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ORIGINAL RESEARCH

Determination of piceid in rat plasma and tissues by high-performance liquid chromatographic method with UV detection

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ABSTRACT: A rapid, sensitive and selective HPLC method was developed and validated for determination of piceid in rat plasma and tissues. The drug was isolated from plasma and tissues by a simple protein precipitation procedure. Chromatographic separation was performed on a C_{18} column with acetonitrile-water (26:74, v/v) as mobile phase. The method was successfully applied to the pharmacokinetics and tissue distribution research after oral administration of a 50 mg/kg dose of piceid to healthy male Wistar rats. The pharmacokinetic parameters showed that piceid was quickly absorbed, distributed and eliminated within 4 h after oral administration. The tissue distribution results showed that, at 10 min, the concentrations of piceid in most tissues reached peak level except in heart and testis. The highest level of piceid was found in stomach, then in small intestine, spleen, lung, brain, testis, liver, kidney and heart. The amount of piceid in testis and heart reached the peak level at 30 min. At 120 min, the amount of piceid in all tissues decreased to a low percentage of the initial concentration. Piceid was absorbed throughout the gastrointestinal tract with considerable absorption taking place in the stomach and small intestine. There was no long-term accumulation of piceid in rat tissues. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: piceid; HPLC; pharmacokinetics; distribution

INTRODUCTION

Piceid (resveratrol-glucoside), *trans*-3,5,4′-trihydroxyl-stilbene-3-mono-D-glycoside, a phenolic compound, exists extensively in medicinal herbs and red wine. Its chemical structure is shown in Fig. 1. The presence of piceid and its aglycone in wine is becoming an important issue due to their claimed relation to a low incidence of cardiovascular diseases and their increasing implication as cancer chemo-preventive agents. It is reported that piceid can enhance the contractility of a single myocardial cell, which may be related to the increase in intracellular calcium concentration (Jin *et al.*, 2000). Other research has suggested that piceid can aslo reduce/inhibit platelet aggregation (Zhang *et al.*, 1995; Shan *et al.*, 1990), reduce thromboxane synthesization (Shan *et al.*, 1990; Wang *et al.*, 1995), inhibit vasoconstriction (Gao *et al.*, 2004), inhibit eicosanoid synthesization (Kimura *et al.*, 1985) and has significant free radical scavenging effects (Cuendet *et al.*, 2000). An anticancer effect has been reported (Jang *et al.*, 1997; Wang *et al.*, 2003). Kimura *et al.* (2000) found in their research that stilbene glucosides isolated from medicinal plants and grapes can inhibit tumor growth and lung metastasis in mice bearing highly metastatic Lewis lung carcinoma (LLC) tumors. They also studied the inhibitory effects of stilbene glucosides on differetiation of human umbilical vein endothelial cells (HUVECs) to form a capillary network. The results suggested that the antitumor and antimetastatic activity of the stilbene glucosides, piceid and 2,3,5,4′-tetrahydroxystilbene-2-*O*-D-glucoside, might be due to the inhibition of DNA synthesis in LLC cells and angiogenesis of HUVECs.

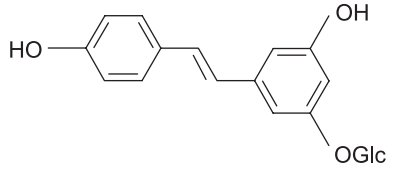

Figure 1. Chemical structure of piceid.

For the determination of piceid in *in vivo* biosamples research, only one HPLC-UV method has been reported (Lin *et al.*, 2001). This method required either a long chromatographic run time or large plasma volumes. In this paper, a more rapid and sensitive RP-HPLC method was described to determine piceid in rat

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Abbreviations used: HUVECs, human umbilical vein endothelial cells; LLC, Lewis lung carcinoma.

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plasma and tissues. After validation, this method was successfully applied to a pharmacokinetics and tissue distribution study of piceid following a single oral dose of 50 mg/kg piceid to healthy Wistar rats.

EXPERIMENTAL

Reagents

Piceid was supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile and methanol were purchased from Tedia (Tedia Company, Inc. USA). Water was purified by double distillation.

