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A capillary gas chromatography-selected ion monitoring mass spectrometry method for the analysis of atractylenolide I in rat plasma and tissues, and application in a pharmacokinetic study

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Abstract

The aim of this paper is to investigate the characteristics of atractylenolide I (AO-I) in the body by a GC–MS method. All bio-samples were cleared up with a liquid–liquid extraction procedure. The calibration curves were linear within a range of 5–1000 ng/mL for plasma samples, 0.06–16.00 μg/g for cerebellum samples, and 0.03–8.00 μg/g for other tissue samples. The limit of quantification (LOQ) for AO-I was 1.0 ng/mL or 1.0 ng/g (S/N ≥ 10) in the bio-samples. In the applications, the main pharmacokinetic parameters were firstly obtained as follows: $T_{max} = 0.37 \pm 0.19$ h, $C_{max} = 0.26 \pm 0.05$ μg/mL, AUC = 1.95 ± 0.30 μg h/mL and $k_a = 10.08 \pm 5.60\ h^{-1}$. The tissue distribution of AO-I in rats after the oral administration of 50.0 mg/kg was from 0.225 to 0.031 μg/g with a decreasing tendency in different tissues like liver > kidney > spleen > cerebellum > heart > cerebrum > lung. The protein binding in rat plasma, human plasma and bovine serum albumin was 80.8 ± 3.9, 90.6 ± 3.1 and 60.9 ± 5.1%, respectively.
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Keywords: Atractylenolide I; Pharmacokinetics; Tissue distribution; Protein binding; GC–MS

1. Introduction

Atractylenolide I (AO-I) is one of the major components considered to be bioactive from Rhizoma *Atractylodes macrocephala* (Baizhu in Chinese), popularly used for the treatment of the spleen and stomach ailments in Asia.

AO-I (as its chemical structure shown in Fig. 1B) is a volatile lactone with a low polarity. Usually, the content of AO-I in Baizhu is about 0.05% (w/w) [1]. AO-I can be isolated from Baizhu herbs using column chromatographic procedures [2]. Pharmacological studies have indicated several activities of AO-I in biological systems such as gastrointestinal inhibitory effects [3], anti-oxidant activity [4], etc. In addition, it was reported that AO-I can improve cachectic cancer patients' appetite and mid-arm muscle circumference, and can significantly depress the levels of serum IL-1, TNF-α, urine PIF, etc. [5]. Recently, some studies [2,6] in our lab have showed that AO-I can selectively antagonize TLR_4 receptor to show an anti-inflammatory effect. There have been some literature reports of the determination of AO-I in Baizhu herbs [1,7] and in related prescriptions [8,9] by HPLC. However, the methods reported were not sensitive enough to determine AO-I in bio-samples because the limit of detection (LOD, S/N ≥ 3) was only about 0.2 μg/mL. In order to quantitatively know the behavior of AO-I in the body, such as its pharmacokinetics, tissue distribution, protein-binding ratio, etc., it is necessary to establish a suitable method for measuring lower concentrations of AO-I in the bio-samples.

In this paper, we describe a sensitive capillary gas chromatography-selected ion monitoring mass spectrometric (GC–MS) method for the studies on the characteristics of AO-I in biological matrices. To improve the accuracy and precision of the method, imperatorin (chemical structure shown in Fig. 1C) was used as an internal standard. This method was applied in a pharmacokinetic study, an investigation of tissue distribution of AO-I, and a protein-binding study.

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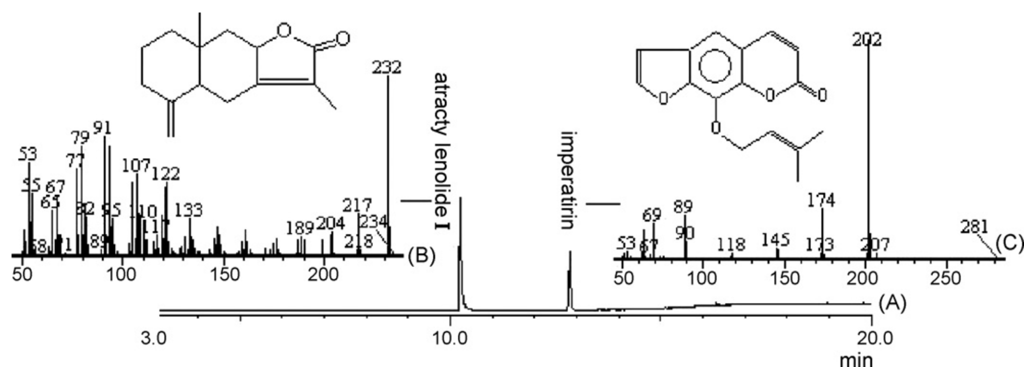

