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Development and validation of a gas chromatography–mass spectrometry method for the determination of isoimperatorin in rat plasma and tissue: Application to the pharmacokinetic and tissue distribution study

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Abstract

Isoimperatorin is one of the major furanocoumarins isolated from the dried root of *Angelica dahuricae* Benth.et Hook. The aim of the present study is to develop a procedure based on gas chromatography–mass spectrometry (GC–MS) to describe the analysis of isoimperatorin in rat plasma and tissue. The method was set up and adapted for the analysis of small biological samples taken from rats. Biological samples were extracted by liquid–liquid extraction. Extracted compounds were acetic ether/light petroleum (1:2). They were separated by GC on a DB-5MS analytical column and determined by a quadrupole mass spectrometer detector operated under selected ion monitoring mode. Excellent linearity was found between 0.027–5.32 μg/mL ($r > 0.99$) for plasma samples and 0.108–21.28 μg/g ($r > 0.99$) for the tissue samples. The limit of detection (LOD) was 1.0 ng/mL or 1.0 ng/g (three times signal/noise ratio). Within- and between-day precisions expressed as the relative standard deviation (RSD) for the method were 2.81–5.22% and 4.72–6.52%, respectively. The method recoveries for all samples were >80%. The main pharmacokinetic parameters obtained were $T_{max} = (1.06 \pm 0.12)$ h, $C_{max} = (0.72 \pm 0.14)$ μg/mL, AUC = (2.11 ± 0.29) h μg/mL and $K_a = (1.76 \pm 0.13)$/h. The concentrations of isoimperatorin in rat liver, heart, cerebellum and cerebrum were higher than those in other organs. The results presented here clearly indicate that this proposed method could be applicable to investigate the pharmacokinetic and tissue distribution of isoimperatorin in rats after administration.

Keywords: Isoimperatorin; GC/MS; Pharmacokinetics; Tissue distribution; *Angelica dahuricae*

1. Introduction

The dried root of *Angelica dahuricae* (Baizhi) Benth.et Hook (Umbelliferae), as an important Chinese herbal medicine [1–3] listed in the Chinese Pharmacopoeia, is used as an antipyretic and analgesic for cold, headaches and toothaches [4]. It is reported to protect against dexamethasone-induced disorders and also to possess liver protective activity, antimicrobial activity, anti-inflammatory activity and antimutagenic activity [5]. *Angelica dahuricae* is known to contain a large number of coumarins and furanocoumarins including coumarin, scopoletin, psoralen, xanthotoxin, bergapten, imperatorin and isoimperatorin [6–8].

Pharmacological studies and clinical practice demonstrated that they have remarkable anticancer, antibacterial and codein effects [9]. They are often used as reference standards in the quality control of *Angelica dahuricae* and its products. A number of studies have been performed on the isolation and identification of the constituents of these furanocoumarins. Analytical studies mostly deal with gas chromatography (GC) coupled with mass spectrometry (MS) methods [10] and high-speed counter-current chromatography [11]. Pharmacological trials have indicated that isoimperatorin, 4-(3-methylbut-2-enyloxy)-7H-furo [3,2-g] chromen-7-one (Fig. 1a), is one of the major active constituents in the essential oil from *Angelica dahurica* [12]. As a result of the efforts to develop isoimperatorin as a potential drug in China, there is the need to evaluate isoimperatorin pharmacokinetics in preclinical models and clinical trials. To support the preclinical pharmacokinetics study, a sensitive method for determination of isoimperatorin in plasma samples is desirable. To our knowledge, no analytical method was reported for the determination of isoimperatorin in biological samples.