Apparatus

The high-performance liquid chromatography system consisted of Waters 1525 pump, Waters UV 2487 dual λ absorbance detector, and a Waters Empower Project workstation.

Chromatographic conditions

A Diamonsil C_{18} column (5 μm, 250 × 4.6 mm i.d.) was used. The column temperature was maintained at 30°C. The mobile phase consisted of an isocratic solvent system of acetonitrile–water (26:74, v/v) at a flow rate of 1.0 mL/min. The wavelength of UV detector was set at 306 nm.

Stock solutions and standards

Piceid stock solution of 15.52 mg/mL were prepared in methanol. Piceid calibrators were prepared by spiking blank biosamples with appropriate amounts of piceid and obtained the relevant concentrations ranging from 0.056 to 1552.00 μg/mL. Low, medium and high quality controls were prepared according to the estimated concentrations in all biosamples. All stock solutions, standards and quality controls were prepared in bulk, aliquoted and stored at –20°C.

Animals

Male Wistar rats (weighing 300 ± 50 g, 5–6 months old, certificate No. DK 0505019) were purchased from Experimental Animal Center of Hebei Province of China. Animals were maintained in an environmental controlled room with a 12 h light–dark illumination cycle, and fed with standard laboratory chow 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.

Drug administration and sampling

Piceid was suspended in 0.5% CMC-Na (sodium carboxymethylcellulose). The suspension was orally administered to rats at a dose of 50 mg/kg. For pharmacokinetic study, blood samples were obtained from the jugular vein according to the specific schedule (5, 10, 15, 25, 30, 45, 60, 90, 120, 150, 180 and 240 min after dose). For tissue distribution study, 18 rats were assigned randomly to three groups and were given piceid orally at 50 mg/kg. Heart, liver, spleen, lung, kidney, stomach, small intestine, brain and testis were collected at 10, 30 and 120 min after administration, respectively.

Biosample preparation

Plasma samples. To 100 μL plasma, 300 μL of methanol were added. The mixture was vortex mixed for 10 s. After being centrifuged at 10,000 rpm for 5 min, 20 μL of the supernatant layer were injected into the HPLC system for analysis.

Tissue samples. Aliquots of 500 mg of tissue samples were homogenized in two volumes of normal saline in an ice bath. A 300 μL quantity of methanol was added into 100 μL of tissue homogenate. Then the tube was vortex mixed for 10 s and the mixture was centrifuged at 10,000 rpm for 5 min. A 20 μL aliquot of the supernatant layer was injected into the HPLC system for analysis.

Pharmacokinetic analysis. Non-compartmental pharmacokinetic analysis of concentration time data was performed. The pharmacokinetic parameters, such as maximum plasma concentration (C_{max}) and time to peak (T_{max}) were obtained directly from the plasma concentration–time plots. The elimination rate constants (K_{el}) were determined by linear regression on the logarithmic transformation of the last four data points of the curve. The apparent terminal half-life ($T_{1/2}$) was calculated by the following equation: $T_{1/2} = 0.693/K_{el}$. The area under the plasma concentration vs time curve up to the last time (t) (AUC_{0-t}) was determined using the trapezoidal rule. The $AUC_{0-\infty}$ values were calculated by adding to AUC_{0-t} the value of C_t/K_{el}. The apparent volume of distribution (V_d) was computed according to the equation: $V_d = D \times K_{el}/AUC$ in which D represents the dose. Total clearance (CL) was calculated by the equation $CL = D/AUC$.

Tissue distribution analysis. Quantification of piceid in tissue was obtained from the peak amplitudes of piceid. The observed concentration (C_o) was calculated by the calibration curves. The mass concentration (C_m) of piceid in tissue samples was computed by the following equation: C_m (μg/g) = C_o (μg/mL) × 2 (mL/g). The column diagram of piceid in tissues at different time after oral administration was drawn according to the data.

RESULTS

Specific

Blank biosample lots containing heparin (n = 6) were tested for the presence of interfering peaks. Figures 2 and 3 show representative chromatograms of blank biosamples and biosamples spiked with piceid reference solution. Piceid was well separated from the endogenous substances in biosamples and had a retention time of approximately 5.8 min.

