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A validated LC-MS/MS method for quantitative analysis of curcumin in mouse plasma and brain tissue and its application in pharmacokinetic and brain distribution studies

Prakash Ramalingam, Young Tag Ko*

College of Pharmacy, Gachon University, Incheon 406-799, South Korea

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ABSTRACT

Curcumin is a well-known multitherapeutic agent widely employed in neurodegenerative disorders and cancer. A selective, fast, and sensitive method employing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was developed and validated for the simultaneous determination of curcumin in mouse plasma and brain tissue, by using salbutamol as an internal standard. Triple quadrupole mass detection with multiple reaction monitoring (MRM) mode was used to monitor the ion transitions, *m/z* of 369 > 285 for curcumin, and *m/z* of 240 > 148 for salbutamol. The method was validated for recovery, accuracy, precision, linearity, and applicability. The lower limits of quantification (LLOQ) in both matrices were 2.5 ng/mL. The inter-day and intra-day precisions and accuracy values were within the Food and Drug Administration (FDA) acceptance criteria, for both matrixes. The method was successfully applied in pharmacokinetics and brain distribution studies of curcumin after intravenous administration of free curcumin and curcumin-loaded solid lipid nanoparticles to mice. Furthermore, the application of this method along with serial blood sampling in mice has led to significant reduction in animal use and dosage and drastic improvement in speed, throughput, and quality of pharmacokinetic parameters.

1. Introduction

Pharmacological induction of neurogenesis in neural stem cells (NSC) may reduce neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [1]. The identification of neurogenesis-inducing therapeutic molecules may help to develop regenerative therapies for neurodegenerative disorders. Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione; also called diferuloylmethane, Fig. 1) is a polyphenolic phytochemical extracted from the root of *Curcuma longa*, commonly called turmeric. Numerous studies have shown the antioxidant, anti-inflammatory, antimalarial, and antitumor properties of curcumin [2,3]. Moreover, several clinical and animal models studies have shown the neuroprotective activity of curcumin in neurodegenerative and neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [4,5].

Despite the potential therapeutic effects of curcumin, clinical applications are restricted by its short systemic retention and poor bioavailability in the brain because of poor aqueous solubility at physiological pH, instability, low absorption, rapid elimination, and limited blood–brain barrier (BBB) permeability [6,7]. Several studies, therefore, have aimed to improve curcumin circulation and retention by using approaches ranging from formulation that use adjuvants, nanoparticles, liposomes, and micelles, to those using phospholipid complexes, derivatives, and analogs [8].

To systemically examine the preclinical plasma pharmacokinetics (PK), and in particular, the brain accumulation of curcumin after *in vivo* administration of various formulations, a highly sensitive and fast assay is necessary. LC-MS/MS is a commonly used analytical tool to quantify drugs from biological matrices for pharmacokinetic evaluation, and it is an established method because of its accuracy, sensitivity, and specificity. LC-MS/MS-based analytical methods have been described for the quantification of curcumin from rat plasma. The sample extraction method in previous studies involved liquid–liquid (LLE) extraction by the addition of 0.1 mL of rat plasma to more than 1 mL of extraction solution [9–11]. Another study showed an LC-MS/MS method for the measurement of curcumin from 0.5 mL of mouse plasma by using with 1.4 mL of acetonitrile extraction solution [12]. However, typically,

* Corresponding author at: College of Pharmacy, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 406-799, South Korea. Tel.: +82 32 899 6417; fax: +82 32 820 4829.
E-mail addresses: youngtakko@gachon.ac.kr, youngtakko@gmail.com (Y.T. Ko).

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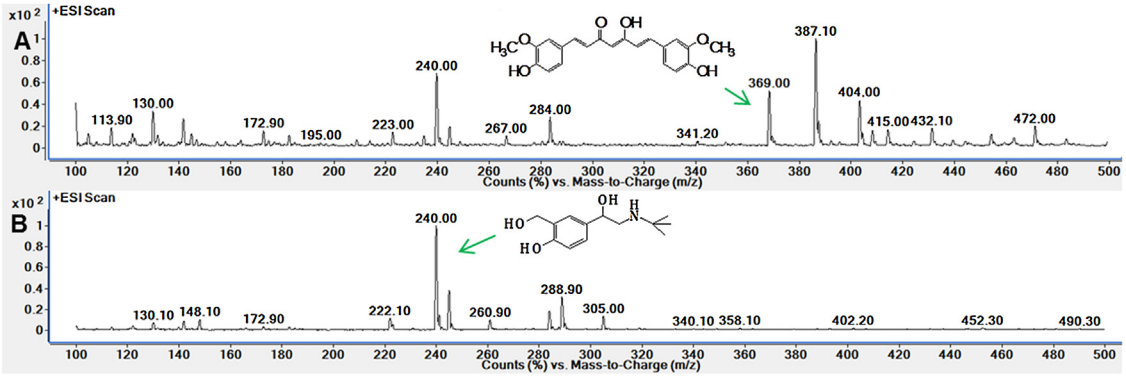

Fig. 1. Full-scan mass spectra of curcumin (molecular weight 369) (A), and salbutamol (molecular weight 240) (B) as obtained in positive ion mode.

pharmacokinetic studies in mice are performed in the traditional manner by using discrete blood sampling, resulting in the dosing and euthanizing of a large number of animals and occurrence of variance in pharmacokinetic parameters because of dosing error and inter-animal variability [13]. Further, pharmacokinetic studies by discrete blood sampling method are time consuming and labor intensive, and therefore, alternatives, such as serial blood sampling methods have been developed and applied [14]. However, only a limited number of studies have quantified curcumin levels in mouse plasma and brain tissues by serial blood sampling.

