



## Short communication

# Plasma pharmacokinetics, tissue distribution and excretion study of 6-gingerol in rat by liquid chromatography–electrospray ionization time-of-flight mass spectrometry

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## ABSTRACT

A rapid resolution liquid chromatography coupled with electrospray ionization (ESI) time-of-flight mass spectrometry method was developed and validated for quantitative analysis of 6-gingerol in plasma and various tissues. Liquid–liquid extraction was employed as sample preparation technique. Biological samples were separated on an Agilent Zorbax StableBond-C<sub>18</sub> column (4.6 mm × 50 mm, 1.8 μm) and detected by TOF/MS with electrospray ionization (ESI) interface in positive ion mode. Calibration curves (1/x<sup>2</sup> weighted) offered satisfactory linearity ( $r^2 > 0.995$ ) within the test range. The lower limit of quantification in different matrices was in a range of 10–100 ng/mL. Inter- and intra-day precision were in the range of 0.91–11.90% and 0.75–10.23%, respectively. Recoveries in plasma, urine and tissues ranged from 72.5% to 90.4%. Glucuronide of 6-gingerol, the major metabolite of 6-gingerol, was further determined after β-glucuronidase hydrolyzation. This developed method was successfully applied to pharmacokinetics, tissue distribution and excretion studies of 6-gingerol after oral or intraperitoneal administration in rats.

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## 1. Introduction

Ginger, the rhizome of *Zingiber officinale* Roscoe, is widely used as a spice in a variety of foods and beverages. In addition, fresh or processed ginger has been used in Traditional Chinese Medicine for many ailments [1]. The major pungent constituent of ginger is 6-gingerol [2,3]. It has been found to possess a variety of effects [4–8], and recent studies in animal models showed that 6-gingerol could suppress carcinogenesis in skin [9], gastrointestinal tract [10] and breast [11]. Although the mechanism of 6-gingerol in carcinogenesis is not yet clear, some studies revealed that nuclear factor-κB is related to the chemopreventive effect of 6-gingerol [12–14].

Comparing the abundant pharmacological studies, few reports were related to the determination of 6-gingerol. High-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) [2,15] was reported for the qualitative analysis of ginger extract. HPLC methods coupled with UV or electrochemical detection were also developed for the determination of 6-gingerol in ginger-containing products [3], plasma [16–18], tissues [19] or simulated gastric and intestinal fluids [20]. Limited sensitivity of

current quantitative methods is the major obstacle for oral pharmacokinetic study, and none of them was applied to the tissue distribution or excretion study of 6-gingerol after oral administration of 6-gingerol. The combination of sub-2 μm material columns with dedicated purpose-built instrumentation (e.g., rapid resolution LC from Agilent and ultra-performance LC from Waters) allows faster separations with excellent peak capacities and daily sample capability for pharmacokinetic research [21,22], metabonomics study [23], and herbal prescription analysis [24]. In our study, a sensitive and specific rapid resolution LC–ESI–TOF/MS method was developed and validated for the estimation of 6-gingerol in different biological matrices to support the development of 6-gingerol. As the phase II metabolism of 6-gingerol may be of importance in pharmacological and toxicological consideration [25,26], we also tried to determine the glucuronide of 6-gingerol in plasma, tissues and urine. This analytical method was successfully applied to plasma pharmacokinetics, tissue distribution and excretion study of 6-gingerol in rats for the first time.

## 2. Materials and methods

### 2.1. Chemicals

Authentic standard of 6-gingerols was purchased from ChromaDex (Santa Ana, CA, USA). Nonivamide (>98%) was provided by Qingdao Haida Chemical Co. Ltd. (Qingdao, China). The purity of

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6-gingerol used in our study was determined to be higher than 98% by HPLC–UV method compared with the standard reference.

Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany), and  $\beta$ -glucuronidase (1,000,000–5,000,000 units/g protein, type IX-A, from *Escherichia coli*) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Solutol HS-15 was kindly provided by BASF (Ludwigshafen, Germany). Distilled, deionized water was produced by a MILLPAK Reagent Water System (Millipore, MA, USA). All solutions were prepared with distilled, deionized water.