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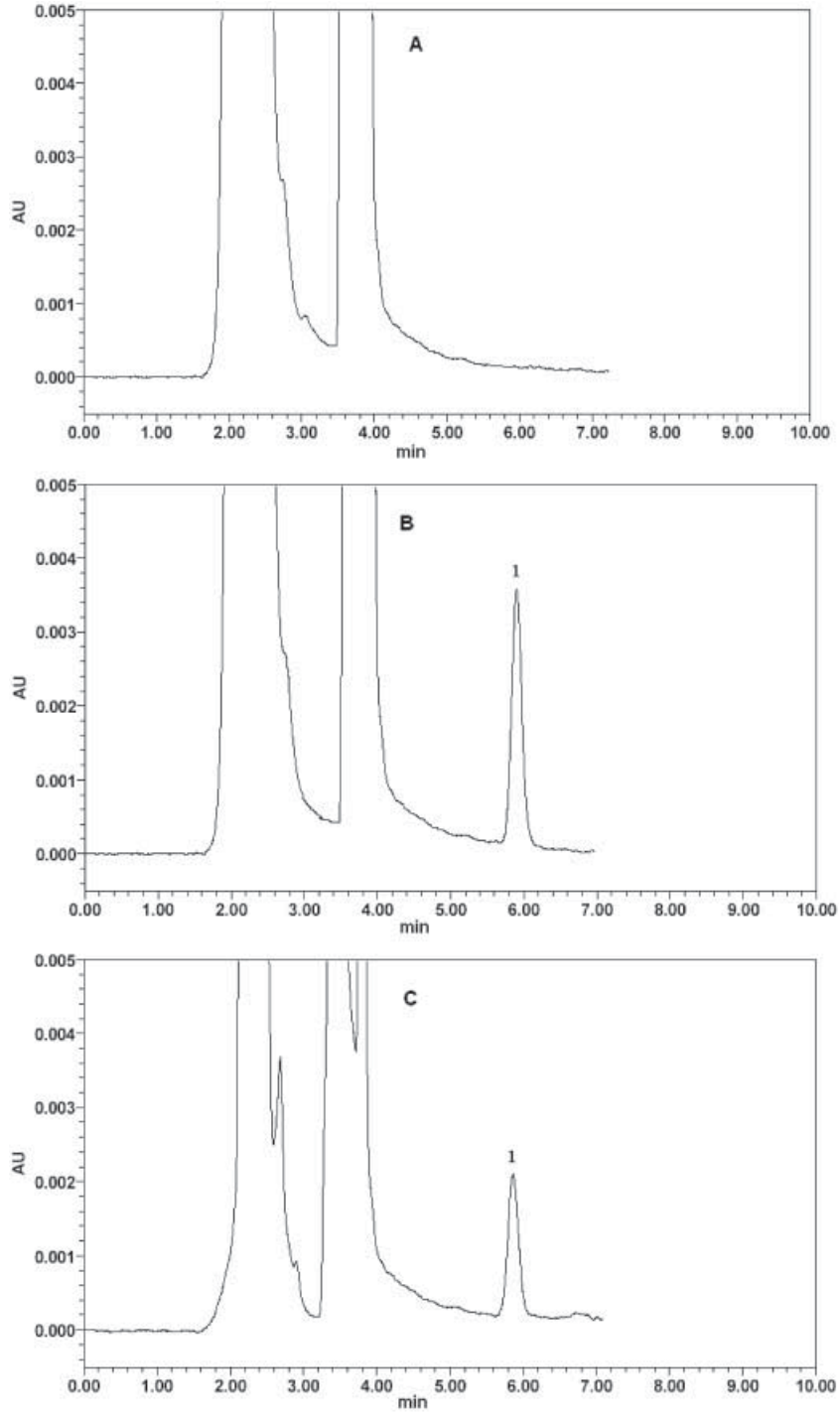

Figure 2. Typical chromatograms of piceid in plasma.

