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Metabolic and pharmacokinetic studies of curcumin, demethoxycurcumin and bisdemethoxycurcumin in mice tumor after intragastric administration of nanoparticle formulations by liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

This paper aims to investigate the metabolism and pharmacokinetics of curcumin, demethoxycurcumin and bisdemethoxycurcumin in mice tumor. To improve water solubility, nanoparticle formulations were prepared as curcuminoids-loaded solid lipid nanoparticles (curcuminoids-SLNs) and curcumin-loaded solid lipid nanoparticles (curcumin-SLNs). After intragastric administration to tumor-bearing ICR mice, the plasma and tumor samples were analyzed by liquid chromatography with ion trap mass spectrometry. We discovered that curcuminoids were mainly present as glucuronides in plasma, whereas in free form in tumor tissue. A validated LC/MS/MS method was established to determine the three free curcuminoids in tumor homogenate. Samples were separated on a Zorbax SB-C_{18} column, eluted with acetonitrile–water (containing 0.1% formic acid), and detected by TSQ Quantum triple quadrupole mass spectrometer in selected reaction monitoring mode. The method showed good linearity (r^2 = 0.997–0.999) over wide dynamic ranges (2–6000 ng/mL). Variations within- and between-batch never exceeded 11.2% and 13.4%, respectively. The extraction recovery rates ranged from 78.3% to 87.7%. The pharmacokinetics of curcuminoids in mice tumor fit two-compartment model and first order elimination. For curcumin-SLNs group, the dosing of 250 mg/kg of curcumin resulted in $AUC_{(0-48\,h)}$ of 2285 ng h/mL and C_{max} of 209 ng/mL. For curcuminoids-SLNs group, the dosing equivalent to 138 mg/kg of curcumin resulted in higher tumor concentrations (AUC = 2811 ng h/mL, C_{max} = 285 ng/mL). It appeared that co-existing curcuminoids improved the bioavailability of curcumin.

1. Introduction

Curcumin, [1,7-bis(4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is a dietary polyphenolic compound isolated from turmeric, the rhizomes of *Curcuma longa* Linn [1]. For a long history, turmeric has been used as a coloring and flavoring agent worldwide. Today, curcumin is considered as a novel, safe and promising anti-cancer agent for both prevention and treatment of cancer. A number of clinical trials are being conducted to confirm its efficacy [2,3]. *In vitro* studies proved that curcumin possessed potent antioxidant and anti-inflammatory activities [4,5]. The mechanism may be that curcumin could suppress the activation of transcription factor NF-κB [6–8] and signaling molecules related to carcinogenesis [9,10]. *In vivo* studies on rodent models further confirmed its anti-diabetes [11,12], anti-Alzheimer's disease [13–17], and anti-tumorigenesis activities [18–23]. In addition, several clinical studies have revealed that curcumin is safe for human even at an oral dosage of 12 g per day [24,25].

The metabolism and pharmacokinetics of curcumin have been studied using HPLC with ultraviolet or mass spectrometry detectors [26–29]. Curcumin was detected at a very low concentration in serum, and was mainly metabolized via conjugation (glucuronidation and sulfation) and reduction [24,30,31]. Most of these studies were performed on healthy animals [26,27,32,33]. As an anti-tumor agent, tumor affinity is highly critical for clinical use of curcumin, and has not yet been studied, so far [34]. Moreover, metabolism of curcumin in pathological animal models, such as tumor-bearing mice, remains unclear. In this paper, a fully validated LC-MS/MS

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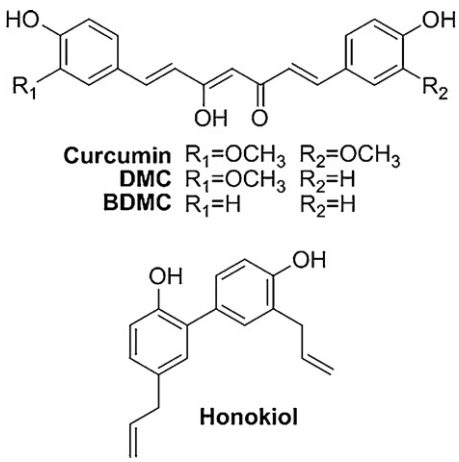

Fig. 1. Chemical structures of curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and honokiol (internal standard).

method was established to study the metabolism and pharmacokinetics of curcuminoids and curcumin in tumor tissues after intragastric administration. In order to improve water solubility, curcuminoids and curcumin were prepared as solid lipid nanoparticles (SLNs) [35,36].

2. Materials and methods

2.1. Chemicals and reagents

Curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and total curcuminoids containing 55.3% curcumin, 17.7% DMC and 27.0% BDMC (w/w) were isolated from the rhizomes of *C. longa* by the authors. Their structures were identified by NMR spectroscopy and mass spectrometry (Fig. 1). The purities were above 98% as determined by HPLC. Honokiol (internal standard, 98% purity) was obtained from China National Institutes for Food and Drug Control (Beijing, China). Soya lecithin and stearic acid were purchased from Beijing Chemical Corporation (Beijing, China). Tween 80 was from Sigma (St. Louis, MO).

HPLC grade acetonitrile and formic acid (Mallinkrodt Baker, Phillipsburg, NJ) were used for LC/MS analysis. De-ionized water was purified by a Milli-Q system (Millipore, Bedford, MA, U.S.A.). Analytical grade acetone and dichloromethane were purchased from Beijing Chemical Corporation (Beijing, China).