We studied various nanocarrier systems such as solid lipid nanoparticles (SLNs) with the objective of developing curcumin formulations that enhance bioavailability and brain accumulation. Further, we developed a highly sensitive and fast LC-MS/MS method for the quantification of curcumin in blood and brain tissues, by using a minimal sample volume of less than 10 μL for systemically examining the PK and, in particular, the brain delivery efficiency of curcumin after *in vivo* administration of various nanocarrier formulations. Eight serial blood samples were collected from a single animal by using lateral saphenous vein puncture, and the whole brains were fractionated into parenchymal and capillary fractions by using capillary depletion methods. The validated LC-MS/MS method was successfully applied to determine the curcumin contents in the plasma and brain fractions, enabling reliable and fast evaluation of *in vivo* behavior and brain delivery of curcumin-loaded nanocarriers. To the best of our knowledge, this is the first study that describes a validated LC-MS/MS method for the quantification of curcumin in a minimal volume of mouse plasma, uses serial blood sampling method, and studies curcumin distribution in the brain tissue after crossing the BBB by using the capillary depletion method.

2. Materials and methods

2.1. Chemicals and reagents

Curcumin (purity >99% by HPLC) was purchased from the TCI Co., Ltd. (Tokyo, Japan). Salbutamol (internal standard [IS], purity >99%, by HPLC), formic acid, and sodium hydroxide were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The mobile phase acetonitrile (Avantor, PA, USA) and water (Fisher Scientific Korea Ltd., Korea) were of HPLC grade. HPLC grade 0.01% formic acid was prepared with de-ionized water, which was purified by Milli-Q purification system (Millipore, MA, USA). All other chemicals and reagents were of analytical grade and used without further purifications.

2.2. Preparation of solid lipid curcumin nanoparticles

Solid lipid curcumin nanoparticles (SLCNs) were produced by the high-shear homogenization and ultrasonication. The lipid phase, consisting of 20 mg of palmitic acid and 2 mg of cholesterol, was heated and melted to a temperature of 5–10 °C above the melting point of the constituents. Curcumin (1 mg) was dispersed in the hot and molten lipid phase. Thereafter, the preheated aqueous solution containing 6 mg of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was added to the lipid phase and a pre-emulsion was formed by using Ultra-Turrax homogenizer (IKA-Werke, Staufen Germany) at 11,000 rpm for 5 min The resulting pre-emulsion was further sonicated using a probe sonicator (Vibracell VCX130; Sonics, USA) for 5 min. The resulting SLCNs nanodispersion was cooled down to room temperature and lyophilized by freeze-drying (Scan Vac, South Korea). The final SLCNs were stored at 4 °C for further experiments.

2.3. Experimental animals, drug administration, and serial sampling

All experimental animal protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Gachon University and performed according to the IAEC guidelines. For *in vivo* studies of the PK and drug distribution in the brain, male Balb/c mice (4 weeks, 20–25 g) were used.

Pharmacokinetic studies: One group of mice received SLCNs at a dose equivalent to 4 mg/kg curcumin by tail vein injection. Free curcumin diluted in 25% Tween 80 was injected in the second group of mice for pharmacokinetic comparison. Serial blood samples (20 μL) were drawn from the saphenous vein at 0.25, 0.5, 0.75, 1, 2, 4, and 8 h after intravenous administration. Blood samples collected in heparinized polythene tubes were centrifuged for 10 min at 4000 rpm and, 4 °C to obtain the plasma. Plasma was separated and stored at −80 °C until further analysis.

Curcumin distribution in the brain: A separate group of mice received tail vein injection of SLCNs and free curcumin at a dose equivalent to 4 mg/kg curcumin. At predefined time intervals of 15, 30, and 60 min after the injection, blood was withdrawn by cardiac puncture. After blood sampling, the mice were decapitated and the whole brains were removed and weighed carefully. Brain tissues were stored and homogenized using capillary depletion method.

2.4. Sample preparation

2.4.1. Plasma

For plasma, 10 μL of mouse plasma sample was spiked with 10 μL of IS working solution (200 ng/mL) containing the mobile

phase followed by 10 μL of 0.5 M sodium hydroxide, for enhancing the curcumin extraction. The mixture was vortexed for approximately 1 min, prior to liquid–liquid extraction (LLE) with 250 μL of ethyl acetate to isolate curcumin. After centrifugation at 4 °C and, 10,000 rpm for 10 min, the organic layer was transferred to another tube and evaporated under vacuum. Finally, the residue was reconstituted in 20 μL of mobile phase (acetonitrile and 0.01% formic acid, 50:50, v/v), vortexed for 1 min, and transferred to a plastic insert with an amber glass autosampler vial. An injection volume of 2 μL was used in the LC-MS/MS system.

2.4.2. Brain tissue

To quantify curcumin content in the brain tissue fractions, including the capillary and brain parenchymal fractions, capillary depletion method was used [15]. In brief, the brains were weighed and homogenized (10 strokes) in 0.8 mL of RHB-buffer (10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM $CaCL_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, and 10 mM D-glucose, pH 7.4) by using an ice-cold glass homogenizer. Afterward, 1.6 mL of 26% dextran was added followed by a second round of homogenization (3 strokes). The homogenate was separated into a supernatant fraction (brain parenchymal fraction) and a pellet fraction (capillary fraction) by centrifugation for 15 min at 4 °C and 5400 × *g* Whole brain homogenate and separated brain fractions were extracted and analyzed for curcumin as described below.

To 40 μL of the whole brain homogenate were added 10 μL of the IS (salbutamol, 200 ng/mL) containing mobile phase and 10 μL of 0.5 M sodium hydroxide. The mixture was vortex-mixed for approximately 1 min. The mixture was subject to the LLE with 250 μL of ethyl acetate. The organic layer was separated after centrifugation at 4 °C and 10,000 rpm for 10 min and evaporated to dryness under vacuum. The dried residue was reconstituted with 20 μL of mobile phase and transferred to an autosampler vial. The injection volume was 2 μL. The same extraction method was followed for 0.1 mL of pellet and supernatant samples.

