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# Development and validation of UPLC method for quality control of *Curcuma longa* Linn.: Fast simultaneous quantitation of three curcuminoids

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ABSTRACT

A new reversed phase ultra performance liquid chromatography (UPLC) method was developed for the rapid quantification of three curcuminoids (curcumin (C), desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC)) in *Curcuma longa* Linn. (*C. longa*) using a Waters BEH Shield RP $C_{18}$, 2.1 mm × 100 mm, 1.7 μm column. The runtime was 2 min. The influence of column temperature and mobile phase on resolution was investigated. The method was validated according to the ICH guideline for validation of analytical procedures with respect to precision, accuracy, and linearity. The limits of detection were 40.66, 49.38 and 29.28 pg for C, DMC and BDMC, respectively. Limits of quantitation for C, DMC and BDMC, were 134.18, 164.44 and 97.50 pg, respectively. Linear range was from 3.28 to 46.08 μg/ml. The mean ± SD percent recoveries of curcuminoids were 99.47 ± 1.66, 99.50 ± 1.99 and 97.77 ± 2.37 of C, DMC and BDMC, respectively. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency and sensitivity. The proposed method was found to be reproducible and convenient for quantitative analysis of three curcuminoids in *C. longa*. This work provided some references for quality control of *C. longa*.



## 1. Introduction

*Curcuma longa* Linn. (*C. longa*), whose rhizome are officially listed in Chinese Pharmacopoeia as "Jianghuang" [1], has long been used in oriental preparations (mainly in China and India). In traditional medicine, *C. longa* is used for multiple pharmacological actions including stop hemorrhage and dissolve clots, against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatic disorders, sprains and swellings caused by injury and sinusitis [2–4]. The use of this herb has been summarized by WHO in the monograph "Rhizoma *Curcumae Longae*" [5].

The major and characteristic active components of the herb are three curcuminoids, namely curcumin (C), desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC). Curcuminoids have been studied for their anti-inflammatory, anti-Alzheimer, anti-cancer, anti-oxidant, hypoglycemic and anti-microbial features [6–11]. The three curcuminoids are the basis for the quality control of *C. longa* and other plant-derived drugs from the herb [12]. The chemical structures of these curcuminoids derivatives are shown in Fig. 1.

Spectrophotometric methods were used to determine the total content of curcuminoids in samples [13]. Commercial curcumin/turmeric products contain mixtures of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin. However, it is not possible to quantify the individual curcuminoids with spectrophotometric methods. Estimation of curcuminoids has been reported by thin-layer chromatography method (TLC) [14], high performance thin-layer chromatography (HPTLC) [15,16], near-infrared spectroscopic analysis [17], microemulsion electrokinetic chromatography [18], LC–ESI-MS/MS [19], capillary electrophoresis [20,21] and supercritical fluid chromatography [22]. But none of the reports above described statistical validation of chromatographic method for simultaneous determination of curcuminoids in *C. longa*. The analysis of individual curcuminoids is possible by using HPLC with modified stationary phases such as amino bonded and styrene divinylbenzene copolymer [23–25], or by using gradient elution [26].

Among the methods mentioned above, HPLC method is the most convenient. But there are many problems in the application of HPLC method: it is difficult to produce ideal separation of three curcuminoids directly (special stationary phase is needed), time-consuming (10 min at least) or requires gradient elution, which

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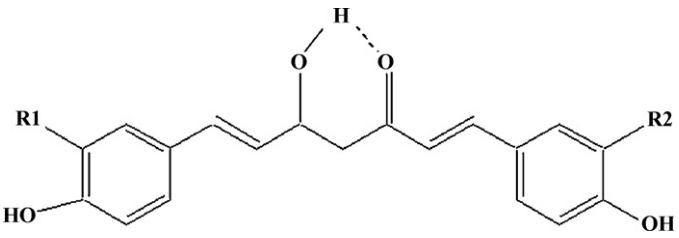


**Fig. 1.** Chemical structure of Curcuminoids. Curcumin: $R_1 = R_2 = OCH_3$; desmethoxycurcumin: $R_1 = H$, $R_2 = OCH_3$; bisdesmethoxycurcumin: $R_1 = R_2 = H$.

usually influences the baseline of chromatogram. It is, therefore, necessary to develop a rapid and reliable method for the simultaneous quantification of three curcuminoids in *C. longa* and its related preparations, which can produce ideal separation of curcuminoids with isocratic elution.