2.2. Preparation and characterization of curcuminoids and curcumin loaded solid lipid nanoparticles (SLNs)

According to previously reported method [37], 900 mg of curcumin and total curcuminoids (including curcumin, DMC and BDMC, see Fig. 2) were dissolved in 50 ml acetone, respectively. An amount of 1.8 g stearic acid and 3.5 g soya lecithin were dissolved in 80 ml dichloromethane. Mix the acetone solution and dichloromethane solution and remove the chemical solvent in a rotary evaporator (EYELA, Japan) in vacuum at 40 °C. The sample was then dissolved in 90 mL of 1% aqueous Tween 80 and then ultrasonicated for 10 min, and were obtained as stable opaque yellow emulsion. Size distribution and polydispersity (PDI) were measured by a laser particle size analyzer (Zeta Sizer, MALVERN, Nano series, UK), and the appearance of nanoparticles was observed using a transmission electron microscope (JEOL, JEM-1230, Japan). For intragastric administration, the SLNs formulations were dispersed in aqueous solution to form a stable emulsion of 10 mg/mL.

2.3. ICR tumor-bearing mice pathological modeling

ICR mice (male, 18–20 g) were obtained from the Laboratory Animal Center of Peking University Health Science Center. The mice were housed in an animal room in a controlled condition (22–24 °C) with free access to food and de-ionized water. All animal treatments were in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals. The experiments were carried out under the approval of Experiment Administration Committee of Peking University Health Science Center. To establish the tumor-bearing mice model, S180 tumor cells (4.0×10^6 per mouse) in 0.9% NaCl solution was subcutaneously inoculated into the right axilla of ICR mice. The bioanalysis was carried out on the 15th day after inoculation when the tumor tissues were approximately 3.0 g. A total of 100 tumor-bearing mice were prepared, and 80 mice with similar tumor size (RSD < 30%) were randomly separated into two groups for pharmacokinetic studies.

2.4. Drug administration and sample collection

For tumor-bearing ICR mice group I, curcuminoids loaded solid lipid nanoparticles (curcuminoids-SLNs) formulations were administered individually by oral gavage at a dose of 250 mg/kg body weight (0.5 mL per mouse), equivalent to 138.25 mg/kg curcumin, 44.25 mg/kg DMC, and 67.50 mg/kg BDMC. Tumor-bearing ICR mice of group II were administered curcumin loaded solid lipid nanoparticles (curcumin-SLNs) formulations intragastrically at the same dose (250 mg/kg). Blank solid lipid nanoparticles (Blank-SLNs) and de-ionized water were orally administered to tumor-bearing ICR mice as blank control.

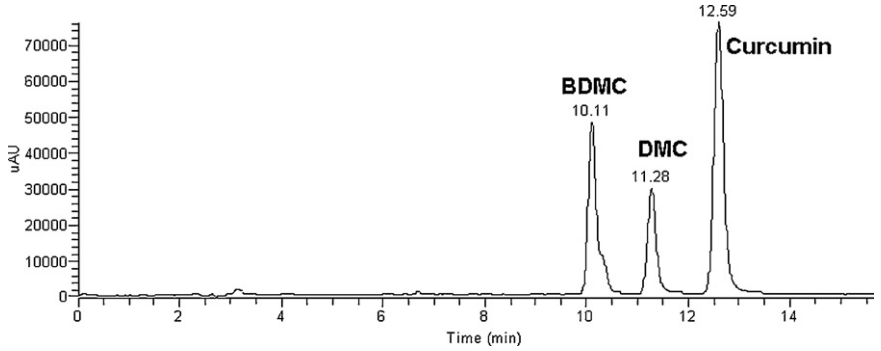

Fig. 2. HPLC profile of total curcuminoids, showing curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). HPLC conditions: YMC Pack-ODS A column (250 mm × 4.6 mm i.d., 5 μm); mobile phase, acetonitrile–0.1% aqueous formic acid (50:50, v/v); flow rate, 1.0 mL/min; column temperature, 35 °C; UV detector, 425 nm.

For each group, five mice were sacrificed at each of the following eight time points: 0.5, 1, 2, 4, 8, 12, 24, and 48 h. Blood and tumor tissues were collected. The blood samples were placed in heparinized tubes and immediately centrifuged to obtain the plasma. Tumor tissue samples were excised and rinsed three times in ice-cold physiological saline. Then samples were dried on filter paper, accurately weighed, cut into chips, and homogenated by Ultra Turrax (T18 basic, German) after adding 0.9% NaCl solution to make a uniform concentration (1 mL/g tissue). The plasma and tumor homogenates were stored at −80 °C until analysis.

2.5. Sample preparation

For metabolites identification, a 500 μL aliquot of plasma sample and tumor homogenate sample collected at 1 h after administration were extracted with 2000 μL acetonitrile by vortex-mixing for 1 min and then ultrasonication for 30 s. After centrifugation at 9500 rpm for 10 min, the upper organic layer was transferred to another tube and dried under a gentle stream of nitrogen at 20 °C. The residue was reconstituted in 100 μL methanol followed by vortex-mixing for 1 min and then ultrasonication for 30 s. After filtering through a membrane (0.22 μm pore size), a 50 μL aliquot was injected into the LC/MS system for analysis.

