Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 793–797
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Determination of oridonin in rat plasma by reverse-phase high-performance liquid chromatography

Jian Ge [a], Wang You-Wei [b,*], Lu Xiao-Cong [c], Cao Ji-Yue [d]

[a] *Wuhan Botanical Garden, The Chinese Academy of Sciences, Wuhan 430074, China*
[b] *College of Pharmacy, Wuhan University, #1 Luojiashan, Wuhan 430072, Hubei Province, China*
[c] *Hubei Center for Disease Control and Prevention, Wuhan 430079, China*
[d] *College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China*

Received 17 June 2006; received in revised form 18 August 2006; accepted 22 August 2006
Available online 2 October 2006

Abstract

A method for quantitative determination of oridonin in rat plasma using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with UV spectrometry was established and the method was applied to a pharmacokinetics study of oridonin in rats. From a variety of compounds and solvents tested, ticolpidine was selected as the internal standard (IS) and ethyl acetate was found to be the best solvent for extracting oridonin from plasma samples. RP-HPLC analysis of the extracts was performed on an analytical column (DIKMA ODS, 200 mm × 4.6 mm; i.d., 5 μm) equipped with a security guard pre-column system. There was a good linearity over the range 0.05–8.0 μg/mL ($r > 0.99$). The recoveries were about 95.0% in plasma, and the intra- and inter-day coefficients of variation were less than 9.0% in all cases. The limit of detection (LOD) was 0.025 μg/mL and the lower limit of quantification (LLOQ) was 0.05 μg/mL. The RP-HPLC method was readily applied to quantitate oridonin in rat plasma within 24 h in a pharmacokinetics study where experimental rats received a single dose of oridonin (12.5 mg/kg) and the result was presented.

Keywords: Oridonin; RP-HPLC; Pharmacokinetics

1. Introduction

Oridonin (Fig. 1A) is a diterpenoid compound obtained from *Rabdosia rubescens* [1]. *R. rubescens* (*Dong Ling Cao*), formerly called *Isodon rubescens*, is the dried entire plant of labiatae plant *R. rubescens* (*Hemsl*) *Hara*, and it has been widely used in China as a herbal medicine. The herb has been used traditionally as a digestant and anti-inflammatory agent, but it also has been used as a folk remedy for carcinomas of the heart and the esophagus in Hunan Province [2]. Oridonin has also been detected in other herbs such as *Isodon trichocarpus* [3], *Isodon Japonicus* [4] and *Isodon shikokianus* [5]. Some studies indicated that oridonin had various pharmacological and physiological effects such as anti-inflammation, anti-bacteria and anti-tumor effects [6,7]. The mechanism underlying the pharmacological effects of oridonin has been studied in cell culture and was reported to be related to blocking DNA synthesis in L1210 tumor and bone marrow cells [8–10] and inducing apoptosis of HeLa, K562 and HL-60 cells [11–13]. The mechanisms of action of oridonin *in vivo* have not been studied in detail, and the development of an analytical method for the measurement of oridonin in biological samples is needed for the further pharmacological and pharmacokinetic studies.

There have been reports describing the determination of active constituents of a lot of herbal medicines by liquid chromatography coupled with UV spectrometry [14], by GC-MS, by LC-MS/MS [15] and by capillary electrophoresis [16]. So far there have been no reports on determination of oridonin in biosamples by reversed-phase high-performance liquid chromatography (RP-HPLC).

Here we report a simple and sensitive RP-HPLC method for determining concentrations of oridonin in rat plasma and its application to a subsequent pharmacokinetics study. This method was fully validated for its specificity, accuracy, precision and sensitivity, and was successfully applied to a pharmacokinetics study of oridonin in rats.