2.5. LC-MS/MS conditions

The LC-MS/MS system comprised of Agilent LC 1100 series (Agilent Technologies, CA, USA) binary pump, vacuum degasser and auto-sampler system connected to a 6490 triple quadrupole MS equipped with electrospray ionization (ESI) source with Agilent jet stream technology. ChemStation was used for system control, data processing, and data acquisition for both LC and mass spectrometry.

Chromatographic separation was achieved by an analytical Sepax BR-C18 (5 μm, 120 Å 1.0 × 100 mm) column. The column temperature was maintained at 30 °C. The temperature of the autosampler was set 4 °C. Sample solutions (2 μL) were injected, and the analytes were eluted using acetonitrile and 0.1% formic acid in water (50:50, v/v) pumped at a constant flow of 0.2 mL/min. The isocratic separation run time was 4 min.

MS/MS system was performed under positive ESI and multiple reactions monitoring (MRM) mode was used to identify the compounds of interest. The MS operational parameters were as follows: argon as a collision gas, capillary voltage at 5 kV, gas flow 15.1 L/min, source temperature at 225 °C, and collision energies of 14 and 12 eV for curcumin and salbutamol, respectively. Detections of the analytes were performed using MRM mode to monitor the precursor-to-product ion transitions of 369 > 285 *m/z* for curcumin and 240 > 148 *m/z* for salbutamol, respectively. Mass data were collected and processed using the MassHunter QQQ Qualitative and Quantitative software (Version B.04.00, Agilent Technologies, Inc., USA).

2.6. Preparation of stock solutions and calibration standards

Stock solutions of curcumin and IS were prepared separately in methanol at a target concentration of 1 mg/mL and aliquoted for storage. Stock working solutions of curcumin were prepared by diluting the stock solutions with mobile phase to calibration standards (2.5–2000 ng/mL) and quality control (QC) samples (1000, 500, 5 and 2.5 ng/mL) in individual biological matrices (blank mouse plasma, and brain homogenate). Working solution of salbutamol was prepared by diluting the stock solution with mobile phase to 200 ng/mL. All the stock and working solutions were stored at −80 °C.

Calibration standards were prepared separately by spiking 10 μL of blank plasma and 40 μL of brain homogenate with 10 μL of the calibration standards containing IS, which was diluted with the matrix to give nine calibration standards in the range of 2.5–2000 ng/mL for plasma, and nine calibration standards in the range of 2.5–2000 ng/mL for brain homogenate. The remaining procedure was the same as that described in Section 2.4.

2.7. Bioanalytical method validation

The method was developed and validated according to the U.S. Food and Drug Administration (FDA) guidelines for Bioanalytical Method Validation, which was published in 2001. The validation method was conducted for curcumin in two biological matrices of mouse: plasma and brain tissue. This analytical method was validated based on specificity, linearity, accuracy, precision, recovery, and stability.

2.7.1. Specificity

The specificity of the analytical method was investigated by checking the mouse plasma and brain homogenate samples to investigate the peak potential interferences at the peak region of curcumin and IS using the established LLE extraction procedure and bioanalytical condition.

2.7.2. Calibration, linearity, and limit of quantification

Calibration standard samples were prepared by spiking IS and different concentration of curcumin to the matrices (drug free mouse plasma and brain homogenate). The nine-point calibration curves ($y = ax + b$) were constructed by plotting peak area ratios of curcumin analyte to the IS (y) *versus* curcumin concentration (x). The linearity of the assay was evaluated with calibration standards over the different concentration range of 2.5–2000 ng/mL. Percentage coefficient of variation (%CV) and percentage relative error (%RE) of nine calibration standards containing mouse plasma and brain homogenate were performed on three separate occasions, respectively. The slope, intercept, and correlation coefficient (r) were measured by $1/x^2$ weighting factor. The limit of quantification (LOQ) was determined as the lower concentration of the calibration standards at which the precision was less than 20% and accuracy was within ±20% of the nominal concentration.

2.7.3. Precision and accuracy

Precision was determined by quality control (QC) samples at three occasions. Intra-day precision of the assay was determined within one day by repeating analysis of the QC samples at three concentration levels (high, medium, and low) ($n = 6$). Inter-day accuracy and precision were determined by repeating analysis of QC samples on three different days. Accuracy of the method was calculated as follows: Accuracy = [(measured concentration − nominal concentration)/nominal concentration] × 100. The acceptable values of accuracy and precision with relative standard deviation (RSD) were as follows: accuracy within ±15% and precision less than 15%.

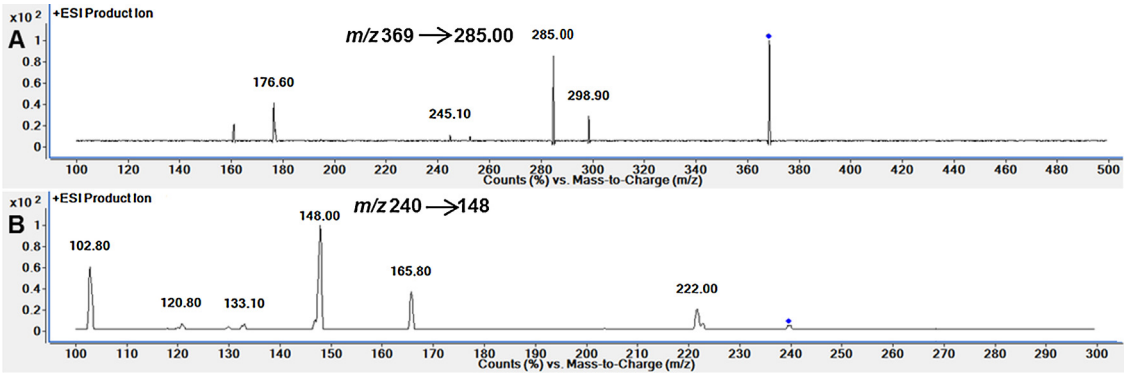

Fig. 2. Product ion spectra of $[M+H]^+$ ion of curcumin (A), and salbutamol (B) as obtained in positive ion mode.