The objective of the current study was to develop a gas chromatography–mass spectrometry method for the determination of isoimperatorin from *Angelica dahurica* in rats. Osthole

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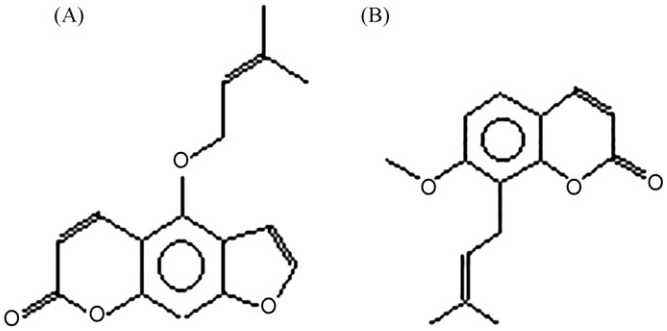

Fig. 1. Chemical structures of isoimperatorin (A) and osthole (B).

is an internal standard (Fig. 1b). The limit of detection was up to 1 ng/mL or 1 ng/g (S/N ≥ 3) of isoimperatorin in biological samples. The procedure is sensitive, accurate and reproducible and should be suitable for the support of this study. The pharmacokinetic parameters and tissue distributional date of isoimperatorin in rats have been obtained first.

2. Experimental

2.1. Chemicals and reagents

Isoimperatorin and osthole were supplied by National Institute for the Pharmaceutical and Biological Products of China. HPLC grade methanol, *n*-hexane, ethyl acetate (AcOEt) and light petroleum were obtained from Fisher Scientific (Pittsburg, USA). Helium (purity, 99.999%) was obtained from Xi'an Analytical Instrument Factory (Xi'an, PR China); other reagents used in the experiment were of analytical grade and from commercial sources. *Angelica dahurica1* (Fisch.ex Hoffm) Benth.et Hook was purchased from the TCM Store (Xi'an, PR China).

Rats (male or female, 200 ± 25 g) were supplied by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, PR China).

2.2. GC–MS conditions and instrumentation

A capillary gas chromatography–mass spectrometry (GCMS-QP2010 Shimadzu, Kyoto, Japan) with a DB-5MS capillary column (30 m × 0.32 mm I.D., 0.25 μm film thickness, Agilent Technologies, USA) was used. The inlet temperature was maintained at 280 °C. The oven temperature was initially held at 140 °C for 2 min and then programmed to 280 °C at 10 °C/min, where it was held constant for 4 min. Helium was used as carrier gas at a constant flow rate of 2.0 mL/min.

The source and electrodes of the quadrupole mass filter were both set to 200 °C. Ionization was carried out in electron impact ionization (EI) mode at 70 eV. Detection was operated under selected ion monitoring (SIM) mode. One qualifying ion was selected for analytes under investigation: *m/z* 244 for osthole and *m/z* 202 for isoimperatorin were abundant and used for quantification. Data were collected using the GC/MS analysis Station software. The data were analyzed using a NIST library (Shimadzu, Kyoto, Japan).

2.3. Preparation of imperatorin

Angelica dahurica1 was ground to 40 mesh and extracted by 95% alcohol. The extracts were extracted by using light petroleum, ethyl ether, AcOEt and *n*-butanol by turns. Then the five parts were obtained.

The light petroleum part was systematically separated by using column chromatographic methods. First, 7 g sample was subjected to a normal phase column chromatography (CC, Silica Gel, 3 × 200 g) using a mixture solution of light petroleum/ethyl ether from (5:1) to (1:1) as a mobile phase to give eight fractions (5 × 100 mL each fraction) after recombination. Fraction 1 (2.7 g) was subjected to further separation, and then two needle crystal substances were obtained (purity: > 98% by HPLC). One was identified as isoimperatorin by means of GC/MS method for the following analysis [13–14].

2.4. Animals and plasma collection

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of Xi'an Jiaotong University. Male pathogen-free Sprague-Dawley rats, weighing 225–275 g, were supplied by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). All rats were maintained under standard conditions with normal access to food and water. There were three sample sets of rats. The first set was used to prepare blank plasma samples, the second for the pharmacokinetic studies and the third for tissue distribution study. The rats were initially anesthetized with ether and remained anesthetized throughout the surgery period. The femoral artery was exposed for further drug administration. The rats were recovering from anesthesia before the test.

