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Original Article

Study on pharmacokinetics and tissue distribution of single dose oral tryptanthrin in Kunming mice by validated reversed-phase high-performance liquid chromatography with ultraviolet detection

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ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form 7 May 2017
Accepted 10 May 2017
Available online 25 May 2017

Keywords:
mouse
natural products
pharmacokinetics
tissue distribution
tryptanthrin

ABSTRACT

Background: Tryptanthrin is a major active constituent of several Chinese herbal plants, such as *Isatidis radix*. Tryptanthrin had been demonstrated to have several beneficial pharmacological effects *in vitro* for human diseases, including antitumor, anti-inflammatory and antibacteria activities. In contrast to the extensive *in vitro* investigations, the *in vivo* disposition process of tryptanthrin was explored limitedly.

Methods: In this study, the pharmacokinetics (PK) and tissue distribution of tryptanthrin in Kunming mice following a single oral dose of 80 mg/kg tryptanthrin were investigated for the first time. Mouse plasma, liver, heart, spleen, lung, kidney and brain were collected and analyzed using a validated reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC–UV) method after biological sample preparation by a simple liquid–liquid extraction.

Results: The chromatographic analysis was performed on a Diamonsil C_{18} column (5 μm, 250 mm × 4.6 mm) and ultraviolet detection was set at a wavelength of 251 nm. The analysis was achieved with a mobile phase of methanol (A) and water (B) (60:40, v/v) at a flow rate of 1.0 mL/min. The method was linear over the concentration range of 4.0–400.0 μg/mL with a lower limit of quantification of 0.10–0.30 μg/mL. Inter- and intraday precisions (relative standard deviations %) were all within 2.93%. Recoveries of tryptanthrin were more than 86.44%. Maximal tryptanthrin concentrations in plasma and tissues of mice were reached within 2.5 hours. The actual highest concentration (C_{max}) in mouse plasma was 3.13 μg/mL, the area under the curve (AUC_{0-t}) was 9.38 h μg/mL, and the terminal half-life was 2.27 hours. The volume of distribution

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http://dx.doi.org/10.1016/j.imr.2017.05.001

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was 343.89 mL, the clearance rate was 204.58 mL/h, and the PK of tryptanthrin in mice after oral administration was fit to 2 compartment 1 st Order. After oral dosing of tryptanthrin to Kunming mice, the analyte was well distributed to the plasma and main tissues. C_{max} was found in the liver with a mean value of 3.54 μg/g, followed by that in the kidney, lung, spleen, heart, and brain.

Conclusion: In this study, a validated RP-HPLC–UV method was developed and successfully applied to PK and tissue distribution of oral tryptanthrin in mice. We confirmed that tryptanthrin was closely related and targeted to plasma, liver, kidney, and lung. These results indicate that tryptanthrin will have a good clinical application in the liver, kidney, or lung. The clinical use of tryptanthrin should focus on its pharmacodynamics and safety study in these tissues.

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1. Introduction

Tryptanthrin as a natural indole quinazoline alkaloid, 6,12-dihydro-6,12-dioxoindolo-[2,1-*b*]-quinazoline, originally isolated from Chinese herbal medicines *Isatidis radix*, *Baphicacanthis Cusiae Rhizoma et Radix*, reportedly has a wide range of pharmacological effects and diverse pharmacological mechanistic pathways without potential cytotoxicity,[1–3] such as antitumor,[4–8] anti-inflammatory[9–11] and antibacterial[12–14] effects in modern pharmacological studies. Its antileukemic activity is considered its most important clinical application. Tryptanthrin has proliferation-attenuating and apoptosis-inducing effects on human chronic myeloid leukemia cells, exerting its antitumor effect on the murine myelomonocytic leukemia cells by causing cell cycle arrest and by triggering cell differentiation.[8] Tryptanthrin as a highly selective cyclooxygenase-2 inhibitor showed inhibitory activity on prostaglandin and leukotriene synthesis, and reduced leukotriene B4 levels with a potency comparable to that of the clinically used 5-lipoxygenase inhibitor[10,11] and so on. Because of its very low content in plants, less than one ten-thousandth (mass/crude drug mass),[2,15] and the fact that it is difficult, laborious, time-consuming, and inefficient in separation and purification of such complex natural samples, targeted synthetic methods were preferred to obtain this potential drug through microbial fermentation,[16] biomimetic synthesis[17] and chemical synthetic methods.[18–20] The pharmaceutical analysis method, an important tool for tryptanthrin drug development and clinical drug evaluation, can timely reflect the problems arising in the drug production process. Using validated analysis methods, drug pharmacokinetics (PK) and tissue distribution can be carried out in the preclinical experimental research stage; these were an effective tool to reduce the failure rate of drug development. Although there were researchers who reported the use of these methods for tryptanthrin, such as a LC-UV method with cinnamaldehyde as internal standard (IS) to determine tryptanthrin in rats,[21,22] or 2-hydroxy acetophenone as IS in rats,[23] no relevant studies of tryptanthrin have been reported in mice. Moreover, the disposition process of tryptanthrin in mice was unknown until now. In this study, a new sensitive and simple reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC–UV) method using 4(3*H*)-quinazolinone as IS, which is most similar to the structure of the analyte, was first established to determine tryptanthrin in plasma and main tissues of Kunming (KM) mice following oral administration. These results are projected to guide subsequent clinical trials of tryptanthrin and its new drug development work.

2. Methods

2.1. Instruments

The LC-100 chromatographic system (WuFeng HPLC, Shanghai, China) consisted of two LC-P100plus pumps, an LC-UV100 plus UV detector and an LC-CO100 column oven. Peak areas were integrated automatically using the WuFeng LC-WS100 chromatographic workstation. Other apparatuses included an SB-5200D ultrasonic device (Scientz, Ningbo, China), a TGL-16G high-speed centrifuge (Anting Scientific, Shanghai, China), an AY-120 electronic balance (Shimadzu, Tokyo, Japan), an SK-1 vortex mixer (JinTan, Jiangsu, China) and a YAZD-5L double-distilled water preparation system (HengXing, Shangyu, China).