2.7.4. Matrix effects

Post extraction spike method was used to evaluate the matrix effects. The matrix effects of analyte and IS were calculated in six different plasma and brain homogenate lots by calculating ratio of the peak area in the presence of mouse plasma or brain homogenate (B) (matrix spiked with HQC, MQC and LQC) to the peak area of the standard curcumin in the reconstitution solvent (A), and expressed as [%ME = $B/A \times 100$]. A value of %ME = 100% is indicative of no matrix effects, a value %ME < 100% suggests ionization suppression and a value %ME > 100% suggests ionization enhancement.

2.7.5. Recovery

The LLE efficiency was determined by comparing the peak areas of post-spiked standard solutions (S_{post}) with areas of pre-spiked standard solutions (S_{pre}). The recovery (RE) of curcumin was calculated as follows: RE (%) = $S_{post}/S_{pre} \times 100$.

2.7.6. Stability

The stability of curcumin in mouse plasma and brain tissue was assessed by maintaining the three different concentrations of QC samples at room temperature for 5 h, in the autosampler for over 24 h, and at −20 °C for 3 weeks, to determine the short and long-term stabilities, respectively. Freeze/thaw stability was assessed after three cycles at −20 °C.

2.8. Data analysis and PK

All quantification and calibration data were processed using Excel spreadsheet software (Microsoft Office 2010, Microsoft, Redmond, USA). Pharmacokinetic parameters for curcumin in mouse plasma were calculated using noncompartmental analysis (NCA) in WinNonlin software (version 2.1, Pharsight, USA). The volume of distribution (V_d) for whole brain, supernatant (brain parenchyma) and pellets (capillary fraction) was calculated by the ratio of the concentration in brain fractions (C_{br}) to plasma concentration (C_{pl}) of drug at different time points, *i.e.*, V_d (l/kg) = C_{br} (ng/kg brain)/C_{pl} (ng/l plasma). The pharmacokinetic parameters, including observed maximum concentration (C_{max}), area under the curve (AUC), and area under the first moment curve (AUMC) were calculated using log-linear trapezoidal rule. The mean residence time (MRT) was determined based on AUMC/AUC. Terminal half-life ($t_{1/2}$) was calculated from 0.693 × MRT.

3. Results and discussion

3.1. Selection of internal standard

Choosing an appropriate internal standard is the key of biological sample analysis. Previous studies have shown that salbutamol is used as an internal standard due to its accessibility, stability under analysis procedure, extraction efficiency, and consistency with the analytes at retention time [10,11]. In order to apply the analytical method for curcumin PK and brain distribution studies, salbutamol was selected as the IS because of its similar chromatographic behavior with curcumin. Salbutamol was found to be practicable and acceptable owing to its good correction for curcumin.

3.2. Optimization of mass spectrometric and chromatographic conditions

The full-scan mass spectrum of curcumin and the IS obtained during quantitative method development displayed a precursor ion $[M+H]^+$ at m/z of 369 and 240, respectively (Fig. 1). The optimal values of collision energies were 14 and 12 eV for producing strong product ions at m/z of 285 and 148 for curcumin and salbutamol, respectively (Fig. 2). Curcumin and salbutamol were detected in positive ESI with MRM mode.

The optimized mobile phase composition produced stable and acceptable peak shapes for curcumin and salbutamol with the 50:50 mixtures of acetonitrile and 0.1% formic acid. The addition of 0.1% formic acid in water to the mobile phase improved the peak shape and accuracy of the analytes. After optimization, the chromatogram of curcumin and salbutamol from both mouse plasma and brain homogenate samples showed good symmetry and high density (Figs. 3 and 4). The retention times of curcumin and salbutamol were 2.2 and 0.6 min, respectively.

3.3. Specificity

The specificity of the analytical method was determined by comparing the chromatograms of curcumin and IS in mouse plasma and brain homogenate samples (standard and sample). Representative chromatograms are shown in Figs. 3 and 4, including blank mouse plasma and brain homogenate samples (Figs. 3A and 4A), plasma and brain homogenate containing curcumin and IS (Figs. 3B, 4B and 3C, 4C), plasma sample collected at 1 h following SLCNs administration (Fig. 3D), and brain homogenate sample obtained at 0.25 h following the administration of SLCNs (Fig. 4D). The method was found to be specific, and no endogenous plasma components or other impurities interferences were observed at the peak region of curcumin and IS.

3.4. Calibration, linearity, and limit of quantification

The calibration curve was linear in peak area ratios over the concentration range of 2.5–2000 ng/mL of curcumin in both mouse plasma and brain homogenate samples, with a correlation