Limit of quantification and linearity

The standard curves of the peak area (Y) to the concentration (C) are listed in Table 1. The calibrations were linear over a certain range in all biosamples with a correlation coefficient (r^2) larger than 0.9900. The lower limit of quantification (LLOQ) of the assay was determined to be 50 ng/mL in plasma, 25 ng/mL in heart, 30 ng/mL in liver, 70 ng/mL in spleen, 40 ng/mL in lung, 75 ng/mL in kidney, 60 ng/mL in stomach, 110 ng/mL in small intestine, 60 ng/mL in brain and 30 ng/mL in testis.

Precision and accuracy

Inter- and intra-assay precision was assessed with low, medium and high quality control samples. Inter-assay

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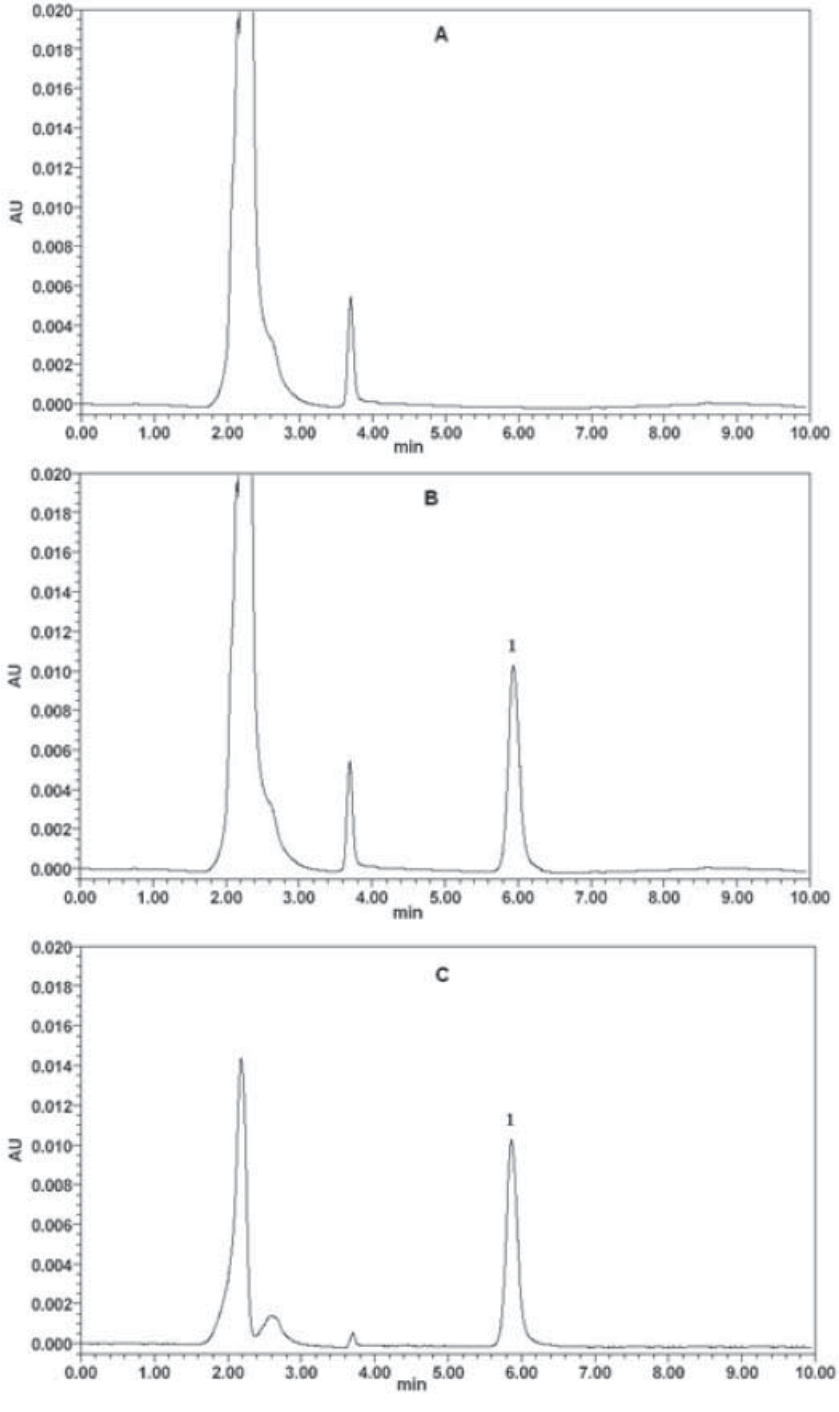