2.2. Chemicals and reagents

Tryptanthrin oral solution (made in our laboratory, lot. no. 20150712), tryptanthrin reference substance (>98%, RN 13220-57-0, provided by the College of Life Sciences, Northwest University, Xi'an, China); 4(3*H*)-quinazolinone (98%; RN 491-36-1, provided by JiuDing, Shanghai, China), heparin sodium (lot. no. 425C0215; Solarbio, Beijing, China), 0.9% sodium chloride injection (lot. no. D15031506, Guizhou KeLun Pharmaceutical Industry Ltd Co., Guizhou Sheng, China), methanol (HPLC-pure; TEDIA Co. Inc. Shanghai, China). The other reagents were analytical grade, double distilled in water.

2.3. Animals

Male♂ KM mice, weighing (18–25) g, were obtained from the Experimental Animal Center of Guizhou Medical University (Guiyang City, Guizhou Province, China), Permit No. SYXK

(Qian) 2012-0001. The animals were kept in an individually ventilated cage system for 5 days prior to starting the experiments, and fed with standard laboratory diet and water. All mice were dosed after an overnight fast except for water.

2.4. *Chromatographic condition*

The analytical separation was performed on a Diamonsil (Diamond 1) C_{18} column (5 μm, 250 mm × 4.6 mm, 5 μm) in a LC-100 chromatographic system (WuFeng, Shanghai, China). The mobile phase was methanol (A) and water (B) (60:40, v/v). The following settings were used: flow rate, 1.0 mL/min; detection wavelength, 251 nm; column temperature, room temperature; injection volume, 20 μL.

2.5. *Sample preparations*

For the plasma samples, a 40-μL plasma sample (or blank plasma for method validation of reference standard solution) was successively added with 10 μL blank methanol (or tryptanthrin standard solution for method validation), 10 μL 70.0 μg/mL IS solution, and 100 μL (1:3, v/v) methanol as protein precipitation reagent. After vortexing for 20 seconds, the samples were centrifuged at 12,000 rpm for 5 minutes. Then, 20 μL of the supernatant liquid was injected into the HPLC system for analysis. For tissue samples, all tissue sample preparations were the same as the plasma sample preparation.

2.6. *Method validation*

We prepared the calibrators by diluting each with 4.0–400.0 μg/mL tryptanthrin in methanol. The stock solutions were added to blank plasma or blank tissue homogenate to provide around 0.2–25.0 μg/mL tryptanthrin. The calibrators in plasma or tissue are described in section "Sample preparations." The calibration curve was obtained by plotting the peak area ratios [tryptanthrin/4(3*H*)-quinazolinone] as a function of the respective concentrations of tryptanthrin, and calculating the linear regression.

The intra- and interday precisions and accuracies of the developed method were evaluated in these biological samples spiked with tryptanthrin. The samples ($n = 5$) that had been spiked at concentrations of 0.25 μg/mL, 2.5 μg/mL, and 12.5 μg/mL for trptanthrin were assayed. Intraday precisions were carried out by repeated analysis of samples at different times during the same day, and the interday precisions were evaluated for 3 continuous days.

The short-term stability in plasma for 8 hours at room temperature, three freeze–thaw cycles in plasma during routine sample preparation, was evaluated using repeated analysis of stored plasma. The long-term stability in plasma was also tested by assaying frozen plasma samples after storage at −20 °C for 15 days.

The matrix effect and recovery yield of tryptanthrin in mouse plasma were evaluated through the standard solutions in mobile phase, which meant that tryptanthrin and IS were prepared directly with mobile phase without any matrix or extraction. Pre-extracted spiked samples which showed tryptanthrin and IS (70.0 μg/mL) were spiked in the extracted matrix of blank mouse plasma to prepare the final concentrations, and postextracted spiked samples that stood for tryptanthrin and IS (70.0 μg/mL) were spiked into blank mouse plasma followed by extraction and high-speed centrifugation. These three kinds of samples were all prepared in triplicate at concentrations of 0.25 μg/mL, 2.5 μg/mL, and 12.5 μg/mL.

2.7. *Drug administration and sampling*

For the pharmacokinetics study, all KM mice were housed at the Experimental Animal Laboratory of Department of Pharmaceutical Analysis, School of Pharmacy, Guizhou Medical University. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Guizhou Medical University, and were in accordance with the Guide for the Care and Use of Laboratory Animals. A total of 36 mice were randomly separated into 12 groups based on predetermined time points, with three mice at each time point. Tryptanthrin was dissolved in 0.5% sodium carboxymethyl cellulose (CMC-Na) aqueous solution at the final concentration of 4.0 mg/mL. The target dose of 80 mg/kg tryptanthrin was administered by oral gavage to the mice; the mice had free access to water after oral administration. Blood samples were obtained from the fossa orbitalis vein according to specific schedules (0.167 hour, 0.333 hour, 0.5 hour, 0.75 hour, 1.0 hour, 1.5 hours, 2.5 hours, 4.0 hours, 6.0 hours, 8.0 hours, 10.0 hours, 12.0 hours). Heparinized blood was immediately centrifuged at 4000 rpm for 10 minutes. The supernatant layer of the blood was thus collected as plasma. For the study of tissue distribution, liver, heart, spleen, lung, kidney, and brain tissues were collected at different time points (0.5 hour, 2.5 hours, and 4.0 hours). After oral dosing, tissue samples were weighed rapidly and placed in a 0.9% sodium chloride solution to remove the blood or content, blotted on a filter paper, and then weighed for wet weight. Small slices of tissues were individually homogenized with normal saline at the rate of 1:4 (weight/volume). All separated plasma and tissue samples were stored at −20 °C prior to the assay, and the LC analysis was finished within 2 weeks. After the completion of this experiment, all remaining mice were euthanized with chloral hydrate followed by cervical dislocation.

2.8. *Analysis of PK and tissue distribution*

The pharmacokinetic model and PK parameters were calculated using the professional PK program, WinNonlin 6.1 (Pharsight). Beta is the elimination rate constant at the terminal phase, and it was calculated as the slope of the linear regression on the terminal data points. C_{max} is the maximal plasma concentration based on the experimental data. Some important PK parameters were also calculated through the noncompartmental analysis. The area under the plasma concentration–time curve from time zero to the last time point (AUC_{0-t}) was calculated using the linear trapezoidal rule. The terminal half-life (Beta_HL) was calculated as 0.693/Beta; the systemic clearance rate (CL_F) was determined as Dose/$AUC_{0-\infty}$; the apparent volume of distribution (V_1_F) was calculated as CL_F/Beta.