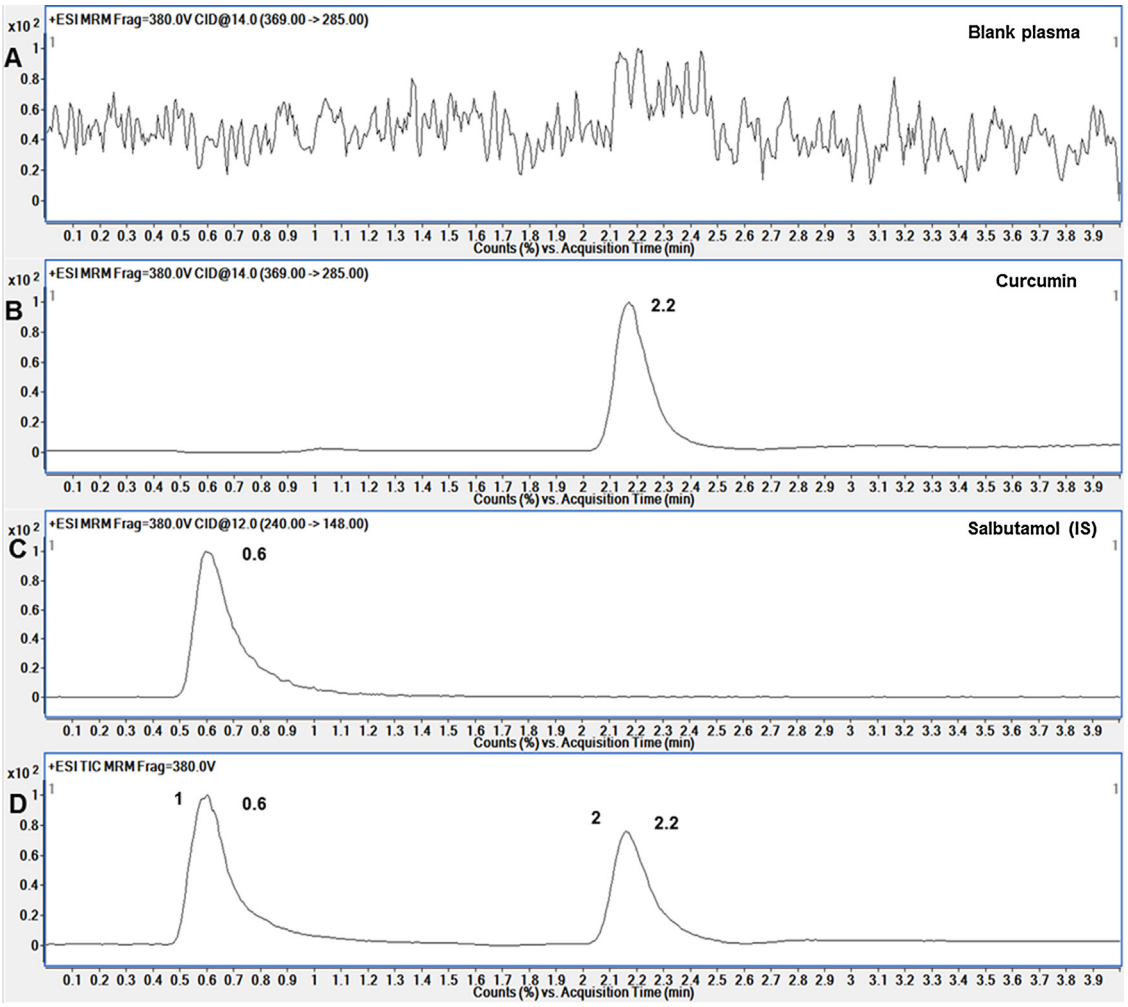

Fig. 3. Representative chromatograms after multiple reaction monitoring (MRM) of curcumin and salbutamol in mouse plasma. Blank plasma (A), blank plasma spiked with the curcumin (B) and salbutamol (C), and typical chromatograms of plasma sample collected at 1 h following SLCNs administration (D) (1) salbutamol, (2) curcumin.

coefficient $r = 0.99$ and consistent slope value when determined using weighting factor of $1/x^2$. %RE and %CV of calibration standards ran on three separate occasions for mouse plasma and brain homogenate are provided in Table 1, respectively. %RE and %CV of calibration standards met the acceptance limits set by the FDA. The lower limit of quantitation (LLOQ) for curcumin in mouse plasma and brain homogenate was 2.5 ng/mL.

3.5. Accuracy and precision

The results of intra-day ($n = 6$) and inter-day ($n = 6$) accuracies and precisions of curcumin and salbutamol estimated by evaluating three concentrations of QC samples (2.5 (LLOQ), 5, 500, 1000 ng/mL) are shown in Table 2. Intra- and inter-day precision in mouse plasma and brain tissue was less than 15% for all concentrations. All bias and RSD values met the acceptance limits set by the FDA guidance Bioanalytical Method Validation (2001).

3.6. Matrix effects

Effects of biological matrix on ion suppression/enhancement were evaluated at three concentration levels (HQC, MQC and LQC) for curcumin and at a single concentration for the IS. The detailed matrix results are shown in Table 3. The matrix effect of curcumin at three QC levels and salbutamol were found to be within the acceptable limits (90–110%). These results indicated that ion suppression/enhancement from the mouse plasma and brain homogenate were negligible under the current method conditions.

3.7. Recovery

The LLE recovery of curcumin was evaluated by comparing the curcumin/IS peak area ratios of extracted QC standards (5, 500, 1000 ng/mL for curcumin) with those from the of nonextracted same-concentration standards for both mouse plasma and brain homogenate. The recovery results are summarized in Table 4. The method provided excellent recovery for curcumin from plasma and brain homogenate samples.

3.8. Stability

Stability of curcumin was investigated using different concentrations of QC plasma and brain homogenate samples. Excellent recoveries of curcumin and salbutamol were observed at different storage conditions as shown in Table 5. No significant loss of curcumin in either plasma or brain homogenate was observed after storing the sample for bench-top stability at room temperature (at least 4 h), 24 h in the autosampler tray, after 2 weeks of storage at −80 °C and three freeze/thaw cycles.