Figure 3. Typical chromatograms of piceid in heart.

precisions (measured as relative standard deviation, or RSD) over one week were 2.4–10% in plasma, 3.7–8.6% in heart, 1.5–5.4% in liver, 3.6–6.5% in spleen, 2.9–8.3% in lung, 1.8–4.3% in kidney, 1.9–9.2% in stomach, 2.5–9.2% in small intestine, 4.6–5.8% in brain and 1.4–7.5% in testis. Intra-assay precisions for low, medium and high quality control samples were all in a valid range. Inter-assay accuracy (measured as RSD) ranged from 1.8 to 7.6% and intra-assay accuracy ranged across all validation runs were 1.6–9.8, 1.1–10.4 and 0.1–8.6% for low, medium and high quality control samples, respectively. All numbers were below the 15% Ch.P threshold (National Commission of Chinese Pharmacopoeia, 2005).

Recovery

Recovery analysis was conducted with spiked piceid biosamples. Recovery was calculated by comparing the piceid peak area in extracted biosamples vs control

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Table 1. Standard curves, correlation coefficients and linear ranges of piceid in biosamples

Biosamples	Standard curves	Correlation coefficients	Linear ranges (μg/mL)
Plasma	$Y = 17.0184C - 166.1259$	0.9983	0.056–11.200
Heart	$Y = 18.9154C - 667.2895$	0.9990	0.075–24.25
Liver	$Y = 17.8260C - 46.2141$	0.9975	0.075–48.50
Spleen	$Y = 20.0912C - 1670.6715$	0.9994	0.075–121.25
Lung	$Y = 19.9521C - 1425.2913$	0.9963	0.075–97.00
Kidney	$Y = 18.4394C - 40.1397$	0.9966	0.075–48.50
Stomach	$Y = 18.2402C + 129.8087$	0.9996	0.606–1552.00
Small intestine	$Y = 18.0042C - 70.1202$	0.9995	0.606–776.00
Brain	$Y = 19.1684C - 794.0444$	0.9977	0.075–48.50
Testis	$Y = 17.9942C - 46.4165$	0.9954	0.075–606.25

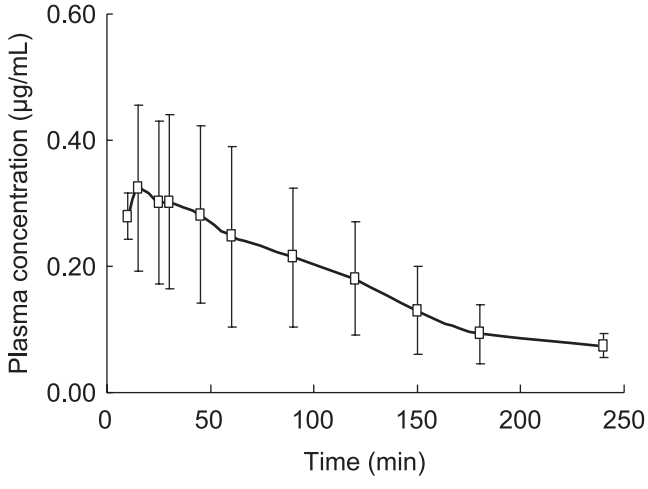

Figure 4. Concentrations vs time profile after oral administration of piceid (mean ± SD).

samples at low, medium, high concentration. The mean recoveries of piceid (mean ± standard deviation) were 83.90 ± 0.45, 88.07 ± 1.86 and 90.31 ± 3.14% ($n = 6$) in plasma and the mean recoveries in all tissue samples were above 85.0%.

Stability

The stability of piceid in biosamples was investigated under a variety of storage and process conditions. The results were compared with a freshly prepared set of quality control samples. The RSD of each set of samples was within the method limits (<15%). In addition, stock solutions of piceid in plasma were stable through three freeze–thaw cycles (data not shown) for at least 6 h.