* Corresponding author.
E-mail address: wyw5618@hotmail.com (W. You-Wei).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.08.016

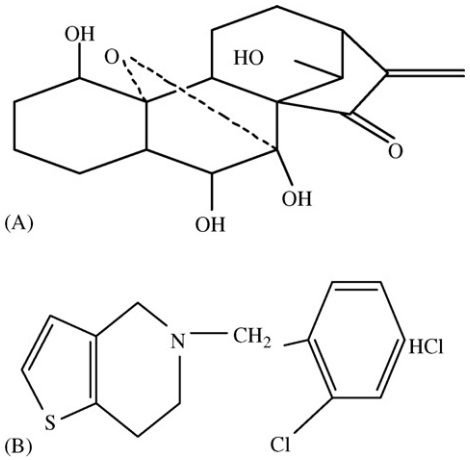

Fig. 1. Chemical structure of (A) oridonin and (B) ticolpidine.

2. Experimental

2.1. Materials, reagents and laboratory animals

Oridonin (Fig. 1A) was isolated from the leaves of *R. rubescens*. HPLC grade acetonitrile was purchased from Tianjin Kermel Chemical Factory (Tianjin, China), and other reagents were of analytical grade. The internal standard (IS) ticolpidine (Fig. 1B) was a generous gift from Dr. Changxiao Liu of Tianjin Institute of Pharmaceutical Research. Concentrations of oridonin were measured by a RP-HPLC equipped with an ultraviolet (UV) detector and a RP-18C column (4.6 mm i.d. × 200 mm) with methanol–water (50:50, v/v) as the mobile phase. The purity of oridonin extracted was determined to be 99.8%. The oridonin injection used in the pharmacokinetics study was manufactured by Wuhan Binhu Double Crane Pharmaceutical CO. LTD.

Male SPF grade Wistar rats weighing 200 ± 20 g were purchased from Hubei Laboratory Animal Centre. Prior to use, all rats were housed in individually ventilated cages (IVC) and provided with sterilized tap water and granule feed *ad libitum*. Room temperature was maintained at 23 ± 1 °C with a relative humidity of 55 ± 10% and a day/night cycle of 12 h. The experimental protocol was approved by the animal care committee of Hubei Laboratory Animal Centre.

2.2. RP-HPLC method

The RP-HPLC shimadzu 10A system consisted of a UV detector and a Zhida N2000 Instrument Workstation. The analytical column was a DIKMA ODS C_{18} column (5 μm, 200 mm × 4.6 mm) which had a DIKMA ODS C_{18} precolumn (4.6 mm × 12.5 mm). The mobile phase was composed of acetonitrile–0.01 M ammonium acetate (0.5% acetic acid) (30:70, v/v). The flow rate was 1.0 mL/min. Column temperature was kept at 25 °C, and the UV detector wavelength was set at 238 nm.

2.3. Assay of oridonin in plasma

A plasma sample (0.2 mL) and a ticolpidine solution (0.1 mL, 25 μg/mL) were both transferred to a 7-mL tapered tube and vortex-mixed for 1 min, then 5 mL ethyl acetate was added. The tube was vortex-mixed for 6 min. After centrifugation (6000 r/min) for 5 min, the organic phase was transferred to a glass test tube and evaporated to dryness at 45 °C under a stream of nitrogen gas. The residue was reconstituted with 0.1 mL mobile phase (acetonitrile–0.01 M ammonium acetate), vortex-mixed for 1 min and centrifuged (10000 r/min) for 2 min. Then 10 μL was injected directly into the RP-HPLC system.

2.4. Method validation

2.4.1. Specificity

The degree of interference to oridonin determination by endogenous co-eluent components was evaluated through comparison of chromatograms derived from blank plasma samples, spiked plasma samples and plasma taken from drug injected rats.

2.4.2. Calibration curve

Stock standard solutions of oridonin were prepared with methanol, and stock standard solution of the internal standard ticolpidine was prepared with the mobile phase. Six concentrations of oridonin solution for the calibration curve were prepared by dilution from the stock solution. The serial solutions (0.2 mL each concentration) were evaporated to dryness at 45 °C under a stream of nitrogen gas. Then 0.2 mL of drug-free plasma and 0.1 mL of the IS solution were added. The calibration range was 0.05–8.0 μg oridonin per milliliter plasma. Calibration standards were extracted and assayed following the method mentioned above. The calibration curve was constructed based on peak-area ratios of oridonin to IS (y) *versus* concentrations of spiked oridonin (x).

