneş et al. in 2024 (6.13 min), our study stands out for simultaneously analyzing three curcuminoids with high resolution, and the peak tailing has been eliminated. With a decreasing flow rate, it allowed for increased interaction time between sample compounds and the stationary phase, resulting in gradual elution and the avoidance of peak tailing. The chromatogram of the curcuminoid sample and standard solution under optimal HPLC conditions is shown in Fig. 3.

Previous studies reported that the column temperature was usually maintained around 35–55 °C (Osoria-Tobon et al. 2016); however, we set it at 25 °C without affecting the active ingredient or leading to poor peak resolution. It also observed a clear trend of narrower peaks and increased peak height than the results in previous studies (Wulandari et al. 2018; Le et al. 2019).

Optimization of the extraction process

All parameters, such as solvent, ratio of sample to solvent, method, and number of extractions, were determined to be the main variables that influence extraction efficiency. To find

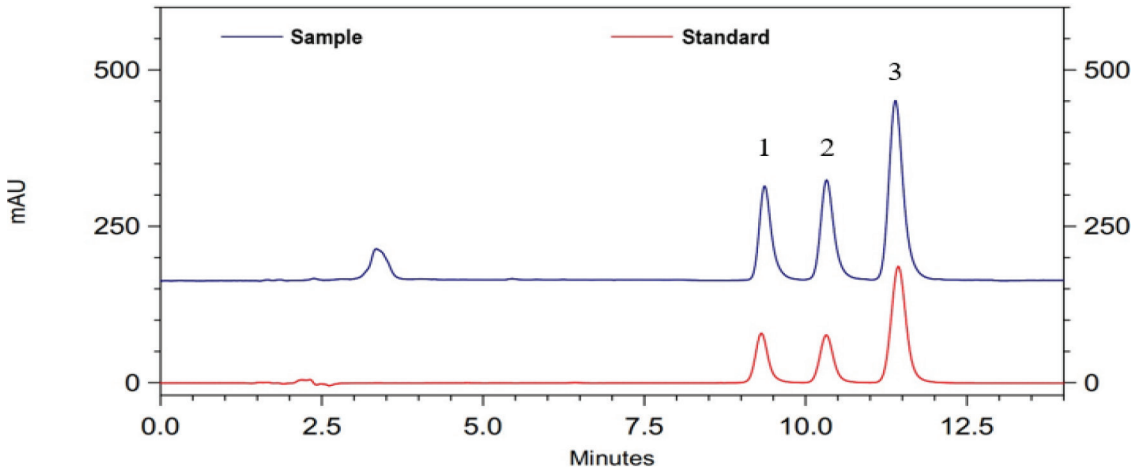

Figure 3. The chromatogram of the curcuminoids sample and standard solution in the optimal HPLC condition. (**1**) bisdemethoxycurcumin; (**2**) demethoxycurcumin; (**3**) curcumin.

the most compatible solvent for turmeric extraction, acetone, ethanol, and methanol were tested in the present study.

Among the three solvents, the figure for the peak area and content of curcuminoids in methanol was the highest. Therefore, methanol was selected for extracting curcuminoids from turmeric. Evaluating optimization of the solid-to-solvent ratio by extraction efficiency compared to the amount of solvent used. The results of the samples showed variations ranging from 1/10 to 1/50 (w/v). It was observed that when the ratio of sample weight to solvent volume was increased from 1/10 to 1/30, there was an obvious increase in the peak area of curcuminoids. From 1/30 to 1/50, the increase was slow compared to other ratios. In this study, we compared the efficiency of two extraction methods: ultrasound-assisted extraction and heat reflux extraction. The results showed that ultrasound-assisted extraction was more effective.

Using methanol as a solvent, a solid-to-solvent ratio of 1/30 (w/v), and ultrasound-assisted extraction for 20 min, nearly all of the curcuminoids were extracted after the third extraction cycle. In addition, the peak area ratio of the fourth extraction cycle to the sum of the third extraction cycle was less than 2%. As the optimal number of extractions, we chose the third extraction cycle for the rest of our extraction process, similar to the previous research data (Sabir et al. 2021). Fig. 4 summarizes the findings from our sample preparation investigation.