Drug-free rat plasma samples were obtained as follows: blood was taken from anesthetized animals by carotid bleeding and collected into heparinized glass tubes. After centrifugation for 10 min at 4000 rpm, the harvested plasma samples were mixed to obtain a homogeneous pool of blank plasma which was stored at −20 °C until analysis.

On the day before the treatment with isoimperatorin, the second sample set of animals were anaesthetized as described above and the femoral vein and artery were catheterized with glass tubes. The catheters were then tunneled subcutaneously and fixed at the back of the neck [15]. The rats were given at least a 24 h recovery period to allow for washout of anesthesia. On the study day, the rats received a 10 mg/kg dose of isoimperatorin by oral administration. The pharmacokinetic study involved serial arterial blood sampling (500 μL) with 11 samples obtained from each animal at 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12 h after administration. The blood samples were transferred to micro tubes containing 5 μL heparin and then centrifuged at 4000 rpm for 10 min. The plasma (200 μL) was separated and frozen immediately at −20 °C until further analysis.

Tissue samples were immediately collected from the heart, liver, spleen, lung, kidney, cerebrum and cerebellum of the third group of rats at 1.5 h after oral administration, and were put into

the physiological saline to exclude the remaining bloodstain. They were weighed, and about 0.5 g of each was taken as analytical sample after drying the moisture with filter paper. Tissue homogenate (4.0 mL of each) was obtained as the tissue sample, transferred to heparinized tube and stored at −20 °C until analysis.

2.5. Extraction procedure for bio-samples

A liquid–liquid extraction method was used for the extraction of isoimperatorin in bio-samples. To each plasma and tissue sample, 10 μL of internal standard (4.68 μg/mL) was added to 200 μL of plasma samples or 20 μL of internal standard (4.68 μg/mL) was added to 500 μL of tissue samples in glass centrifuge tubes. Next, 1.0 mL (for plasma samples) and 2.5 mL (for tissue samples) acetic ether/light petroleum (1:2) was added and the mixture was vortexed for 5 min. After centrifugation for 10 min at 4000 rpm, the upper organic layer was transferred to a clean tube. The organic solution was evaporated under a stream of nitrogen at 40 °C. To the residue, 0.1 mL of acetic ether/light petroleum (1:1) was added, and centrifugation procedure was repeated. Aliquot (1 μL) of the supernatant was injected into the GC/MS systems for analysis.

2.6. Standard stock solutions

The standard stock solutions were prepared by dissolving 11.3 mg of isoimperatorin and 11.7 mg osthole in 10 mL acetic ether/light petroleum (1:2) to yield nominal concentrations of 1.13 and 1.17 mg/mL. And the solutions were kept at −20 °C before use. The stock solutions were diluted with acetic ether/light petroleum (1:2) to obtain calibration solutions (range 0.027–5.32 μg/mL). Internal standard solution was prepared by dilution of the stock solution to a concentration of 4.68 μg/mL, osthole.

2.7. Method validation

Isoimperatorin standard plasma samples (0.027, 0.133, 0.266, 0.532, 1.33 and 5.32 μg/mL) were prepared by spiking 200 μL of blank plasma with 20 μL of standard stock solution and 10 μL of the internal standard solution (4.68 μg/mL) prepared above. Isoimperatorin standard tissue samples (0.027, 0.133, 0.266, 0.532, 1.33 and 5.32 μg/mL) were prepared by spiking 500 μL of blank tissue homogenate with 50 μL of standard stock solution and 20 μL of the internal standard solution (4.68 μg/mL) prepared above.

Samples were processed as described above, and peak area ratios of isoimperatorin/osthole were calculated. Quality control samples to determine the accuracy and precision of the method were independently prepared by standard biology solution at low (0.027 μg/mL), medium (0.532 μg/mL) and high (5.32 μg/mL) concentrations. Extraction yields were determined by comparing the peak area ratios after extraction from biology samples with the peak area ratios of not extracted standards. All samples were stored at −20 °C until analysis. Analyst stability in plasma was evaluated by analyzing three replicates of QCs exposed to different conditions of time and temperature subjected to long-term storage at room temperature or −20 °C. Stability in the reconstitution solvent was assessed by re-pretreated samples and comparing the results with those of freshly extracted ones.

