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Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC–MS/MS

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Abstract

This study presents a validated liquid chromatography technique coupled with tandem mass spectrometry (LC–MS/MS) to measure curcumin in rat plasma and provide curcuminoids analysis from the extract of *Curcumin longa* L. This method was applied to investigate the pharmacokinetics of curcumin in a freely moving rat. The analytes were separated by a reversed phase C18 column (150 × 4.6 mm I.D., particle size 5 μm) and eluted with acetonitrile-1 mM HCOOH mobile phase (70:30, v/v) with a flow rate of 0.8 ml/min in rat plasma and herbal extracts. Multiple reaction monitoring (MRM) was used to monitor the transition of the deprotonated molecule *m/z* of 367 $[M-H]^-$ to the product ion 217 for curcumin, a *m/z* of 337–217 for demethoxycurcumin and a *m/z* of 265–224 for honokiol (internal standard) analysis. The limit of detection (LOD) and quantification (LOQ) of curcumin in the rat plasma were 1 and 5 ng/ml, respectively. The method was linear in the range of 5–1000 ng/ml with a coefficient of correlation greater than 0.996 in the rat plasma. After curcumin (500 mg/kg, p.o.) administration, the maximum concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were 0.06 ± 0.01 μg/ml and 41.7 ± 5.4 min, respectively. The elimination half-life ($t_{1/2,\beta}$) were 28.1 ± 5.6 and 44.5 ± 7.5 min for curcumin (500 mg/kg, p.o.) and curcumin (10 mg/kg, i.v.), respectively. The oral bioavailability was about 1%.

Keywords: Bioavailability; *Curcuma longa*; Curcumin; Herbal analysis; Pharmacokinetics

1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Fig. 1) is a phenolic substance derived from spice herb *Curcuma longa* L., which is widely used in the food industry as a natural food coloring agent and curry powder [1]. Curcumin and its analogs are similar to other polyphenolic compounds that have received wide attention as antioxidants [1] having the potential to prevent cancer and other chronic diseases [2]. The anti-cancer effect of curcumin may be related to the specific inhibition of cyclooxygenase-2 (COX-2) expression in HT-29 human colon cancer cells [3] or the disruption of mitotic spindle structures in MCF-7 breast cancer cells [4]. Due to its pharmacological effects including wound-healing, antiviral, and anti-infectious activities [5], and anti-amyloidogenic effects, its use for treatment of Alzheimer disease has also been suggested [6].

In 1953, a liquid chromatographic method with spectrophotometric detection was developed to separate and quantify curcuminoids from *C. longa* L. [7]. Curcuminoid pigment has been described by paper or thin layer chromatographic methods [8,9]. Tonnesen and Karlsen (1983) used the Nucleosil amino stationary phase to separate the three major curcuminoids from turmeric curcuminoids [8]. Gas chromatography was used for the measurement of curcumin [10]. Capillary electrophoresis was used for the quantitative determination of curcuminoids in Curcuma rhizomes with photodiode array detection [11] or amperometric detection [12]. Another liquid chromatographic method was developed to measure curcumin

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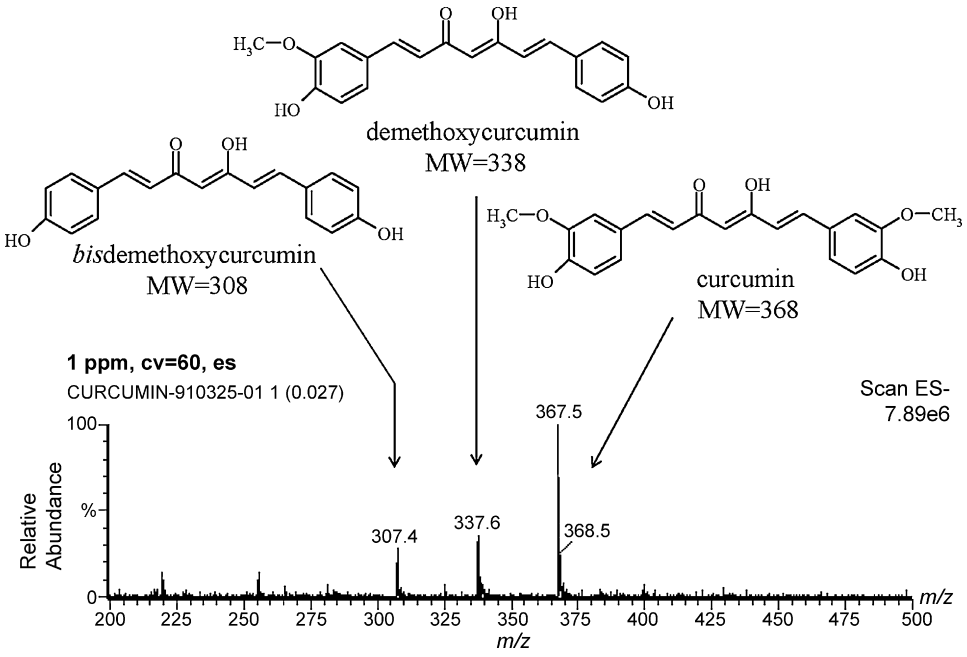