Recently, the commercially available technique of ultra performance liquid chromatography (UPLC) has been proven to be one of the most promising developments in the area of fast chromatographic separations [27]. The columns of UPLC system utilize sub-2 μm particles as stationary phase, and those very small particles allow a drastic improvement of the resolution per time unit, because chromatographic efficiency and optimal mobile phase velocity are both inversely proportional to the particle size. Therefore, due to the high efficiency of sub-2 μm particles, the column length can be decreased to obtain equivalent resolution in a sharply reduced analysis time [28–30]. Because of its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis [31,32]. In fact, Marczylo et al. [33] had reported a UPLC coupled with MS detector to analyze curcumin and its metabolites in rat biomatrices in 12 min, which would be compared in this paper.

So, in this paper, we reported here, the development of an UPLC method for simultaneous quantification of three curcuminoids in *C. longa*. The aim of this study is (1) to simultaneously and fast quantify multi-component *C. longa* with ideal separation and (2) to evaluate the internal quality of this medicinal material with different sources and preparation technologies.

## 2. Experimental

### 2.1. Materials and reagents

Samples of *C. longa* were collected from different places in Sichuan, Yunnan (two provinces in south-western China) and Burma. All samples were identified by one of the authors (Prof. Xiao X.H.) and deposited in the China Military Institute of Chinese Materia Medica. Methanol and acetonitrile were HPLC grade (Fisher Scientific); phosphoric acid and acetic acid were purchased from Beijing Chemical Regents Company (Beijing, China); deionized water was prepared by using a Millipore Milli-Q system (Millipore, MA, USA). The standards (Fig. 1) of curcumin (C), desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC) (purity > 98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing China) and Shunbo Bioengineering Co. Ltd. (Shanghai). These reference compounds were dissolved in acetonitrile/water mixture and filtrated through a 0.22-μm membrane filter prior to injection into UPLC/HPLC system.

### 2.2. High performance liquid chromatography

The HPLC system used for initial chromatographic development was Agilent 1100 separation module with a photodiode array detector. A Kromasil $C_{18}$, 250 mm × 4.6 mm, 5 μm column was used for separation. Mobile phase consisting of a mixture of 0.05% aqueous acetate acid and methanol in the ratio of 15:85 (v/v) with the flow rate of 1 ml/min was employed. The injection volume was 10 μl while detector was set at 420 nm. The column was maintained at 25 °C.

### 2.3. Ultra performance liquid chromatography

UPLC was performed using a Waters Acquity system equipped with binary solvent delivery pump, auto sampler and photodiode array detector. The chromatographic separation was performed on the following four types of $C_{18}$ columns: Waters Acquity® BEH $C_{18}$, HSS BEH $C_{18}$, HSS T3 and BEH Shield RP $C_{18}$ (Waters Corp., MA). The mobile phase consisting of a mixture of 0.05% aqueous phosphoric acid and acetonitrile in the ratio of 34:66 (v/v) with the flow rate of 0.4 ml/min was employed. The detector wavelength was set at 420 nm. The injection volume was 1 μl while the column was maintained at 30 °C.

### 2.4. Standard solution preparation

The standard stock solutions of C, DMC and BDMC (about 100 μg/ml of each) were prepared in acetonitrile/water mixture. These solutions were stored at 4 °C and were stable for at least 1 month. Working standard solutions contain C (5.76 μg/ml), DMC (4 μg/ml) and BDMC (3.68 μg/ml). Each standard was freshly prepared by diluting suitable amounts of the above-mentioned mixed solutions before injection.

### 2.5. Sample preparation

Samples were prepared as follows: 1.0 g (rhizome)/5.0 g (root tuber) powder of dried material was accurately weighed and placed into a 100 ml vial, with precisely 100 ml of methanol added in. The vial was weighed again and recorded. Then, the sealed vial was shaken 40 times and extracted by ultrasonication at room temperature. After cooling, methanol was added into the vial to make up to the initial weight. 2 ml of supernatant fluid was diluted into 10 ml volumetric flask and methanol was used to compensate the rest volume. Samples were finally filtrated through a 0.22-μm membrane filter prior to the injection into UPLC system.