## 2.2. LC–TOF/MS analysis

Analysis was performed with an Agilent 1200 series RRLC system (Agilent, Germany) equipped with a binary pump (G1312B) and a thermostatically controlled column apartment (G1316B). Chromatographic separation was carried out at 25 °C on an Agilent Zorbax StableBond-C<sub>18</sub> column (4.6 mm × 50 mm, 1.8  $\mu$ m). The mobile phase consisted of 0.1% formic acid water (A) and ACN (B) using an isocratic elution of 42% B (v/v) at 0–6 min. The flow rate was 0.5 mL/min.

Detections were performed by an Agilent orthogonal TOF/MS (Agilent, USA) equipped with an ESI source. The TOF/MS analysis worked in positive mode, and mass range was set at  $m/z$  100–1200. The conditions of ESI source were as follows: drying gas (N<sub>2</sub>) flow rate, 9.0 L/min; drying gas temperature, 325 °C; nebulizer, 35 psig; capillary voltage, 4000 V; fragmentor 125 V; skimmer voltage, 60 V. All the acquisition and analysis of data were controlled by Agilent LC–MS TOF Software (Agilent, USA) and Applied Biosystems/MDS–SCIEX Analyst QS Software (Frankfurt, Germany), respectively. Tuning mix (G1969–85000, containing fluorocarbons) was used for lock mass calibration in our assay.

## 2.3. Animals

Male Sprague–Dawley rats (250 ± 40 g) were purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University (Nanjing, China) and protocol was approved by the Animal Ethics Committee of this institution.

## 2.4. Drug administration and sample preparation

### 2.4.1. Plasma kinetics

Eighteen rats were divided into three groups. A single dose of 6-gingerol dissolved in 2.5% of solutol was given orally (30 mg/kg and 120 mg/kg) or intraperitoneally (30 mg/kg) to six rats. Blood samples were collected at 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 12 h, and then centrifuged at 3000 × g for 10 min. All plasma samples were stored at –70 °C until analysis. Each sample was mixed with 5  $\mu$ L of IS (40  $\mu$ g/mL), then extracted with 300  $\mu$ L of ethyl acetate twice. The organic layer was evaporated to dryness at 40 °C under nitrogen and dissolved in 600  $\mu$ L of methanol for analysis. For the determination of 6-gingerol glucuronide, 100  $\mu$ L of plasma samples were incubated with 5  $\mu$ L of  $\beta$ -glucuronidase (>625 unit) at 37 °C for 2 before extraction. Equivalent plasma samples of the same extraction procedure but without the treatment of  $\beta$ -glucuronidase were analyzed in parallel. They were used as the background for the determination of 6-gingerol glucuronide. An aliquot of 1  $\mu$ L of the mixture was injected into HPLC.

### 2.4.2. Tissue distribution studies

Forty male Sprague–Dawley rats were given a single oral administration of 30 mg/kg 6-gingerol. Tissues (heart, liver, spleen, lung,

kidney, and brain) of five rats were removed at 0.5, 1, 2, 3, 4, 6, 8 and 12 h after dosing and washed with normal saline. Each tissue sample was weighted and stored at –70 °C. Before analysis, tissue sample was diluted with 3 mL of saline and homogenized. The homogenate mixed with 10  $\mu$ L of IS (40  $\mu$ g/mL) was incubated with 5  $\mu$ L of glucuronidase (>625 unit) for 2 h at 37 °C, and extracted by 3 mL of ethyl acetate twice. Organic layer was evaporated to dryness at 40 °C under nitrogen and dissolved in 600  $\mu$ L of methanol for the analysis. An aliquot of 2  $\mu$ L of the solution was injected into HPLC.

### 2.4.3. Elimination studies

Six male rats receiving a single oral administration of 30 mg/kg 6-gingerol were placed in separate metabolic cages and urine samples were collected in 0–24 h after dosing. The volume of urine samples were measured prior to storage at –20 °C. For the determination of 6-gingerol glucuronide, 5  $\mu$ L of glucuronidase (>625 unit) was added to 200  $\mu$ L of urine samples spiked with 5  $\mu$ L of IS (10.8  $\mu$ g/mL), and the mixture was incubated at 37 °C for 2 h. Methanol (800  $\mu$ L) was added to urine samples, and the mixture was centrifuged at 13,800 g for 5 min. An aliquot of 5  $\mu$ L of the mixture was injected into HPLC.

