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Short communication

Pharmacokinetics and tissue distribution study of orientin in rat by liquid chromatography

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Abstract

A simple HPLC–UV method was established for the determination of orientin in plasma and different tissues of rat (heart, liver, spleen, lung, kidney, brain, stomach and small intestine). The separation was achieved by HPLC on a C_{18} column with a mobile phase composed of acetonitrile–0.1% acetic acid (20:80, v/v), UV detection was used at 348 nm. Good linearity was found between 0.250–50.0 μg/ml ($r^2 = 0.9966$) for plasma samples and 0.050–50.0 μg/ml ($r^2 \geq 0.9937$) for the tissue samples, respectively. Within- and between-day precisions expressed as the relative standard deviation (R.S.D.) for the method were 2.3–9.6% and 3.0–7.4%, respectively. The relative recoveries of orientin ranged from 95.4 to 100.6% for plasma and 93.1 to 107.9% for tissue homogenates. The developed method was successfully applied to the pharmacokinetics and tissue distribution research after intravenous administration of a 20 mg/kg dose of orientin to healthy Sprague–Dawley rats. The main pharmacokinetics parameters obtained presented that orientin was quickly distributed and eliminated within 90 min after intravenous administration. The tissue distribution results showed that liver, lung and kidney were the major distribution tissues of orientin in rats, and that orientin had difficulty in crossing the blood–brain barrier. It was also found that there was no long-term accumulation of orientin in rat tissues.

Keywords: Orientin; HPLC; Plasma; Pharmacokinetics; Tissue distribution

1. Introduction

Orientin is one of flavonoid glycosides isolated from the dry flowers of *Trollius ledebouri* Reichb [1] which is widely used in the treatment of colds, high fevers, chronic tonsillitis and acute tympanitis [2]. As a main active compound, orientin demonstrated antiviral, antimicrobial, antioxidant, radioprotection activities [3,4]. The structure of orientin is shown in Fig. 1.

Analytical techniques applied to the quantification of orientin from various herbal medicines and foods included liquid chromatography–mass spectrometric method (LC–MS) [5–8], high-performance liquid chromatography (HPLC) with UV detection [9–13], thin-layer chromatography (TLC) [14], and so on. With the growing significance of a potential beneficial role of orientin in human health, there is an increasing demand for analyzing it in vivo and researching on its pharmacokinetics. However, to our knowledge, only one report had addressed the quantification of orientin in rabbit plasma samples [15], there has been no research on the pharmacokinetics and tissue distribution in rat after administration of pure orientin. It is well known that the pharmacokinetics and tissue distribution can help us explain and predict a variety of events related to the efficacy and toxicity of herbal preparations. Therefore, it is necessary for an intensive investigation on pharmacokinetics of pure orientin for a better understanding of the mechanism of action and facilitating further research and development of *T. ledebouri*.

In the present paper, we developed and validated a rapid and sensitive RP-HPLC method to determine orientin in rat plasma and tissues. The method was successfully applied to a pharmacokinetics and tissue distribution study after intravenous administration of 20 mg/kg orientin to healthy rats.

2. Experimental

2.1. Materials and reagents

The reference standard of orientin was extracted and purified from *T. ledebouri* Reichb in our laboratory. Its chemical

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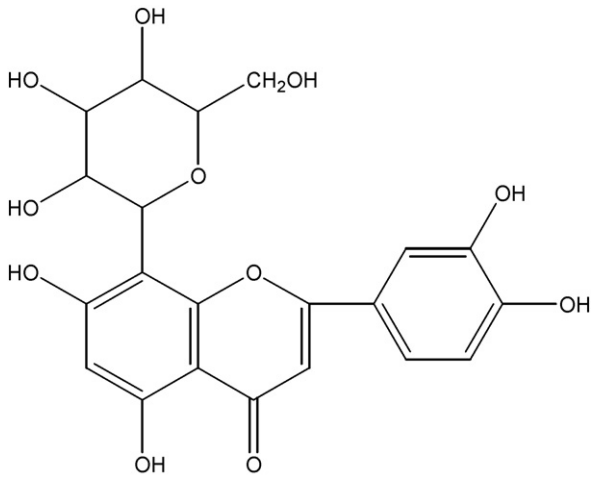