### 2.6. Validation of UPLC method

The UPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [34]. Assay method precision was determined using six independent test solutions. The intermediate precision of the assay method was also evaluated using different analysts on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from samples. Three different quantities (low, medium, high; see Table 4) of the authentic standards were added to the known real sample. The mixtures were extracted as described in Section 2.5, and were analyzed using the developed UPLC method. Linearity test solutions were prepared by diluting the mixed standards stock solution. Mean values of symmetry factor, plate count and resolution were calculated for all components (USP method). The LOD and LOQ for three curcuminoids were estimated by injecting a series of dilute solutions at known concentration. Precision study was also carried at the LOQ level.

## 3. Results and discussions

### 3.1. HPLC method transfer to UPLC

Curcumin and its naturally occurring demethoxy analogues are powerful complexing agents, which can form inter- and intra-

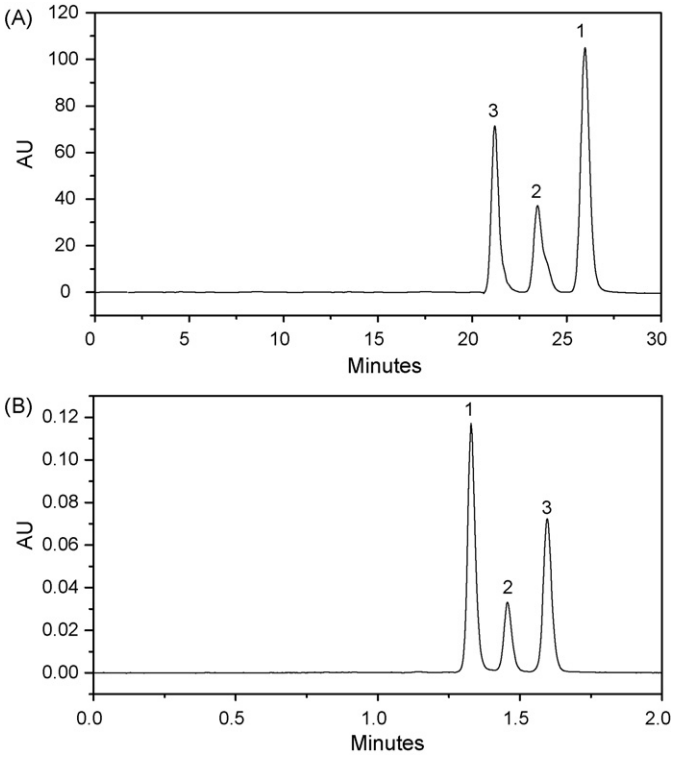


**Fig. 2.** Comparison of chromatograms of mixed standard obtained from (a) HPLC and (b) UPLC. Key: (1) curcumin; (2) desmethoxycurcumin; (3) bisdesmethoxycurcumin.

molecular bonds. In fact, Asakawa and his associate reported in 1981 that HPLC system based on $C_{18}$ stationary phases could not completely separate the three curcuminoids [35]. The separation of curcuminoids could be achieved by using an amino-bonded stationary phase with a critical condition; the water content of mobile phase would influence the separation of curcuminoids severely, and with little alteration of which would take as long as 8 h to reach equilibrium. Even in the original condition, the peak tailing was still observed [24].

Jayaprakasha and his associate reported a HPLC method using gradient elution to separate three curcuminoids [31]. The resolutions of curcuminoids' chromatographic peaks were not reported, but it could be observed that three curcuminoids were not separated completely from the given chromatograms.

According to previous reports, only Jadhav had reported a HPLC method [17] for the separation and quantification of curcuminoids with ideal resolutions (peak Rs > 2, asymmetry factors < 1.2) using amino-bonded stationary phase and 1% trifluro-acetic acid in mobile phase, which indicated by the author was the main reason for the good peak resolutions.