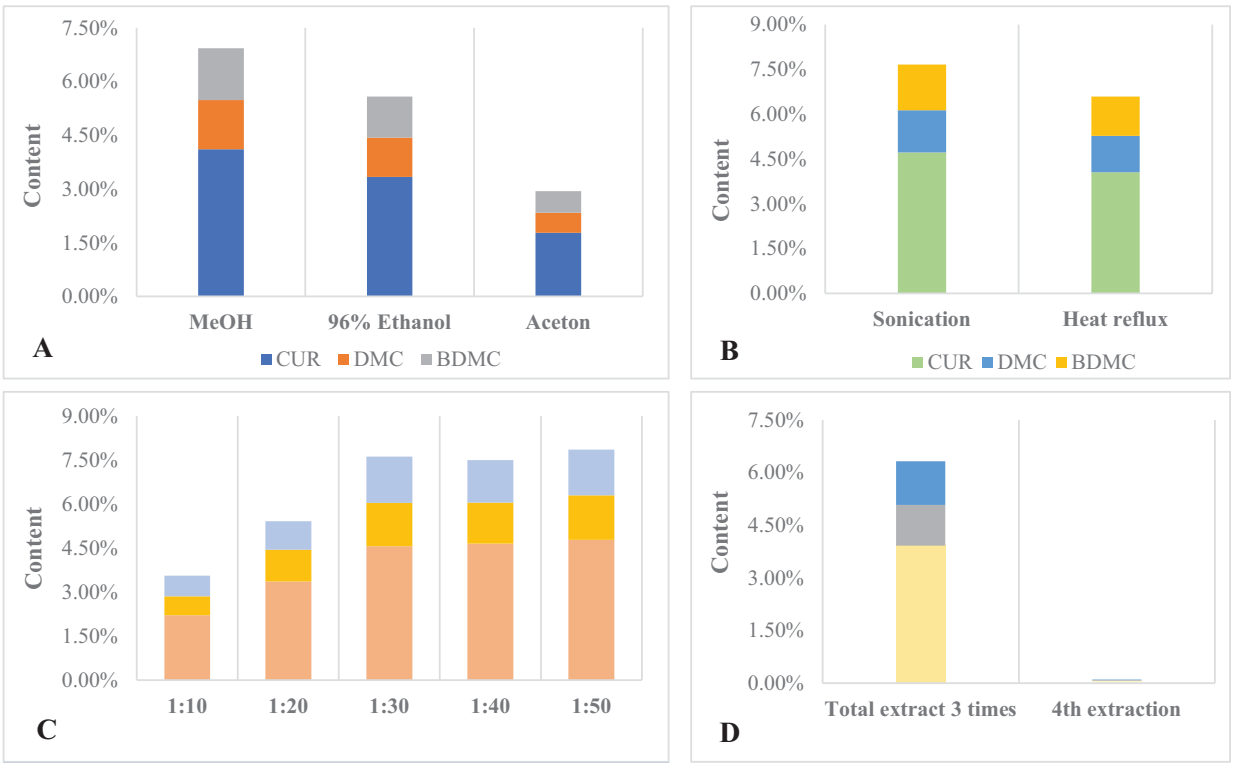

Figure 4. Curcuminoid content was obtained during the optimization of extraction conditions. **A.** Extraction solvent; **B.** Method of extraction; **C.** Ratio of sample solvent; **D.** Number of extractions.

The curcuminoid extraction method employed in this study presents a notable improvement in efficiency, with a remarkably shorter duration of approximately 1 hour, in comparison to the experiments conducted by Slaček et al. (2023) (1.5 hours) and Widmann et al. (2022) (48 hours). Through meticulous optimization of key extraction parameters and the strategic use of methanol as the extraction solvent, supplemented by ultrasound assistance, this method has effectively streamlined the extraction process. Despite the reduced time and simplified procedure, the method ensures accurate analysis and quantification of CUR, DMC, and BDMC levels concurrently. In contrast to the findings of Slaček et al. (2023), where quantification was limited to CUR with low resolution, this approach offers both efficiency and precision, positioning it as a valuable tool for practical research and applications in quantification and testing.

Method validation

System suitability

The results for the system suitability parameters are shown in Table 2. RSD values for all parameters quantified in the system suitability tests were found to be less than 2%, which indicates that this method is very well suited for achieving the target separation of the three curcuminoids.

Table 2. The result of the system suitability test performed on the analytical method employed for the simultaneous quantification of curcuminoids.