2.4.3. Accuracy and precision

Accuracy and precision of the method were assessed by assaying five replicate quality control (QC) samples, respectively. The QC samples were prepared in the same way as the samples for calibration at low (0.05 μg/mL plasma), medium (0.4 μg/mL plasma) and high (2.0 μg/mL plasma) concentrations. Intra-day accuracy and precision were evaluated at different times during the same day. Inter-day accuracy and precision were determined over five different days.

2.4.4. Extraction recovery

QC samples at low (0.05 μg/mL), medium (0.4 μg/mL) and high (2.0 μg/mL) concentrations were used. Recovery of oridonin with ethyl acetate extraction was determined by comparing observed peak-area ratios in extracted biosamples to those of non-processed standard solutions.

2.4.5. Stability

The stabilities of oridonin in plasma were evaluated. The stabilities of oridonin in plasma were assessed using spiked QC samples at the levels of 0.05, 0.4 and 2.0 μg/mL under three

conditions (RT 10 min, RT 12 h and −20 °C 12 h). Stability was expressed as a percentage of the nominal concentration.

2.5. Application of RP-HPLC in a pharmacokinetics study

Six male Wistar rats per each time point received a single dose of oridonin of 12.5 mg/kg via the intravenous route. Blood samples were collected in tapered tubes (containing sodium heparin) via the eye venous plexus before and at 5 min, 30 min, 1 h, 1.5 h, 3 h, 6 h, 12 h and 24 h after drug administration, respectively. The blood samples were centrifuged at 6000 r/min for 3 min at room temperature, and then the plasma samples were transferred to new tubes, respectively. Oridonin extraction were performed with these plasma samples within 10 min and then determined by the RP-HPLC as described above. The concentration of oridonin in each plasma sample was calculated using the calibration curve. The parameters of pharmacokinetics were calculated using the 3P97 computer program written by the Chinese Society of Mathematical Pharmacology.

3. Results and discussion

3.1. Specificity of chromatograms

The RP-HPLC chromatogram peak of oridonin and IS as assayed using the standard solution was at 8.907 and 13.249 min, respectively (Fig. 2A). No significant interfering peaks appeared for normal rat plasma samples (Fig. 2B). A rat plasma sample spiked with oridonin and IS yielded two respective chromatogram peaks identical to that from the standard solution (Fig. 2C). A chromatogram of a plasma sample taken at 24 h after oridonin administration showed identifiable oridonin peak (Fig. 2D) where the IS peak was clear and strong.

This result indicated an excellent specificity for determining oridonin using RP-HPLC. The peaks for oridonin and IS were sharp, clean and well separated and there were no interfering peaks from plasma components. This RP-HPLC method shall have higher specificity than a previously reported radioactive isotope labeling method for quantitating oridonin contained