Fig. 1. The full scan mass spectrum of curcuminoid mixture, containing curcumin, demethoxycurcumin and bisdemethoxycurcumin. The molecular ion peaks of these three major ingredients are marked. The chemical structures of curcumin, demethoxycurcumin and bisdemethoxycurcumin and their molecular weights are illustrated on the top of mass spectrum.

and its related compounds [13]. The methods for measuring curcumin in biological samples have already been established with high-performance liquid chromatography (HPLC)-UV detection using liquid–liquid extraction from pig plasma and the UV wavelength set at 430 nm [14]. Health et al. reported a reversed phase LC–UV system with a UV absorbance of 262 nm for the measurement of curcumin from human plasma and urine [15]. Recently, curcumin and metabolites in biological matrices were measured by matrix-assisted laser desorption ionization time-of-flight mass spectrometry [16].

To the best of our knowledge, the use of LC–MS/MS for the determination of the content of curcuminoids in *Curcuma longa* L. and in rat plasma has not been demonstrated. We therefore develop a LC–MS/MS system for the determination and identification of the herbal ingredients curcumin and demethoxycurcumin from *C. longa* L. and present its pharmacokinetics and oral bioavailability application.

2. Material and methods

2.1. Chemicals and reagents

The curcuminoid mixture purchased from Sigma (C1386, St. Louis, MO, USA) was identified as curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 1). The high lipophilic curcuminoid mixture was purified by a normal phase silica gel column using acetone/$CHCl_3$ (5/95, v/v) as eluent in an ascending progress mode to yield curcumin and demethoxycurcumin. The structures of curcumin and demethoxycurcumin were identified by spectroscopic analysis (^{1}H-, ^{13}C- and 2D-NMR, UV, and MS) and compared with data from the literature [17]. Internal standard honokiol was also purified from our lab [18]. The authentic curcuminoids and honokiol were dissolved in methanol at a concentration of 1 mg/ml in brown glass vials and stored at 4 °C. Liquid chromatographic grade solvents and reagents were obtained from Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Herbal preparation

The crushed roots of *C. longa* L. were purchased from drug store in Taipei and identified by a research fellow C.J. Chou, National Research Institute of Chinese Medicine, Taipei, Taiwan. The grounded herbal root (1 g) was extracted with 10 ml of water, 50% ethanol and 95% ethanol boiling in water bath for 3 min and the least volume of solvent was added to the given aliquot. Each sample set was centrifuged at 8000 × *g* for 10 min, then the supernatant was filtered with a syringe filter (0.45 μm). The filtrates were dried and reconstituted with acetonitrile to 1 mg/ml for LC–MS/MS analysis.

2.3. Experimental animal

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Yang-Ming University. Male specific pathogen-free Sprague-Dawley rats were obtained from the Laboratory Animal Center of the National Yang Ming University. The animals had free access to food (Laboratory rodent diet 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time only food was removed. Six Sprague-Dawley rats (280–320 g) were initially anesthetized with pentobarbital (50 mg/kg, i.p.). During

the period of surgery, the body temperature of rats was maintained at 37 °C with a heating pad. After surgery, the rats were installed in the experimental cage and allowed to recover for 1 day.