## 2.5. Method validation

### 2.5.1. Specificity

For specificity, three different batches of drug-free rat plasma were analyzed for the exclusion of any endogenous co-eluting interferences at the peak region of 6-gingerol or IS.

### 2.5.2. Calibration curve and lower limit of quantification (LLOQ)

A methanol stock solution of 6-gingerol was serially diluted to the desired concentrations. Aliquot (5  $\mu$ L) of each diluted solution was spiked into blank plasma, urine or tissue homogenates to give concentrations ranging from 0.02 to 25  $\mu$ g/mL for urine or plasma and 0.1–5  $\mu$ g/mL for tissue samples, respectively. The resultant samples were mixed thoroughly, then treated and analyzed in the same manner as described. All solutions were stored at 4 °C before and between uses. Samples of each concentration were analyzed in triplicate. The concentration of free 6-gingerol is directly calculated according to corresponding calibration curve, and the concentration of 6-gingerol glucuronide was determined by the difference between  $\beta$ -glucuronidase treated and untreated samples. Calculated concentrations of analytes in body liquid samples were expressed in  $\mu$ g/mL, while tissue concentrations of 6-gingerol glucuronide were converted into  $\mu$ g/g. The LLOQ was defined as the lowest concentration of spiked samples where both the precision and accuracy were less than 20% by analyzing three replicates of analytes.

### 2.5.3. Precision and accuracy

Stock solution of 6-gingerol was spiked into blank plasma, urine or tissue homogenates to give quality control (QC) samples of three concentrations. High, medium and low levels of QC samples (25  $\mu$ g/mL, 10  $\mu$ g/mL and 0.02  $\mu$ g/mL for urine or plasma samples, 5  $\mu$ g/mL, 1  $\mu$ g/mL and 0.1  $\mu$ g/mL for tissue samples) were chosen to determine intra- and inter-day precision of the method. The intra-day precision was determined by analyzing the three levels of QC samples for three times within one work day. While for inter-day precision test, the testing samples were determined in 3 consecutive days. The concentrations were calculated from corresponding calibration curve. R.S.D. and percentage difference between amounts spiked and determined were taken as measures of precision and accuracy.

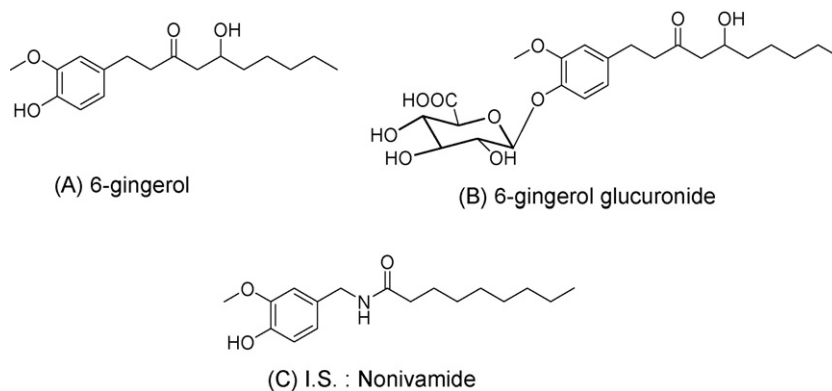


Fig. 1. The chemical structures of 6-gingerol, 6-gingerol glucuronide and nonivamide (IS).

#### 2.5.4. Extraction recovery and matrix effect

Recoveries were evaluated by high, medium and low levels of QC samples. The preparation of blank biological matrix procedure was the same as Section 2.3. The extraction recovery was determined by calculating the ratio of the amounts of QC samples finally obtained against those originally dissolved with biological matrix extract. The matrix effect was determined by the ratio of the amounts of 6-gingerol dissolved with blank matrix extract against those dissolved with menthol. The procedure was repeated three times.