Fig. 1. Total ion current chromatogram of AO-I and imperatorin (A), mass-spectrogram and structure formula of AO-I (B) and mass-spectrogram and structure formula of imperatorin (C).

2. Experimental

2.1. Chemicals and reagents

The dried roots of *A. macrocephala* were bought from the civilian's drugstore (Xi'an, China) and identified by the pharmacognosy laboratory of the School of Medicine, Xi'an Jiaotong University (Xi'an, China). Imperatorin was supplied by the National Institute for the Pharmaceutical and Biological Products of China. HPLC grade methanol, *n*-hexane, ether, methanol and acetic ether were obtained from Fisher Scientific (Pittsburg, PA). Helium (purity, 99.999%) was from Xi'an Analytical Instrument Factory (Xi'an, China). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO). Human plasma was obtained from a healthy volunteer. Other reagents used in the experiment were analytical grade and from commercial sources.

2.2. Instrumentation and GC–MS conditions

The HPLC system consisted of a SPECTRA P200 chromatographic pump, a SPECTRA 100 detector (Thermo Separation Products, Fremont, CA, USA), a 7125 hand-sampling valve (Rheodyne Company, Berkeley, USA), and an ANASTAR chromatographic workstation (AOTAI Technology Ltd., Tianjin, China). SFE was carried out by using a HA 220-50-06 extraction system (Hua'an SFE Ltd., Nantong, Jiangsu, China).

A capillary GC–MS instrument (GCMS-QP2010 Shimadzu, Kyoto, Japan) with a DB-5MS capillary column (30 m × 0.32 mm I.D., 0.25 μm film thicknesses, Agilent Technologies, Palo Alto, CA, USA) was used. The inlet temperature was maintained at 280 °C. The oven temperature was initially held at 140 °C for 2 min and was 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. The detection was operated under selected ion monitoring (SIM) mode and the time program: from 3 to 12.0 min the *m*/*z* was 232 (AO-I) and 12.0–20 min the *m*/*z* was 202 (imperatorin). The ions of *m*/*z* 232 and *m*/*z* 202 were the most abundant and were selected for quantitation. Data were collected and analyzed using an NIST library (Shimadzu, Kyoto, Japan).

2.3. Isolation and purification of AO-I

The dried roots of *A. macrocephala* were powdered (about 60 mesh). 2 kg of the powder was put into 5 L supercritical extraction pan and the temperature control apparatus was switched on. When the temperature of extraction pan reached 50 °C, compressor pump was turned on to keep the pressure and the temperature of extraction pan at 20.0 MPa and 50 °C and that of separation pan at 10.0 MPa and 30 °C. The powder was cyclically extracted for 3 h with a 40 kg/h flow rate of CO_2. The supercritical CO_2 fluid extraction of *A. macrocephala* was a yellow oil. The extraction yield was 2.5%.

50 g of the yellow oil was dissolved in Et_2O and adsorbed with 65 g of silica gel. After 300 g of silica gel was packed into a column (600 mm × 100 mm) by dry column-packing method, the sample was added at the upper end of the column and eluted with solvent systems of light petroleum, light petroleum/Et_2O (1:1) and Et_2O, respectively. The fraction of light petroleum/Et_2O (1:1) (10 g) was collected and subjected to column chromatography over C_{18} silica gel (600 mm × 30 mm), and eluted with MeOH/H_2O (60:40–65:35). The fractions were analyzed by HPLC. An ODS column (150 mm × 4.6 mm, 5 μm) was used in the analytical chromatographic separation. The mobile phase was MeOH/H_2O (67:33) with a flow rate of 1.0 mL/min at a column temperature of 37 °C, and the detection wavelength was set at 220 nm. The fractions containing AO-I were combined and the MeOH was retrieved. The remaining aqueous solution was extracted with Et_2O. The fractions of Et_2O were combined and the Et_2O was retrieved. White-needle-like crystals were formed and separated by a filter. The structure of the crystal was identified as AO-I by comparing the spectral data (^{1}H, ^{13}C NMR, and MS) with reports according to the literature [10]. The purity of AO-I was greater than 99% as determined by HPLC equipped with an UV detector.