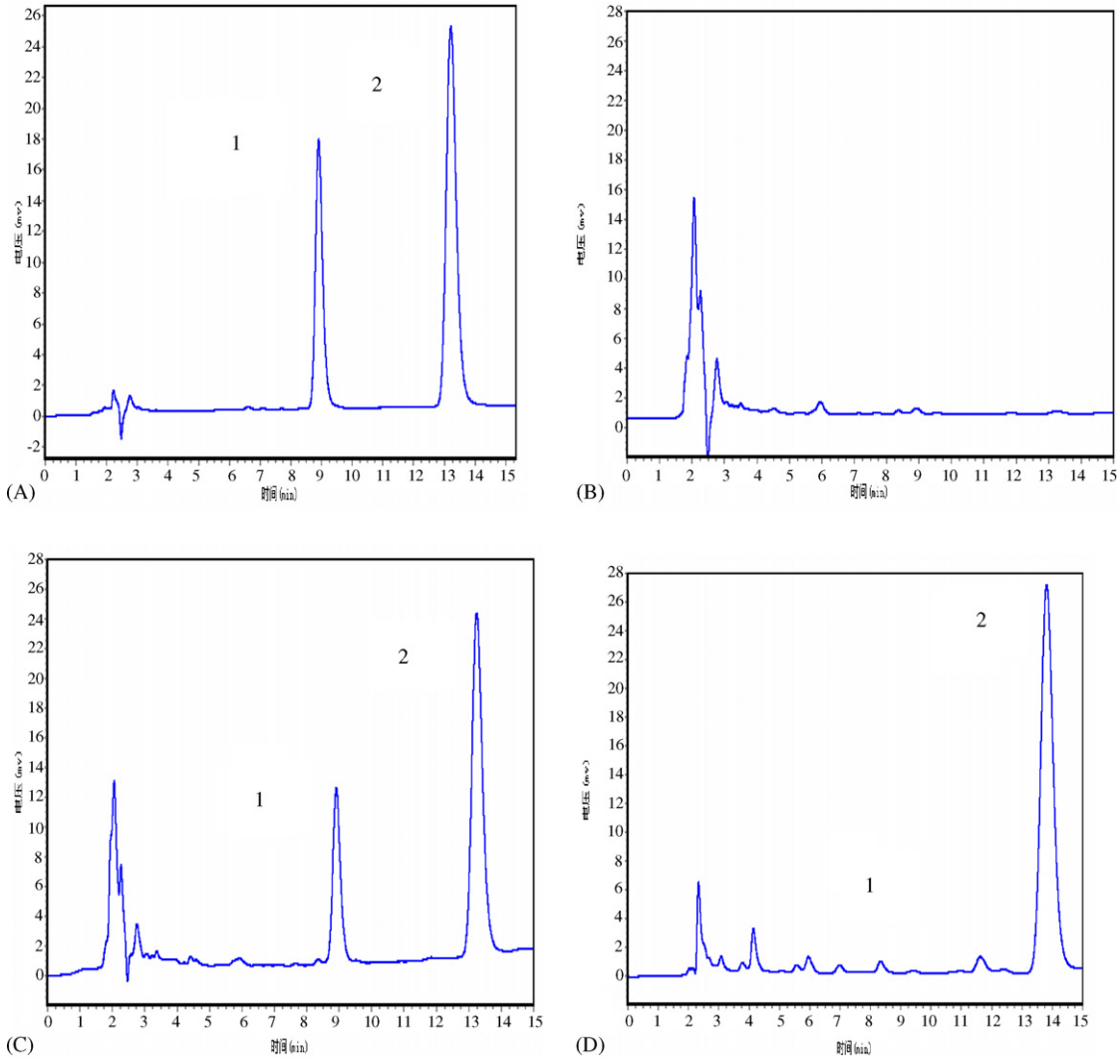

Fig. 2. Chromatograms of (A) a standard solution of oridonin and ticolpidine (IS) at 8 and 25 μg/mL, (B) a blank rat plasma, (C) a rat plasma spiked with ordionin and IS at 8 and 25 μg/mL, respectively, and (D) a rat plasma sample collected 24 h after intravenous administration of 12.5 mg/kg oridonin. Peak 1, oridonin; peak 2, ticolpidine.

in biosamples [17], where the total radioactivity detected may include radioactive metabolites and thus may not represent the overall concentration of oridonin.

3.2. Method development

Initially, several mobile phases were selected to separate oridonin and IS in plasma, including methanol:water (50:50, v/v), acetonitrile:water (25:75, v/v), methanol:water (55:45, v/v), methanol:0.01 mol/L ammonium acetate (0.5% acetic acid) (50:50, v/v) and acetonitrile:0.01 mol/L ammonium acetate (0.5% acetic acid) (30:70, v/v) (data not shown). However, only when acetonitrile:0.01 mol/L ammonium acetate (0.5% acetic acid) (30:70, v/v) was used as mobile phase, oridonin and IS in plasma could be well separated by the RP-HPLC.

A number of extraction solvents were tried to extract oridonin from rat plasmas samples, including ethyl acetate, diethyl ether, chloroform, acetonitrile and methanol. Ethyl acetate gave a high extraction recovery for oridonin without any significant interference. Methanol and acetonitrile dissolved many endogenous co-eluents and resulted in a high back pressure and a short column life. Diethyl ether and chloroform both extracted oridonin with little interference but with low recovery. Therefore, ethyl acetate was chosen as the most suitable extraction solvent.