2.4. LC–MS/MS

The LC–MS/MS analysis was performed using a Waters 2690 with a 996 photodiode assay (PDA) detector together with an automatic liquid chromatographic sampler and an autoinjection system hyphenated to a Micromass Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. The separation was achieved using a ZORBAX Extend-C18 column (150 × 4.6 mm I.D.; 5 μm, Agilent, Palo Alto, CA, USA). The system delivered a constant flow of 200 μl/min and the mobile phase consisted of 70% acetonitrile and 30% 1 mM HCOOH. The volume of injection was 10 μl.

For the operation in MS/MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface (ESI) was used. The analyte infusion experiment was performed using a Mode 22 multiple syringe pump (Harvard, Holliston, MA, USA). During the analyses, the ESI parameters were set as follows: capillary voltage, 2.5 kV for negative mode; source temperature, 80 °C; desolvation temperature, 250 °C; cone gas flow, 100 l/h; and desolvation gas flow, 500 l/h. The cone voltage of *m/z* 367 was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 60 V and the collision voltage was also adjusted to optimize the product ion signals as 15 eV for curcumin analysis. The cone voltage of *m/z* 337 was 79 V and the collision voltage was 11 eV for demethoxycurcumin analysis. The MRM used to monitor the transition of the deprotonated molecule *m/z* 367 $[M-H]^-$ to the product ion 217 for curcumin analysis and *m/z* 337 → *m/z* 217 for demethoxycurcumin analysis. All LC–MS/MS data were processed by the MassLynx version 3.5 NT Quattro data acquisition software.

2.5. Method validation procedures

All calibration curves were required to have a correlation value of at least 0.996. The intra-assay and inter-assay variabilities were determined by quantitating six replicates at concentrations of 5, 10, 25, 100, 500, 1000, 5000 and 10000 ng/ml using the LC–MS/MS method described above on the same day and six consecutive days, respectively. The accuracy (bias%) was calculated from the mean value of observed concentration (C_{obs}) and the nominal concentration (C_{nom}) as follows: accuracy: (bias%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: precision (RSD %) = [standard deviation (SD)/C_{obs}] × 100.

2.6. Recovery of herbal extracts procedures

Four sets of grounded root (1 g/set) of *C. longa* L. were prepared to evaluate the recovery of herbal extracts. Except the control group, the other three sets were individually added appropriate quantity of curcumin and demethoxycurcumin. Each 10 μg of curcumin and demethoxycurcumin was added in set 1,and 20 μg of analytes in set 2, and 40 μg in set 3. All sets were extracted with 10 ml 95% EtOH boiling in water bath for 3 min and filtered through a 0.45 μm Millipore filter to get the extracts. The extracts were analyzed by the procedures described above. The recovery of curcumin and demethoxycurcumin from the herbs was calculated as follows: recovery = [(measured concentration − basal concentration)/added concentration] × 100.

2.7. Blood sampling and sample preparation

Two groups of rats were used for the experiments. One group of rats was given orally curcumin (500 mg/kg), and the other group was intravenously injected curcumin (10 mg/kg) through the femoral vein. The automated blood sampling system DR-II (Eicom Corp., Kyoto, Japan) has been applied for blood sampling in conscious and freely moving rats. A 150 μl blood sample was withdrawn from the jugular vein into a heparin-rinsed vial with fraction collector according to a programmed schedule at 10, 20, 30, 40 and 50 min, 1, 1.5, 2, 2.5 and 3 h after dosing. Each blood sample was centrifuged at 3000 × *g* for 5 min. The resulting plasma sample (50 μl) was vortex-mixed with 100 μl of internal standard (honokiol, 0.1 μg/ml) solution. The denatured protein precipitate was separated by centrifugation at 8000 × *g* for 5 min. An aliquot (20 μl) of the supernatant was directly injected onto the LC–MS/MS for analysis. Data from these samples were used to construct pharmacokinetic curves of curcumin concentration in plasma versus time. The same sample handling process was used for the determination of precision and accuracy.