2.4. Standard solutions

Stock solutions (1.0 mg/mL) of AO-I and imperatorin (internal standard, I.S.) were prepared in methanol and stored at

−20 °C. The AO-I stock solution was further diluted with methanol to obtain a set of standard solutions with a concentration range of 0.005–1.00 μg/mL for preparation of spiked AO-I in blank bio-samples. A 5.0 μg/mL solution of imperatorin was prepared by further diluting its stock solution with methanol.

2.5. Sample collection

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Experimentation Committee of the National Research Institute of Chinese Medicine. Male pathogen-free Sprague–Dawley rats, weighing 230–280 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 groups of rats. The first group was used to prepare blank plasma samples, the second one for the pharmacokinetic studies, and the third one for a tissue distribution study. The rats were initially anesthetized with ether, and remained anesthetized throughout the surgical period. The rats were given at least a 24 h recovery period to allow for washout of anesthesia.

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 at 1500 × *g* for 10 min, the blank plasma was obtained and stored at −20 °C until used.

Before the treatment with AO-I, the second group of animals was anesthetized as described above. A cannula was implanted in the left femoral artery to collect the blood sample. On the day of the experiment, rats received a 50.0 mg/kg dose of AO-I by oral administration. The pharmacokinetic study involved serial arterial blood sampling (250 μL) with 10 samples obtained from each animal at 0, 5, 10, 15, 30 min, 1, 3, 6, 12 and 48 h after the administration. The blood samples were transferred to heparinized micro tubes and then centrifuged at 1500 × *g* for 10 min. The resulting plasma (100 μL) was separated and frozen immediately at −20 °C until further analysis.

The third group of rats was sacrificed at 0.8 h after the administration and the tissues were immediately isolated, including the heart, liver, spleen, lung, kidney, cerebrum and cerebellum. They were weighed and about 0.5 g of each tissue was taken as analytical samples after drying excess moisture with filter paper. Tissue homogenate (2.0 mL for each) was obtained from the tissue samples, transferred to heparinized tube and stored at −20 °C until sample extraction.

2.6. Extraction procedure for bio-samples

Accurately measured 100 μL aliquots of plasma samples or 500 μL aliquots of tissue samples were each added to 10 mL glass centrifuge tubes followed by the addition of 20 μL of imperatorin solution (5.0 μg/mL). Then, 1.0 mL (for plasma sample) or 2.0 mL (for tissue sample) ethyl ether was added into the tubes. The mixture was vortexed for 5 min and centrifuged at 1500 × *g* for 10 min. The upper organic layer was transferred to a clean tube and evaporated to dryness under a gentle nitrogen stream at 40 °C. The residue was dissolved in 100 μL of *n*-hexane/ether (1:1) solution. Duplicate 1 μL aliquots of this solution were injected into the GC–MS systems for analysis.

2.7. Method validation

AO-I standard plasma samples (0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 μg/mL) were prepared by spiking 80 μL of blank plasma with 20 μL of standard solution and 20 μL of imperatorin solution (5.0 μg/mL) prepared above. AO-I standard tissue samples (0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 μg/mL) were prepared by spiking 450 μL of blank tissue homogenate with 50 μL of standard stock solution and 20 μL of imperatorin solution (5.0 μg/mL).

Samples were processed as described above and peak area ratios of AO-I/imperatorin were calculated. Quality control samples to determine the accuracy and precision of the method were independently prepared by blank plasma at low (0.005 μg/mL), medium (0.10 μg/mL) and high (1.0 μg/mL) concentrations. Extraction yields were determined by comparing the peak area ratios after extraction from biological samples with the peak area ratios of non-extracted standards. All samples were stored at −20 °C until analysis. Analyst stability in plasma and tissue was evaluated by analyzing three replicates of QCs exposed to different conditions of time and temperature subjected to long-term storage at room temperature. Stability for freeze at −20 °C and three freeze–thaw cycles for 4 weeks in the reconstitution solvent were assessed by re-extracted samples and comparing the results with those of freshly extracted ones.