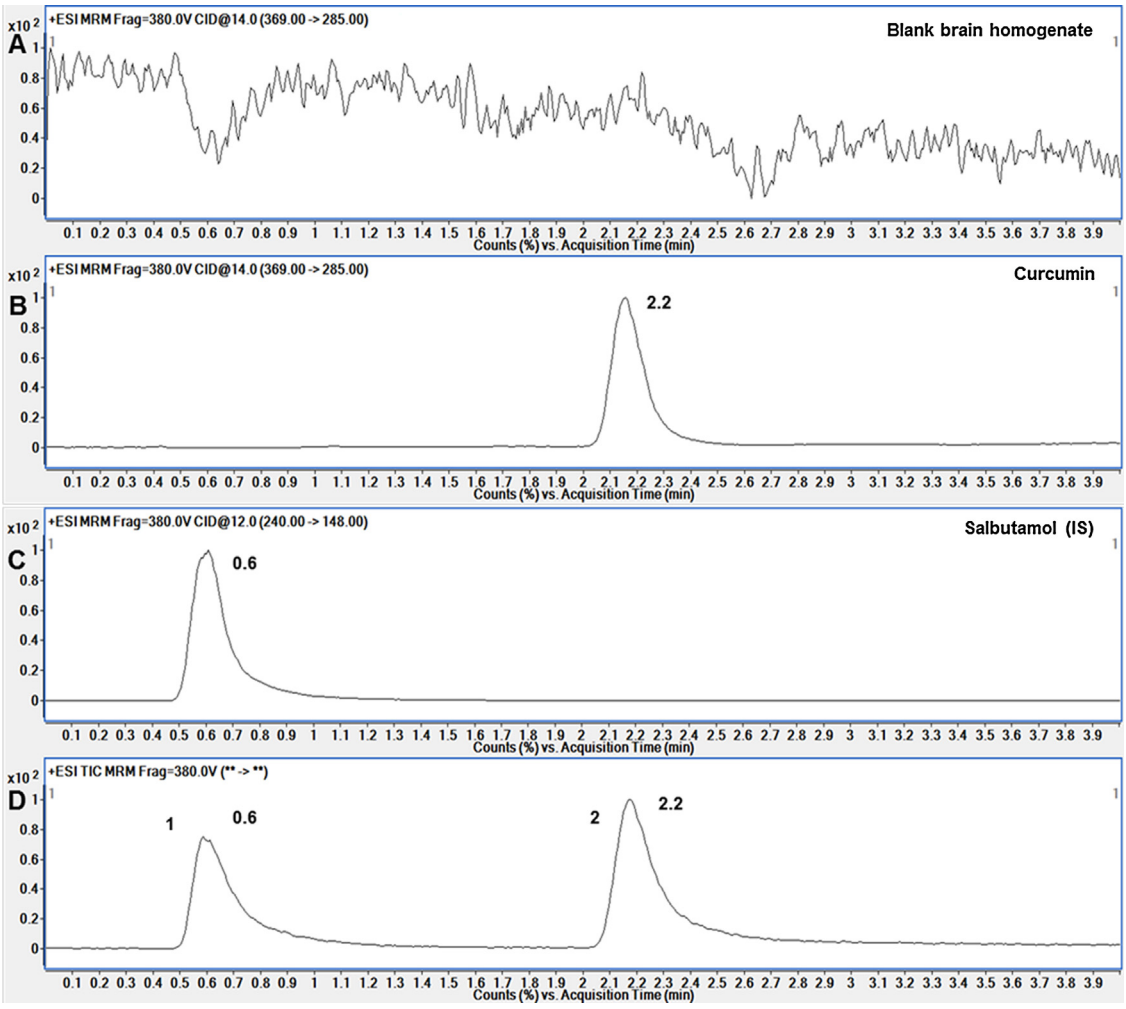

Fig. 4. Representative MRM chromatograms of curcumin and salbutamol in mice brain homogenates. Blank brain homogenates (A), blank brain homogenate spiked with curcumin (B) salbutamol (C), and typical chromatograms of brain homogenate sample collected at 0.25 h following the administration of solid lipid nanoparticles (SLCNs) (D) (1) salbutamol, (2) curcumin.

Table 1
Back calculated curcumin concentration of mouse plasma and brain homogenate calibration standards at three different occasions ($n = 6$).

Nominal concentration (ng/mL)	Plasma			Brain		
	Measured concentration (ng/mL)	% RE	%CV	Measured concentration (ng/mL)	% RE	%CV
2000	2040.6 ± 33.8	−2.0	1.4	2034.1 ± 31.2	−1.7	1.5
1000	1018.7 ± 21.5	−1.87	2.1	1015.0 ± 20.4	−1.5	2.1
500	514.0 ± 14.8	−2.8	2.9	509.3 ± 14.3	−1.8	2.8
250	249.6 ± 8.8	0.1	3.5	246.7 ± 9.0	1.2	3.6
100	100.1 ± 3.6	−0.1	3.7	100.3 ± 5.0	−0.4	5.1
50	49.7 ± 2.2	0.6	4.5	49.4 ± 3.2	1.1	6.6
25	25.3 ± 0.5	−1.3	2.3	25.1 ± 0.5	−0.5	2.0
5	5.0 ± 0.3	−0.6	6.4	4.8 ± 0.3	3.3	7.2
2.5	2.5 ± 0.3	−1.3	12.7	2.4 ± 0.2	2.6	10.3

Table 2
Precision and accuracy of LC-MS/MS analysis of curcumin in mouse plasma and brain homogenates samples ($n = 6$).

Matrix	Nominal (ng/mL)	Intra-day[a]			Inter-day[b]		
		Measured (ng/mL)	RSD (%)	Accuracy (%)	Measured (ng/mL)	RSD (%)	Accuracy (%)
Plasma	1000 (HQC)	1043.3 ± 18.6	1.8	4.3	989.7 ± 17.6	1.7	−1.0
	500 (MQC)	496.3 ± 13.0	2.6	−0.7	503.5 ± 11.6	2.3	0.7
	5 (LQC)	5.2 ± 0.1	2.9	4.7	4.8 ± 0.2	5.2	−3.3
	2.5 (LLOQ)	2.4 ± 0.3	12.3	−1.3	2.3 ± 0.3	13.1	−6.6
Brain	1000 (HQC)	997.4 ± 13.8	1.4	−0.2	1007.6 ± 21.3	2.1	0.7
	500 (MQC)	500.1 ± 5.0	1.0	0.1	500.7 ± 5.4	1.1	0.1
	5 (LQC)	4.9 ± 0.3	6.5	−1.3	5.3 ± 0.4	7.6	6.6
	2.5 (LLOQ)	2.4 ± 0.2	8.3	−4	2.6 ± 0.2	9.5	5.3

RSD, relative standard deviation;
The analyses were performed on the same day[a] and three different days [b].

Table 3
Matrix effects on ionization suppression/enhancement of curcumin and salbutamol ($n = 6$).