2.8. Pharmacokinetic application

Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic software WinNonlin Standard Edition Version 1.1 (Pharsight Corp., Mountain View, CA, USA) by noncompartmental method. The area under the drug concentration–time curve (AUC) is here used as a measure of the total amount of unaltered drug that reaches the systemic circulation. The oral bioavailability (*F*) is defined as the fraction of unchanged drug reaching the systemic circulation following administration through the oral route. The absolute oral bioavailability of a drug is generally measured by comparing the respective AUCs after oral and intravenous administration according to the following equation:

$$F = \frac{AUC_{p.o.}/Dose_{p.o.}}{AUC_{i.v.}/Dose_{i.v.}}$$

3. Results and discussion

3.1. LC–MS/MS

We used the full scan in negative ion modes (scan range from *m/z* 200 → *m/z* 500) to identify the analytes. With full scan mass spectra for the determination of curcumin (precursor

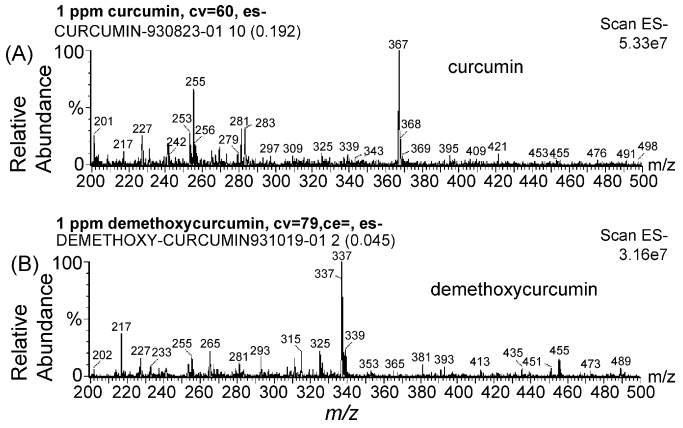

Fig. 2. Full scan mass spectra of (A) curcumin (molecular weight 368) and (B) demethoxycurcumin (molecular weight 338).

ion is 367 $[M-H]^-$) and demethoxycurcumin (precursor ion is 337 $[M-H]^-$), we applied the two cone voltages of 60 and 79 V, respectively (Fig. 2). Collision energies of 15 and 11 eV were optimal to produce the main product ion at *m/z* 217 of curcumin and demethoxycurcumin which are shown in Fig. 3. The analytes were detected in negative ionization mode by monitoring the precursor-product combination in MRM mode. After optimization, the mass transitions were *m/z* 367 → *m/z* 217 for curcumin and *m/z* 337 → *m/z* 217 for demethoxycurcumin with good symmetry and high intensity, as shown in Fig. 4. The retention time of curcumin and demethoxycurcumin were 2.46 min and 2.36 min, respectively. In the initial method development, a small signal and tailing were observed in chromatograms when the mobile phase consisted of water–acetonitrile (30:70, v/v) at a flow rate of 0.8 ml/min. In order to improve the peak shapes, formic acid (1 mM) was added to the mobile phase.

3.2. Method validation

Linear least-square regression analysis of the calibration graph on six different days demonstrated linearity between the response and the nominal concentration of curcumin and

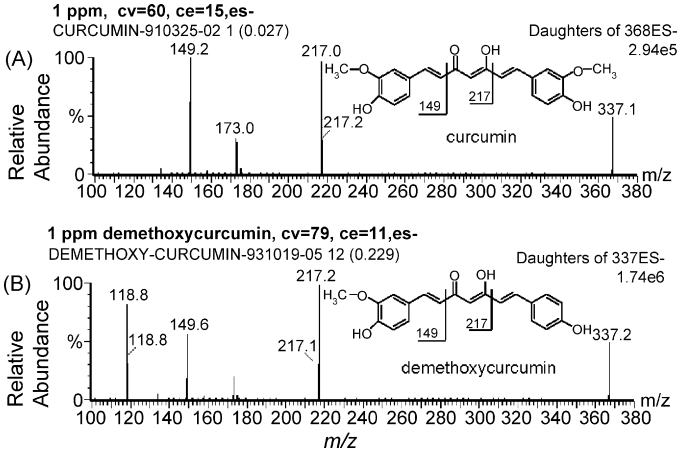

Fig. 3. Product ion scan spectra of (A) curmumin and (B) demethoxycurcumin to monitor the fragmentation transitions of *m/z* 367 → *m/z* 217 and *m/z* 337 → *m/z* 217, respectively.