2.8. Statistical analysis

The pharmacokinetic parameters of isoimperatorin in rats were calculated by the 3p97 software supplied by the Pharmacological Society of China (Beijing, China). The tissue distributions of imperatorin in rat's tissue were evaluated by measuring the concentrations of isoimperatorin at 1.5 h after the oral administration (at about 30 min after the time of T_{max}). All data were expressed as means ± standard deviation. The statistical differences were estimated with Student's test.

3. Results and Discussion

3.1. Chromatography

A typical gas chromatography–total ion current (GC–TIC) mass spectrogram chromatogram and structure formula obtained for isoimperatorin standard and osthole standard are shown in Fig. 2 as analyzed by GC–MS in the electron impact (EI) mode. The retention time of isoimperatorin was 11.8 min and osthole was 10.8 min. It is obvious that the GC–TIC chromatogram and mass-spectrogram can provide the elective ion and the time program: from 3 to 11.5 min, the *m/z* was 244 (osthole) and 11.5–20 min the *m/z* was 202 (isoimperatorin).

The gas chromatography–selected ion monitoring (GC–SIM) chromatograms obtained for blank samples, isoimperatorin standard and osthole standard, blank tissue samples and tissue samples are shown in Fig. 3 and plasma and tissue samples after administration of isoimperatorin in Fig. 4 as analyzed by GC–MS. The presence of specific fragmentations, such as *m/z* of 244 and 202 is shown in Fig. 2. It is obvious that the GC–SIM method simplifies the chromatogram very efficiently and provides a single peak for identification.

3.2. Linearity and limit of detection

Linear calibration curves were obtained in the given concentration range of isoimperatorin in plasma samples and each tissue sample, respectively. Standard curves were fitted to a first-degree

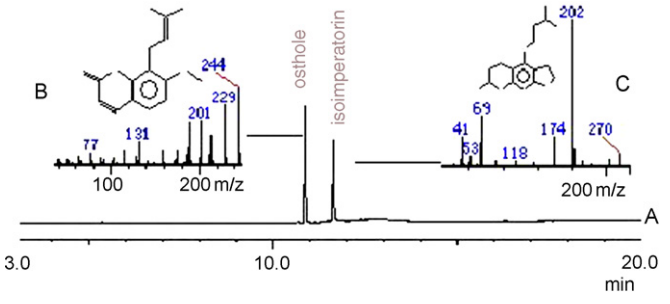

Fig. 2. Total ion current chromatogram of isoimperatorin and osthole (A), mass-spectrogram and structure formula of osthole (B) and mass-spectrogram and structure formula of isoimperatorin (C).

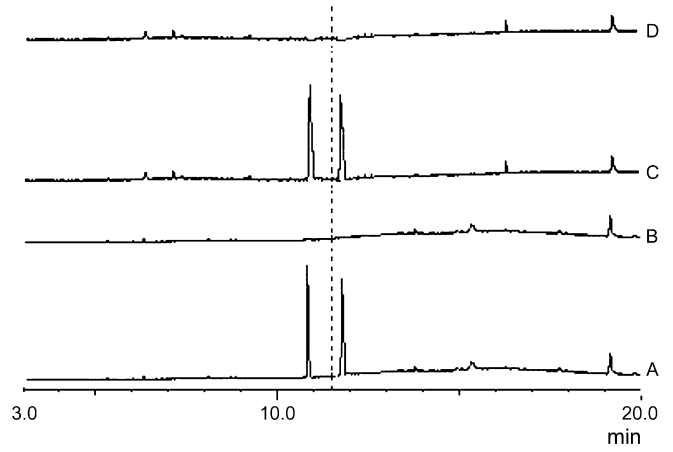

Fig. 3. Selected ion monitoring chromatograms of an extract of blank plasma spiked with isoimperatorin (0.532 μg/mL) and osthole (A), blank plasma (B), blank heart homogenate spiked with isoimperatorin (tissue 2.128 μg/g) and osthole (C) and blank heart homogenate (D).

polynomial, $y = ax + b$, where y is the peak area of imperatorin/osthole, a and b are constants and x is isoimperatorin concentration (μg/mL). Typical values for the regression parameters and the concentration range of the different sample are listed in Table 1. The limits of detection (LOD) of the method were measured to be up to 1 ng/mL or 1 ng/g of isoimperatorin (S/N ≥ 3).