Fig. 1. Chemical structure of orientin.

structure was confirmed by ^{1}H and ^{13}C-nuclear magnetic resonance spectroscopy and its purity was over 99% by HPLC analysis. Orientin purity administrated to rats was over 95%. Acetonitrile and methanol were of HPLC grade and obtained from Tedia (Tedia, Fairfield, USA). The distilled water was prepared from demineralized water and used throughout the study.

2.2. HPLC conditions

The HPLC system mainly consisted of a Waters 515 pump and a Waters 2487 dual λ absorbance detector (Waters Assoc., Milford, MA, USA). Data was collected by a HPLC chromatography workstation (Intelligence and information institute of Zhejiang University). The analytical column was a Diamonsil C_{18} column (5 μm, 250 mm × 4.6 mm i.d.; Diamonsil, USA). The separation was carried out with the mobile phase consisting of acetonitrile–0.1% acetic water (20:80, v/v) at a flow rate of 1.0 ml/min. Chromatograms were monitored at 348 nm and the temperature of column was kept at 35 °C.

2.3. Animals

Male and female Sprague–Dawley rats (250 ± 20 g) were obtained from the Hebei Laboratory Animal Center (Shijiazhuang, People's Republic of China). They were kept in an environmentally controlled breeding room for 5 days before starting the experiments and fed with standard laboratory food and water ad libitum. On the day the rats were treated, chow was removed from the cages 8 h before the experiment. All protocols and procedures were approved by our Institutional Animal Care and Use Committee.

2.4. Drug administration and sampling

Orientin was dissolved in 1,2-propanediol-redistilled water (1:5, v/v) to the concentration of 4 mg/ml and filtered through a 0.22 μm filter before experiment. For pharmacokinetics study, the orientin solution was administered to rats ($n = 6$) via a tail vein injection at a dose of 20 mg/kg. Blood samples were obtained from the fossa orbitalis vein according to the specific schedule (2, 4, 8, 12, 16, 20, 25, 30, 45, 60, 90 min) and then centrifuged at 4000 × g for 20 min. The separated plasma was frozen at −20 °C before assay. Blank plasma was collected by the same method before rats were administrated. For tissue distribution study, 30 rats were assigned randomly to six groups and were given orientin solution via a tail vein injection at doses of 20 mg/kg. Heart, liver, spleen, lung, kidney, stomach, small intestine, and brain were obtained at 5, 15, 30, 45, 60 and 90 min after administration, respectively. Tissue samples were weighed rapidly and put into normal saline solution to remove the blood or content, blotted on filter paper, and then weighed for wet weight and homogenized in saline solution (500 mg/ml). The obtained tissue homogenates were stored at −20 °C until analysis performed using the procedure described below.

2.5. Sample extraction procedure

To 100 μl of the above plasma or tissue homogenates, 500 μl of methanol were added. The resulting solution was thoroughly vortex-mixed for 1 min. After centrifugation at 12,000 × g for 10 min, the supernatant was collected and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was then reconstituted with 100 μl mobile phase, and centrifuged at 12,000 × g for 5 min, and an aliquot (20 μl) of the supernatant was injected into the HPLC system.

2.6. Preparation of standard and quality control samples

Stock solution of orientin was prepared in methanol to give a final concentration of 500 μg/ml. A series of standard solutions with concentration in the range of 0.100–100.00 μg/ml was obtained by further dilution of the stock solution with methanol. All the solutions were stored at −20 °C and were brought to room temperature before use. To prepare the standard calibration samples and quality control samples, several orientin standards in the concentration range of 0.250–50.0 μg/ml in plasma or 0.050–50.0 μg/ml in homogenates were prepared by adding 50 μl of orientin stock solutions to 100 μl plasma or different tissue homogenates of untreated rat. The quality control (QC) samples, which were used in the validation, were prepared in the same way as the standard calibration samples.