Pharmacokinetics

The plasma concentration vs time curve for piceid is shown in Fig. 4. Maximum concentration of piceid was reached at 20.83 ± 8.61 min. The curve showed a slow distribution phase and a slow terminal elimination phase lasting up to 240 min after administration. The peak plasma concentration of piceid was 0.364 ± 0.151 μg/mL. The main pharmacokinetic parameters of piceid in rats after oral administration are summarized in Table 2.

Tissue distribution

Piceid was detected in various organs at 10 min. The concentration of piceid reached the peak level in all tissues except heart and testis. The highest level was found in stomach, then in small intestine, followed by spleen, lung, brain, testis, liver, kidney and heart. At 30 min, the highest concentration of piceid was still detected in stomach. However, the concentration dropped sharply from 168.79 ± 77.45 to 72.76 ± 32.68 μg/g. Piceid in small intestine also decreased to almost 28.03% of the initial concentration. The amount of piceid in testis reached a peak level of 29.08 ± 12.12 μg/g. In the other organs, the content of piceid decreased to some degree at 30 min. At 120 min, piceid was still present in the stomach in relatively high concentrations. The concentrations of piceid in the various organs are shown in Table 3. The column diagram of piceid in tissues at different times after oral administration is shown in Fig. 5.

DISCUSSION

An HPLC assay for piceid in biosamples was developed and validated. The present bioanalytical method includes a simple protein precipitation procedure and reversed-phase HPLC analysis with UV detection, and demonstrated adequate specificity and sensitivity for the determination of piceid in rat plasma and tissue samples following a single oral administration of a 50 mg/kg dose.

At present, the studies of the pharmacokinetics and distribution of piceid have been poorly documented, and no study has been reported about the oral administration. In this study, the pharmacokinetic research obtained the main parameters about piceid in rats after

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Table 2. Pharmacokinetics parameters of piceid after single oral administration of piceid to rats

Parameters	C_{max} (μg/mL)	T_{max} (min)	$T_{1/2}$ (min)	K_{el} (min^{-1})	V_d (L/kg)	CL (L/kg/min)	AUC_{0-t} (μg min/mL)	$AUC_{0-\infty}$ (μg min/mL)
Piceid	0.364 ± 0.151	20.83 ± 8.16	100.57 ± 17.05	0.016 ± 0.003	73.61 ± 35.89	1.20 ± 0.37	41.78 ± 15.15	52.26 ± 16.18

C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; $T_{1/2}$, elimination half-life; K_{el}, elimination constant; V_d, apparent volume of distribution; CL, total clearance; AUC_{0-t}, area under the concentration vs time curve from 0 to the last sampling time; $AUC_{0-\infty}$, area under the concentration vs time curve from 0 to infinity. Data are expressed as means ± SD (n = 6).

Table 3. Absolute quantification of piceid in digested organs of mice given a single oral dose of piceid

	Concentration (μg/g)		
Tissues	10 min	30 min	120 min
Heart	0.50 ± 0.26	4.95 ± 2.54	0.23 ± 0.07
Liver	4.47 ± 2.51	0.53 ± 0.33	0.27 ± 0.11
Spleen	28.03 ± 13.81	3.74 ± 1.51	0.47 ± 0.18
Lung	10.42 ± 3.86	2.88 ± 1.04	0.72 ± 0.37
Kidney	2.58 ± 1.19	0.82 ± 0.25	0.52 ± 0.29
Stomach	168.79 ± 77.45	72.76 ± 32.68	5.52 ± 2.87
Small intestine	108.66 ± 29.79	30.46 ± 16.63	2.72 ± 1.34
Brain	6.07 ± 2.85	4.14 ± 2.72	0.33 ± 0.13
Testis	5.30 ± 2.40	29.08 ± 12.12	0.56 ± 0.28