#### 2.5.5. Stability

The stability of 6-gingerol in plasma, urine and tissue was determined under different storage or handling conditions using high, medium and low QC samples. Short-term temperature stability was assessed by analyzing QC samples kept at ambient temperature (25 °C) for 6 h. Freeze–thaw stability and long-term stability (–70 °C) was checked through three cycles. The QC samples were

stored at –70 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for over 24 h under the same conditions and thawed unassisted at room temperature. The freeze–thaw cycles were repeated three times in 4 weeks, and then analyzed on the third cycle. The obtained results were compared with the nominal concentration of 6-gingerol (Fig. 1).

### 3. Results and discussion

#### 3.1. HPLC–MS analysis

The MS spectra of 6-gingerol, 6-gingerol glucuronide and IS are shown as Fig. 2. The most abundant fragmentation ions of 6-gingerol at  $m/z$  277.17 ([M+H–H<sub>2</sub>O]<sup>+</sup>) was selected for quantification under extracted ion chromatogram (XIC) mode in a mass window of  $m/z$  277.13–277.22. HPLC–MS analysis of the blank and spiked

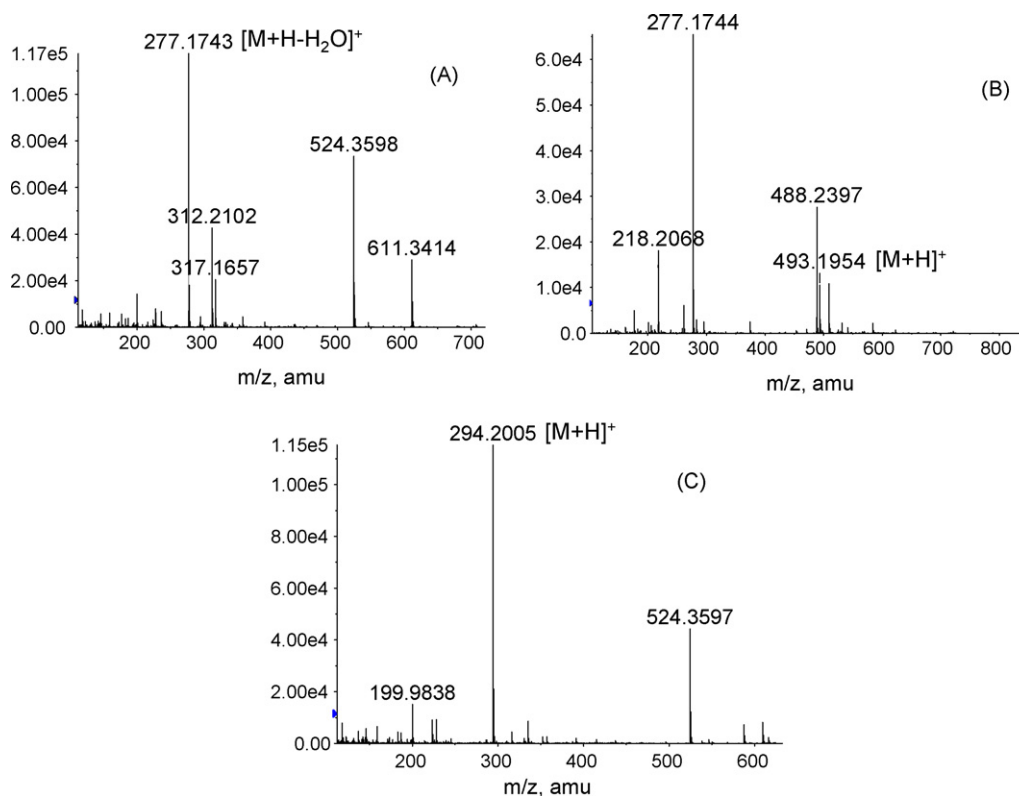
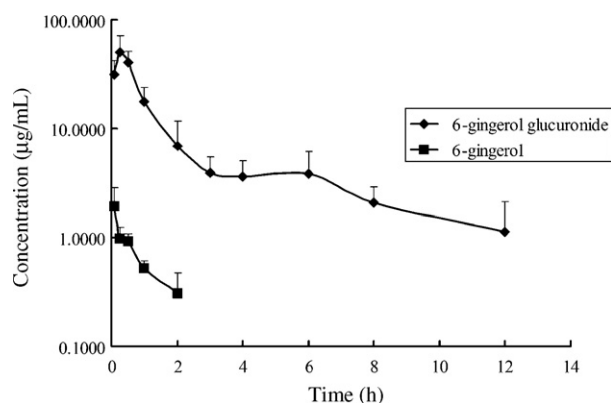