Analyte	BDMC	DMC	CUR
Retention time (min)			
Mean	9.353	10.343	11.448
RSD%	0.58%	0.47%	0.39%
Peak area			
Mean	3650550	4125305	13815704
RSD%	1.96%	1.33%	1.46%
Capacity factor			
Mean	8.353	9.343	10.448
RSD%	0.65%	0.52%	0.42%
Resolution			
Mean	-	2.805	2.823
RSD%	-	1.08%	0.76%
Asymmetry			
Mean	1.167	1.108	1.117
RSD%	0.87%	1.41%	0.73%
Theoretical plate number			
Mean	12356.68	12469.00	12344.17
RSD%	1.24%	1.37%	1.80%

Specificity

As a result, the retention time of the curcuminoid peaks in the sample was equivalent to the standard. The chromatogram of the spiked sample showed a clear increase in the height and peak area of the curcuminoid peaks. In addition, the sample solvent and mobile phase solvent do not appear to have peaks with retention times equivalent to the retention times of curcuminoid peaks in the standard sample. Therefore, the specificity of this method was accepted. The spectra of the CUR standard and CUR in the sample CL is presented in Fig. 5.

Linearity of calibration curves, LOD, and LOQ

The analytical data for linearity, LOD, and LOQ are shown in Table 3. The linear ranges of CUR (7.5–90 μg/mL), DMC (2.5–30 μg/mL), and BDMC (2.5–30 μg/mL) are suitable for the analysis of curcuminoids. Satisfactory linearity was found in the correlation coefficient value greater than 0.9950 for all analytes within the test ranges. The results for LOD and LOQ were found to be between 0.25 and 0.75 μg/mL, respectively. The low values of LOD and LOQ indicated that the proposed method provides good sensitivity.

Accuracy and precision

The percentage recovery of curcumin for the accuracy test obtained was 99.36–106.09% for CUR, 86.54–98.40% for DMC, and 94.53–105.43% for BDMC. RSD values for the inter-day precisions range from 2.10% to 4.98% (RSD < 5.30%). These values are in agreement with accuracy and precision in AOAC. It suggested that the proposed method is well-validated and suitable for quantitatively detecting curcuminoids. The intra- and inter-day precision and accuracy for the analytes from three concentrations are summarized in Table 4.

Determination of curcuminoids

The HPLC-DAD-based method has been developed and validated for the simultaneous quantification of curcuminoids. About 19 samples of CL collected from various locations were analyzed, and each sample was analyzed in triplicate to determine the mean amount of each curcuminoid. The content (%, w/w) in these sample solutions was calculated according to the procedure of sample preparation described above, expressed as milligrams of curcumin per 150 mg of dry material. Additionally, the total curcuminoid content was calculated based on the sum of the three compounds. The content of CUR, DMC, and BDMC in all

Table 3. Linearity, LOD, and LOQ for the HPLC/PDA method validation.

		Linearity, LOD, and LOQ		
		CUR	**DMC**	**BDMC**
Concentration (μg/mL)		7.5–90	2.5–30	2.5–30
R^2		0.9969	0.9966	0.9960
Calibration curve		Y = 616404X + 625053	Y = 680707X + 196762	Y = 674564X + 100881
LOD (n = 6)	Conc (μg/mL)	0.25	0.25	0.25
	S/N	4.026	3.553	3.538
LOQ (n = 6)	Conc (μg/mL)	0.75	0.75	0.75
	S/N	13.018	13.983	15.112

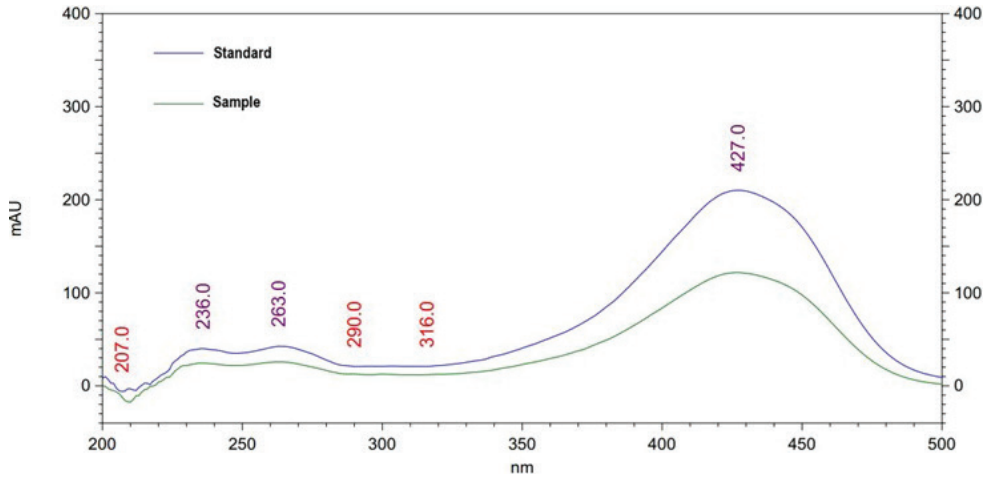

Figure 5. Comparison of the spectra of the CUR standard and CUR in the sample CL.

