

Simultaneous Measurement of Tadehaginoside and its Principal Metabolite in Rats by HPLC–MS/MS and its Application in Pharmacokinetics and Tissue Distribution Study

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Abstract

Background

Tadehaginocide, an active ingredient isolated from *Tadehagi triquetrum* L., exhibited various biological activities. However, the pharmacokinetics and tissue-distribution which affects tadehaginocide's therapeutic actions and application remain elusive.

Methods

To clarify the metabolism of tadehaginocide *in vivo*, a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was established to detect the level of tadehaginocide in plasma and eleven tissues (brain, heart, liver, spleen, lungs, kidneys, stomach, small intestine, skeletal muscle, body fat, and testes). Besides, this validated method was also successfully applied to the quantitative determination of its metabolite, *p*-hydroxycinnamic acid (HYD) in plasma. The pharmacokinetic and tissue-distribution of tadehaginocide were investigated by this developed method.

Results

The pharmacokinetic study indicated that tadehaginocide in plasma of rats with intragastric administration showed relatively low concentration may be due to the formation of its metabolite, and the quick absorption of tadehaginocide was detected following intravenous administration. Tissue-distribution study indicated that kidney and spleen were the major distribution organs for tadehaginocide in rats.

Conclusions

These results could provide clues for exploring the bioactivity of tadehaginocide based on its pharmacokinetic characteristics.

Background

Tadehagi triquetrum, widely distributed in the tropical region of China, has been investigated by chemists and pharmacologists. As a traditional Chinese medicine in the southwest of China, *T. triquetrum* has been used extensively in treatment for cold and fever, sore throat, lung sputum, jaundice, hepatitis, nephritis, and enteritis [1–3]. Tadehaginocide was obtained from this plant and assigned as its main active constituent, which comprises a glucosyl, a phloroglucinol, and a *trans-p*-hydroxycinnamoyl moiety (Fig. 1A). Recent studies have revealed that tadehaginocide displayed a broad spectrum of biological activities and deemed as a lead compound for further drug development. For example, it has been demonstrated that tadehaginocide could reduce CCl₄-induced oxidative damage and inflammatory through the Nrf2 signaling pathway and NF-κB pathway [4]. In addition, tadehaginocide displayed the ability in increasing glucose uptake by up-regulating PPARγ and glucose transporter-4 in C2C12 Myotubes [5]. Besides, tadehaginocide exhibited biological activities in decreasing the accumulation of lipid in HepG2 cells [6]. Moreover, tadehaginocide showed potent activities in curing metabolic disease and could be used as a therapeutic agent against obesity, diabetes, and atherosclerosis [6, 7].

Our previous study partially explored the identification of tadehaginocide and its metabolites, and preliminarily determined that *p*-hydroxycinnamic acid (HYD) was the major metabolite of tadehaginocide in rats (Fig. 1B) [8]. It is reported that HYD also showed strong antidiabetic and antihyperlipidemic effects by modulating the level of glucose, triglyceride, and total cholesterol [9, 10]. Tadehaginocide and its metabolite HYD are similar in pharmacological

activities, but it is still unclear that which one plays the major role *in vivo* after treatment with tadehaginoside. Due to the novel chemical structure and potent biological abilities of tadehaginoside and HYD, their characteristic *in vivo* should be clarified. Up to now, the pharmacokinetics and tissue-distribution of tadehaginoside in rats have never been carried out. Studying pharmacokinetics and tissue-distribution can help to predict and clarify events related to drug efficacy and toxicity, and is an essential part of the drug development process [11, 12]. Accordingly, the *in vivo* pharmacokinetics and tissue-distribution of tadehaginoside in an applicable model and exploration of its properties would be extremely helpful.

Therefore, this work focused on establishing a specific and reliable HPLC-MS/MS method so that it can be used in quantitative determining the tadehaginoside and its metabolite in rats. This work analyzed the pharmacokinetic properties of tadehaginoside and its metabolite, which could provide a reference foundation for assessing the pharmacological activities of tadehaginoside and its metabolites.