The KM mice were administered with a single oral dose of tryptanthrin at 80 mg/kg. Tissue distribution was assessed at

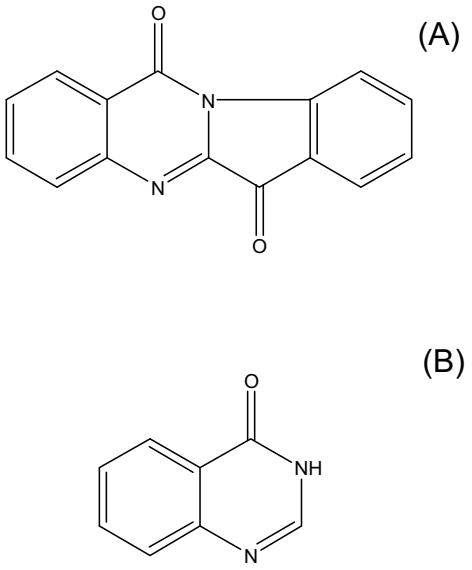

Fig. 1 – Chemical structures. A) Tryptanthrin. B) Internal standard 4(3*H*)-quinazolinone.

three different time points (0.5 hour, 2.5 hours, and 4.0 hours) after oral gavage administration. Three male mice each time point were sacrificed. Various tissues including liver, heart, spleen, lung, kidney, and brain tissues of each mouse were promptly removed at these three time points for determination of tryptanthrin. Several additional mice were sacrificed in predose to provide blank plasma and control tissues for analysis.

3. Results

3.1. *Method validation*

The chemical structure of tryptanthrin contains the heterocyclic structure, quinazolinone (Fig. 1), which drove us to search out similar structure compounds as a suitable IS in this study. 4(3*H*)-Quinazolinone was finally selected as the IS, not only because it provided the quinazolinone structure, but also because it was very cheap and easy to obtain, and there was no significant direct interference from endogenous substances in IS and the analyte. Typical chromatograms resulting from the analysis of various plasma samples or tissue homogenate are shown in Figs. 2–8 . Tryptanthrin and 4(3*H*)-quinazolinone appeared as well as resolved peaks with retention times of 20.1 minutes and 4.6 minutes, respectively.

Calibration samples were prepared using mouse blank plasma or tissue homogenate, which were spiked with tryptanthrin at known concentrations (4.0–400.0 μg/mL) and were assayed as described above. The least-squares linear regression evaluation of the relationship of peak area ratios (tryptanthrin/IS) (*y*) versus tryptanthrin concentration (*x*), correlation coefficients, limits of detection, and lower limits of quantification are all listed in Table 1. Precisions were determined for 15 spiked plasma samples with three different tryptanthrin concentrations. In the intraday precision assay, the relative standard deviations (RSDs) of precision were less

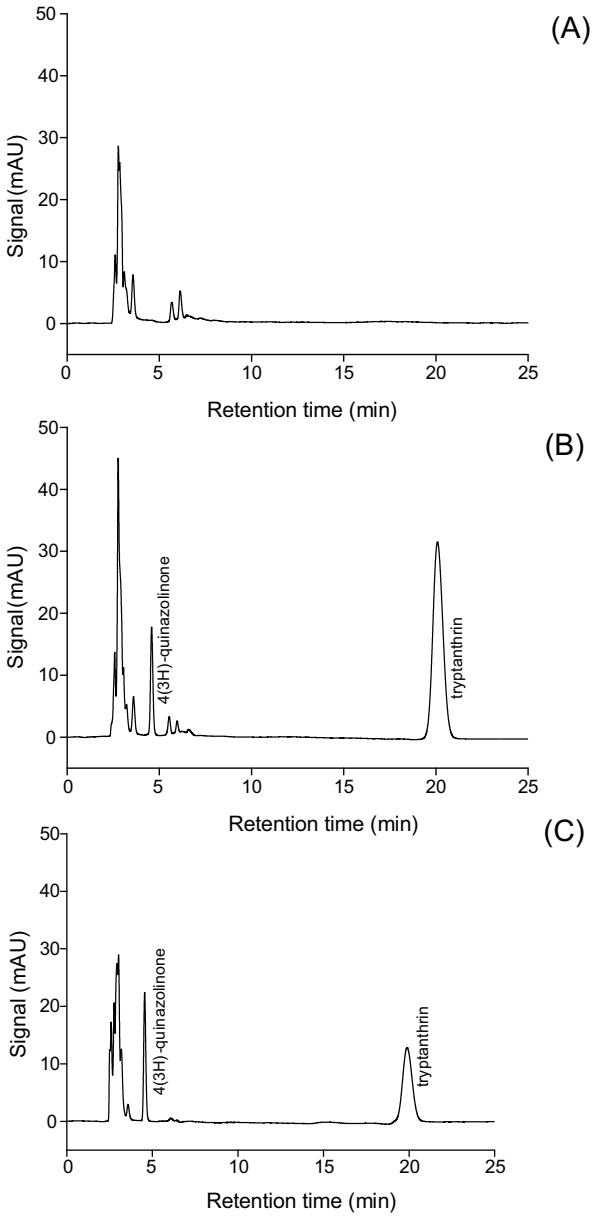

Fig. 2 – HPLC chromatograms. A) Mouse blank plasma. B) Mouse blank plasma spiked with tryptanthrin and IS (70 μg/mL). C) A mouse plasma sample spiked with IS (70.0 μg/mL) after single tryptanthrin IV injection C). HPLC, high-performance liquid chromatography; IS, internal standard.

than 3%—between 1.05% and 2.37% for all tryptanthrin concentrations (0.25 μg/mL, 2.5 μg/mL, 12.5 μg/mL); the accuracies were within ±15% ($n = 5$). In the interday precision assay (0.25 μg/mL, 2.5 μg/mL, 12.5 μg/mL), RSDs of 1.81–2.93% and accuracies of 86.25–105.61% also met the requirements of the biological sample analysis method ($n = 5$).

For the stability test of plasma samples, stored plasma spiked with 4.0 μg/mL, 40.0 μg/mL, and 200.0 μg/mL was evaluated at final concentrations of 0.25 μg/mL, 2.5 μg/mL, and 12.5 μg/mL, respectively, in mouse plasma ($n = 3$). The short-term stability test for 8 hours at room temperature

Table 1 – Linear regression equations, linearity ranges and method sensitivities of tryptanthrin in biological samples.