The above sample preparation method was modified and employed for pharmacokinetic studies. To a 330 μL aliquot of tumor homogenate sample, 20 μL of the IS working solution was added. Samples were then vortex-mixed for 30 s and extracted with 1400 μL acetonitrile by vortex-mixing for 1 min and ultrasonication for 30 s. After centrifugation at 9500 rpm for 10 min, the upper organic layer was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen at 20 °C. The residue was reconstituted in 200 μL methanol followed by vortex-mixing for 1 min and ultrasonication for 30 s. After filtering through a membrane (0.22 μm pore size), a 5 μL aliquot was injected into the LC/MS/MS system for analysis.

2.6. Chromatographic and mass spectrometric conditions

Liquid chromatography with ion trap mass spectrometry (LC/IT-MS) was used for metabolites identification. A Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFisher, San Jose, CA, USA) was connected to an Agilent 1100 HPLC system via an ESI source in a post-column splitting ratio of 3:1. Chromatography was performed on a YMC Pack-ODS A C_{18} reversed-phase column (250 mm × 4.6 mm i.d., 5 μm) and an Agilent Zorbax SB-C_{18} guard column (12.5 mm × 4.6 mm i.d., 5 μm). The mobile phase consisted of acetonitrile (A) and 0.1% aqueous formic acid (B). The gradient elution program was as follows: 0 min, 5:95 (A–B, v/v); 30 min, linear gradient to 45:55; 50 min, 95:5; 55 min, 95:5. The flow rate was 1 mL/min. The column temperature was 30 °C. The optimized MS ionization parameters were as follows: negative ESI mode; capillary temperature, 330 °C; capillary voltage, 35 V; tube lens offset voltage, −40 V.

Quantitative analysis was carried out on an LC/MS/MS system. The HPLC system consisted of a Finnigan Surveyor LC instrument (ThermoFisher, CA, USA) with an Agilent Zorbax SB-C_{18} column (150 mm × 2.1 mm i.d., 3.5 μm) equipped with an Agilent Zorbax SB-C_{18} guard column (12.5 mm × 4.6 mm i.d., 5 μm). The HPLC was connected to a Finnigan TSQ Quantum triple quadrupole mass spectrometer via ESI source. The mobile phase consisted of acetonitrile (A) and 0.1% aqueous formic acid (B). The following linear gradient elution program was used: 0 min, 50:50 (A–B, v/v); 6 min, 85:15; 7 min, 95:5; 8 min, 95:5. The flow rate was 0.2 mL/min. The column temperature was 40 °C. The ESI source was operated in negative ion mode. High purity argon was used as the collision gas (1.0 mTorr). Other ESI parameters: source temperature, 330 °C; spray voltage, 4.0 kV; capillary offset, 35 V, source fragmentation voltage, 10 V. The mass spectrometer scanned in Selected Reaction Monitoring (SRM) mode. The SRM ion pair transitions and collision energy levels of analytes and IS are given in Table 1.

Table 1
Optimized SRM (selected reaction monitoring) parameters for curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and honokiol (IS).

Analyte	Precursor ion	Product ion	Collision energy (V)	Tube lens offset (V)
Curcumin	367.3	149.2	29	96
DMC	337.1	119.2	48	103
BDMC	307.2	119.1	50	91
Honokiol	265.2	224.1	42	120

2.7. Stock solutions, working solutions, and quality control (QC) samples

The standard stock solutions of curcumin, DMC, BDMC, and honokiol (IS) stock solutions were individually prepared in methanol to a final concentration of 500 μg/mL. Mixed working solutions of three standard compounds were prepared by further mixing and diluting the stock solutions with methanol to 30,000, 10,000, 3000, 1000, 300, 100, 30 and 10 ng/mL of curcumin, DMC, and BDMC, respectively. The working solution of IS was prepared by diluting the stock solution of honokiol with methanol to 10 μg/mL. All the working solutions were stored at −20 °C.

Calibration curves were prepared by spiking 300 μL of blank tumor homogenate with 40 μL of the above mentioned mixed working solutions of three standard compounds and 20 μL of the IS working solution. The other treatments were the same as described in Section 2.5 for pharmacokinetics samples. The linear concentration ranges were 2–2000 ng/mL for curcumin and DMC, and 2–6000 ng/mL for BDMC, respectively.

The quality control (QC) samples were prepared by adding the stock solution of three analytes into blank tumor homogenates to obtain final concentrations of 60, 300, and 900 ng/mL for each analyte, which represented low, medium, and high concentration QC samples, respectively.

2.8. Data analysis

All calibration and quantitation data were processed with Xcalibur 2.0.7 software (ThermoFisher, CA, USA). Pharmacokinetic modeling was performed with WinNonlin® software (version 6.1, Pharsight, USA). The area under the tumor homogenate concentration–time curve from zero to last point (AUC_{0-t}) were calculated using the Gauss-Newton (Levenberg and Hartley) method.

3. Results and discussion

3.1. Metabolites identification in mice plasma and tumor by LC/IT-MS

To improve the solubility of curcumin and curcuminoids, lipid nanoparticles (curcuminoids-SLNs and curcumin-SLNs) were prepared. The average particle size was 118.9 ± 14.8 nm (n = 3), and the average polydispersity (PDI) was 0.41 ± 0.16 (n = 3). The particle size was confirmed by transmission electron microscopy (Fig. 1S). The curcuminoids-SLNs contained 55.3% curcumin, 17.7% demethoxycurcumin (DMC), and 27.0% bisdemethoxycurcumin (BDMC).

According to previous reports, glucuronidation is the major phase II metabolic reaction after intragastric administration of curcumin in rats [24,31]. In our study, glucuronides of curcumin, DMC and BDMC were also detected as major metabolites in mice plasma.