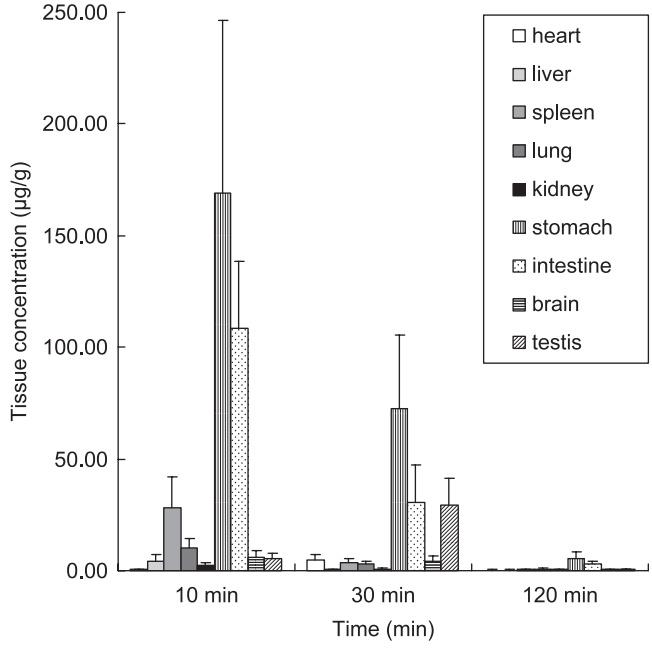

Figure 5. Piceid concentrations in tissues at different times after administration (mean ± SD, n = 6).

oral administration. Time to reach the maximum concentration was relatively short at 20.83 ± 8.16 min. The maximum concentration was 0.364 ± 0.151 μg/mL. The average eliminate half life ($T_{1/2}$) was 100.57 ± 17.05 min. The mean apparent volume of distribution is 73.61 ± 35.89 L/kg, which is much larger than the average total body fluid of rats. It suggested that the drug–plasma protein binding ratio of piceid was low and a great quantity of piceid may bind with tissues. The tissue distribution results confirmed this deduction. The elimination half life ($T_{1/2}$) and total clearance were 100.57 ± 17.05 min, 1.20 ± 0.37 L/kg/min, respectively, which showed rapid elimination of piceid from rats. AUC_{0-t} was 41.78 ± 15.15 μg·min/mL and $AUC_{0-\infty}$ was 52.26 ± 16.18 μg·min/mL.

The whole tissue distribution condition showed that piceid was diffused to most tissues immediately after oral administration, and the amount greatly diminished in all the tissues within hours. The peak level of piceid was detected at 10 min after administration in most tissues, except the heart and testis. The maximum level of piceid was detected in stomach, 168.79 ± 77.45 μg/g at 10 min, which was the administration site. At 30 min, the level was approximately 43.11% of the initial concentration, whereas, 2.50% remained 120 min after administration. At 10 min, the level of peceid in small intestine was also relatively high among the other tissues at 10 min and dropped to almost 28.03% at 30 min, which may mainly be attributed to the method of oral administration. Among the non-gut tissues, the level of piceid in spleen was high with a concentration of 28.03 ± 13.81 μg/g at 10 min. The distribution of piceid in brain showed that it had the ability to cross the blood–brain barrier after oral administration, which confirms the reports that piceid shows protective effects against brain injury (Huang *et al.*, 2005; Tian *et al.*, 2003). The concentration of piceid in testis reached a peak level at 30 min, which showed that it took more time for piceid to cross the blood–testis barrier.

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CONCLUSION

An HPLC assay for piceid in biosamples was developed and validated, which was successfully applied to the pharmacokinetics and tissue distribution research. The present bioanalytical method includes a simple protein precipitation procedure and reversed-phase HPLC analysis with UV detection, and demonstrated adequate specificity and sensitivity for the determination of piceid in rat plasma and tissue samples following a single oral administration of a 50 mg/kg dose. The short run time and low level of detection are ideal for large-scale subject analysis, especially when larger sample volumes are unattainable.

Piceid was absorbed, distributed and eliminated quickly after oral administration. The major distribution organs of piceid in rats were gastrointestinal tract (stomach, small intestine) and spleen. Piceid was able to cross the blood–brain barrier and blood–testis barrier. There was no long-term accumulation of piceid in rat tissues.

Acknowledgements

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