3. Results and discussion

3.1. Chromatography

In the present study, the mobile phase comprised of acetonitrile and 0.1% acetic water (20:80, v/v). If acid was not used, the chromatogram of orientin had a phenomenon of tailing. Phosphoric acid and acetic acid was tested to improve the peak shapes and eliminate peak tailing. Though sharp and symmetric peak was found with these two acids, acetic acid was used for the consideration of column protection. A wavelength of 348 nm was chosen according to the maximum absorption spectrum of

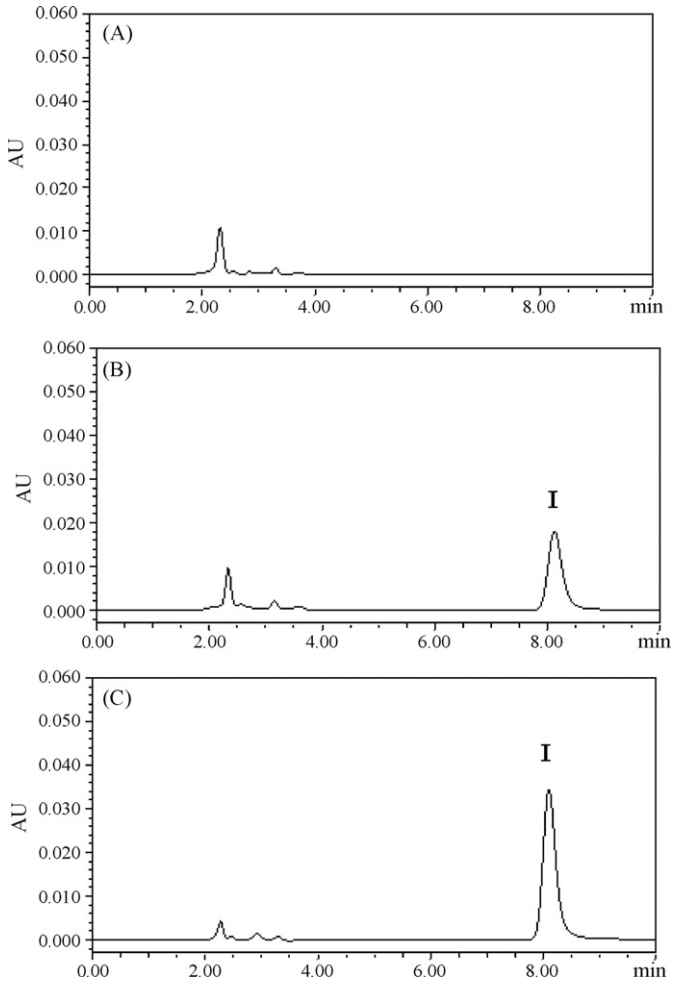

Fig. 2. Representative chromatograms of blank plasma (A), plasma sample spiked with orientin (B) and a plasma sample collected from a rat at 12 min after a single *i.v.* administration of orientin at a dosage of 20 mg/kg (C). No interferences were observed at the retention times of the analytes. Peak I: orientin.