Matrix effect (ME)	Nominal concentration (ng/mL)	Plasma (%ME)	Brain (%ME)
Curcumin	1000	103.5 ± 4.3	94.6 ± 4.7
	500	101.9 ± 3.9	96.2 ± 3.3
	5	102.7 ± 2.4	93.7 ± 3.1
Salbutamol	500	98.1 ± 3.4	95.2 ± 4.2

Table 4
Extraction recoveries of the curcumin in mouse plasma and brain homogenates ($n = 6$).

Drug	Nominal (ng/mL)	Recovery ($n = 6$) Mean (%) ± SD (%)	
		Plasma	Brain
Curcumin	1000	84.1 ± 3.9	72.45 ± 2.17
	500	80.1 ± 6.2	51.59 ± 5.78
	5	77.7 ± 10.7	57.25 ± 5.59

3.9. Application of the validated method to pharmacokinetic study

The developed and validated method was subsequently applied to analyze the pharmacokinetic profiles in mouse plasma samples. We also determined curcumin distribution in the brain after it crosses the BBB on intravenous administration of free curcumin and SLCNs. The plot of the plasma concentration–time profiles of curcumin after intravenous administration of the 4 mg/kg formulation and free curcumin in mice is shown in Fig. 5. The calculated pharmacokinetic parameters are summarized in Table 6. In the curcumin group, the AUC of curcumin in plasma was lower, indicating that the systemic circulation of curcumin is limited. The SLCN formulation was able to significantly increase curcumin concentrations in the plasma because of increased AUC and MRT of curcumin. The enhanced MRT of curcumin in the plasma implies that the SLCN formulation prolonged the retention time of curcumin, which may be because of the sustained release of curcumin from SLCNs.

The BBB is a unique, selective barrier formed by the capillary endothelial cells, which protects the brain from harmful substances in the blood stream. The BBB is semipermeable and is the rate-limiting factor for therapeutic drug permeation to the brain. The BBB allows only the entry of therapeutic drug molecules with low molecular weight; however, hydrophobic molecules can easily cross the BBB. In our formulations, curcumin and carrier SLCNs have high lipophilicity, enabling the drug to cross BBB and enter the brain tissue. It can be discussed that the total brain accumulation of curcumin after i.v. injection may not completely reflect curcumin that has crossed the BBB and may relatively represent curcumin content onto or into vascular system without transcytosis into the post capillary compartments. Transcytosis across BBB can be determined by capillary depletion method or microdialysis method. In this study, we used capillary depletion method to determine the accurate transport of drug across the cerebral endothelium [15].

Brain distribution of curcumin in mice after intravenous administration of free curcumin and SLCNs is shown in Fig. 6A and B. At 15 min after intravenous administration of SLCNs, no significant change of curcumin distribution in brain fractions was noted. However, at 30 and 60 min after administration of SLCNs, the curcumin distribution in the brain significantly increased. Curcumin tended to remain in the brain fractions for long durations, possibly because SLCNs are resistant to liver metabolism resulting in slower decrease of curcumin plasma concentrations than of free curcumin. The curcumin content from SLCNs in the brain tissue fractions showed that a significantly higher level of curcumin crossed the BBB than that from free curcumin.

According to the results of the pharmacokinetic and brain distribution analyses, curcumin-containing SLCNs could significantly enhance the AUC, AUMC, MRT, and $t_{1/2}$ of curcumin in the plasma. Curcumin distribution from SLCNs in brain fractions, including the whole brain, capillary fraction (the pellet), and supernatant fraction of the brain parenchyma, showed that a significantly higher level of curcumin is accumulated in the brain. The pharmacokinetic data suggest that therapeutic activity after the use of SLCNs might be better compared to that in the presence of free curcumin. The results provide information about dose determination, and time of administration to enable effective in preclinical and clinical therapeutic researches. All the results obtained strongly suggest that our validated method would be useful for clinical pharmacokinetic and brain distribution analyses.

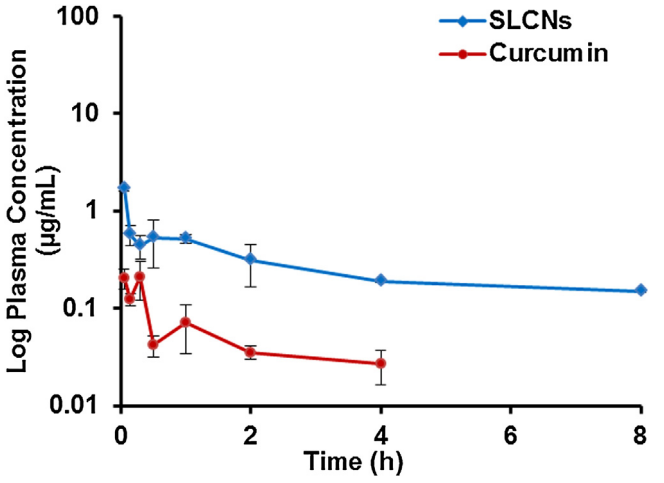

Fig. 5. Method of application: Plasma concentration–time profile of curcumin in mice after intravenous administration of curcumin and solid lipid nanoparticles (SLCNs) at a dose of 4 mg/kg. Values are presented as mean ± standard error ($n = 3$).

Table 6
Pharmacokinetic parameters of curcumin in mouse plasma after intravenous administration of curcumin solution and solid lipid nanoparticles (SLCNs) at 4 mg/kg.

PK parameters	Curcumin solution	SLCNs
$[AUC]_{0-t}$ (μg h/mL)	0.21 ± 0.02	2.23 ± 0.67
$[AUC]_{0-\alpha}$ (μg h/mL)	0.24 ± 0.02	3.27 ± 0.81
$[AUMC]_{0-t}$ (μg h/mL)	0.25 ± 0.02	6.13 ± 1.68
$t_{1/2}$ (h)	1.48 ± 0.11	4.23 ± 1.37
MRT (h)	1.25 ± 0.07	2.79 ± 0.33

Data presented as mean ± SE ($n = 3$).

Table 5
Stability of curcumin in mouse plasma and brain homogenates ($n = 6$).