3.3. Precision, accuracy and recovery

Obviously, our method provides accuracy and selectivity for determination of isoimperatorin. However, for an accuracy quantization, it is necessary to estimate the recovery under different situations. Table 2 shows a summary of recoveries for rats' biology samples spiked with different concentrations of isoimperatorin (0.027, 0.532 and 5.32 μg/mL, $n = 5$) from five normal rats (blank experiments). The recoveries for all samples were >80%, except for liver samples which were >70%. The between-day precision ranged from 2.81–5.22% and within-day precision ranged from 4.72 to 6.52%.

3.4. Analyte stability

The stability tests were designed to cover the anticipated conditions that real samples may experience. The results are summarized in Table 3. Ambient temperature storage of the QCs for up to 2 h prior to pretreatment appeared to have little effect on the quantification. QCs stored in a freezer at −20 °C remained stable through the course of 4 weeks.

Table 1
Linear regression of peak area ratios of isoimperatorin/osthole and concentrations for isoimperatorin in biological samples

Sample	Calibration curve	Correlation coefficient	Linear range (μg/g)
Plasma	$y = 0.996x + 0.073$	0.9986	0.027–5.32*
Heart	$y = 0.139x + 0.013$	0.9970	0.108–21.28
Liver	$y = 0.158x - 0.127$	0.9996	0.108–21.28
Spleen	$y = 0.171x - 0.172$	0.9997	0.108–21.28
Lung	$y = 0.189x - 0.243$	0.9956	0.108–21.28
Kidney	$y = 0.168x - 0.135$	0.9982	0.108–21.28
Cerebrum	$y = 0.155x - 0.110$	0.9941	0.108–21.28
Cerebellum	$y = 0.078x - 0.189$	0.9986	0.216–42.56

* Unit is μg/mL.