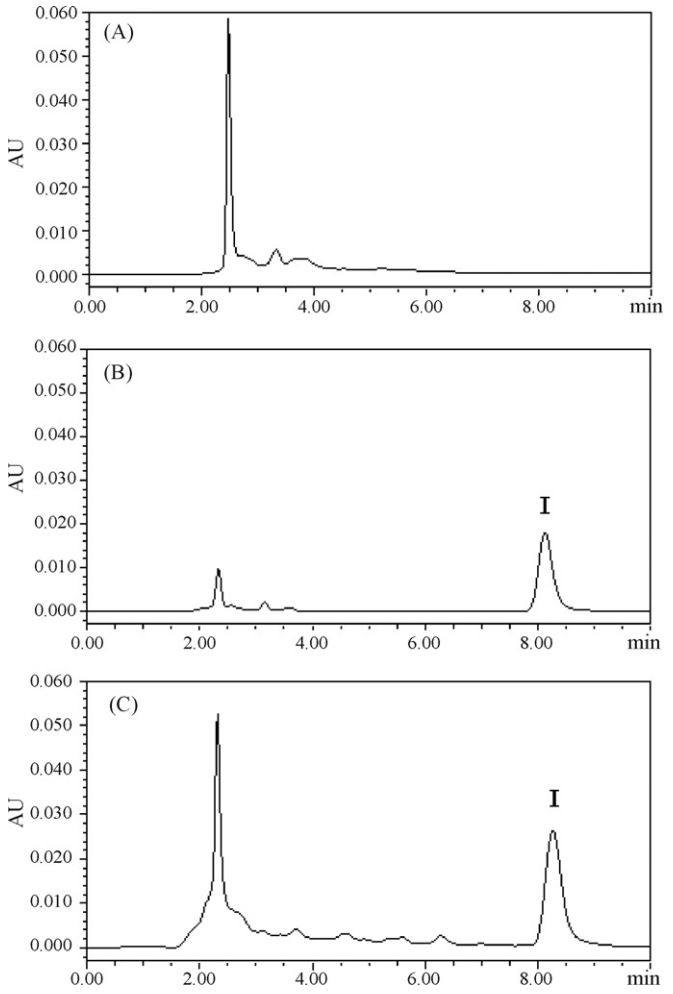

Fig. 3. Representative chromatograms for determination of orientin in rat lung. Blank tissue (A); blank tissue spiked with orientin (B); tissue samples at 15 min after administration orientin (C). Peak I: orientin.

orientin to achieve a high selectivity against endogenous compounds in the chromatograms. Compared to the previous study [15], the present method had a relatively simple elution system and a relatively short chromatography run time (the analysis time was shortened from 17.5 to 10 min).

3.2. Sample preparation

Due to multiple hydroxyl groups presented in the structure, orientin has strong polarity which resulted in its low solubility in low polarity solvent. Therefore, the higher polarity of solvent, *n*-butanol was employed as extraction solvent. However, the extraction recoveries obtained were unsatisfactory. Hence protein precipitation prior to solvent extraction was chosen as a simple and effective sample pretreatment method. The precipitating agents, acetonitrile, methanol and 6% perchloric acid were tested. To get a good separation and protect the HPLC column, methanol was chosen as the best protein precipitant.

In the previous method [15], the biosamples were also prepared based on a simple protein precipitation and the supernatant after precipitation was directly injected to HPLC system for analysis. The LOQ of the method was relatively high (0.53 μg/ml). However, orientin could not be detected in some biosamples in our present work with the same sample preparation method resulting to the failure of studying pharmacokinetics and tissue distribution of orientin after intravenous administration, which may be caused by the different experimental animal species.

Table 1
Calibration curves for orientin in plasma and tissues

Biosamples	Calibration curves	Correlation coefficients (r^2)	Linear ranges (μg/ml)
Plasma	$Y = 25184X - 2026.42$	0.9966	0.25–50.00
Kidney	$Y = 21037X + 1654.86$	0.9948	0.05–25.00
Liver	$Y = 23193X - 654.88$	0.9954	0.05–25.00
Lung	$Y = 25914X + 2524.34$	0.9954	0.50–25.00
Small intestine	$Y = 21052X + 10.85$	0.9964	0.05–2.50
Spleen	$Y = 22939X - 1090.37$	0.9946	0.05–2.50
Heart	$Y = 21062X - 1023.43$	0.9991	0.05–2.50
Stomach	$Y = 24354X - 1114.42$	0.9937	0.05–2.50
Brain	$Y = 21123X - 168.54$	0.9957	0.05–2.50