2.8. Protein-binding ratio

Plasma protein binding of AO-I was determined by an ultrafiltration technique using a micropartition system (Centrifree, Millipore, Bedford, MA, USA). 1.0 mL of rat or human plasma or aliquots of bovine serum albumin solution (buffer pH 7.4: phosphate buffer 0.03 mol/L with 0.1 mol/L NaCl; protein concentration: 42.5 g/L) containing various concentrations of AO-I (0.005, 0.05 and 0.5 μg/mL) were incubated 4 h at 37 °C. Then, the aliquots (500 μL) were transferred to the ultrafiltration tubes with a filter pore size of 10 kDa and centrifuged at 3000 × *g* for 20 min at 22 °C. Accurately measured 100 μL aliquots of the filtrate were each added to 10 mL glass centrifuge tubes followed by the addition of 20 μL of imperatorin solution (5.0 μg/mL). Measurement of AO-I was the same as the plasma samples. The fraction of unbound drug was determined by the following equation: $f_u = C_u/C_t$. Where f_u is the fraction of unbound drug in plasma and C_u and C_t are the unbound and total concentrations of the drug in plasma, respectively.

2.9. Statistical analysis

The pharmacokinetic parameters of AO-I in rats were calculated by 3p97 software supplied by the Pharmacological Society of China (Beijing, China). The tissue distribution of AO-I in rat tissues was evaluated by determining the concentrations of AO-I at 0.8 h after the oral administration (at about 30 min after the time of T_{max}). All data were expressed as means ± standard devi-

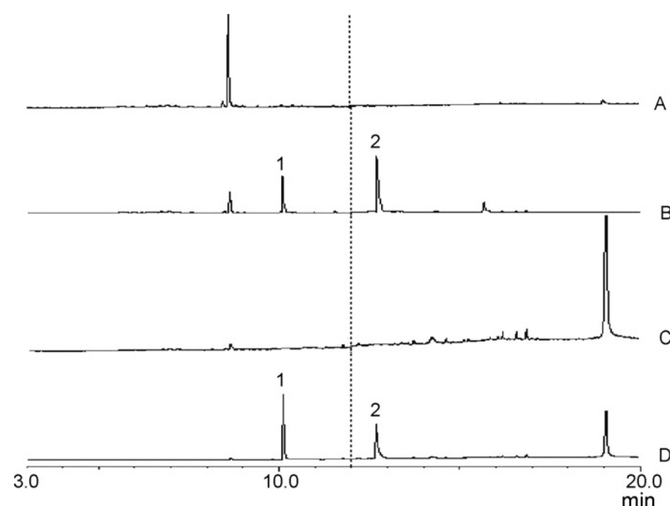

Fig. 2. Selected ion monitoring chromatograms of an extract of blank plasma (A), blank plasma spiked with AO-I (0.10 μg/mL) and imperatorin (1.0 μg/mL) (B), blank heart homogenate (C), blank heart homogenate spiked with AO-I (0.10 μg/mL) and imperatorin (0.2 μg/mL) (D). 1. AO-I, 2. Imperatorin.

ation. The statistical differences were estimated with Student's *t*-test.

3. Results and discussion

3.1. Chromatography

Typical GC–MS chromatograms obtained for AO-I standard and imperatorin standard are shown in Fig. 1. The retention time of AO-I was 10.1 min and imperatorin was 12.7 min. The GC–MS chromatograms obtained for blank samples, AO-I standard and imperatorin standard, blank tissue samples and tissue samples are shown in Fig. 2 and plasma and tissue samples after administration of AO-I in Fig. 3. The presence of specific fragmentations, such as *m*/*z* of 232 and 202 was shown in Fig. 1. The GC-selected-ion monitoring method simplifies the chromatogram and provides a single peak for identification.