Table 4. The data on accuracy and precision for the HPLC/PDA method validation.

Analyte	Level	Concentration (µg/mL)	Intra-day (n = 3)			Inter-day (n = 9)		
			Measured Conc (Mean ± SD, µg/mL)	RSD (%)	Accuracy (%)	Measured Conc (Mean ± SD, µg/mL)	RSD (%)	Accuracy (%)
BDMC	80%	6.4	6.75 ± 0.20	3.02	105.43	6.46 ± 0.30	4.58	100.94
	100%	8.0	7.85 ± 0.31	4.01	98.16	7.56 ± 0.38	4.98	94.53
	120%	9.6	9.38 ± 0.31	3.29	97.73	9.22 ± 0.42	4.54	96.05
DMC	80%	6.4	5.54 ± 0.24	4.37	86.54	5.61 ± 0.25	4.54	87.68
	100%	8.0	7.65 ± 0.15	2.01	95.58	7.87 ± 0.26	3.34	98.40
	120%	9.6	8.37 ± 0.41	4.92	87.23	8.38 ± 0.38	4.52	87.32
CUR	80%	24	25.46 ± 0.05	0.20	106.09	25.06 ± 0.71	2.83	104.44
	100%	30	31.15 ± 0.52	1.68	103.84	29.81 ± 1.41	4.73	99.36
	120%	36	37.79 ± 0.76	2.02	104.98	37.59 ± 0.79	2.10	104.43

samples ranged from 0.77–10.30%, 0.33–6.92%, and 0.03–3.23%, respectively. CUR was identified as the predominant compound in the majority of the samples examined, whereas BDMC was consistently found to be present at the lowest levels. As shown in Fig. 6, the lowest content of curcuminoid was present in sample CLR-13 (1.12%) and the highest amounts in sample CLR-08 (16.59%). The content of each compound varied significantly, likely influenced by factors such as environmental growth conditions, harvest timing, post-harvest processes, and the origin of the samples.

Curcuminoid levels vary among provinces because of several factors. Differences in geography and climate, including variations in altitude, temperature, rainfall, humidity, and sunlight exposure, influence how curcuminoids grow and develop. Moreover, variations in soil nutrients, from peat to clay and sandy soil, affect the absorption and maturity of curcuminoids. Additionally, variations in agricultural practices, such as fertilizer and pesticide usage, also play a role in curcumin production. Collectively, these geographical, climatic, soil, and ag-

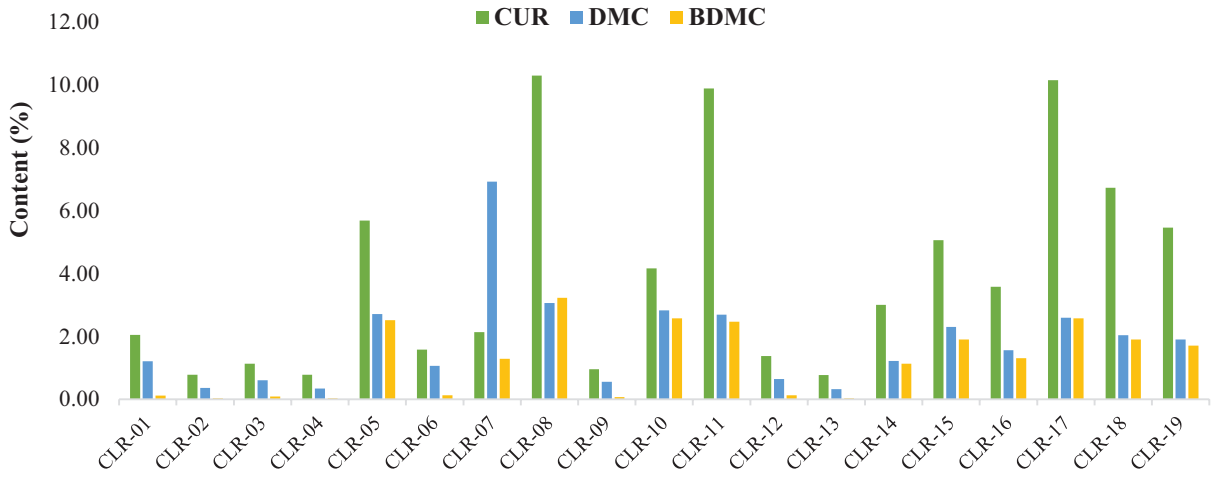

Figure 6. Curcuminoid content in 19 samples with three replicate measurements for each sample.

ricultural factors contribute to observed variations in curcuminoid levels among provinces. As a result, Tra Vinh (9.8127°N, 106.2993°E), Soc Trang (9.6025°N, 105.9739°E), and Dong Nai (11.0686°N, 107.1676°E) are characterized by a hot and humid climate, ample rainfall, and soil enriched with organic matter from silt deposition. These factors contribute to the substantial presence of curcuminoids in turmeric cultivated in these areas.