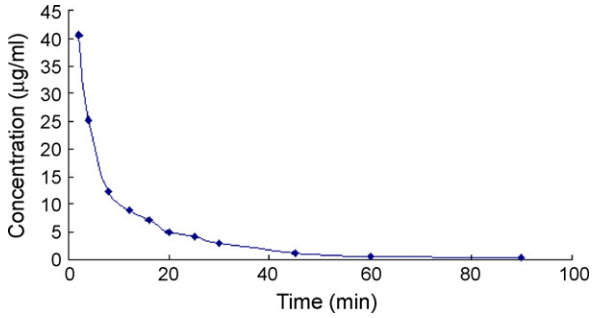

Fig. 4. Mean plasma concentration–time profiles of orientin after a single *i.v.* administration of 20 mg/kg orientin to rats.

Rabbits were used in the lecture [15], while rats were applied in this study. In order to improve the sensitivity of the method, the supernatant after protein precipitant was concentrated using nitrogen blowing. Accordingly, the LOQ of the present method was improved to 0.25 μg/ml in plasma, 0.50 μg/ml in lung and 0.05 μg/ml in other tissues.

Some compounds such as rutin, naringenin, puerarin were also tested as internal standard in order to improve the accuracy and precision for analysis. Although, no suitable internal standard was found, the method used here was feasible and can satisfy the requirement of experiment.

Table 3
Pharmacokinetics parameters of orientin (mean ± S.D., $n = 6$) after a single *i.v.* administration of orientin

Parameters	Values
$t_{1/2\alpha}$ (min)	1.48 ± 0.14
$t_{1/2\beta}$ (min)	7.22 ± 0.87
$t_{1/2\gamma}$ (min)	25.74 ± 3.05
V_c (l/kg)	0.234 ± 0.006
CL (l/(kg min))	0.040 ± 0.001
$AUC_{(0-t)}$ (mg min/l)	495.9 ± 10.1
$AUC_{(0-\infty)}$ (mg min/l)	513.7 ± 19.8
$MRT_{(0-t)}$ (min)	15.38 ± 0.15

$t_{1/2\alpha}$: half-life of rapid distribution phase; $t_{1/2\beta}$: half-life of slow distribution phase; $t_{1/2\gamma}$: half-life of elimination phase; V_c: apparent volumes of distribution of the central compartments; CL: total body clearance; AUC: area under the plasma concentration vs. time curve; MRT: mean residence time.

3.3. Method validation

3.3.1. Specificity

The degree of interference by endogenous substances was assessed by inspection of chromatograms derived from processed blank and rat samples. It was indicated that orientin was well separated and no interferences were detected from endogenous substances or metabolites. The representative chro-

Table 2
Precision and accuracy data for orientin in plasma and tissues ($n = 3$ days, triplicate per day)

Sample matrix	Added concentration (μg/ml)	Found concentration (μg/ml)	Accuracy (%)	Precision (%)	
				Within-day	Between-day
Plasma	1.00	0.95	95.4	2.3	6.8
	5.00	5.28	105.7	4.5	3.0
	25.00	25.12	100.6	3.9	2.8
Liver	0.10	0.11	107.9	5.3	6.2
	1.00	1.01	100.8	8.1	5.3
	10.00	9.76	97.7	5.4	4.2
Kidney	0.10	0.09	94.5	5.7	4.2
	1.00	0.93	93.4	4.3	3.8
	10.00	9.79	97.8	6.6	6.9
Lung	1.00	0.95	95.5	6.5	4.0
	5.00	4.85	97.3	3.4	5.0
	25.00	25.33	101.0	6.3	5.8
Spleen	0.10	0.10	96.2	3.2	5.8
	0.50	0.47	94.1	6.8	5.6
	2.00	1.96	98.3	3.9	4.8
Stomach	2.00	1.89	94.7	6.7	7.4
	0.50	0.48	95.1	8.3	5.2
	0.10	0.10	95.3	4.9	3.7
Heart	0.10	0.09	94.4	4.7	4.3
	0.50	0.47	93.1	2.6	3.6
	2.00	1.91	95.5	6.2	7.0
Small intestine	0.10	0.09	93.6	6.3	4.9
	0.50	0.52	103.6	9.6	5.4
	2.00	1.96	97.8	5.3	6.9
Brain	0.10	0.09	94.4	6.5	4.2
	0.50	0.47	93.2	2.9	5.8
	2.00	1.94	96.8	4.8	6.5