3.2. Linearity and LOQ

Linear calibration curves were obtained in the given concentration range of AO-I in plasma samples and each tissue sample, respectively. Standard curves were fitted to a first degree polynomial, $y = ax + b$, where y is the peak area of AO-I/imperatorin, a and b were constants, and x is AO-I concentration (μg/mL or μg/g). Typical values for the regression parameters and the concentration range of the different sample were listed in Table 1. The LOQ of the method was measured to be up to 1 ng/mL or 1 ng/g of AO-I (S/N ≥ 10).

3.3. Precision, accuracy and recovery

Table 2 shows a summary of recoveries for 5 blank rat samples spiked with different concentrations of AO-I (0.005, 0.10 and 1.00 μg/mL). The recoveries for all samples were >80%, except for liver samples which were >75%. The between-day precision ranged from 1.0 to 6.4% and within-day precision ranged from 3.3 to 7.3%.

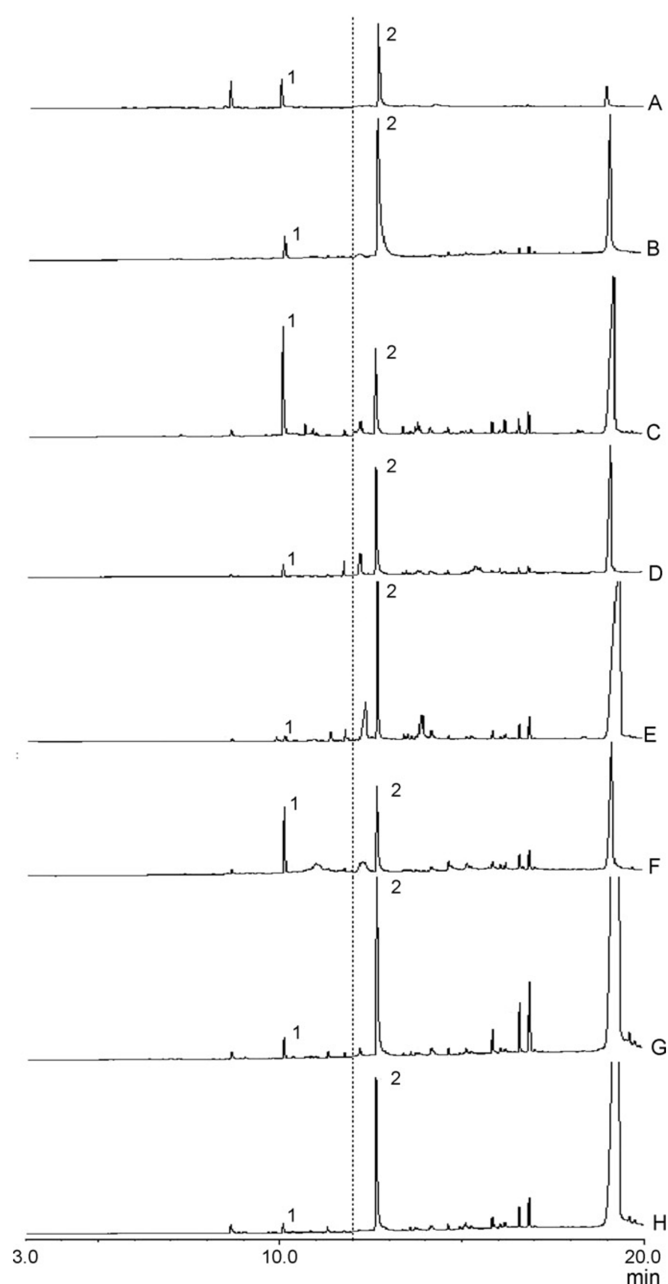

Fig. 3. Chromatograms of rat plasma and tissue samples after administration of AO-I. Plasma 0.235 μg/mL (A), heart 0.073 μg/mL (B), liver 0.291 μg/mL (C), spleen 0.046 μg/mL (D), lung 0.019 μg/mL (E), kidney 0.103 μg/mL (F), cerebrum 0.050 μg/mL (G), cerebellum 0.088 μg/mL (H). 1. AO-I, 2. Imperatorin.

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 and subjected to three freeze–thaw cycles remained stable through the course of 4 weeks.