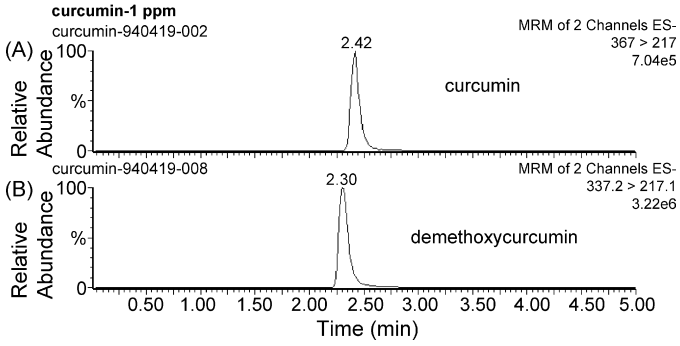

Fig. 4. LC–MS/MS chromatograms showing (A) standard curcumin (1 μg/ml) and (B) standard demethoxycurcumin (1 μg/ml).

demethoxycurcumin over the range of 2.5–10000 ng/ml. The results of linear regression analysis showed that the correlation coefficients of all standards curves were better than 0.996. The data show excellent reproducibility. The limit of detection (LOD) of curcumin was determined to be 1 ng/ml at a signal-to-noise ratio of 3. The lower limit of quantitation (LLOQ) was 2.5 ng/ml, which was defined as the lowest concentration of the linear ranges.

The intra- and inter-assay precision and accuracy values of curcumin and demethoxycurcumin standard solution are presented in Table 1 and all % of bias and RSD values were within 19%. The extraction recovery of curcumin and demethoxycurcumin in 95% ethanol has been determined at three different concentration levels of 1000, 2000 and 4000 ng/ml, respectively. This method gave good recovery for herbal extracts and the absolute recoveries of curcumin and demethoxycurcumin from 95% ethanol ranged from 91.1 to 111.6% and from 89.9 to 99.3%, as shown in Table 2.

Table 1
Inter- and Intra-day precision (RSD) and accuracy (Bias) of the curcumin and demethoxycurcumin in stock solution by LC–MS/MS

Nominal concentration (ng/ml)	Observed concentration (ng/ml)	Precision (RSD%)	Accuracy (Bias%)
Curcumin			
Intra-assay			
2.5	2.8 ± 0.5	18	12
100	99.9 ± 0.4	0.4	−0.1
10000	9500 ± 90	1.0	−5.0
Inter-assay			
2.5	2.7 ± 0.5	19	8.0
100	100.1 ± 0.4	0.4	0.1
10000	10060 ± 48	0.5	0.6
Demethoxycurcumin			
Intra-assay			
2.5	2.8 ± 0.5	18	12
100	100.3 ± 0.2	0.2	0.3
10000	9960 ± 55	0.6	−0.4
Inter-assay			
2.5	2.9 ± 0.5	17	16
100	100.2 ± 0.2	0.2	0.02
10000	10080 ± 27	0.3	0.8

Observed concentration data are expressed as mean ± SD ($n = 6$).

Table 2
Recovery (%) of curcumin and demethoxycurcumin were measured by adding these herbal ingredients in *Curcuma longa* L. for further extraction

Added concentration (ng/ml)	Measured concentration (ng/ml)	Recovery (%)
Curcumin		
1000	2248.6 ± 80.8	111.6 ± 10.4
2000	3122.2 ± 194.8	99.5 ± 9.4
4000	4774.9 ± 694.4	91.1 ± 6.3
Demethoxycurcumin		
1000	1985.3 ± 24.4	95.6 ± 5.8
2000	2828.1 ± 115.7	89.9 ± 3.5
4000	3970.6 ± 115.9	99.3 ± 2.7

The basal curcumin and demethoxycurcumin concentrations were 1132.6 ± 52.5 ng/ml and 1029.4 ± 3.1 ng/ml, respectively. Data are expressed as mean ± S.E.M. (three individual extraction was performed). Recovery = [(Measured concentration − basal concentration)/Added concentration] × 100.