Table 4
Distribution of orientin in tissues after a single *i.v.* administration to rats

Tissues	Concentration (μg/g, $\bar{X} \pm$ S.D., $n=5$)					
	5 min	15 min	30 min	45 min	60 min	90 min
Kidney	60.74 ± 3.88[a]	14.22 ± 2.06	0.84 ± 0.12	0.52 ± 0.02	0.82 ± 0.04	0.25 ± 0.03
Liver	48.21 ± 4.56	20.35 ± 2.45	6.16 ± 0.42	4.30 ± 0.23	2.35 ± ±0.13	0.93 ± 0.08
Heart	1.00 ± 0.04	0.44 ± 0.02	0.25 ± 0.03	0.14 ± 0.01	0.23 ± 0.02	–
Lung	45.07 ± 6.03	15.49 ± 1.35	4.16 ± 0.26	2.01 ± 0.17	3.73 ± 0.08	1.03 ± 0.12
Spleen	1.56 ± 0.09	0.93 ± 0.07	0.54 ± 0.05	0.50 ± 0.03	0.34 ± 0.03	0.21 ± 0.01
Stomach	1.28 ± 0.11	1.01 ± 0.05	0.33 ± 0.03	0.41 ± 0.04	0.67 ± 0.06	0.24 ± 0.03
Small intestine	4.29 ± 0.27	2.65 ± 0.12	0.18 ± 0.00	0.68 ± 0.03	0.84 ± 0.04	0.45 ± 0.05
Brain	0.13 ± 0.01	0.39 ± 0.02	0.22 ± 0.02	0.11 ± 0.01	–	–

[a] Sample was diluted when detected.

matograms for determination of orientin in plasma and tissues are shown in Figs. 2 and 3, respectively. The typical retention time for orientin was approximately 8.3 min.

3.3.2. Linearity, limit of quantification and limit of detection

The standard curves of the peak area (Y) to the concentration (X) are listed in Table 1. The calibrations were linear over a certain range in all biosamples with a correlation coefficient (r^2) larger than 0.9937. The limit of quantification (LOQ) was defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%. The current assay offered an LOQ of 0.25 μg/ml in plasma, 0.50 μg/ml in lung and 0.05 μg/ml in other tissues ($n=6$), respectively. Usually, the limit of detection (LOD) could be determined at a signal-to-noise ratio (S/N) of 3. The LOD of current assay based on S/N = 3 was 20, 22, 20, 17, 19, 16, 18, 18 and 18 ng/ml in plasma, heart, liver, spleen, lung, kidney, stomach, small intestine and brain, respectively.

3.3.3. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of orientin to blank rat plasma and tissues and the results are summarized in Table 2. The validation of the sample preparation and HPLC procedure in plasma and different tissues demonstrate that the method is accurate and precise with coefficients of variation within- and between-day below 10% for all the samples. The relative recoveries of orientin ranged from 95 to 106% for plasma and 93 to 108% for tissue homogenates.