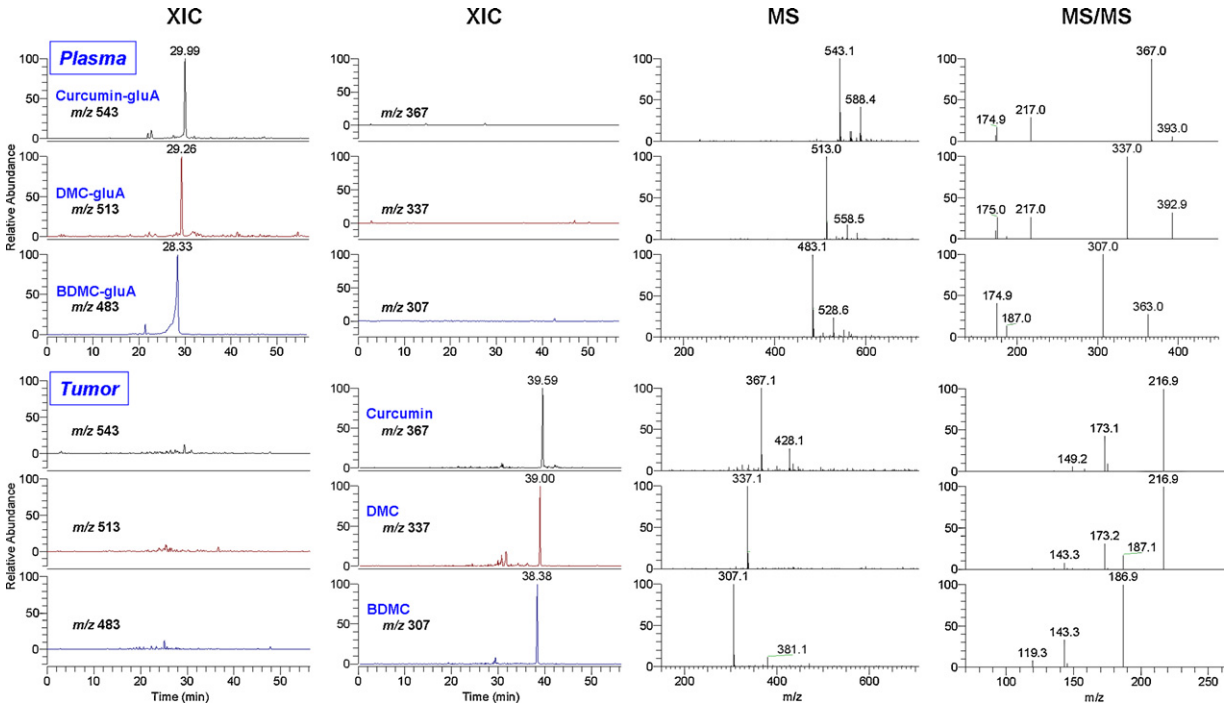

Fig. 3. XIC chromatograms and mass spectra of curcuminoids and their glucuronides in mice plasma and tumor homogenate. DMC, demethoxycurcumin; BDMC, bisdemethoxycurcumin. For XIC chromatograms, the *y* scales were around 2.0E6.

They gave $[M-H]^-$ ions at *m/z* 543, 513, and 483, and yielded major fragments at *m/z* 367, 337, and 307, respectively, by losing 176 mass units [30,33] (Fig. 3). In contrast, free curcuminoids were not detected in plasma by ion trap mass spectrometer.

Interestingly, free curcuminoids, i.e. curcumin, DMC and BDMC were detected in mice tumor tissues at high amounts, whereas no glucuronides were detected. The free curcuminoids were identified by both comparing with reference standards and by analyzing their MS^n spectra, *m/z* 367 → 217 → 173 for curcumin, *m/z* 337 → 217 → 173 and *m/z* 337 → 187 → 143 for DMC, and *m/z* 307 → 187 → 143 for BDMC (Fig. 3). The above characteristic fragment ions were in accordance with previous reports [38].

Our study indicated that orally administered curcuminoids were rapidly converted into their glucuronides, which were detected at high concentrations in mice plasma. These glucuronides were then transformed into the original free curcuminoids in tumor tissues. The metabolic pathway of curcuminoids was proposed in Fig. 4. To the best of our knowledge, this is the first report that curcuminoids are mainly present as glucuronides in mice plasma, and are present in free form in tumor tissue. Given that tumor is the target tissue for anti-cancer activities of curcuminoids, the concentrations of free curcuminoids in tumor tissues could be more critical than the concentrations of curcuminoids or their glucuronides in plasma. Moreover, it could be deduced that free curcuminoids, instead of their glucuronides, might be the active form to suppress tumor *in vivo*, albeit the anti-tumor activities of free and conjugated curcuminoids have not been carefully compared, so far.

3.2. Quantitative analysis of curcuminoids in tumor by LC/MS/MS

Honokiol has similar chemical structure, chromatographic behavior, and ionization response in ESI source with curcuminoids, and was reported for good stability [26]. In this study, honokiol was chosen as the internal standard for quantitation of curcuminoids.

The triple quadrupole mass spectrometer was monitored in the negative ESI mode as all the three curcuminoids and honokiol (IS) showed good ionization efficiency. The following SRM transitions were chosen for quantitation: *m/z* 367.3 → 149.2 for curcumin, *m/z* 337.1 → 119.2 for DMC, *m/z* 307.2 → 119.1 for BDMC, and *m/z* 265.2 → 224.1 for honokiol (Fig. 5). Parameters including tube lens offset voltage and collision energy were optimized to obtain maximal signal intensities.