In this study, UPLC method and HPLC method were established for simultaneous quantification of three curcuminoids. Typical UPLC and HPLC chromatogram was depicted in Fig. 2. When developing the UPLC method, four different RP $C_{18}$ columns: BEH $C_{18}$, HSS BEH $C_{18}$, HSS T3 and BEH Shield RP $C_{18}$ (all the columns were designed for UPLC from Waters Corp., MA) were tried in this study. The former three columns could not separate curcuminoids well. The UPLC chromatogram with BEH Shield RP $C_{18}$ column is depicted in Fig. 2. Typical chromatograms of UPLC with BEH $C_{18}$, HSS BEH $C_{18}$, HSS T3 columns are depicted in Fig. 3.

As shown in Fig. 1, curcuminoids have two tautomeric forms: the keto- and enol-form, the mixture of these two forms of curcuminoids might be the reason of peak tailing in chromatograms. Peak tailing along with elevation of baseline was observed in

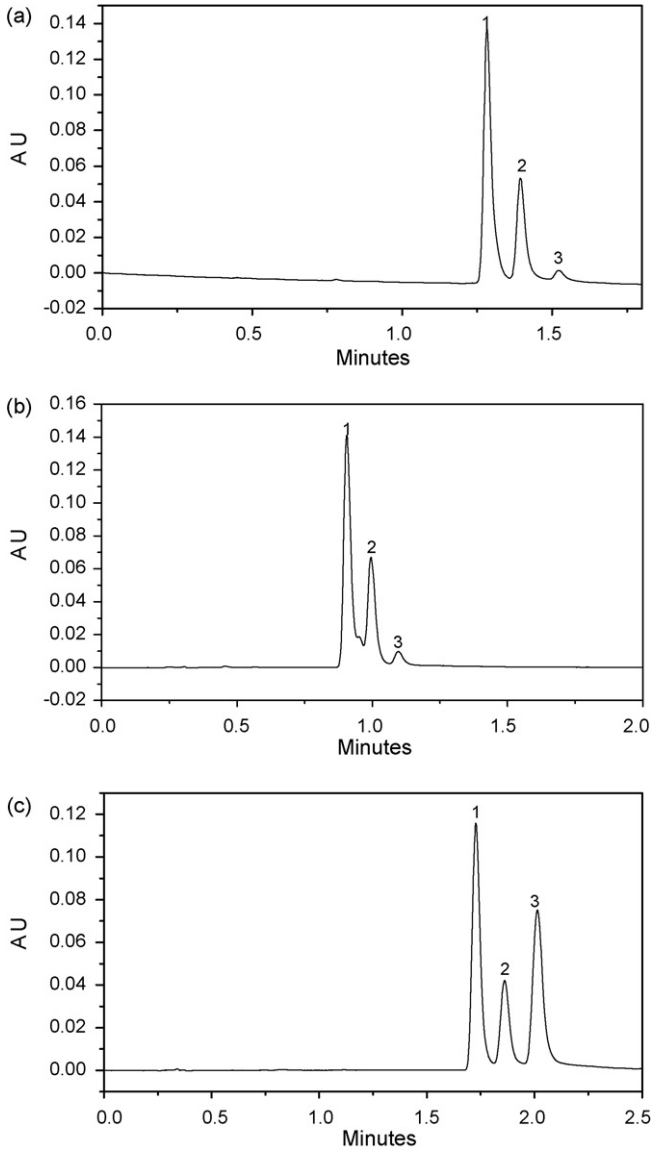


**Fig. 3.** UPLC chromatograms of curcuminoids with different columns. (1) Curcumin; (2) desmethoxycurcumin; (3) bisdesmethoxycurcumin. (a) HSS T3 column; (b) HSS BEH $C_{18}$ column; (c) BEH $C_{18}$ column. Chromatographic conditions: flow rate = 0.4 μl. A = 0.05% aqueous phosphoric acid, B = acetonitrile. (a) 40%A:60%B; (b) 34%A:66%B; (c) gradient elution: 0 min, 35%B; 0–1 min, 40%B, 1–3.5 min 45%B; monitored at 420 nm.

chromatograms. With BEH $C_{18}$ column, using isocratic or gradient elution (flow rate = 0.4 μl; A = 0.05% aqueous phosphoric acid, B = acetonitrile. a: 40%A:60%B; b: 34%A:66%B; c: gradient elution: 0 min, 35%B; 0–1 min, 40%B, 1–3.5 min 45%B), the best resolution of curcuminoids was less than 2(USP method). Excellent separation was achieved only with the BEH Shield RP $C_{18}$ column. Though, all the columns are reversed phase $C_{18}$, the reason for the different chromatographic behavior might be due to the difference in specific surface area of stationary phase or end capping of columns. The BEH Shield RP $C_{18}$ column incorporated an embedded carbamate group into the bonded phase which increased the retention of phenolic compounds, which made the symmetry peak and good separation possible.