In vivo samples	Linear regression equation	Linearity range (μg/mL)	*r*	LOD (μg/mL)	LLOQ (μg/mL)
Plasma	$y = 0.7301x - 0.3541$	0.2–25.0	0.9994	0.04	0.20
Liver	$y = 0.9895x + 0.0873$	0.2–10.0	0.9970	0.04	0.20
Heart	$y = 0.8010x + 0.2502$	0.1–10.0	0.9993	0.02	0.10
Spleen	$y = 1.1312x - 0.0528$	0.1–10.0	0.9997	0.04	0.10
Lung	$y = 1.6959x + 0.0993$	0.15–10.0	0.9990	0.03	0.15
Kidney	$y = 1.7214x - 0.3422$	0.3–10.0	0.9992	0.06	0.30
Brain	$y = 1.6209x + 0.1574$	0.2–10.0	0.9975	0.04	0.21

LOD, limit of detection; LLOQ, lower limit of quantification.

Table 2 – Matrix effect and recovery yield of tryptanthrin in mouse plasma ($n = 5$).

Concentration (μg/mL)	Matrix effect		Recovery yield	
	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
0.25	85.6	8.36	86.44	3.98
2.5	96.1	5.84	95.87	2.28
12.5	93.2	3.28	96.41	2.12

RSD, relative standard deviation.

yielded RSDs of 2.17%, 1.26%, and 2.34%, respectively; three freeze–thaw cycles test in plasma yielded RSDs of 3.76%, 2.51%, and 2.09%, respectively; long-term stability test after storage at −20 °C for 15 days yielded RSDs of 4.64%, 2.93%, and 3.38%, respectively.

Based on the data shown in Table 2, the matrix effect and recovery yield of 0.25 μg/mL, 2.5 μg/mL, and 12.5 μg/mL tryptanthrin in mouse plasma were from 85.6% to 96.1% and from 86.44% to 96.41%, respectively ($n = 5$). The matrix effect and recovery yield were all within ±15%, which was generally accepted to carry out the analysis of biological samples containing the drug. In short, the above method validation results can ensure that our follow-up study was correct and reliable.

3.2. *Application to pharmacokinetic study*

The plasma mean concentration–time curves of tryptanthrin in KM mice after oral administration of 80 mg/kg tryptanthrin is shown in Fig. 9. It demonstrated that tryptanthrin was absorbed and eliminated relatively quickly in the plasma. The pharmacokinetic model and the parameters were calculated using the PK software. The main PK parameters of tryptanthrin in KM mice after oral administration are summarized in Table 3. PK model, 2 compartment 1 st Order was given the best fit to the plasma concentration–time curves obtained in KM mice, whereas the weighting coefficient (w) was 1/c·c for the two-compartment model. The minimum AIC, Akaike Information Criterion was −49.79, and SBC, Schwarz Bayesian Criterion was −47.36. The C_{max} that appeared at 1.50 hours after dosing was 3.14–2.53 μg/mL; the plasma concentration after T_{max} dropped drastically owing to fast and vast distribution into tissues, and then it followed a long terminal phase probably because of the contribution of tryptanthrin in the tissue back to plasma. The analyte was detectable up to 12 hours in the mouse plasma.

Table 3 – Main pharmacokinetic parameters of oral tryptanthrin in KM mice.

PK parameters	Unit	Value
AIC	–	−49.79
SBC	–	−47.36
V_1_F	mL	343.89
K_{01}	1/h	1.17
K_{10}	1/h	0.59
K_{12}	1/h	0.29
K_{21}	1/h	0.61
AUC_{0-t}	h μg/mL	9.38
$AUC_{0-\infty}$	h μg/mL	9.57
Alpha	1/h	1.19
Beta	1/h	0.30
Alpha_HL	h	0.57
Beta_HL	h	2.27
A	μg/mL	−208.93
B	μg/ml	2.60
CL_F	mL/h	204.58
T_{max}	h	1.50
C_{max}	μg/mL	3.13
MRT	h	3.06

AIC, Akaike Information Criterion ; Alpha_HL, alpha half life ; AUC_{0-t}, plasma concentration–time curve from time zero to the last time point; Beta_HL, terminal half-life; CL_F, systemic clearance rate; C_{max}, maximal plasma concentration; KM, Kunming; MRT, mean retention time ; SBC, Schwarz Bayesian Criterion ; T_{max}, highest temperature; V_1_F, apparent volume of distribution.

3.3. *Application to tissues distribution study*

In our results, the content changes of tryptanthrin in the main tissues of KM mice was similar to its time–concentration trend in plasma after the oral administration of tryptanthrin, which was unlike the changes of plasma drug concentrations after intravenous administration. Through the oral gavage to KM mice, the C_{max} of tryptanthrin was reached among plasma and tissues within 2.5 hours after dosing. The concentration–time point histograms of liver, heart, spleen, lung, kidney, and brain are presented in Fig. 10. The analyte was detectable up to