3.3. Herbal analysis

Due to the similar chemical property of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, they are hardly separated to a pure stage. Bisdemethoxycurcumin is a less component in the curcumoid mixture. After repeatedly column chromatography yield curcumin and demethoxycurcumin standards, but bisdemethoxycurcumin also polluted with other curcumoid and not suit for standard use in this stage. Fig. 4 shows the chromatograms of standards of (A) curcumin and (B) demethoxycurcumin. Representative chromatogram of curcumin and demethoxycurcumin from crude extract of *C. longa* L. with 95% ethanol are shown in Fig. 5. These results indicate that the contents of curcumin in *C. longa* L. extracted by water, 50% ethanol and 95% ethanol are 0.11 ± 0.03, 14.4 ± 0.9 and 165.3 ± 2.1 mg/kg, respectively. The contents of demethoxycurcumin in *C. longa* L. extracted by water, 50% ethanol and 95% ethanol were 0.13 ± 0.01, 3.9 ± 0.2 and 43.7 ± 0.3 mg/kg, respectively (Table 3). The curcumin content in *C. longa* L. was higher than demethoxycurcumin in all extracted organic solvents, whereas the water extracts were not significantly different ($p = 0.21$; *t*-test), which may be due to the low water solubility of cuurcumin and demethoxycurcumin.

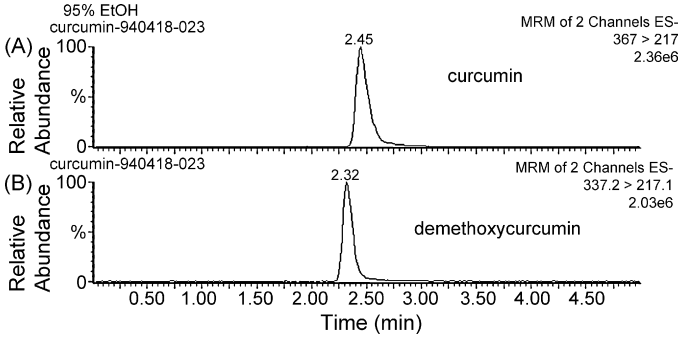

Fig. 5. LC–MS/MS chromatograms showing the contents of (A) curcumin; (B) demethoxycurcumin in 95% ethanol extract of *C. longa*.

Table 3
The extraction yields of the curcumin and demethoxycurcumin from *Curcuma longa* L.

Solvents	curcumin (mg/kg)	Demethoxycurcumin (mg/kg)
Extract with water	0.11 ± 0.03	0.13 ± 0.01
Extract with 50% ethanol	14.4 ± 0.9	3.9 ± 0.2*
Extract with 95% ethanol	165.3 ± 2.1	43.7 ± 0.3*

Data are expressed as mean ± S.E.M. ($n = 3$).
* Significant difference compared with curcumin content ($p < 0.05$; *t*-test).

Table 4
Inter- and Intra-day precision (RSD) and accuracy (Bias) of the curcumin in rat plasma by LC–MS/MS

Nominal concentration (ng/ml)	Observed concentration (ng/ml)	Precision (RSD%)	Accuracy (Bias%)
Intra-assay			
5	5.1 ± 0. 6	12	2.0
100	107 ± 13.4	13	7
10000	10075 ± 410	4.1	0.8
Inter-assay			
5	5.1 ± 0.6	12	2.0
100	101.6 ± 8.3	8.2	1.6
10000	10289 ± 629	6.1	2.9

Observed concentration data are expressed as mean ± SD ($n = 6$).

3.4. Plasma sample

The capacity factor (k') of curcumin is estimated as 3 ($t_0 \sim 0.6$ s). When the elution time is so long, the matrix effect is usually negligible. Besides, data in Table 1 and Table 4 show high recovery in both spiked herb extract and plasma samples, which proves the matrix species don't cause any interference. Fig. 6A and B show the chromatogram of a drug-free plasma extract, with mass transitions of *m/z* 367 → *m/z* 217 for curcumin and *m/z* 265 → *m/z* 224 for internal standard (honokiol), respectively, illustrating a clean baseline with no interference peaks eluted within 5 min. Fig. 7A and B show the chromatogram of a standard of curcumin (5 ng/ml) and internal

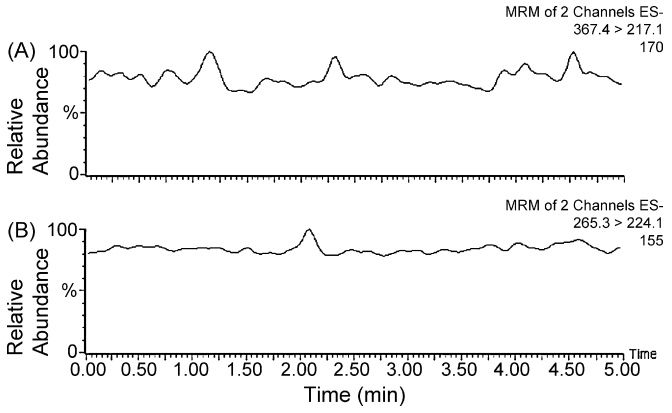

Fig. 6. LC–MS/MS chromatograms of drug-free plasma extracts acquired with monitoring the fragmentation transition of (A) curcumin (*m/z* 367 → *m/z* 217) and (B) internal standard honokiol (*m/z* 265 → *m/z* 224).