In Marczylo's [33] report, they had used BEH $C_{18}$ column to separate curcuminoids with gradient elution in 12 min. The detail of curcuminoids' resolution was not given in their paper. In our study, it is difficult to get an ideal resolution with BEH $C_{18}$ column, so we

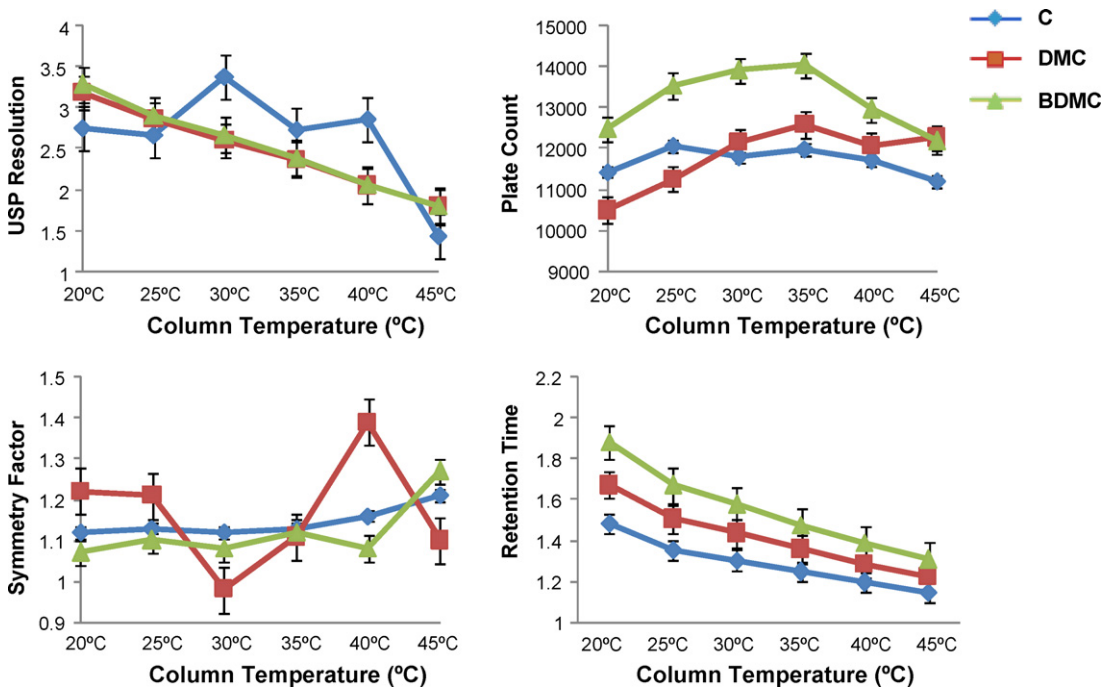


**Fig. 4.** Influence of column temperature on UPLC chromatographic performance ($n = 3$, chromatographic conditions: flow rate = 0.4 μl, 0.05% aqueous phosphoric acid:acetonitrile: 34:66; monitored at 420 nm).

have changed the column to BEH Shield RP $C_{18}$ column, which could get an ideal separation of curcuminoids in 2 min.