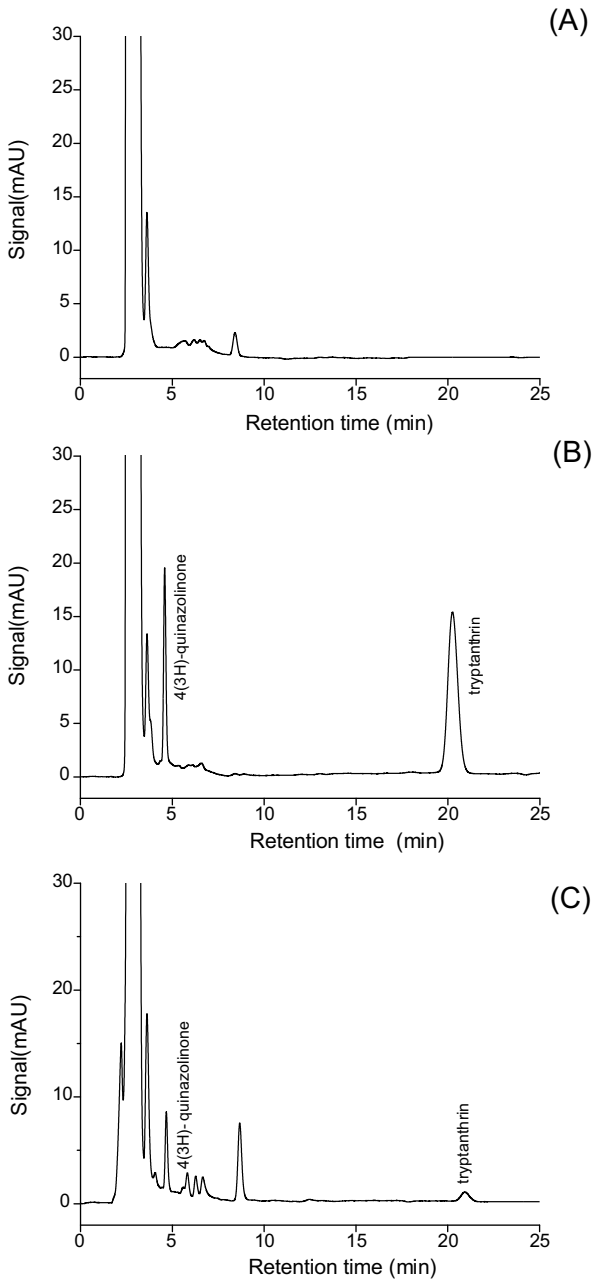

Fig. 3 – HPLC chromatograms. A) Mouse blank liver tissue homogenate. B) Mouse blank liver tissue homogenate spiked with tryptanthrin and IS (70 μg/mL). C) a mouse liver tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration.
HPLC, high-performance liquid chromatography; IS, internal standard.

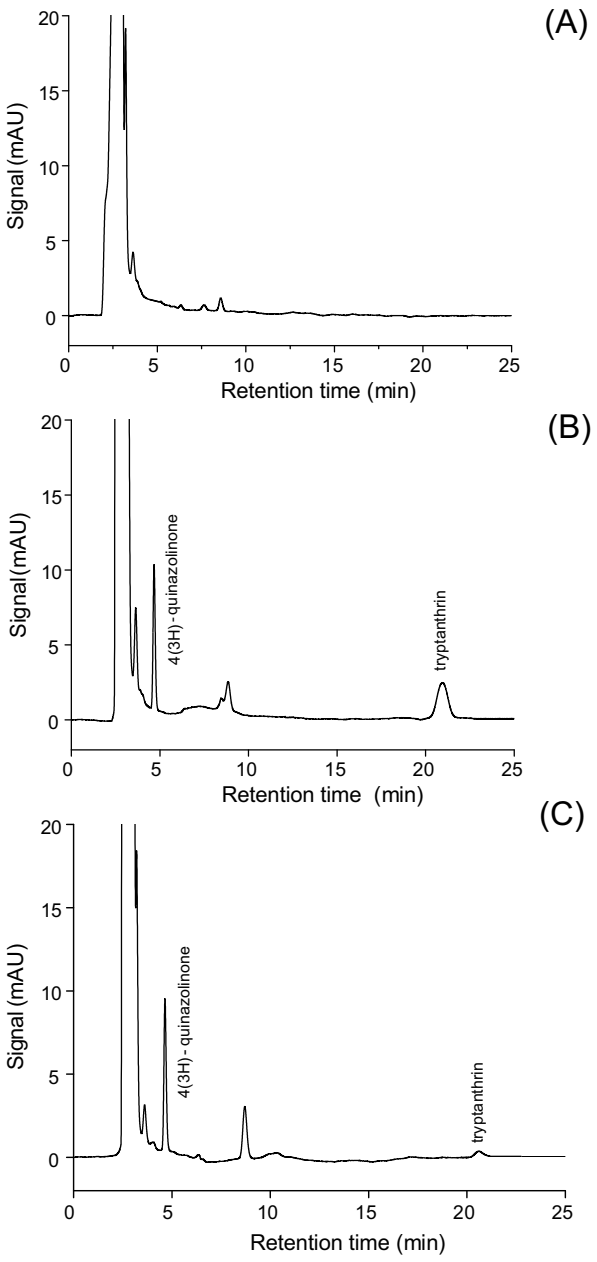

Fig. 4 – HPLC chromatograms of mouse blank heart tissue homogenate A), mouse blank heart tissue homogenate spiked with tryptanthrin and IS (70 μg/mL) B), and a mouse heart tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration C).
HPLC, high-performance liquid chromatography; IS, internal standard.

4 hours in the liver, heart, spleen, lung, kidney, and brain. Through the established RP-HPLC–UV method, the highest tissue C_{max} at 2.5 hours was found in the liver with a mean value of 3.54 μg/g, followed by that in the kidney (2.12 μg/g), lung (1.46 μg/g), and spleen, heart, and brain (all less than 1.0 μg/g). Levels of tryptanthrin in the liver and kidney were markedly higher than those in other tissues. Other tissues did not have C_{max} higher than that in the plasma except for liver, which ranged from 0.64- to 1.26-fold of that in the plasma. This indicated that the clinical application of tryptanthrin should focus on its pharmacodynamics and safety study in liver, kidney, and lung tissues at least. The content of tryptanthrin in the mouse brain was the lowest (less than 0.2 μg/g), suggesting that it may be difficult for tryptanthrin to cross the *in vivo* blood–brain barrier of KM mice,[24] so that the development of tryptanthrin drug therapy method based solely on the nature of tryptanthrin itself is impractical for curing brain diseases.