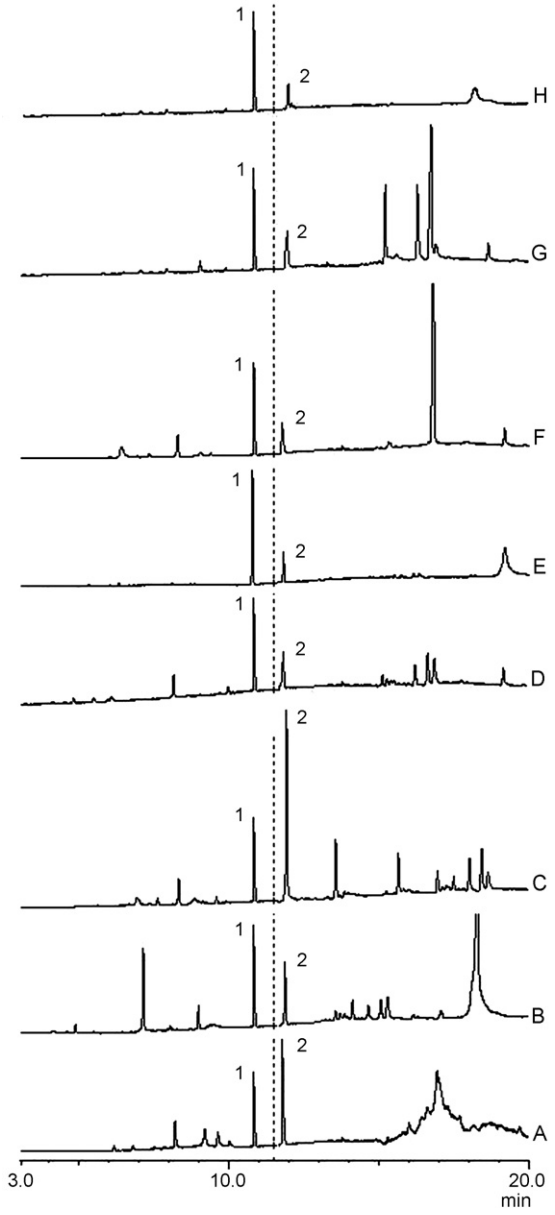

Fig. 4. Chromatograms of rat plasma and tissue samples after administration of isoimperatorin (A) plasma, (B) heart, (C) liver, (D) spleen, (E) lung, (F) kidney, (G) cerebrum and (H) cerebellum, (1) osthole (2) isoimperatorin.

3.5. Pharmacokinetics analysis

The concentration of isoimperatorin in plasma sample was determined by the same method. The plasma isoimperatorin concentration–time curves were analyzed using 3p97 program to determine the compartment model, and the pharmacokinetics parameters calculated. The plasma isoimperatorin concentration–time curve conformed to the one-compartment with the first absorption model. Fig. 5 shows representative

Table 2
Within-day and between-day precision and recovery of isoimperatorin from biological samples

Sample	Added (μg/g)	Intra-day			Inter-day		
		Found (μg/g)	RSD (%)	Recovers (%)	Found (μg/g)	RSD (%)	Recovers (%)
Plasma*	0.027	0.023	3.91	84.81	0.022	4.76	83.23
	0.532	0.459	5.22	86.32	0.464	6.52	87.21
	5.32	4.64	4.83	87.29	4.63	5.12	86.99
	0.216	0.189	3.99	87.55	0.185	5.14	85.70
Heart	2.128	1.854	4.35	87.12	1.779	4.85	83.58
	10.64	8.865	4.78	83.32	8.900	5.81	83.65
	0.216	0.162	3.26	75.06	0.156	5.00	72.22
Liver	2.128	1.608	3.39	75.55	1.580	4.79	74.27
	10.64	8.609	5.11	80.91	8.210	6.04	77.16
	0.216	0.181	3.64	83.94	0.179	4.60	83.10
Spleen	2.128	1.813	4.41	85.20	1.809	4.82	85.02
	10.64	9.380	4.67	88.16	9.187	5.37	86.34
	0.216	0.190	4.38	88.15	0.182	5.20	84.18
Lung	2.128	1.792	2.81	84.23	1.770	4.39	83.20
	10.64	9.315	4.44	87.55	9.193	5.18	86.40
	0.216	0.190	3.21	87.79	0.190	5.96	87.76
Kidney	2.128	1.904	4.51	89.48	1.871	5.44	87.92
	10.64	9.337	3.81	87.75	8.956	5.07	84.17
	0.216	0.190	3.04	87.87	0.185	4.12	85.82
Cerebrum	2.128	1.939	4.41	91.13	1.895	4.90	89.05
	10.64	8.873	4.66	83.39	8.829	4.69	82.98
	0.216	0.187	3.32	86.76	0.183	4.60	84.91
Cerebellum	2.128	1.857	3.83	87.27	1.781	4.27	83.68
	10.64	8.999	4.34	84.58	8.796	5.69	82.67

* Unit is μg/mL.

plasma concentration–time profiles for an oral administration of 10 mg/kg isoimperatorin. The pharmacokinetics parameters are summarized in Table 4. A peak concentration of isoimperatorin of (0.72 ± 0.14) μg/mL was achieved within about 1 h after administration. Plasma levels then declined rapidly. The area under the plasma concentration–time curve (AUC) was (2.11 ± 0.29) h μg/mL.