3.3. Method validation

3.3.1. Specificity

The specificity was evaluated by comparing blank, spiked and routinely prepared tumor mice homogenate samples (Fig. 6). Curcumin, DMC, BDMC and IS were eluted at 5.63, 5.31, 5.02 and

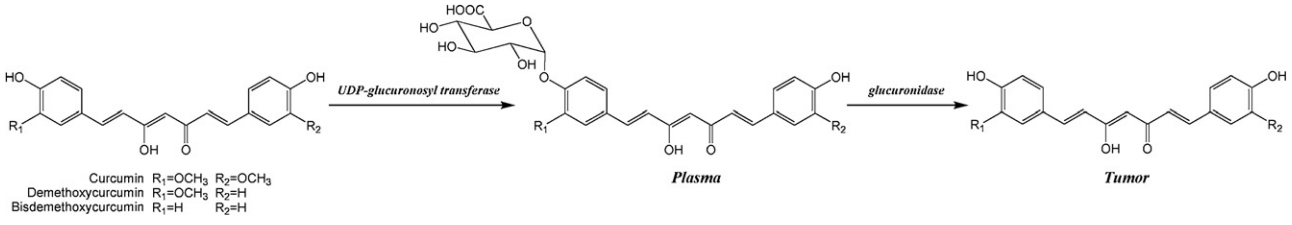

Fig. 4. A proposed metabolic pathway of curcuminoids in mice after intragastric administration.

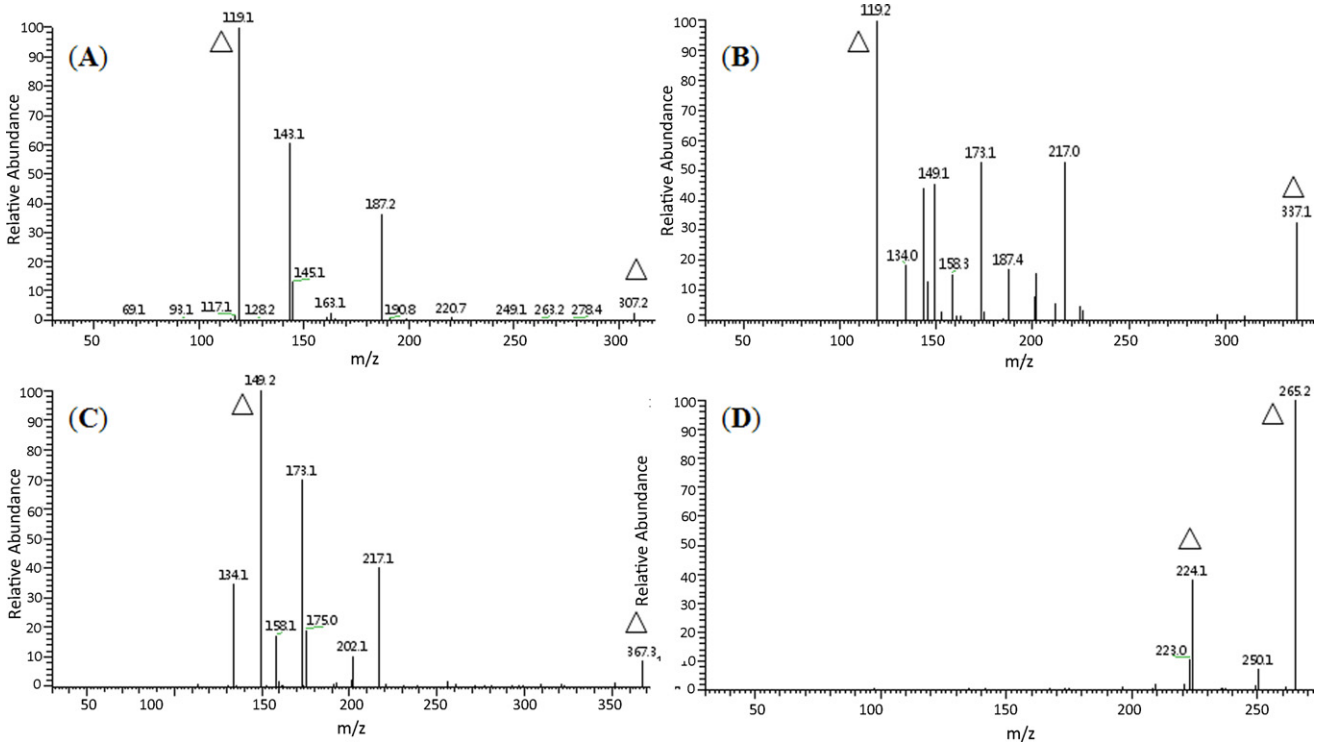

Fig. 5. (−)-ESI-MS/MS spectra of bisdemethoxycurcumin (A), demethoxycurcumin (B), curcumin (C), and honokiol (D, internal standard).

6.76 min, respectively. No apparent interference was observed in the matrix.

3.3.2. Calibration curves and limit of detection (LOD)

Calibration curves were established by analyzing a series of spiked samples as described in Section 2.7. The calibration curves ($y = ax + b$) were constructed by plotting peak area ratios (y) of each analyte to IS against analyte concentrations (x), and $1/x^2$ weighting power was applied. All the three calibration curves exhibited good linearity with correlation coefficients (r^2) of 0.997–0.999 in broad dynamic ranges (Table 2). The limit of detection (LOD) was determined at a signal-to-noise ratio (S/N) of 3:1. LODs for curcumin, DMC and BDMC were 0.02, 0.20 and 0.06 ng/mL, respectively. The lower limit of quantitation (LLOQ) was determined as the lower end of the calibration range. The LLOQs of the three curcuminoids were 2.0 ng/mL.