Fig. 2. Typical MS spectra of 6-gingerol (A), 6-gingerol glucuronide (B) and IS (C) in rat plasma.

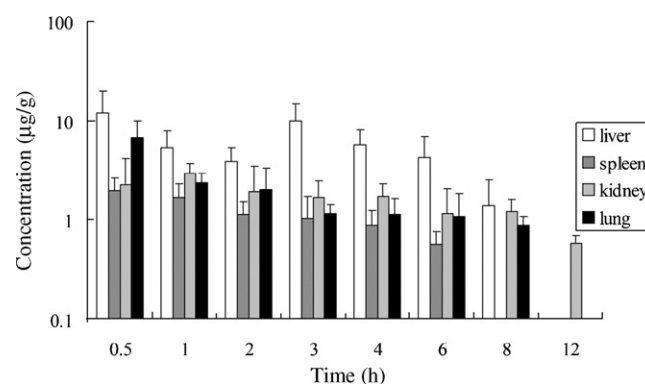


**Fig. 3.** Mean plasma concentration–time profiles of 6-gingerol and 6-gingerol glucuronide in rats following oral administration of 120 mg/kg 6-gingerol ( $n = 6$ ).

plasma samples showed no endogenous peak interference with the quantification of 6-gingerol and the IS. In order to improve ionization efficiency and peak shape, 0.1% formic acid was added to water phase (Figs. 3 and 4).

### 3.2. Validation of the HPLC method

The HPLC method was demonstrated to be suitable for the quantification of 6-gingerol in plasma samples, tissue samples and urine samples. The calibration model was selected based on the data obtained by linear regression with  $1/x^2$  weighting factor. The calibration curves for all matrices showed good linearity ( $r^2 > 0.9951$ ) over the concentration ranges tested. Both the intra- and inter-day precision in different matrices were less than 15% (0.91–11.9% and 0.75–10.23%, respectively). The recoveries of different matrices ranged from 72.5% to 90.4%. The data showed acceptable reproducibility, precision and recovery. The LLOQ for 6-gingerol in rat plasma or urine was 10 ng/mL, and fell in the range of 10–100 ng/mL



**Fig. 4.** Mean tissue concentration–time histogram of 6-gingerol glucuronide in rats following oral administration of 30 mg/kg 6-gingerol.

in different tissues. Matrix effects were found to be acceptable in different matrices (76.49–97.27%). The detail data are shown in Table 1.

After storage at ambient temperature (25 °C) for 6 h, the concentrations of analytes in different matrices deviated less than  $\pm 15\%$  from their nominal concentrations (1.86–12.7%). In freeze–thaw stability and long-term stability test, the concentrations obtained were higher than 85% of their nominal concentrations (86.1–99.5%). The data suggested no significant analyte loss during sample storage and processing procedure.

### 3.3. Plasma pharmacokinetics of 6-gingerol

The calculated pharmacokinetic parameters of 6-gingerol and its glucuronide after different routes or dosages of administration are summarized in Table 2. Data were analyzed using the Drug and Statistics version 2.0 program (Anhui Provincial Center for Drug Clinical Evaluation, China).

After oral administration of 6-gingerol at low dose (30 mg/kg), free 6-gingerol was not observed in plasma, but its glucuronide was

**Table 1**

Intra- or inter-day precision, accuracy, recovery and matrix effect for 6-gingerol in rat plasma, tissues and urine.