Table 1
Summary of linearity (range, slope, r^2, and intercept values) AO-I in biological samples

Analyte	Linearity				SOQ (μg/mL or g)
	Range (μg/mL or g)	r^2	Slope ± S.D.	Intercept ± S.D.	
Blood	0.005–1.00	0.9995	0.359 ± 0.014	0.006 ± 0.001	0.001
Heart	0.03–8.00	0.9998	0.198 ± 0.008	0.183 ± 0.007	0.001
Liver	0.03–8.00	0.9942	0.137 ± 0.006	0.259 ± 0.008	0.010
Spleen	0.03–8.00	0.9998	0.243 ± 0.009	0.274 ± 0.012	0.008
Lung	0.03–8.00	0.9990	0.217 ± 0.003	0.262 ± 0.005	0.004
Kidney	0.03–8.00	0.9994	0.238 ± 0.009	0.256 ± 0.010	0.009
Cerebrum	0.03–8.00	0.9997	0.176 ± 0.006	0.164 ± 0.007	0.001
Cerebellum	0.06–16.00	0.9996	0.087 ± 0.004	0.219 ± 0.011	0.009

3.5. Pharmacokinetics analysis

Plasma AO-I concentration–time curves from the pharmacokinetic study were analyzed using 3p97 program to determine the compartment model, and the pharmacokinetic parameters were also calculated. The plasma AO-I concentration–time curve conformed to the two-compartment model with first order absorption. Fig. 4 shows representative plasma concentration–time profiles for an oral administration of 50 mg/kg AO-I. The pharmacokinetic parameters are summarized in Table 4. A peak concentration of AO-I of 0.26 ± 0.05 μg/mL was achieved within about 0.37 h after administration. Plasma levels then declined rapidly. The area under the plasma concentration–time curve (AUC) was 1.95 ± 0.30 μg h/mL.

3.6. Tissue distribution analysis

There was a wide tissue distribution of AO-I in rats at 0.8 h after oral administration and shown in Fig. 5. The observed distribution in order of AO-I concentration was as follows: liver > kidney > spleen > cerebellum > heart > cerebrum > lung.

3.7. Protein-binding ratio

Plasma protein binding of AO-I was determined by the ultrafiltration method. The bound fractions of AO-I were calculated

Table 2
Within-day and between-day precision and recovery of AO-I from biological samples ($n = 6$)

Sample	Added (μg/mL or g)	Intra-day			Inter-day		
		Found (μg/mL or g)	Recovery (%)	R.S.D. (%)	Found (μg/mL or g)	Recovery (%)	R.S.D. (%)
Plasma	0.02	0.018	90.9	1.3	0.018	92.0	5.6
	0.10	0.090	90.4	5.9	0.095	94.6	6.9
	1.00	0.916	91.6	3.0	0.919	91.9	4.0
Heart	0.04	0.036	90.3	1.0	0.036	89.8	3.3
	0.80	0.709	88.7	4.4	0.709	88.6	4.9
	8.00	7.282	91.0	2.5	7.012	87.7	3.7
Liver	0.04	0.032	80.4	6.4	0.032	79.2	7.3
	0.80	0.633	79.1	3.9	0.618	77.3	4.8
	8.00	6.439	80.5	5.1	6.493	81.2	6.0
Spleen	0.04	0.038	93.9	3.6	0.037	93.1	4.6
	0.80	0.762	95.2	4.4	0.712	89.0	4.8
	8.00	7.053	88.2	4.7	6.907	86.3	5.4
Lung	0.04	0.035	88.2	4.4	0.034	84.2	5.2
	0.80	0.754	94.2	5.8	0.714	89.2	4.4
	8.00	7.004	87.6	4.4	6.912	86.4	5.2
Kidney	0.04	0.035	87.8	3.2	0.035	87.8	6.0
	0.80	0.716	89.5	4.5	0.703	87.9	5.4
	8.00	7.020	87.8	3.8	7.214	90.2	5.1
Cerebrum	0.04	0.035	87.9	3.0	0.036	89.8	4.1
	0.80	0.729	91.1	4.4	0.712	89.1	4.9
	8.00	7.471	93.4	4.7	7.438	93.0	4.7
Cerebellum	0.08	0.077	96.8	4.3	0.076	94.9	4.6
	1.60	1.428	89.3	3.8	1.499	93.7	4.3
	16.00	15.133	94.6	4.3	14.827	92.7	5.7