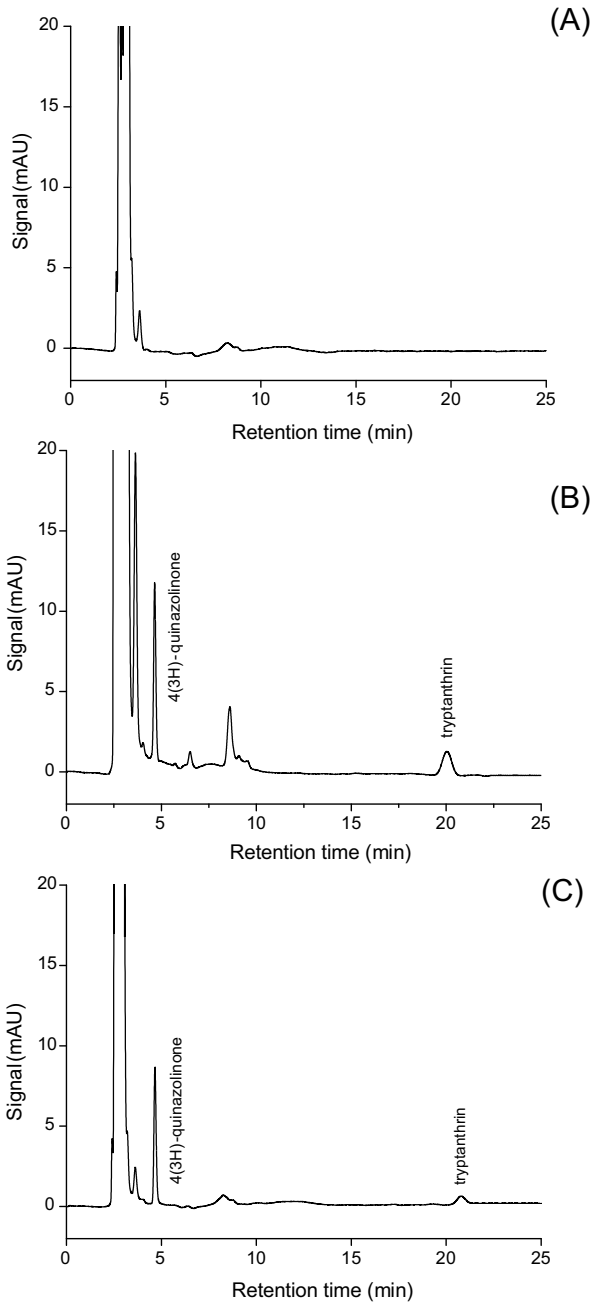

Fig. 5 – HPLC chromatograms. A) Mouse blank spleen tissue homogenate. B) Mouse blank spleen tissue homogenate spiked with tryptanthrin and IS (70 μg/mL). C) A mouse spleen tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration.
HPLC, high-performance liquid chromatography; IS, internal standard.

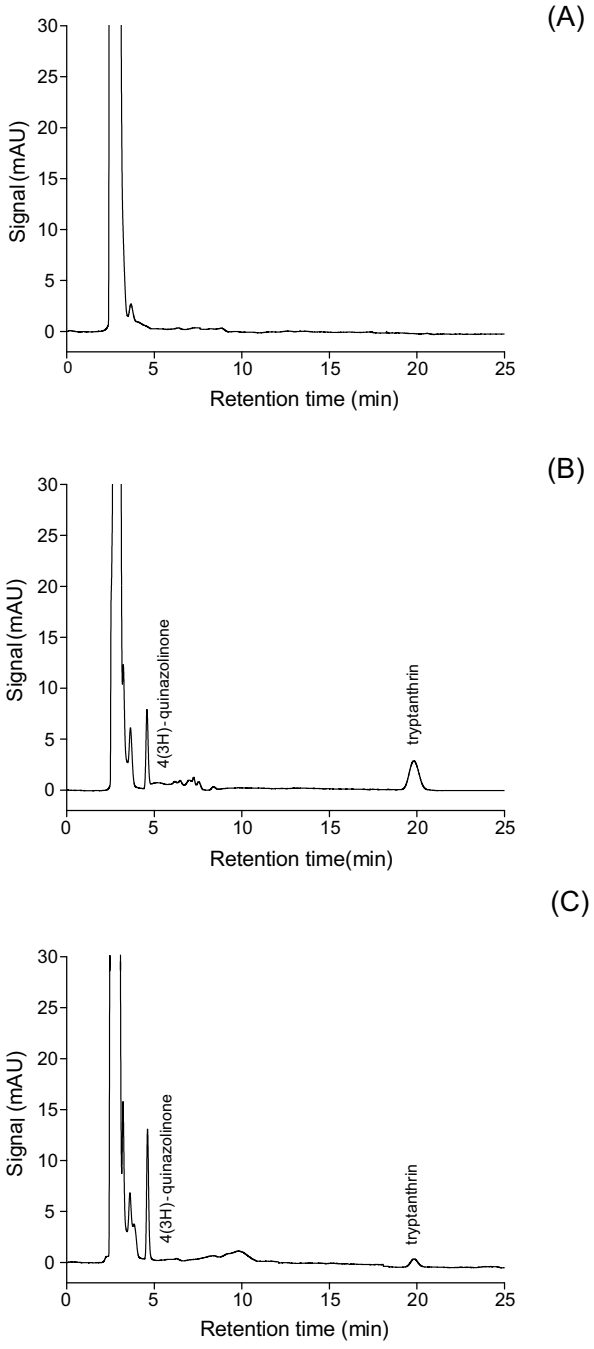

Fig. 6 – HPLC chromatograms. A) Mouse blank lung tissue homogenate. B) Mouse blank lung tissue homogenate spiked with tryptanthrin and IS (70 μg/mL). C) A mouse lung tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration.
HPLC, high-performance liquid chromatography; IS, internal standard.

4. Discussion

The established RP-HPLC–UV method in this study is a simple, accurate, and selective quantification method of tryptanthrin to warrant further PK study in mouse. It can be used as an alternative analytical method for tryptanthrin in all biological samples. The data given above about this quantification method revealed that the proposed method was validated and practical, requiring inexpensive reagents and analytical equipment. KM mouse as a kind of popular experimental mouse in modern pharmacological research was used in this study for the first time to explore PK and tissue distribution