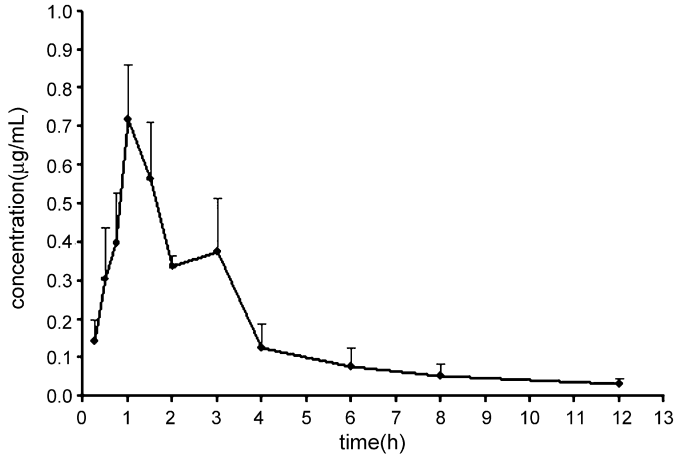

Fig. 5. The mean plasma concentration–time curve of isoimperatorin in rats after oral administration isoimperatorin (10 mg/kg, $n = 5$).

3.6. Tissue distribution analysis

There was a wide tissue distribution of isoimperatorin in rats at 1.5 h after oral administration as shown in Fig. 6. The observed distribution consequence in isoimperatorin concentration was as follows: cerebrum > cerebellum > liver > heart > spleen > lung > kidney.

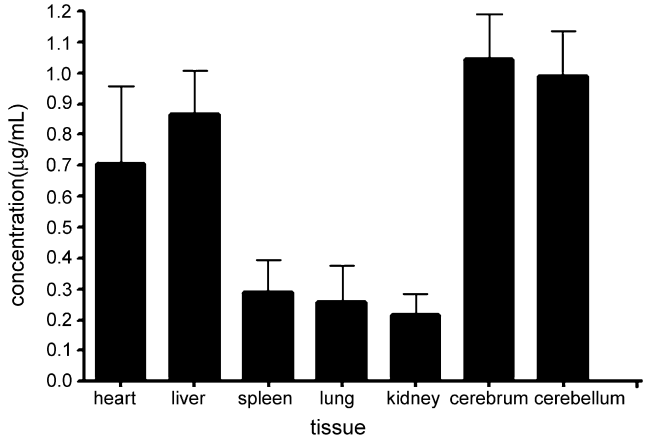

Fig. 6. The mean tissue distributions of isoimperatorin in rat heart, liver, spleen, lung, kidney, cerebrum and cerebellum at 1.5 h after oral administration isoimperatorin (10 mg/kg, $n = 5$).

Table 3
Summary of the stability of isoimperatorin in rat plasma and tissue samples

Samples	Added (μg/g)	25 °C for 2 h			−20 °C for 4 weeks		
		Found (μg/g)	RSD (%)	Recovers (%)	Found (μg/g)	RSD (%)	Recovers (%)
Plasma*	0.027	0.023	6.22	85.19	0.024	5.69	88.89
	0.532	0.465	3.13	87.41	0.452	4.18	84.96
	5.32	4.85	2.59	91.17	4.83	3.21	90.79
	0.216	0.186	7.76	86.24	0.181	6.01	83.69
Heart	2.128	1.826	5.12	85.81	1.736	6.72	81.57
	10.64	8.726	4.55	82.01	8.686	4.68	81.64
	0.216	0.153	6.03	70.75	0.149	6.87	69.21
Liver	2.128	1.516	6.16	71.24	1.495	4.66	72.26
	10.64	7.795	5.88	74.26	7.996	5.91	70.15
	0.216	0.178	5.41	82.63	0.175	4.47	81.09
Spleen	2.128	1.785	4.18	83.89	1.766	4.69	83.01
	10.64	9.241	3.44	86.85	8.973	4.24	84.33
	0.216	0.188	6.15	86.84	0.177	7.07	82.17
Lung	2.128	1.765	3.58	82.92	1.728	4.26	81.19
	10.64	9.176	6.21	86.24	8.979	4.05	84.39
	0.216	0.187	5.98	86.48	0.185	4.83	85.75
Kidney	2.128	1.876	3.28	88.17	1.828	4.31	85.91
	10.64	9.197	5.58	86.44	8.742	3.94	82.16
	0.216	0.187	4.81	86.56	0.181	4.99	83.81
Cerebrum	2.128	1.911	3.18	89.82	1.852	3.77	87.04
	10.64	8.733	3.43	82.08	8.615	5.56	80.97
	0.216	0.185	4.09	85.45	0.179	3.47	82.9
Cerebellum	2.128	1.829	3.16	85.96	1.738	3.14	81.67
	10.64	8.860	3.11	83.27	8.582	4.56	80.66

* Unit is μg/mL.