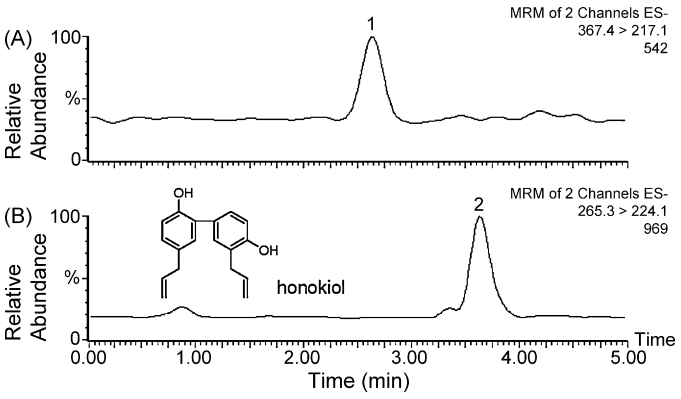

Fig. 7. LC–MS/MS chromatograms showing the rat plasma sample spiked with (A) curcumin (5 ng/ml); (B) internal standard (honokiol, 100 ng/ml).

standard (honokiol 100 ng/ml) spiked in plasma, respectively. Fig. 8A and B show the chromatogram of a plasma sample containing curcumin (53.5 ng/ml) collected from a rat plasma after curcumin administration (500 mg/kg, p.o.) and no peak distortions were visible. Each determination is completed within 5 min and no carry-over peaks were detected in subsequent chromatograms of plasma samples.

Table 4 shows the precision and accuracy of curcumin in rat plasma, and all % of bias and RSD values were within 8.2%. Mean drug plasma recovery was calculated by comparing the peak area ratios of extracted plasma samples with those obtained from unextracted calibrators with the same amount of curcumin. The extraction recovery values for curcumin in rat plasma were $97.6 \pm 4.6\%$ and $96.0 \pm 3.8\%$ for plasma at the concentration of 2.5 and 10 ng/ml.

To optimize the chromatographic condition, 1 mM formic acid was added to the mobile phase to improve peak shapes in the chromatogram. The precursor and product ions of the analytes were monitored in the multiple reaction mode as deprotonated molecule *m/z* of 367 $[M-H]^-$ to the product ion 217 for curcumin analysis and a *m/z* of 337–217 for demethoxycurcumin.

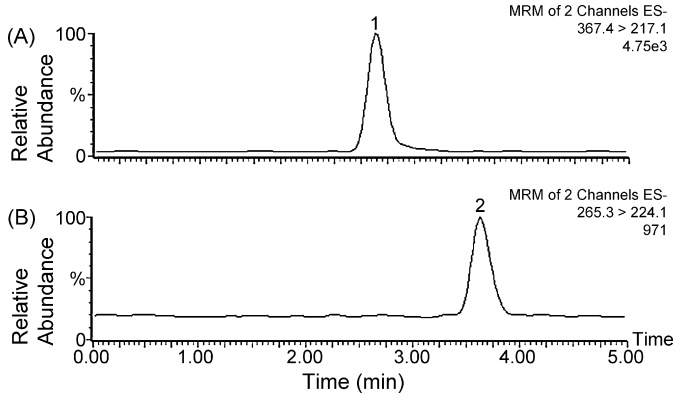

Fig. 8. LC–MS/MS chromatograms of rat plasma samples (A) containing curcumin (53.5 ng/ml) collected from rat plasma after curcumin administration (500 mg/kg, p.o.); (B) rat plasma spiked with internal standard (honokiol, 100 ng/ml). These LC–MS/MS chromatograms were acquired with monitoring the fragmentation transitions of curcumin (*m/z* 367 → *m/z* 217) and internal standard honokiol (*m/z* 265 → *m/z* 224).