Sample matrix	Spiked concentration ( $\mu\text{g/mL}$ )	Intra-day precision (R.S.D., %)	Inter-day precision (R.S.D., %)	Accuracy (mean $\pm$ S.D., %)	Recovery (mean $\pm$ S.D., %)	Matrix effect (mean $\pm$ S.D., %)
Plasma	0.02	2.41	6.88	106 $\pm$ 4.23	93.2 $\pm$ 1.56	92.71 $\pm$ 4.27
	10	8.74	7.51	91.5 $\pm$ 0.46	81.2 $\pm$ 3.28	90.61 $\pm$ 2.99
	25	1.93	2.40	107 $\pm$ 7.95	97.0 $\pm$ 3.84	97.27 $\pm$ 0.46
Urine	0.02	11.4	5.49	101 $\pm$ 2.40	89.1 $\pm$ 2.31	88.16 $\pm$ 0.32
	10	10.9	2.68	96.7 $\pm$ 7.51	87.4 $\pm$ 7.43	80.64 $\pm$ 1.82
	25	7.80	5.00	87.0 $\pm$ 3.77	91.7 $\pm$ 1.64	82.69 $\pm$ 2.24
Heart	0.1	8.21	10.23	104 $\pm$ 2.00	80.6 $\pm$ 1.66	76.49 $\pm$ 2.76
	1	2.20	6.68	106 $\pm$ 6.51	80.8 $\pm$ 0.59	78.77 $\pm$ 1.62
	5	1.30	2.12	94.8 $\pm$ 1.58	76.3 $\pm$ 4.99	80.16 $\pm$ 2.92
Liver	0.1	7.15	2.07	106 $\pm$ 3.99	71.7 $\pm$ 3.54	77.99 $\pm$ 1.34
	1	1.92	3.73	91.0 $\pm$ 5.17	78.1 $\pm$ 1.85	79.64 $\pm$ 1.35
	5	2.30	1.84	106 $\pm$ 6.94	67.6 $\pm$ 8.10	87.03 $\pm$ 3.16
Spleen	0.1	0.63	6.38	96.4 $\pm$ 0.46	85.3 $\pm$ 1.19	81.12 $\pm$ 2.82
	1	1.43	1.06	107 $\pm$ 2.82	83.1 $\pm$ 1.28	79.12 $\pm$ 2.33
	5	1.33	1.70	101 $\pm$ 0.81	80.7 $\pm$ 1.43	83.11 $\pm$ 2.69
Lung	0.1	5.60	3.84	110 $\pm$ 4.26	74.0 $\pm$ 2.12	76.95 $\pm$ 2.73
	1	2.59	1.97	97.2 $\pm$ 3.40	84.1 $\pm$ 6.44	81.10 $\pm$ 4.61
	5	4.76	1.83	89.6 $\pm$ 0.88	75.2 $\pm$ 4.68	80.98 $\pm$ 4.72
Kidney	0.1	11.9	5.44	96.9 $\pm$ 0.34	73.7 $\pm$ 3.57	80.56 $\pm$ 1.83
	1	3.55	2.65	109 $\pm$ 3.95	83.8 $\pm$ 5.42	83.35 $\pm$ 1.92
	5	1.50	1.70	92.4 $\pm$ 5.80	80.0 $\pm$ 9.01	79.29 $\pm$ 1.98
Brain	0.1	9.70	5.12	104.6 $\pm$ 2.26	76.4 $\pm$ 2.12	86.82 $\pm$ 2.24
	1	2.26	2.03	93.7 $\pm$ 3.36	90.9 $\pm$ 5.16	86.99 $\pm$ 1.38
	5	0.91	0.75	110 $\pm$ 5.25	75.6 $\pm$ 8.90	85.84 $\pm$ 2.84

**Table 2**

Plasma pharmacokinetic parameters of 6-gingerol in rats Plasma pharmacokinetic parameters of 6-gingerol glucuronide in rats.