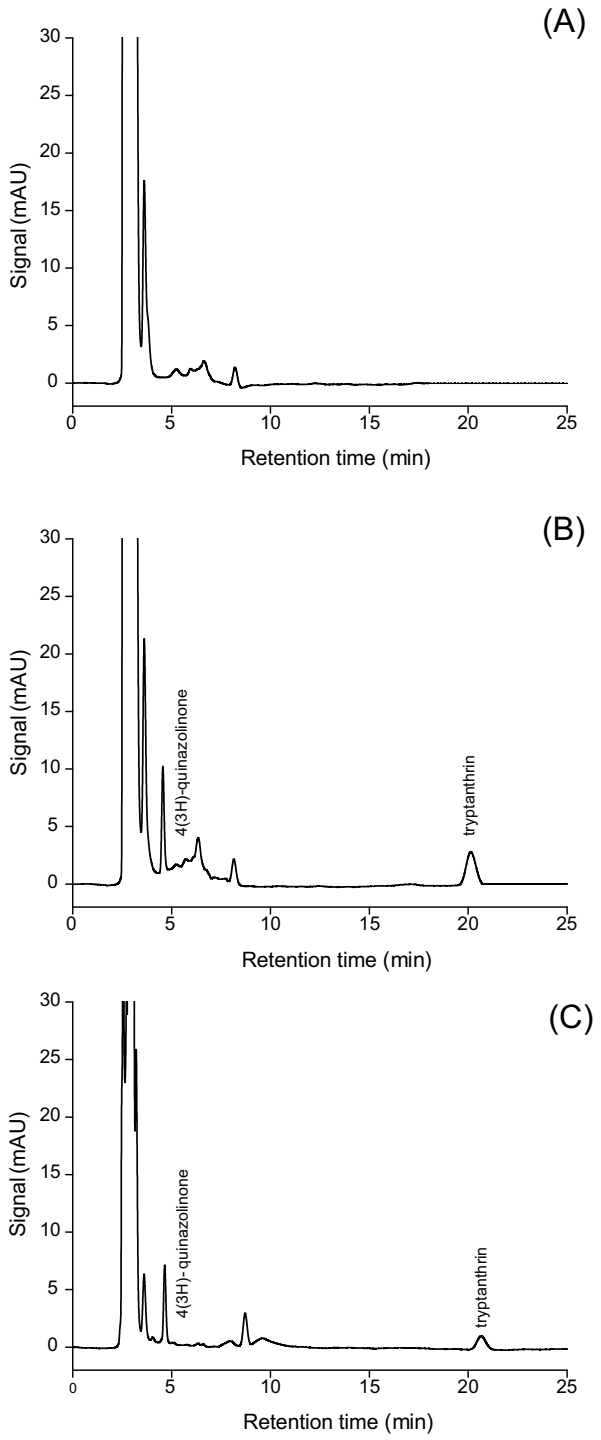

Fig. 7 – HPLC chromatograms. A) Mouse blank kidney tissue homogenate. B) Mouse blank kidney tissue homogenate spiked with tryptanthrin and IS (70 μg/mL). C) A mouse kidney tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration. HPLC, high-performance liquid chromatography; IS, internal standard.

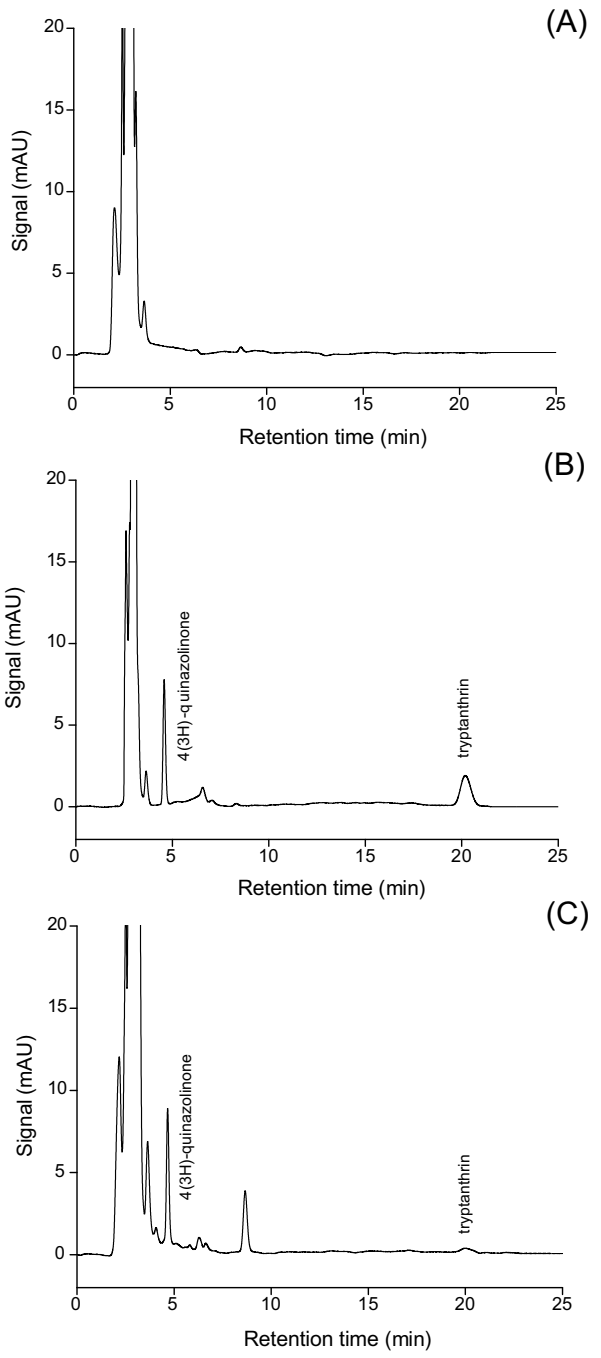

Fig. 8 – HPLC chromatograms. A) Mouse blank brain tissue homogenate. B) Mouse blank brain tissue homogenate spiked with tryptanthrin and IS (70 μg/mL). C) A mouse brain tissue homogenate spiked with IS (70.0 μg/mL) after single tryptanthrin oral administration. HPLC, high-performance liquid chromatography; IS, internal standard.