3.3.4. Extraction recovery and stability

The extraction recovery analysis was conducted with spiked orientin biosamples at three QC levels and calculated by comparing the orientin peak area in extracted biosamples with those found by direct injection of standard solutions at the same concentration. The mean recoveries of orientin in plasma at three different concentrations (1.00, 5.00 and 25.00 μg/ml) were 68.1, 71.4 and 72.9% and the mean recoveries in all tissue samples were above 66.0%.

The stability of orientin in biosamples was investigated under a variety of storage and process conditions: for storage stability, samples (five replicates at each QC concentration) were prepared and stored at −20 °C for 15 days. On day 15, all samples were thawed and analyzed along with the freshly prepared set of quality control samples; for freeze–thaw stability testing, the QC were determined after three freeze–thaw cycles and the concentrations were compared to their nominal concentra-

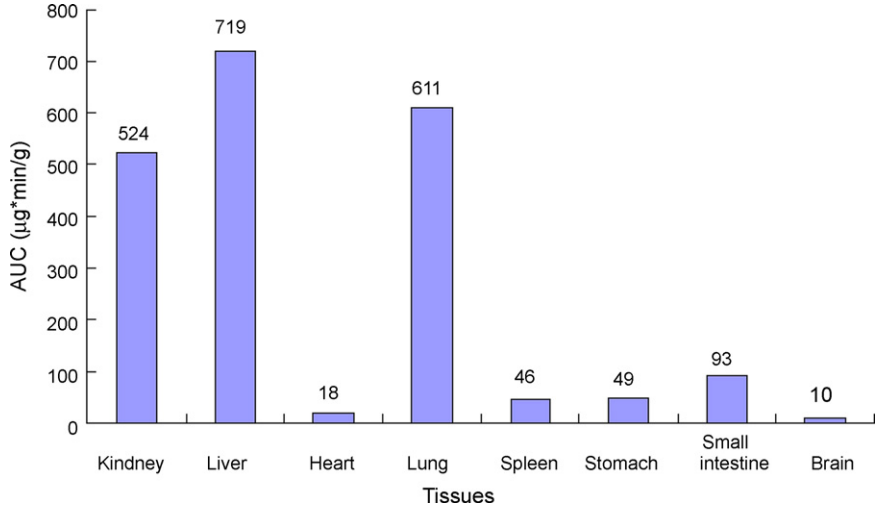

Fig. 5. The area under curve (AUC) of orientin in various tissues.

tions. Analysis of these samples consistently afforded values that were nearly identical to those of freshly prepared QC samples (R.S.D. < 15%), thus confirming the overall stability of orientin in plasma and tissues under frozen storage.

3.4. Pharmacokinetics study

The pharmacokinetics model and the parameters were calculated by the practical pharmacokinetics program Version 97 (3P97) edited by the Committee of the Mathematic Pharmacology, the Chinese Society of Pharmacology. The compartment model was established by the methods of the survival square sum (SUM), the Akaike's information criterion (AIC) and the fitted degree (r^2). The plasma concentration–time curves of orientin in rats following intravenous administration of 20 mg/kg orientin is shown in Fig. 4, demonstrating that orientin is eliminated rapidly from the plasma. The plasma concentration of orientin was detectable only up to 90 min in rats using this analytical method. A three-compartment open model (weight = $1/c^2$) gave the best fit to the plasma concentration time curves obtained in rats. The main pharmacokinetics parameters of orientin in rats after intravenous administration are summarized in Table 3.

In addition, we also tried to conduct pharmacokinetics study of orientin given to rats by oral route. However, the concentration of orientin in plasma was too low to be detected even the oral dose was up to five times as inject one, which indicated that orientin might have a poor absorption from gastrointestinal tract in rat.