Different compounds, including ticolpidine, anthranilic acid and trimethoprim, were tried as internal standard in order to improve the precision of RP-HPLC quantification which was especially important for assaying biological samples. Ticolpidine turned out to be the most suitable internal standard since it exhibited a similar behavior to oridonin during the sample preparation procedure.

3.3. Calibration and validation

Evaluation of the assay was performed with a calibration curve over the concentration range 0.05–8.0 μg oridonin per mL plasma. The slope and intercept of the calibration graph were calculated by weighted least squares linear regression. The regression equation of the curve and the correlation coefficients (r) were calculated as followed. Good linear relationship between the peak-area ratios and the concentrations was observed.

$$Y = (0.1563 \pm 0.0023)X - (0.0024 \pm 0.0002), \quad r = 0.9996$$

The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curve that can be determined with acceptable precision. The LLOQ was found to be 0.05 μg/mL for oridonin in rat plasma. The accuracy and precision of the method were evaluated with quality control samples at concentrations of 0.05, 0.4 and 2.0 μg/mL. The intra- and inter-day precisions were less than 9.0% for all three concentrations (Table 1). The data proved good precision and accuracy of the RP-HPLC method developed.

Extraction recovery of oridonin at concentrations of 0.05, 0.4 and 2.0 μg/mL in plasma was 95.01 ± 9.01, 94.25 ± 9.38 and 94.84 ± 5.30% ($n = 5$), respectively, which were nearly identical and approaching 100%, suggesting little oridonin was lost in the recovery procedure for a wide concentration range.

Table 1
Intra- and inter-day precision and accuracy of the method in plasma ($n = 5$)

Spiked concentration (μg/mL)	Accuracy (%)		R.S.D. (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
0.05	96.35 ± 6.26	102.46 ± 3.28	6.88	8.92
0.4	101.43 ± 6.09	109.10 ± 3.11	7.24	8.38
2.0	107.82 ± 4.15	104.79 ± 5.42	4.05	2.67

Table 2
Stability of oridonin in plasma ($n = 5$)

Nominal concentration (μg/mL)	Recovered (%)		
	Room temperature (10 min)	Room temperature (12 h)	−20 °C (12 h)
0.05	98. 8 ± 8.3	0[a]	0[a]
0.4	96.5 ± 5.8	38.1 ± 3.6	49.8 ± 4.4
2.0	100.9 ± 7.3	41.4 ± 3.3	47.1 ± 3.8

[a] No oridonin was detected.

Stability of oridonin in test materials is a prerequisite for obtaining valuable data. The stability of oridonin in rat plasma was studied under three situations of RT 10 min, RT 12 h and −20 °C 12 h, respectively (Table 2). Oridonin in plasma was stable within 10 min but not for 12 h storage either at RT or −20 °C. Therefore, all the plasma samples collected from the experimental animals were extracted in ethyl acetate within 10 min to ensure full recovery of oridonin and accurate determination.

3.4. Application in a pharmacokinetics study

This RP-HPLC analytical method was applied to the quantification of oridonin concentrations in rats in a pharmacokinetics study. After tail vein injection of oridonin at 12.5 mg/kg dose, oridonin in plasma were determined for a series of time points within 24 h. The plasma oridonin concentration–time curve was fitted to a two-compartment open model (Fig. 3). The $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated to be 0.12 h and 6.06 h, respectively, indicating that oridonin distributed very fast and was eliminated slowly in rats (Table 3). The value of V_d was 1.83 L/kg,