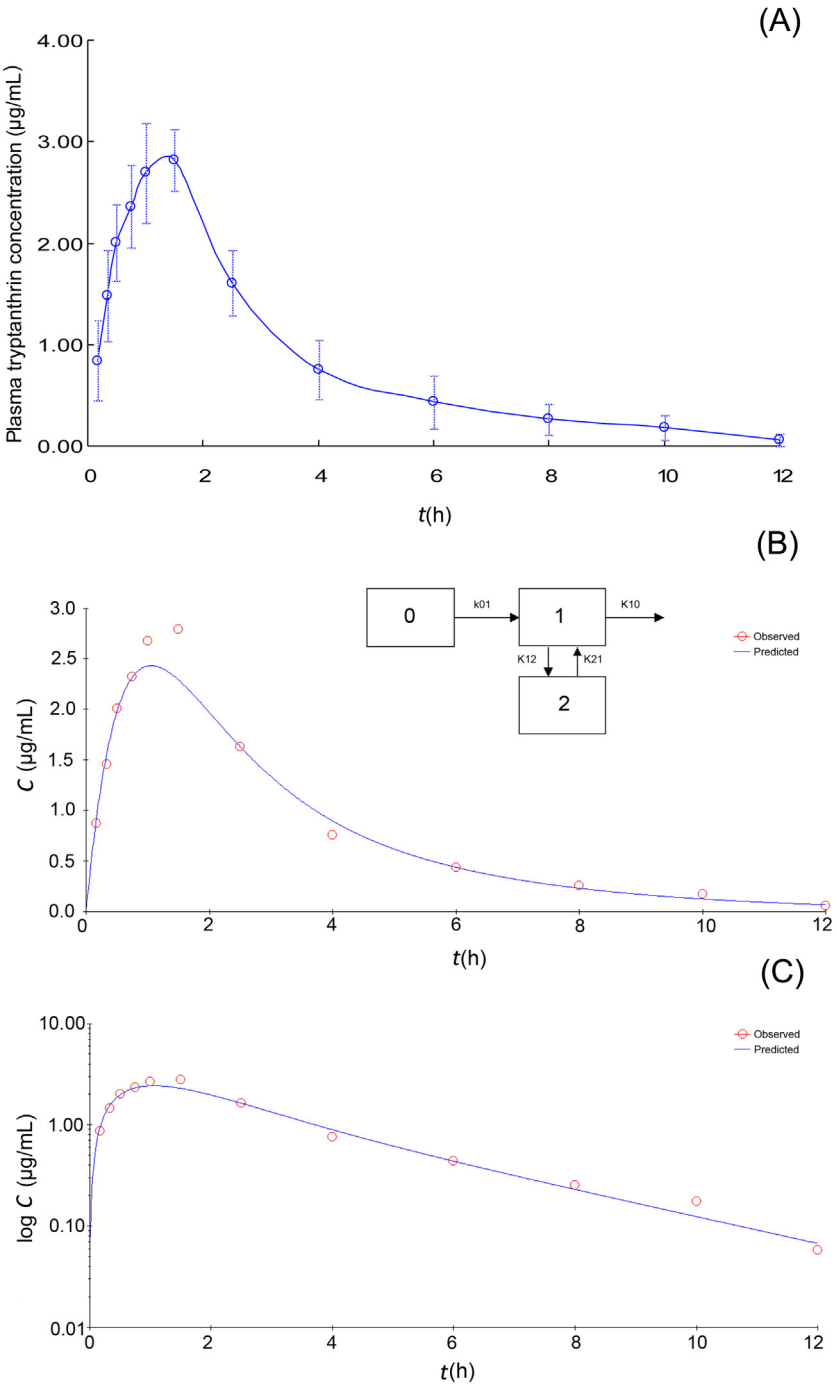

Fig. 9 – Plasma tryptanthrin concentration-versus-time curve after single oral tryptanthrin solution in Kunming (KM) mice. A) Actual plasma tryptanthrin. B) Mean plasma tryptanthrin C–t. C) Log C–t ($n = 3$).

of tryptanthrin *in vivo*. It was very suitable to do tissue or organ distribution analysis of certain drugs compared to rats and other animals. Through this RP-HPLC–UV method, the study described the PK of tryptanthrin in KM mice following 80 mg/kg oral dose for the first time, and established the tissue distribution profile and *in vivo* PK parameters.

In this experiment, KM mice were administered oral tryptanthrin in the PK study. Then tryptanthrin was distributed rapidly in plasma, liver, kidney, lung, and other tissues within 4 hours. The drug concentration in these tissues was gradually reduced after drug administration. The concentration at the 12th hour was one-forty-eighth of the highest concentration (C_{max}) at the 1.5th hour. This meant that there was no obvious accumulation of tryptanthrin in the body, but its treatment dose, minimum toxic, or side effect dose were all unclear because we did not have an additional experiment here to examine its effective dose. The pharmacodynamics and safety study of tryptanthrin in liver, kidney, and lung tissues warrant follow-up study.

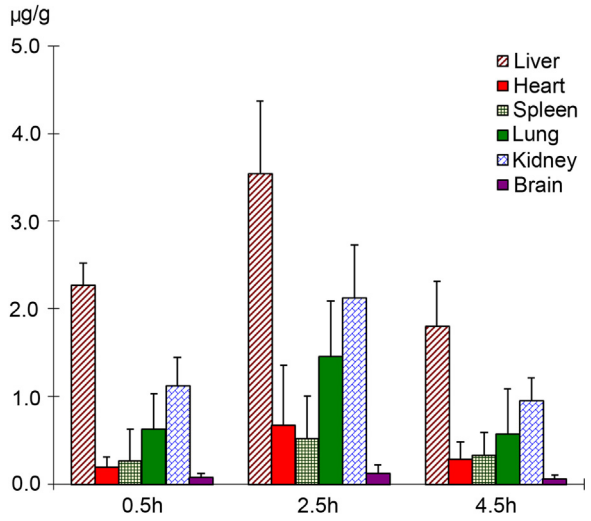

Fig. 10 – Concentrations of tryptanthrin in Kunming (KM) mice tissues after oral administration at the dose of 80 mg/kg ($n = 3$).

As well known, tryptanthrin was reported to show significant antitumor and antileukemia effects, which made it a very promising antitumor drug. From results of this PK and tissue distribution research, we confirmed that tryptanthrin is closely related and targeted to plasma, liver, kidney, and lung. This may explain why tryptanthrin has good curative effects on leukemia and other blood diseases. The results also indicated that tryptanthrin will have good clinical application in treatment of tumors in the liver, kidney, or lung. Our group also had reported its antioxidative effect on human retina cells recently,25 which will be another future research direction of tryptanthrin. This research provided further basis for drug–drug interaction studies, dosing regimen, physiologically based PK modelling, and prediction in clinical trials in the future.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was financially supported by National Innovation and Entrepreneurship Training Project for College Students, China (No. 201410660004), Guizhou Provincial International Cooperation Project (No. G[2014]7010), Guizhou Provincial Innovation and Entrepreneurship Training Project for College Students (No. 201510660024), Doctoral Fund of Guizhou Medical University (No. J[2014]006) and Guizhou Provincial Engineering and Technology Research Center for Research and Development of Chemical Synthetic Drugs (No. QKH2016-5402).

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