Ionization suppressant was thought to provide acceptable chromatographic separations. The separation of curcuminoids was definitely improved after acid being added to mobile phase. Effect of different ionization suppressing agents such as acetic acid (up to 4% (v/v) pH 2.47), phosphoric acid (up to 0.05% (v/v) pH 2.3) and trifluoroacetic acid (TFA up to 0.1% (v/v)) had been studied. Jadhav and his associate studied different types of acid in mobile phase and reported that only with TFA could achieve good peak shape and resolutions due to the partial deactivation of stationary phase by highly electronegative fluorine ions of TFA [17]. In this study, the results were different from theirs, in that, the peak shape and resolutions were affected by the acidity of mobile phase, not the type of acid. Mobile phase with each types of acid could get ideal resolution and symmetry factor in appropriate acidity. So when it needs to be coupled with a mass spectrometer, the acetic acid could be used to replace phosphoric acid in mobile phase. To achieve total resolution of the curcuminoids and shortest possible runtime, the final mobile phase was chosen as described in Section 2.3.

To improve detector sensitivity and to achieve equivalent system performance compared with conventional HPLC at same sample concentration UPLC instrument manufacturer recommends to use low injection volume (see Acquity UPLC Columns Calculator software (Version 1.1.1) of Waters Corporation).

Attempts to improve the chromatographic performance were made by altering the column temperature. The results are shown in Fig. 4. The flow rate was set at 400 μl/min when column temperature was altered.

The retention time of curcuminoids decreased with the increase of column temperature; resolution, theoretical plates and symmetry factor obtained for C, DMC and BDMC showed some improvement with the increase of column temperature. The plate count of curcuminoids showed a declining trend above 35 °C. The resolution of curcumin declined above 35 °C. The best symmetry factor of three curcuminoids was achieved at 30 °C. Hence, the column temperature of 30 °C was preferred. At this column temperature and the flow rate of 400 μl/min, a satisfactory and rapid separation was achieved in 2 min with a backpressure of 6000 psi.

### 3.2. Comparison study of chromatographic performance

Comparative data on chromatographic performance of HPLC and UPLC have been obtained by injecting a solution of mixed standards (5 μg/ml each). The performance parameters of both systems are shown in Table 1. The runtime of UPLC was reduced by 10-fold to that of HPLC. The retention behavior of curcuminoids was changed on different columns: the eluted sequence of curcuminoids on BEH Shield RP $C_{18}$ column (UPLC) was C, DMC, BDMC while it was BDMC, DMC, C on Kromasil $C_{18}$ column (HPLC). The UPLC method showed higher analysis efficiency than HPLC method. Typical HPLC chromatograms and UPLC chromatograms are depicted in Fig. 2.

### 3.3. UPLC method validation

#### 3.3.1. Calibration and linearity

To determine the linearity of UPLC response, standard solutions of C, DMC and BDMC were prepared as described earlier. Good linear correlations were obtained between peak areas and concentration in the selected ranges of C, DMC and BDMC. Characteristic parameters for regression equations and correlation coefficients are given in Table 2. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression graph.

#### 3.3.2. Sensitivity

The LOD and LOQ of three curcuminoids for UPLC are summarized in Table 2, with RSD 2.4% at injection volume of 1 μl. The LOQ concentration for HPLC was found to be 150 ng/ml, with RSD 4.8% at injection volume 10 μl. The parameters of LOD and LOQ are given in Table 2.

#### 3.3.3. Precision

Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions in triplicates at three differ-

**Table 1**
Comparison of system performance of HPLC and UPLC.

| Components | Elution time (min) | | USP resolution | | symmetry factor | | USP plate count | | plate height | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HPLC | UPLC | HPLC | UPLC | HPLC | UPLC | HPLC | UPLC | HPLC | UPLC |
| BDMC | 13.087 | 1.576 | 17.42 | 2.67 | 0.89 | 1.08 | 15,495 | 13,912 | 0.0161 | 0.0071 |
| DMC | 14.390 | 1.435 | 2.90 | 2.60 | 0.97 | 0.95 | 11,372 | 12,143 | 0.0219 | 0.0082 |
| C | 15.851 | 1.304 | 2.88 | 3.38 | 0.91 | 1.12 | 14,596 | 11,788 | 0.0171 | 0.0085 |

**Table 2**
Calibration curves, LOD and LOQ of the investigated components ($n = 3$).

| Components | Regression equation ($y = ax + b$)[a] | | $R^2$ | Linear range (μg/ml) | LOD (pg) | LOQ (pg) |
|---|---|---|---|---|---|---|
| | a | b | | | | |
| C | 19,144 ± 20.11 | 17,906 ± 18.02 | 0.999 | 5.76–46.08 | 40.66 | 134.18 |
| DMC | 8025 ± 15.37 | 5974 ± 8.49 | 0.999 | 4.00–32.00 | 49.38 | 164.44 |
| BDMC | 20,567 ± 38.66 | 6663 ± 17.92 | 0.999 | 3.28–26.24 | 29.28 | 97.50 |

[a] $y$ is the peak area, $x$ is the concentration, $a$ is the slope and $b$ is the intercept of the regression line, respectively.