Matrix	Nominal	% Recovery Fresh	4 h at room temperature	Autosampler 24 h	1 week at 20 °C	3 freeze–thaw cycles
Plasma	1000	99.56 ± 1.53	97.05 ± 4.57	98.37 ± 4.19	102.53 ± 1.01	96.41 ± 7.72
	500	100.39 ± 0.46	100.05 ± 1.52	98.41 ± 2.59	101.32 ± 0.39	101.74 ± 12.10
	5	91.35 ± 11.51	92.27 ± 9.99	93.73 ± 12.03	111.44 ± 3.95	96.58 ± 21.47
Brain	500	106.09 ± 3.17	104.72 ± 3.22	101.52 ± 3.56	96.34 ± 8.57	102.95 ± 2.24

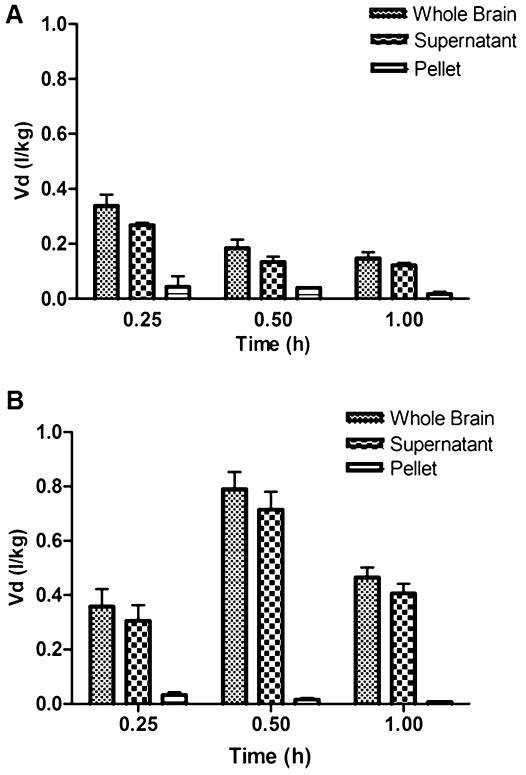

Fig. 6. Brain distribution of curcumin in mice after intravenous administration of curcumin (A) and solid lipid nanoparticles (SLCNs) (B) at a dose of 4 mg/kg. Values are presented as mean ± standard error ($n = 3$).

4. Conclusion

In this study, we developed and validated a highly sensitive and specific LC-MS/MS method for the quantitative analysis of curcumin in mouse plasma and brain tissue samples. The validated method was successfully applied to pharmacokinetic and brain distribution studies of SLNs, following intravenous administration in mice. The use of the serial blood sampling method in mice, capillary depletion method for brain homogenization, use of less volumes of plasma and brain tissue, and simple LLE method described in this study would lead to significant reductions in animals and doses used and would drastically improve the speed, throughput, and the quality of the analyzed pharmacokinetic parameters. Further, the short extraction time, reduced solvent consumption, and low retention time will allow this method to be easily applied for the quantification of curcumin in large numbers of plasma and brain homogenate samples.

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References

[1] S.K. Tiwari, S. Agarwal, B. Seth, A. Yadav, S. Nair, P. Bhatnagar, M. Karmakar, M. Kumari, L.K.S. Chauhan, D.K. Patel, ACS Nano 8 (2014) 76.
[2] R.K. Maheshwari, A.K. Singh, J. Gaddipati, R.C. Srimal, Life Sci. 78 (2006) 2081.
[3] H. Tang, C.J. Murphy, B. Zhang, Y. Shen, E.A. Van Kirk, W.J. Murdoch, M. Radosz, Biomaterials 31 (2010) 7139.
[4] A.S. Darvesh, R.T. Carroll, A. Bishayee, N.A. Novotny, W.J. Geldenhuys, C.J. Van der Schyf, Expert Opin. Invest. Drugs 21 (2012) 1123.
[5] M.A. Hickey, C. Zhu, V. Medvedeva, R.P. Lerner, S. Patassini, N.R. Franich, P. Maiti, S.A. Frautschy, S. Zeitlin, M.S. Levine, Mol. Neurodegener. 7 (2012) 12.
[6] J. Sun, C. Bi, H.M. Chan, S. Sun, Q. Zhang, Y. Zheng, Colloids Surf. B: Biointerfaces 111 (2013) 367.
[7] Y.-M. Tsai, C.-F. Chien, L.-C. Lin, T.-H. Tsai, Int. J. Pharm. 416 (2011) 331.
[8] P. Anand, A.B. Kunnumakkara, R.A. Newman, B.B. Aggarwal, Mol. Pharmaceut. 4 (2007) 807.
[9] V. Kakkar, S. Singh, D. Singla, S. Sahwney, A.S. Chauhan, G. Singh, I.P. Kaur, J. Chromatogr. B 878 (2010) 3427.
[10] A. Liu, H. Lou, L. Zhao, P. Fan, J. Pharm. Biomed. Anal. 40 (2006) 720.
[11] N.M. Khalil, T.C.F.d. Nascimento, D.M. Casa, L.F. Dalmolin, A.C.d. Mattos, I. Hoss, M.A. Romano, R.M. Mainardes, Colloids Surf. B: Biointerfaces 101 (2013) 353.
[12] R. Li, X. Qiao, Q. Li, R. He, M. Ye, C. Xiang, X. Lin, D. Guo, J. Chromatogr. B 879 (2011) 2751.
[13] S.X. Peng, B.A. Rockafellow, T.M. Skedzielewski, N.D. Huebert, W. Hageman, J. Pharm. Sci. 98 (2009) 1877.
[14] V. Kurawattimath, K. Pocha, T.T. Mariappan, R.K. Trivedi, S. Mandlekar, Eur. J. Drug Metab. Pharmacokinet. 37 (2012) 23.
[15] S. Wohlfart, A.S. Khalansky, S. Gelperina, D. Begley, J. Kreuter, J. Control. Release 154 (2011) 103.