3.3.3. Precision and accuracy

Precisions were determined by analyzing three different concentrations of QC samples (six replicates for each concentration) for 3 consecutive days. Within- and between-batch precisions were evaluated by relative standard deviation (RSD), which ranged from 3.3–11.2% and 3.6–13.4%, respectively (Table 3). Accuracy was calculated as relative error (RE) by the following formula: $RE\% = [(C_{det} - C_{nom})/(C_{nom})] \times 100$, where C_{nom} represented the nominal concentration and C_{det} represented the mean value of detected concentrations. The calculated accuracy values for three curcuminoids were 5.5–14.3%. The above data met the FDA requirements for bioanalytical method validation, indicating this method was acceptable for pharmacokinetic studies [39].

3.3.4. Recovery

Extraction recoveries were determined by comparing routinely prepared QC samples with routinely prepared blank samples spiked with QC solutions. Recoveries were calculated by the formula: recovery (%) = concentration found/concentration spiked × 100%. Extraction recoveries determined for curcumin, DMC and BDMC after protein precipitation ranged from 78.3% to 87.7% (Table 3). The extraction recovery of IS was above 85%. The extraction recoveries were acceptable for bioanalysis.

3.3.5. Stability

Stability of the three analytes in tumor homogenate was tested at three QC concentration levels and by a series of experiments: (a) freeze and thaw stability, stability of tumor homogenate after three cycles of freezing at −20 °C and thawing at room temperature; (b) short-term room temperature stability, stability of tumor homogenate at room temperature for 4 h during sample pretreatment process; (c) long-term stability, stability of tumor homogenate at −20 °C for 1 month; and (d) post-preparation stability, stability of the extracted samples in autosampler (10 °C) for 24 h. In addition, the stability of three analytes and IS in stock and working solutions at 1–4 °C for 1 month was also evaluated. The three analytes were proved stable both in methanol (at −4 °C) and in tumor homogenate (at −20 °C) after long term storage for 1 month. Degradation of the analytes after three freeze–thaw cycles, at room temperature storage (4 h), or at autosampler storage (24 h) was below 15%, 10%, and 10%, respectively. These data indicated the analytes were stable during sample preparation and chemical analyses.

3.3.6. Matrix effect

Matrix effect was evaluated by comparing the signals produced by the same QC concentration in blank methanol and in blank mice tumor homogenate. The matrices were pretreated following the routine method, and were reconstituted with the same QC solution. No significant difference between the two matrices was observed. The results indicated the absence of obvious matrix effect for quantitative analysis.