Table 4
Pharmacokinetic parameters of isoimperatorin in rats

	K_e (h^{-1})	K_a (h^{-1})	$T_{1/2k\alpha}$ (h)	$T_{1/2ke}$(h)	AUC (μg mL^{-1} h^{-1})	CL (mL g^{-1} h^{-1})	T_{peak} (h)	Vd (mL g^{-1})	C_{max} (μg mL^{-1})
Average	1.34	1.76	0.40	1.72	2.11	5.16	1.03	11.56	0.72
±SD	0.44	0.13	0.13	0.38	0.29	0.36	0.12	0.18	0.14

4. Conclusions

A gas chromatography–selected ion monitoring (SIM) mass spectrometry method for the qualitative and quantitative analysis of isoimperatorin in rat plasma and tissue is described. This method has been demonstrated to be usable in pharmacokinetic and tissue distribution studies of isoimperatorin in *Angelica dahurica* and its prescriptions. The pharmacokinetic and tissue distribution results achieved may be useful for further study of the bioactive mechanism of isoimperatorin.

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References

[1] L.M. Perry, Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses, The MIT Press, Cambridge, 1980.
[2] H.Y. Hsu, Y.P. Chen, S.J. Shen, C.C. Hsu, H.C. Chang, Oriental Materia Medica: A Concise Guide, Oriental Healing Arts Institute, 1986.
[3] Q.Z. Zhang, X.Y. Xu, X.L. Zhang, Chin. J. Ethnomed. Ethnopharm. 2 (2002) 98.
[4] W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin, Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer, Berlin, 1992.
[5] C.M. Kim, M.Y. Heo, H.P. Kim, K.S. Sin, P. Pachaly, J. Arch. Pharm. Res. 14 (1991) 87.
[6] Y. Saiki, K. Morinaga, O. Okegawa, S. Sakai, Y. Amaya, A. Ueno, S. Fukushima, J. Yakugaku Zasshi 53 (1971) 1313.
[7] H.S. Ban, S.S. Lim, K. Suzuki, S.H. Jung, S. Lee, Y.S. Lee, K.H. Shin, K. Ohuchi, J. Planta Med. 69 (2003) 408.
[8] Ola Bergendorff, Kim Dekermend Jian, Mogens Nielsen, J. Phytochem. 44 (1997) 1121.
[9] Y.B. Ji, Pharmacological Action and Application of Available Composition of Traditional Chinese Medicine, Heilongjiang Science &Technology Press, Haerbin, 1995.
[10] I. Takano, I. Yasuda, N. Takahashi, T. Hamano, T. Seto, K. Akiyama, J. Kenkyu, Nenpo-Tokyo-toritsu Eisei Kenkyusho 41 (1990) 62.
[11] Y. Wei, Y. Ito, J. Chromatogr. A 1052 (2004) 223.
[12] F.Q. Yang, T.Y. Zhang, Q.H. Liu, G.Q. Xu, Y.B. Zhang, S. Zhang, Y.C. Ito, J. Chromatogr. A 883 (2000) 67.
[13] R.M. Liu, A.F. Li, A.L. Sun, J. Chromatogr. A 1052 (2001) 223.
[14] Eric Frerot, Erik Decorzant, J. Agric. Food Chem. 52 (2004) 6879.
[15] P.N. Gueye, S.W. Borron, P. Risede, C. Monier, F. Buneaux, M. Debray, F.J. Baud, Toxicol. Sci. 65 (2002) 107.