**Table 3**
Intra-day and inter-day variation of curcuminoids by the proposed method ($n = 3$).

| Components | Contents (μg/ml) | Intra-day | | | Inter-day |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| C | 5.76 | 100.02 ± 0.86 | 99.38 ± 0.65 | 101.22 ± 0.39 | 101.12 ± 0.16 |
| | 11.52 | 99.75 ± 0.84 | 98.36 ± 0.93 | 100.17 ± 0.86 | 100.93 ± 0.68 |
| | 17.28 | 100.67 ± 0.69 | 101.24 ± 0.81 | 102.03 ± 0.94 | 100.45 ± 0.34 |
| DMC | 4.00 | 100.29 ± 0.22 | 100.34 ± 0.98 | 99.68 ± 0.91 | 101.76 ± 0.13 |
| | 8.00 | 98.96 ± 0.25 | 100.82 ± 0.46 | 101.06 ± 0.67 | 100.87 ± 0.32 |
| | 12.00 | 99.35 ± 0.85 | 99.19 ± 0.63 | 101.45 ± 0.32 | 99.56 ± 0.66 |
| BDMC | 3.68 | 98.76 ± 0.18 | 100.23 ± 0.12 | 100.83 ± 0.21 | 99.98 ± 0.38 |
| | 7.36 | 99.32 ± 0.47 | 98.89 ± 0.71 | 98.91 ± 0.62 | 99.01 ± 0.42 |
| | 11.04 | 100.24 ± 0.32 | 100.87 ± 0.63 | 101.03 ± 0.54 | 101.03 ± 0.15 |

**Table 4**
Accuracy of the investigated components ($n = 3$).

| Components | Contents (μg/ml) | Quantity added (μg/ml) | Theoretical amount (μg/ml) | Recorded amount (μg/ml) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|---|
| C | 18.27 | 11.52 | 29.79 | 29.55 | 97.9 | 3.17 |
| | 18.27 | 23.04 | 41.31 | 41.59 | 101.2 | 2.24 |
| | 18.27 | 34.56 | 52.83 | 52.58 | 99.3 | 4.16 |
| DMC | 11.12 | 6.00 | 17.12 | 17.02 | 98.3 | 2.73 |
| | 11.12 | 12.00 | 23.12 | 23.33 | 101.8 | 3.29 |
| | 11.12 | 18.00 | 29.12 | 28.84 | 98.4 | 2.62 |
| BDMC | 3.98 | 1.84 | 5.82 | 5.76 | 96.5 | 4.01 |
| | 3.98 | 3.68 | 7.66 | 7.68 | 100.5 | 3.59 |
| | 3.98 | 5.52 | 9.50 | 9.29 | 96.3 | 2.10 |