Table 3
Summary of the stability of AO-I in biological samples

	Add (μg/mL)	Found (μg/mL)	Recovers (%)	R.S.D. (%)
Plasma				
25 °C for 2 h	0.02	0.018	90.5	5.3
	0.10	0.092	90.1	3.3
	1.00	0.915	91.3	1.1
−20 °C for 4 w	0.02	0.018	90.1	7.7
	0.10	0.091	90.1	4.4
	1.00	0.901	90.1	0.1
Freeze–thaw (three cycles)	0.02	0.018	89.8	5.6
	0.10	0.090	89.9	6.3
	1.00	0.912	91.2	4.8
Heart				
25 °C for 2 h	0.04	0.032	79.0	7.8
	0.80	0.643	80.4	5.1
	8.00	6.705	83.8	4.6
−20 °C for 4 w	0.04	0.032	80.2	6.0
	0.80	0.633	79.1	6.7
	8.00	6.171	77.1	4.7
Freeze–thaw (three cycles)	0.04	0.032	81.0	6.2
	0.80	0.664	83.0	6.0
	8.00	6.311	78.9	3.4
Liver				
25 °C for 2 h	0.04	0.037	91.8	6.0
	0.80	0.755	94.4	6.2
	8.00	7.343	91.8	5.9
−20 °C for 4 w	0.04	0.036	89.6	6.9
	0.80	0.708	88.5	4.7
	8.00	7.002	87.5	5.9
Freeze–thaw (three cycles)	0.04	0.037	93.0	7.8
	0.80	0.711	88.9	6.0
	8.00	6.897	86.2	4.6
Spleen				
25 °C for 2 h	0.04	0.034	84.1	5.4
	0.80	0.713	89.1	4.2
	8.00	6.902	86.3	3.4
−20 °C for 4 w	0.04	0.035	87.6	4.5
	0.80	0.702	87.8	4.7
	8.00	7.203	90.0	4.2
Freeze–thaw (three cycles)	0.04	0.036	89.7	6.8
	0.80	0.711	88.9	6.5
	8.00	7.428	92.9	5.1
Lung				
25 °C for 2 h	0.04	0.038	94.8	6.2
	0.80	0.748	93.6	3.6
	8.00	7.403	92.5	6.2
−20 °C for 4 w	0.04	0.037	91.4	7.1
	0.80	0.721	90.1	4.3
	8.00	7.083	88.5	4.1
Freeze–thaw (three cycles)	0.04	0.036	90.9	7.6
	0.80	0.642	80.2	4.3
	8.00	7.261	90.8	2.7
Kidney				
25 °C for 2 h	0.04	0.036	90.3	6.0
	0.80	0.732	91.4	3.3
	8.00	7.211	90.1	5.6
−20 °C for 4 w	0.04	0.035	88.5	4.8
	0.80	0.727	90.9	4.3
	8.00	6.418	80.2	3.9
Freeze–thaw (three cycles)	0.04	0.036	89.0	5.1
	0.80	0.643	80.4	4.9
	8.00	7.505	93.8	4.7

Table 3 (*Continued*)

	Add (μg/mL)	Found (μg/mL)	Recovers (%)	R.S.D. (%)
Cerebrum				
25 °C for 2 h	0.04	0.038	95.1	4.8
	0.80	0.704	88.0	3.2
	8.00	7.042	88.0	3.4
−20 °C for 4 w	0.04	0.038	94.1	5.0
	0.80	0.699	87.4	3.8
	8.00	7.013	87.7	5.6
Freeze–thaw (three cycles)	0.04	0.036	89.4	4.5
	0.80	0.701	87.6	3.8
	8.00	7.019	87.7	3.0
Cerebellum				
25 °C for 2 h	0.04	0.036	91.0	4.1
	0.80	0.746	93.3	3.2
	8.00	7.175	89.7	3.1
−20 °C for 4 w	0.04	0.036	88.9	3.5
	0.80	0.743	92.9	3.1
	8.00	7.582	94.8	4.6
Freeze–thaw (three cycles)	0.04	0.037	93.7	5.6
	0.80	0.740	92.7	4.4
	8.00	7.556	94.6	1.7