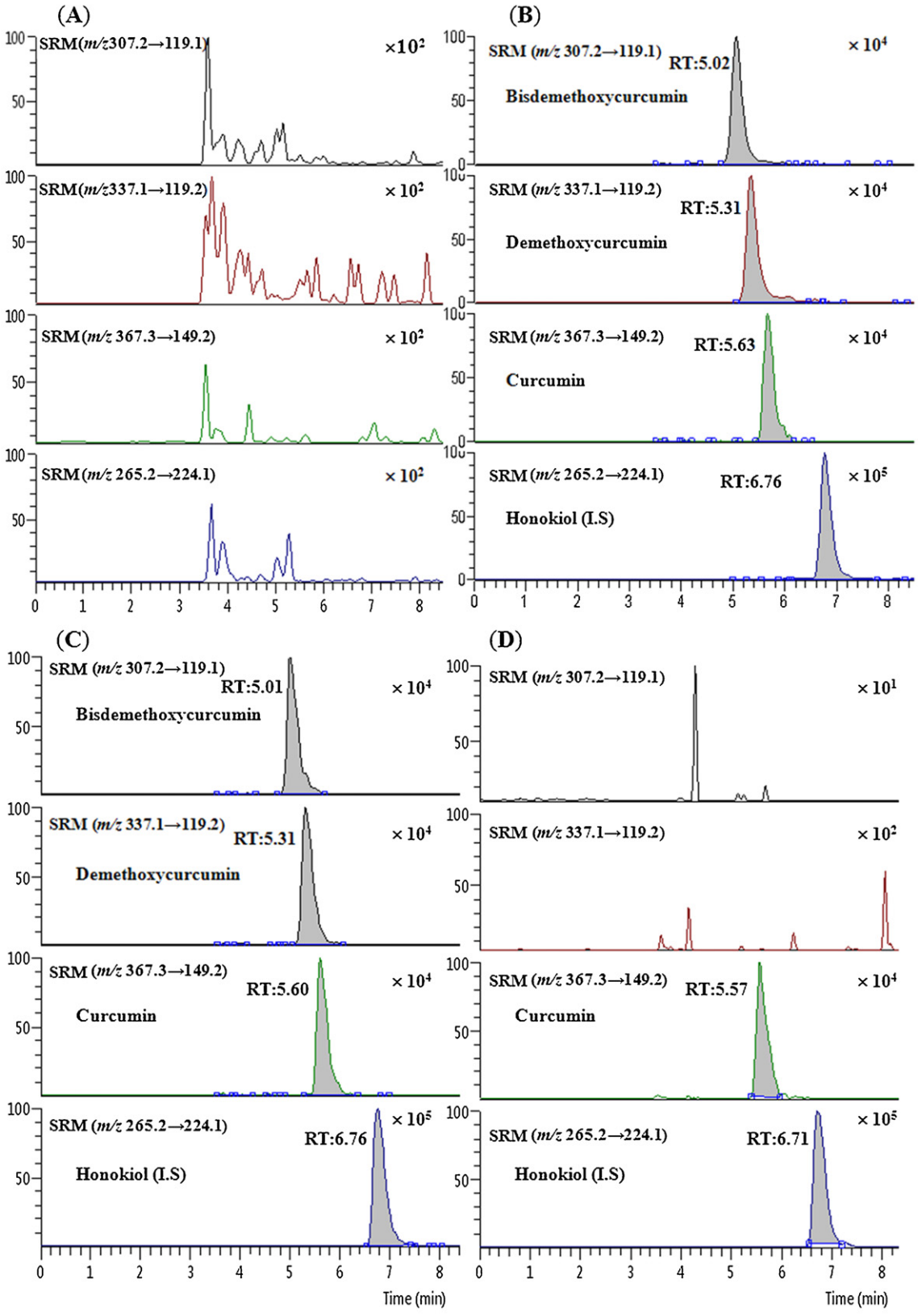

Fig. 6. Representative SRM chromatograms of curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and honokiol (IS): (A) blank tumor homogenate; (B) blank tumor homogenate spiked with the three analytes and IS; (C) tumor homogenate sample obtained 1 h after intragastric administration of curcuminoids-SLNs at a dose of 250 mg/kg; (D) tumor homogenate sample obtained 1 h after intragastric administration of curcumin-SLNs at a dose of 250 mg/kg.

Table 2
Regression equations and LODs of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC).

Analytes	Regression equation	Linear range (ng/mL)	Correlation coefficient (r^2)	LOD (ng/mL)
Curcumin	$y = 5.15 \times 10^{-4}x - 5.72 \times 10^{-4}$	2.0–2000.0	0.9993	0.02
DMC	$y = 4.89 \times 10^{-4}x + 1.14 \times 10^{-4}$	2.0–2000.0	0.9980	0.20
BDMC	$y = 7.66 \times 10^{-4}x + 4.17 \times 10^{-4}$	2.0–6000.0	0.9978	0.06

Note: In the regression equation $y = ax + b$, x refers to the concentration of three analytes (ng/mL); y, the peak area ratio of analyte to internal standard; r^2, the correlation coefficient. LOD, limit of detection.

Table 3
Precision, accuracy, and recovery of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC).

Analyte	Concentration added (ng/mL)	Within-batch ($n = 6$)			Between-batch ($n = 3$)			Extraction recovery (%, mean ± SD)
		Concentration measured (ng/mL, mean ± SD)	Precision (%, RSD)	Accuracy (%, RE)	Concentration measured (ng/mL, mean ± SD)	Precision (%, RSD)	Accuracy (%, RE)	
Curcumin	60.0	59.4 ± 6.7	11.2	−1.0	56.9 ± 7.0	12.3	−5.2	81.1 ± 3.6
	300.0	302.0 ± 31.9	10.6	0.7	315.2 ± 42.4	13.4	5.1	85.1 ± 1.7
	900.0	949.9 ± 51.8	5.5	5.5	931.1 ± 102.9	11.1	3.5	87.4 ± 3.6
DMC	60.0	51.4 ± 3.9	7.5	−14.3	52.1 ± 3.4	6.6	−13.2	78.3 ± 5.6
	300.0	264.9 ± 8.9	3.4	−11.7	267.0 ± 10.2	3.8	−10.9	84.1 ± 3.4
	900.0	791.5 ± 36.0	4.6	−12.1	782.3 ± 41.9	5.4	−13.1	85.3 ± 1.2
BDMC	60.0	52.9 ± 2.6	4.8	−11.9	52.0 ± 3.4	6.5	−13.4	83.6 ± 1.7
	300.0	258.1 ± 12.3	4.8	−14.0	257.8 ± 11.0	4.3	−14.1	83.2 ± 3.6
	900.0	791.7 ± 26.4	3.3	−12.0	784.7 ± 27.9	3.6	−12.8	87.7 ± 4.1

3.4. Determination of curcuminoids in tumor homogenate samples

The established LC/MS/MS method was used to analyze tumor homogenate samples collected at different time points after drug administration. The calculated concentrations are shown in Table 1S ($n = 5$). Pharmacokinetic curves of the three curcuminoids in mice tumor tissues after administration of curcumin-SLNs or curcuminoids-SLNs were plotted as illustrated in Fig. 7.

By examining the 0–48 h tumor concentrations of the analytes and their concentrations in curcuminoids-SLNs, we found the ratio of the three analytes changed remarkably. In the curcuminoids-SLNs formulation, curcumin, DMC and BDMC accounted for 55.3%, 17.7%, and 27.0% of total curcuminoids, respectively. However, the percentage of curcumin increased to around 75% (an average of eight time points), whereas BDMC decreased to 10%, and DMC remained at 15% of total curcuminoids in the tumor tissues (Fig. 2S). These results indicated that curcumin showed higher bioavailability and tumor tissue affinity than the other two curcuminoids. Interestingly, Sandur et al. had reported that curcumin possessed more potent NF-κB suppression activity than DMC and BDMC [40]. In addition, curcumin is the predominant curcuminoid of turmeric. Taken together, it appears that curcumin could be more promising as a potential anti-tumor drug than the other two curcuminoids.