This LC–MS/MS method offered several advantages over conventional LC–UV since it has higher sensitivity and shorter analytical run times to measure curcumin and demethoxycurcumin. Other methods have been described for the measurement of curcumin in aqueous solution and in biological samples [7–15]. The LC–UV detection [15] shows the LLOD of curcumin in plasma and urine to be 0.063 and 0.091 μg/ml, respectively. Recently, tetrahydrocurcumin, one of the major metabolites of curcumin has been detected in plasma and urine by LC–UV with high sensitivity of 0.05 μg/ml [19]. It is well known that tandem mass spectrometric methods provide superior sensitivity to that of spectrophotometric methods. In general, mass spectrometric methods do not require any chemical modifications on the analyte compounds [20,16]. The present paper demonstrates the high speed of analysis and excellent specificity of this LC–MS/MS method, which was used to monitor curcumin and demethoxycurcumin with retention times less than 3 min. Furthermore, the short run time analysis allows increased sample throughput for routine purposes and additional pharmacokinetic application.

3.5. Pharmacokinetic application

This analytical method was developed for the application in pharmacokinetic study of curcumin in a freely moving rat. Fig. 9 illustrates the concentration versus time profiles of curcumin with a single intravenous and oral dose administration to six individual rats for each group. The areas under the concentration versus time curves were 7.2 ± 1.2 min μg/ml and 3.6 ± 0.6 min μg/ml for intravenous (10 mg/kg) and oral (500 mg/kg) doses, respectively (Table 5). The oral bioavailability of curcumin in a freely moving rat was about 1%. Recent search indicates that intragastric gavage of the rats with 60 mg/kg curcumin for 4 consecutive days led to a down-regulation of the intestinal P-glycoprotein (P-gp) level, up-regulation of hepatic P-gp level, but the renal P-gp level was unaffected [21]. Addition with hydrophobic property of curcumin led to

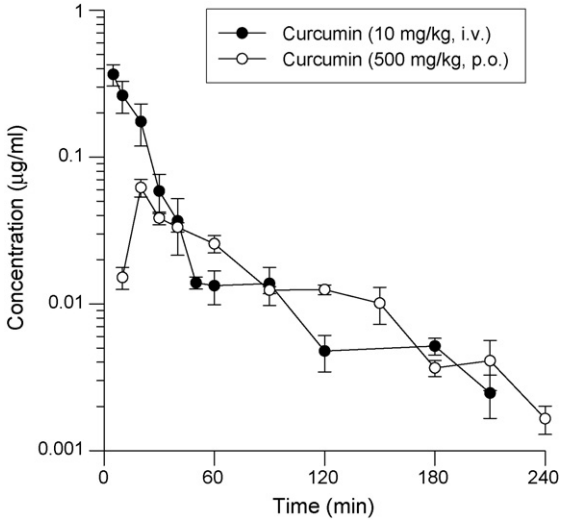

Fig. 9. Concentration vs. time curves of curcumin after drug administration (500 mg/kg, p.o. and 10 mg/kg, i.v.) in rats.

Table 5
Pharmacokinetic data after curcumin administration (10 mg/kg, i.v. and 500 mg/kg, p.o.) in rats

Parameters	Curcumin	
	10 mg/kg, i.v.	500 mg/kg, p.o.
AUC (min μg/ml)	7.2 ± 1.2	3.6 ± 0.6
$t_{1/2,\beta}$ (min)	28.1 ± 5.6	44.5 ± 7.5
C_{max} (μg/ml)	0.36 ± 0.05	0.06 ± 0.01
T_{max} (min)		41.7 ± 5.4

Data are expressed as mean ± S.E. mean from six individual experiments for each group.

the limitation of oral bioavailability. A novel formulation of curcumin–phospholipid complex was developed to improve the oral absorption and liver protection of curcumin in rats [22]. LC–MS/MS should be an excellent tool for pharmacokinetic study of curcumin.

In summary, we have developed a sensitive, specific and reliable LC–MS/MS assay for the determination of curcumin and demethoxycurcumin in herbal extracts and curcumin in rat plasma. The method is rapid, accurate and precise, so it can be used as an analytical tool for the quality control of various species of turmeric herbs. In addition, the method was applied in the pharmacokinetic study in freely moving rat and its oral bioavailability was about 1%.

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