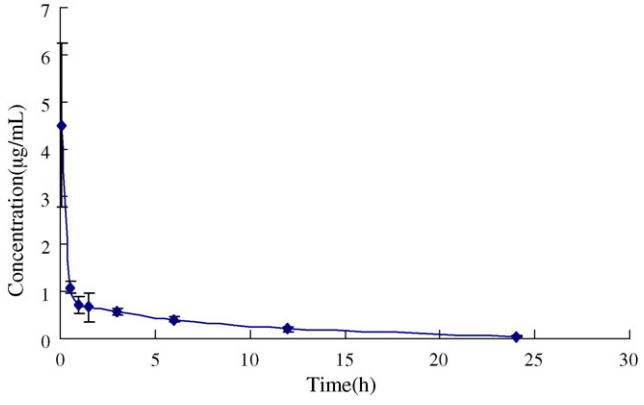

Fig. 3. Mean plasma concentration–time curve in rats after a single intravenous dose of oridonin (12.5 mg/kg). Each point represents the mean oridonin concentration of six rats.

Table 3
Pharmacokinetics parameters calculated from the i.v. oridonin study in rats

Parameter	Value
$t_{1/2\alpha}$ (h)	0.12
$t_{1/2\beta}$ (h)	6.06
CL (L/kg/h)	1.56
AUC (μg h/mL)	7.96
V_d (L/kg)	1.83

indicating that oridonin might accumulate in certain organ or had been metabolized in the plasma.

The RP-HPLC method satisfied the requirement of this study and demonstrated its general suitability for pharmacokinetics studies of oridonin in rats.

4. Conclusion

A specific, simple, sensitive and accurate RP-HPLC method has been developed and validated for the quantitative determination of oridonin in rat plasma. In comparison with the method of radioactive isotope labeling, this RP-HPLC method is more specific, accurate as well as simpler and safer. The method has been successfully applied to a pre-clinical pharmacokinetics study.

Acknowledgement

This work was supported by National "863" Project (2002AA2Z3237).

References

[1] H.D. Sun, Z. Wen Lin, C.Q. Qin, J. Yunnan Bot. Res. 3 (1981) 95–100.
[2] M.Y. Chang, Anticancer Medicinal Herbs, Hunan Science and Technology Publishing House, Changsha, 1992.
[3] E. Fujita, T. Fujita, J. Pharm. Soc. Jpn. 87 (1967) 1150–1153.
[4] E. Fujita, T. Fujita, M. Shibuya, Tetrahedron Lett. 27 (1966) 3153–3162.
[5] T. Kubota, I. Kubo, Bull. Chem. Soc. Jpn. 42 (1969) 1778.
[6] I. Kubo, M. Taniguchi, Y. Satomura, T. Kubota, Agric. Biol. Chem. 38 (1974) 55–56.
[7] E. Fujita, Y. Nagao, K. Kaneko, S. Nakazawa, J. Kuroda, Chem. Pharm. Bull. 24 (1976) 2118–2127.
[8] E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa, H. Kuroda, Experientia 32 (1976) 203–206.
[9] M.Y. Wang, C. Lin, T.M. Zhang, Zhongguo Yao Li Xue Bao (Acta Pharmacol. Sin.) 8 (1987) 164–165.
[10] M.G. Shou, M.Y. Wang, Q. Wang, et al., Zhongguo Yao Li Xue Bao (Acta Pharmacol. Sin.) 8 (1987) 83–86.
[11] C.W. Feng, X.L. Pan, J.H. Zhou, et al., J. Shan Dong Univ. TCM 24 (2000) 225–226.
[12] J. Wang, Q.Y. Liu, H.Y. Hu, et al., Chin. Pharmacol. Bull. 17 (2001) 402–404.
[13] J.J. Guo, X.L. Pan, C.W. Feng, et al., J. Anhui TCM Coll. 19 (2000) 34–36.
[14] Y.B. Li, Z.L. Xiong, F.M. Li, J. Chromatogr. B 821 (2005) 235–239.
[15] H.Y. Wang, J. Jiang, P. Hu, J. Chromatogr. B 834 (2006) 155–162.
[16] S. Rudaz, S. Cherkaoui, J.Y. Gauvrit, P. Labteri, J.L. Veuthey, Electrophoresis 22 (2001) 3316–3326.
[17] C. Li, Q.Y. Zhang, W.K. Wu, S.H. Cao, D.X. Wang, M.G. Yi, Acta Pharmacol. Sin. 4 (1983) 57–60.