3.5. Pharmacokinetic modeling and PK parameters

According to WinNonlin software modeling, the pharmacokinetics of curcuminoids in mice tumor tissues fit two-compartment model and first order elimination (Fig. 3S). The pharmacokinetic parameters are given in Table 4.

For tumor-bearing ICR mice administered with curcuminoids-SLNs (group I), $AUC_{0-48\,h}$ values in tumor tissues were 2811.8, 506.6 and 303.6 ng h/mL for curcumin, DMC and BDMC, respectively. T_{max} values were 1.5 h, 1.2 h, and 1.6 h, respectively. The C_{max} values were 285.2, 85.8 and 24.2 ng/mL, respectively. The $t_{1/2}{}^{\alpha}$ values were 1.9 h, 2.1 h, and 3.2 h, and $t_{1/2}{}^{\beta}$ values were 41.8 h, 39.7 h, and 19.3 h, respectively (Table 4). For those mice administered with curcumin-SLNs (group II), the $AUC_{0-48\,h}$ (2285.5 ng h/mL) and C_{max} (209.3 ng/mL) values of curcumin were slightly smaller than those of group I. Considering that the original dosage of curcumin in group I (138.2 mg/kg) was much lower than group II (250 mg/kg), it appeared that co-existing DMC and BDMC could improve the absorption of curcumin, and thus enhance its

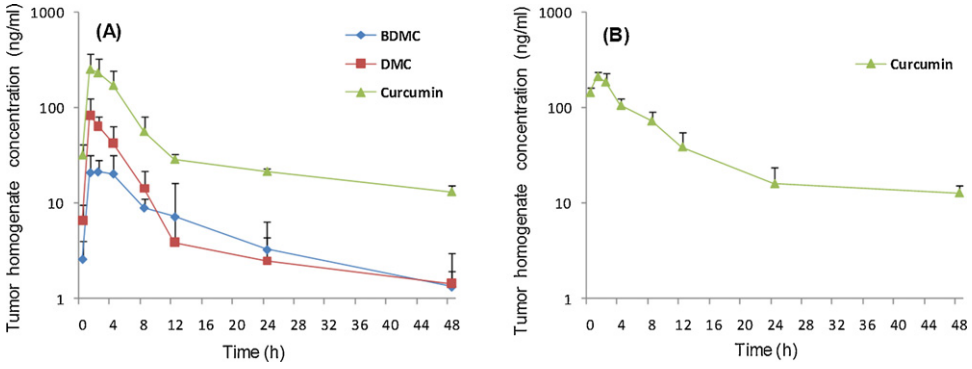

Fig. 7. Tumor concentration–time profiles of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in tumor-bearing ICR mice after intragastric administration of curcuminoids-SLNs (A) and curcumin-SLNs (B), respectively.

Table 4
Pharmacokinetic parameters of curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) in tumor tissues after intragastric administration of curcuminoids-SLNs and curcumin-SLNs to ICR mice.

Parameters	Curcuminoids-SLNs			Curcumin-SLNs
	Curcumin	DMC	BDMC	Curcumin
AUC_{0-48} (ng h/mL)	2811.8	506.6	303.6	2285.5
$t_{1/2}{}^{\alpha}$ (h)	1.9	2.1	3.2	3.1
$t_{1/2}{}^{\beta}$ (h)	41.8	39.7	19.3	28.6
V1_F (mL)	6784.5	8045.2	45,697.7	22,364.6
V2_F (mL)	31,592.2	32,992.0	43,587.8	38,197.9
CL_F (mL/h)	983.4	1747.0	4447.0	2187.7
CLD2_F (mL/h)	1313.0	899.7	3533.7	2115.3
C_{max} (ng/mL)	285.2	85.8	24.2	209.3
T_{max} (h)	1.5	1.2	1.6	0.8

exposure to tumor. According to previous reports, no evidences support that DMC or BDMC could be converted into curcumin through metabolism [41]. However, Ampasavate et al. reported that DMC could inhibit P-glycoprotein, an important efflux transporter closely related to drug absorption [42]. This may be a mechanism for co-existing curcuminoids to enhance the bioavailability of curcumin.

We also compared tumor concentrations of curcumin when it was administered in free form or as nanoparticles. Surprisingly, although nanoparticles remarkably improved the water solubility of curcumin, this formulation did not virtually improve its concentration in tumor tissues. Curcumin was administered to tumor-bearing mice at the same dosage in free form or as nanoparticles, respectively. The tumor concentrations of curcumin at 0.5, 1 and 4 h were 160.6, 122.6 and 128.3 ng/mL for free curcumin and 144.7, 212.9 and 106.1 ng/mL for curcumin-SLNs, respectively ($n = 2$, details not shown here). No significant difference was observed between these two groups. Based on this preliminary result, whether nanoparticles could improve the bioavailability of curcumin warrants further evaluation.

4. Conclusions

A tumor-bearing ICR mice model was established for metabolites identification and pharmacokinetic study of curcumin, demethoxycurcumin and bisdemethoxycurcumin in nanoparticle formulations by a fully validated LC/MS/MS method. The curcuminoids were mainly present as glucuronides in mice plasma, but in free form in tumor tissues. The pharmacokinetics of curcuminoids in mice tumor fit two-compartment model and first order elimination. Moreover, we found that co-existing curcuminoids improved the concentration of curcumin in tumor tissues. This is the first pharmacokinetic study of curcuminoids in mice tumor. The results could help understand the anti-tumor mechanism of curcuminoids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.07.042.

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