**Table 5**
Contents (μg/g) of curcuminoids in *C. longa* ($n = 3$).

| Source | Curcumin | Desmethoxycurcumin | Bisdesmethoxycurcumin |
|---|---|---|---|
| Jiajiang, Sichuan | 14.88 ± 0.20 | 9.23 ± 0.05 | 3.35 ± 0.01 |
| Mabian, Sichuan | 24.84 ± 0.06 | 18.56 ± 0.14 | 7.99 ± 0.02 |
| Emei, Sichuan | 19.46 ± 0.21 | 14.92 ± 0.16 | 5.98 ± 0.01 |
| Renshou, Sichuan | 31.01 ± 0.26 | 22.00 ± 0.52 | 8.55 ± 0.03 |
| Chongzhou, Sichuan | 21.60 ± 0.15 | 17.97 ± 0.11 | 8.30 ± 0.03 |
| Qianwei, Sichuan | 17.75 ± 0.17 | 13.03 ± 0.19 | 4.79 ± 0.01 |
| Chengdu, Sichuan | 20.09 ± 0.42 | 18.76 ± 0.38 | 7.30 ± 0.04 |
| Tingjiang, Sichuan | 15.28 ± 0.20 | 13.19 ± 0.23 | 6.68 ± 0.02 |
| Sanjiang, Sichuan | 15.22 ± 0.35 | 11.32 ± 0.18 | 4.78 ± 0.05 |
| Qingxi, Sichuan | 12.68 ± 0.21 | 9.77 ± 0.13 | 3.89 ± 0.01 |
| Muchuan, Sichuan | 22.00 ± 0.19 | 2.05 ± 0.06 | 7.49 ± 0.06 |
| Leshan, Sichuan | 27.35 ± 0.30 | 18.51 ± 0.64 | 6.93 ± 0.03 |
| Yibin, Sichuan | 20.82 ± 0.34 | 14.26 ± 0.26 | 5.67 ± 0.02 |
| Yunnan | 5.65 ± 0.11 | 4.88 ± 0.15 | 0.45 ± 0.01 |
| Burma | 24.17 ± 0.25 | 39.92 ± 0.67 | 2.25 ± 0.03 |

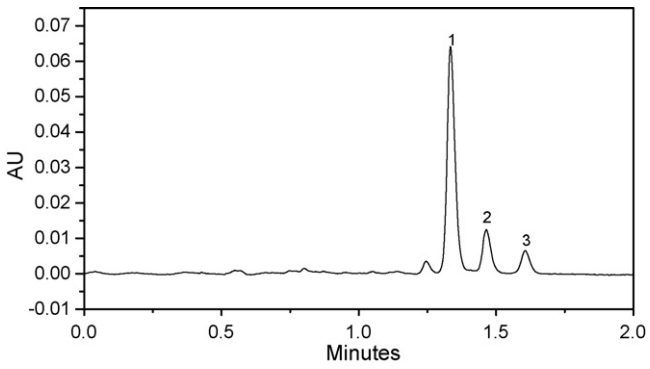


**Fig. 5.** Typical UPLC-UV chromatogram of *C. longa* (sample from Jiajiang Sichuan). (1) Curcumin; (2) desmethoxycurcumin; (3) bisdesmethoxycurcumin (chromatographic conditions: flow rate = 0.4 μl, 0.05% aqueous phosphoric acid:acetonitrile: 34:66; monitored at 420 nm).

ent concentrations. Inter-day precision was evaluated by using freshly prepared solution in triplicates on three different days. These results are summarized in Table 3.

### 3.3.4. Accuracy

The accuracy of the method was determined by spiking known amount of mixed standards in known *C. longa* samples in triplicate at levels 50%, 100% and 150% of the specified limit. The recoveries of three curcuminoids were calculated and given in Table 4. The recovery of the investigated components ranged from 96.3% to 101.8%, and their RSD values were all less than 5.0%, characterizing good reliability and accuracy of the method.

### 3.4. Application

The developed UPLC method was applied to the simultaneous determination of three curcuminoids in *C. longa*. Target components were identified by comparing the retention times and UV spectra with those presented in the chromatogram of the mixture standard solution. The peak purity of target components in these samples was verified using photodiode array (PDA) detector. The chromatograms are depicted in Fig. 5, and the results are presented in Table 5. As shown in Table 4, the contents of three curcuminoids in *C. longa* were quite variable, contents of three curcuminoids in most *C. longa* samples were curcumin > desmethoxycurcumin > bisdesmethoxycurcumin, except the sample collected from Buma, which might indicate the differences in *C. longa*'s quality and bioactivity. Therefore, it is recommendable to control the contents of the individual active components rather than their total content.

## 4. Conclusion

This work first developed an UPLC method for fast simultaneous quantitation of Curcumin, Desmethoxycurcumin and Bisdesmethoxycurcumin in *C. longa*. The UPLC method reported here was found to be capable of giving faster analysis with good resolution than that achieved with conventional HPLC. The method was completely validated showing satisfactory data for all the parameters tested. This method is also eco-friendly for its low consumption of organic solvents as compared to other analytical techniques. Overall, it is suitable for rapid and accurate quality control of *C. longa* and its related medicinal materials or preparations.

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