	Parameter	Oral administration		Intraperitoneal administration
		30 mg/kg	120 mg/kg	30 mg/kg
6-Gingerol	$AUC_{(0-t_n)}$ ( $\mu\text{g h/mL}$ )	–	$1.33 \pm 0.47$	$4.06 \pm 1.30$
	$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	–	$1.90 \pm 0.97$	$5.96 \pm 1.89$
	$T_{\text{max}}$ (h)	–	0.083	0.083
	$T_{1/2}$ (h)	–	$0.766 \pm 0.615$	$0.381 \pm 0.297$
6-Gingerol glucuronide	$AUC_{(0-t_n)}$ ( $\mu\text{g h/mL}$ )	$23.49 \pm 10.56$	$76.62 \pm 16.07$	$30.96 \pm 14.88$
	$C_{\text{max}1}$ ( $\mu\text{g/mL}$ )	$15.14 \pm 9.98$	$51.99 \pm 19.30$	$18.88 \pm 11.35$
	$T_{\text{max}1}$ (h)	0.083	0.25	0.083
	$C_{\text{max}2}$ ( $\mu\text{g/mL}$ )	$2.88 \pm 2.30$	$3.83 \pm 0.83$	$1.63 \pm 0.83$
	$T_{\text{max}2}$ (h)	3	6	4
	$T_{1/2}$ (h)	$0.495 \pm 0.257$	$0.923 \pm 0.348$	$0.645 \pm 0.531$

detected as major metabolite of 6-gingerol, indicating an extensive first-pass metabolism. In the case of oral administration of 6-gingerol at high dose (120 mg/kg), plasma concentration–time profile and pharmacokinetic parameters of both 6-gingerol and its glucuronide could be obtained. 6-Gingerol was rapidly absorbed but also rapidly cleared in plasma. After intraperitoneal administration of 30 mg/kg, 6-gingerol was rapidly absorbed and cleared in plasma, with a  $C_{\text{max}}$  of  $5.97 \pm 1.89 \mu\text{g/mL}$  at 0.083 h. Plasma concentration–time profile of 6-gingerol glucuronide after different route or dosage of administration all exhibited a double-peak curve, and plasma concentration level of 6-gingerol glucuronide finally dropped below LLOQ after 12 h. The  $AUC_{(0-t_n)}$  of 6-gingerol glucuronide was substantially higher than 6-gingerol in plasma.

### 3.4. Tissue distribution

The tissue concentrations of 6-gingerol glucuronide determined at 0–12 h after oral administration of 30 mg/kg 6-gingerol is shown in Fig. 4, and 6-gingerol was not distributed to tissues. Data were not meaningful to heart and brain because the concentration levels of 6-gingerol or its glucuronide were below LLOQ. This phenomenon could be explained by the polarity increase of introducing a glucuronic acid group into the molecule. Experimental data showed that 6-gingerol glucuronide was mainly distributed into abundant blood-supply tissues, which implied that blood flow or perfusion rate of an organ is the key factor affecting the distribution of 6-gingerol glucuronide. Mean  $C_{\text{max}}$  of 6-gingerol glucuronide for all the tissues analyzed were obtained at 0.5 h after administration, and the highest concentration of 6-gingerol glucuronide appeared in liver. Meanwhile, the high level in kidney and liver demonstrated that they were both responsible for excretion of 6-gingerol. The double-peak concentration–time curve in plasma and liver indicates that the glucuronide of 6-gingerol could return to the blood circulation after administration, probable by hepato-enteric circulation.

### 3.5. Elimination

Following oral administration of 6-gingerol, total mean recovery of 6-gingerol in urine within 24 h was about  $5.36 \pm 0.80\%$  in the form of glucuronide. Free 6-gingerol was not observed in urine.

## 4. Conclusion

In our research, a rapid resolution LC–ESI–TOF/MS method was developed and validated for quantitative analysis of 6-gingerol in plasma, urine and tissue samples for the first time, and the LLOQ was better than current HPLC–UV methods. An extensive

first-pass metabolism was observed. The plasma concentration and  $AUC_{(0-t_n)}$  of 6-gingerol glucuronide were substantially higher than 6-gingerol without reference to administration dosage or manner, while nearly all the publications related to the effect and mechanism of 6-gingerol did not consider the glucuronidation procedure adequately. The toxicological and pharmacological features of 6-gingerol glucuronide should be considered in the near future as the potential value of 6-gingerol for human health.

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