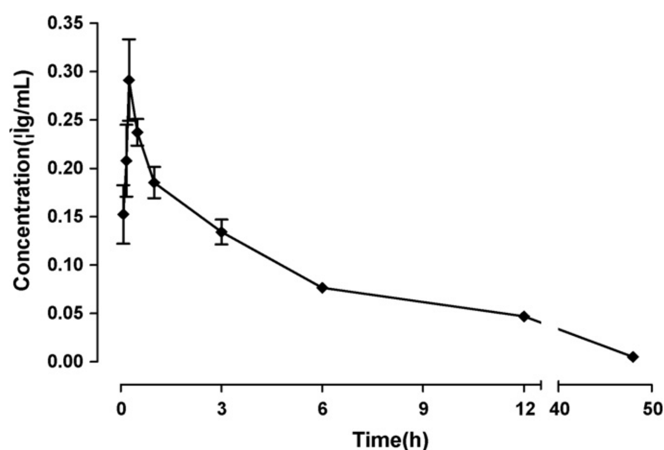

Fig. 4. The mean plasma concentration–time curve of AO-I in rats after oral administration of 50 mg/kg ($n = 5$).

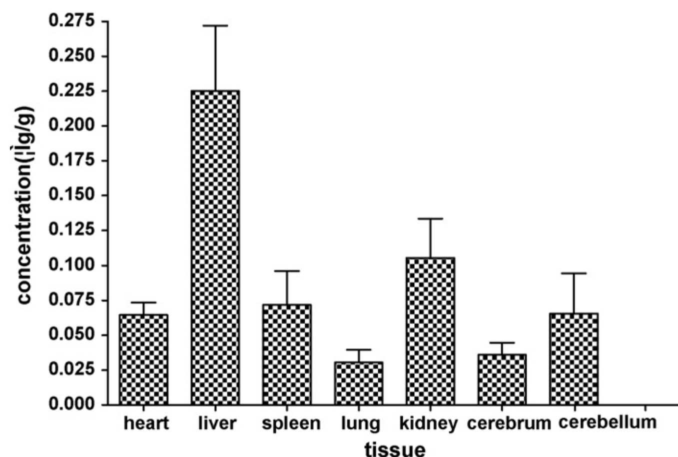

Fig. 5. The mean tissue distributions of AO-I in rat heart, liver, spleen, lung, kidney, cerebrum and cerebellum at 0.8 h after oral administration of 50 mg/kg ($n = 5$).

Table 4
Relevant pharmacokinetic parameters of AO-I in rats after oral administration of 50 mg/kg ($n = 5$)

	Average	S.D.
k_a (1/h)	10.08	5.60
$T_{1/2\alpha}$ (h)	0.92	0.65
$T_{1/2\beta}$ (h)	9.74	1.71
AUC (μg h/mL)	1.95	0.30
CL (L/h)	6.80	0.92
T_{max} (h)	0.37	0.19
Vd (mL/g)	0.19	0.09
C_{max} (μg/mL)	0.26	0.05

Table 5
Protein binding of AO-I with rat and human plasma, bovine serum albumin ($n = 3$)

	Total concentrations (ng/mL)	Protein binding (%)	Mean ± S.D. (%)
Rat plasma	5	81 ± 7	81 ± 4
	50	83 ± 1	
	500	79 ± 1	
Human plasma	5	92 ± 3	91 ± 3
	50	92 ± 2	
	500	87 ± 1	
Bovine serum albumin	5	56 ± 5	61 ± 5
	50	65 ± 4	
	500	61 ± 2	

and shown in Table 5. The protein-binding rates of AO-I with rat plasma, human plasma and bovine serum albumin were 81 ± 4, 91 ± 3 and $61 \pm 5\%$, respectively. Binding to the plasma is linear over the concentration range of 5–500 ng/mL. The rates of AO-I with rat and human plasma were higher than with bovine serum albumin.

4. Conclusions

A GC–MS method for the determination of AO-I in rat plasma and tissues has been demonstrated. Using this method, it was found that the AO-I in rats was easily absorbed and slowly eliminated after oral administration. As for tissue distribution, there was a decreasing tendency: liver > kidney > spleen > cerebellum > heart > cerebrum > lung. With regard to protein-binding ratio, differences were observed between rat plasma, human plasma and bovine serum albumin. The results will provide a basis for the further understanding of the behavior of AO-I in vivo.

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