3.5. Tissues distribution study

Concentrations of orientin were determined in various tissues of rat such as heart, liver, lung, spleen, kidney, brain, stomach and small intestine, respectively. At 5 min after administration of orientin to rats, the highest level of orientin was observed in tissues except brain. And at 90 min, orientin was few or undetectable in all collected tissues. Table 4 shows the concentrations of the orientin in rat tissues at 5, 15, 30, 45, 60 and 90 min after *i.v.* dose of orientin.

In tissues distribution study, the AUC of orientin was also calculated (Fig. 5.). It was showed that orientin was mainly distributed in abundant blood-supply tissues such as liver, kidney and lung, which implied that the distribution of orientin was depended on the blood flow or perfusion rate of the organ. Nevertheless, the level of orientin in heart was much lower than that in above-mentioned tissues, which indicated that orientin might bind tightly with some target proteins in the heart. The high distribution in lung, kidney and liver confirms the reports that orientin has good curative effects on respiratory, urinary system infection and liver protection [16,17]. Meanwhile, the high level in kidney demonstrated that kidney might be the primary excretion organ of prototype orientin. The lowest level founded in brain implied that orientin had difficulty crossing the blood–brain barrier because of its high polarity. At 90 min, the concentrations of orientin were low or undetected in all collected tissues, that is, there was no long-term accumulation after *i.v.* dose of orientin.

4. Conclusion

In conclusion, the assay procedure presented in this report provides a simple, rapid and sensitive procedure for the determination of orientin in biological samples including plasma and tissues. The achieved pharmacokinetics and tissue distribution results may be useful for further study of the bioactive mechanism of orientin.

Acknowledgement

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References

[1] W.Z. Huang, L. Wang, J.A. Duan, Chin. Trad. Herb. Drugs 31 (2000) 731–732.
[2] Flora Reipublicae Popularis Sinicae, Tomus, vol. 27, Science Press, Beijing, 1979, p. 85.
[3] Y.L. Li, S.C. Ma, Y.T. Yang, S.M. Ye, P.P. But, J. Ethnopharmacol. 79 (2002) 365–368.
[4] B. Vrinda, P.U. Devi, Mutat. Res./Gen. Toxicol. Environ. Mutagen. 498 (2001) 39–46.
[5] X. Li, Z. Xiong, X. Ying, L. Cui, W. Zhu, F. Li, Anal. Chim. Acta 580 (2006) 170–180.
[6] R. Colombo, J. Yariwake, E. Queiroz, K. Ndjoko, K. Hostettmann, Phytochem. Anal. 17 (2006) 337–343.
[7] G.C. Kite, E.A. Porter, F.C. Denison, R.J. Grayer, N.C. Veitch, I. Butler, M.S.J. Simmonds, J. Chromatogr. A 1104 (2006) 123–131.
[8] C. Pereira, J. Yariwake, M. McCullagh, Phytochem. Anal. 16 (2005) 295–301.
[9] Y. Zhang, B. Bao, B. Lu, Y. Ren, X. Tie, Y. Zhang, J. Chromatogr. A 1065 (2005) 177–185.
[10] W.Z. Huang, X. Liang, Chin. Pharm. J. 35 (2000) 658–659.
[11] L. Bramati, F. Aquilano, P. Pietta, J. Agric. Food Chem. 51 (2003) 7472–7474.
[12] A.M. Wang, Y.M. Wang, L.N. Liu, Y.J. Li, China J. Chin. Mater. Med. 31 (2006) 1047–1049.
[13] Z. Liu, L. Wang, W. Li, Y. Huang, Z.C. Xu, China J. Chin. Mater. Med. 29 (2004) 1049–1051.
[14] L.J. Su, Ch. Li, L.J. Liu, Chin. Trad. Herb. Drugs 28 (1997) 151–152.
[15] X. Li, T. Huo, F. Qin, X. Lu, F. Li, J. Chromatogr. B 853 (2007) 221–226.
[16] P. Wang, Chin. Trad. Patent Med. 18 (1996) 50.
[17] P. Wang, Chin. Trad. Patent Med. 17 (1995) 51.