HPLC fingerprint analysis of *Curcuma longa* L. in Vietnam

The optimized HPLC method was applied to the 19 samples, and representative chromatograms from selected samples are shown in Table 5. and showed good similarity with HPLC profiles. A fingerprint approach with normalization of retention time and peak area was used by calculating the relative retention time (RRT) and relative peak area (RPA) for each common peak relative to the reference peak. Peaks identified in the standard fingerprint were designated as "characteristic common peaks" to represent the sample's distinctive features. Peak CUR (no. 3), which is one of the most important active constituents in *Curcuma longa*, was selected as the reference peak (S) because this peak had a considerably high content in the total area. The RRT and RPA for all characteristic common peaks relative to this reference peak were computed (Table 5).

To begin with, the RRT values of DMC and BDMC were 0.91 ± 0.003 and 0.82 ± 0.006, respectively (mean ± SD). The resulting RRTs for each common peak were relatively consistent, which indicated that the RRT was a suitable parameter for the identification of the samples. In contrast, the RPA values were significantly different, highlighting the crucial role of fingerprint analysis. The sample from Tra Vinh province had the greatest CUR, followed by samples from Dong Nai and Soc Trang. DMC and BDMC had the greatest content in Hau Giang and Tra Vinh, respectively. CUR had the lowest content in Long An province. Generally, based on the data from the fingerprint analysis, it indicates a good-quality source for the raw material in CL.

Table 5. RRT and RPA of common peaks in the 19 batches of *Curcuma longa* L.

Peak name	CUR (S)		DMC		BDMC	
	RRT	RPA	RRT	RPA	RRT	RPA
CRL-01	1	1	0.90	0.65	0.82	0.06
CRL-02	1	1	0.91	0.52	0.82	0.04
CRL-03	1	1	0.91	0.59	0.83	0.08
CRL-04	1	1	0.91	0.47	0.83	0.05
CRL-05	1	1	0.91	0.53	0.83	0.48
CRL-06	1	1	0.91	0.75	0.82	0.09
CRL-07	1	1	0.9	3.57	0.81	0.66
CRL-08	1	1	0.91	0.33	0.82	0.34
CRL-09	1	1	0.91	0.64	0.82	0.08
CRL-10	1	1	0.91	0.75	0.82	0.68
CRL-11	1	1	0.91	0.30	0.82	0.27
CRL-12	1	1	0.91	0.52	0.83	0.10
CRL-13	1	1	0.91	0.47	0.83	0.04
CRL-14	1	1	0.91	0.45	0.83	0.41
CRL-15	1	1	0.91	0.50	0.82	0.41
CRL-16	1	1	0.91	0.48	0.83	0.40
CRL-17	1	1	0.91	0.28	0.83	0.28
CRL-18	1	1	0.91	0.33	0.82	0.31
CRL-19	1	1	0.91	0.38	0.82	0.34
Mean	**1**	**1**	**0.91**	**0.66**	**0.82**	**0.27**
SD	**0**	**0**	**0.00**	**0.72**	**0.01**	**0.21**
RSD (%)	**0**	**0**	**0.37**	**109.22**	**0.73**	**76.52**

RRT: Relative retention time; RPA: Relative peak area; S: The reference peak.

Conclusion

This study developed the optimized extraction procedure and the simultaneous determination of curcuminoids in *Curcuma longa* L. by HPLC with good system suitability, specificity, linearity, precision, and accuracy. To our best knowledge, the chromatographic fingerprint analysis method for quality evaluation of *Curcuma longa* L. rhizomes is the first study in Vietnam, and it provides a reliable and high-precision source of data. These results help to select and develop raw material areas with characteristics suitable for production and consumption.

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Supplementary material 1

List of supplementary materials

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Data type: docx

Explanation note: **A.** Species identification; **B.** Optimization of lc condition; **C.** Optimization of extraction process; **D.** Method validation; **E.** Fingerprint analysis.

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