Methods

Reagents

The aerial parts of *T. triquetrum* plant were collected from the Lingshui, Hainan province of China. The plant was identified by Professor Niankai Zeng (School of Pharmaceutical Science, Hainan Medical University, Hainan, China). Tadehaginoside (the purity is over 98%) was separated and purified from *T. triquetrum* by our research group. HYD (the purity is over 98%) was from Beijing Bailingwei Technology Co., Ltd. (Beijing, China). Quercetin, purchased from the National Institute for Food and Drug Control (Beijing, China), was used as the internal standard (IS, purity = 99.1%) for LC-MS/MS analysis. Ascorbic acid, CH₃OH, and HCOOH were of HPLC grade and were obtained from Aladdin Reagents (Shanghai, China). Deionized water was sourced from Hangzhou Wahaha (Hangzhou, China).

Instruments and LC-MS/MS conditions

The HPLC system was equipped with a SIL-20AC_{XR} autosampler, two LC-20AD_{XR} pumps, an online degasser, and a CTO-20A column oven, and they were all purchased from Shimadzu (Kyoto, Japan). The chromatographic column was Synergi™ Fusion-RP 80 Å C₁₈ (4 μm, 2.10 mm i.d × 50 mm, Phenomenex, Torrance, CA, USA), the temperature was maintained at 40°C during analysis. The aqueous solution containing 0.1% formic acid and methanol with 0.1% formic acid made up the mobile phase. The gradient elution was 10% B at 0-0.29 min, 90% B at 0.30-3.00 min, and 10% B at 3.01-4.00 min. The flow rate was set at 0.5 mL/min and the injection volume was 5 μL.

An AB Sciex Triple Quad™ 5500 system (Applied Biosystems, Foster City, CA, USA) was operated in the electrospray negative ionization mode (ESI⁻). To separate and determine tadehaginoside, HYD, and IS efficiently, the MS analysis detection was optimized when the collision energy at -22 V for tadehaginoside, -18 V for HYD and -29 V for IS, respectively. The optimized declustering potential was -120 V for tadehaginoside, 120 V for HYD, -120 V for IS. Temperature, 550°C; curtain gas, 25 psi; nebulizer gas, 55 psi; heater gas, 50 psi; ion spray voltage, -4500 V. The ion pairs of *m/z* 433.3→125.2 (tadehaginoside), *m/z* 162.8→119.0 (HYD) and *m/z* 301.1→151.0 (IS) were used for the quantitative analysis while undergoing multiple reactions monitoring (MRM).

Animals

Animal studies were conducted with 180-240 g male Sprague-Dawley rats, which were purchased from Hunan Slack Jingda Experimental Animals (Hunan, China; approval number: SCXK (Xiang) 2016-0002). All rats were placed in cages in a room with a relative humidity of 50% and temperature at $23 \pm 2^\circ\text{C}$ and were exposed to a 12 h light-dark cycle for a week before experiments. Animals were fasted for 12 h before drug administration, and water could be obtained *ad libitum*. Experimental procedures on animals were undertaken following the National Guidelines and were approved by the animal ethics committee of Hainan Medical University (reg. no. 201506017/HMU).

Preparation of IS and samples

Preparation of stock solutions and working solutions

A certain amount of tadehaginoside, HYD, and IS was dissolved in methanol to obtain the stock solution at a concentration of 1 mg/mL. The working solution of IS was further handled by dilution with methanol of stock solution to a final concentration of 10 $\mu\text{g/mL}$. Next, a linear concentration gradient (1, 5, 10, 100, 1000, 5000, 10000, 20000 ng/mL) of tadehaginoside stock working solution was serially diluted with methanol for pharmacokinetic studies and 50, 100, 1000, 5000, 10000 and 20000 ng/mL for tissue-distribution studies. Moreover, the concentrations of working solutions in plasma were 10, 50, 100, 500, 1000, 5000, 10000, 20000 ng/mL for HYD. All solutions were kept refrigerated at 4°C .

Preparation of calibration standards and quality control (QC) samples

Calibration standards and QC samples were prepared by mixing blank plasma or tissues with the working solutions as mentioned in section 2.4.1. The concentrations of calibration standards were ranged from 1 ng/mL to 2000 ng/mL (1, 10, 100, 500, 1000 and 2000 ng/mL) for tadehaginoside in plasma, and 5 ng/mL to 2000 ng/mL (5, 10, 100, 500, 1000 and 2000 ng/mL) in tissues. Similarly, calibration curve was prepared in the range of 10 ng/mL to 2000 ng/mL at six concentration levels (10, 50, 100, 500, 1000, 2000 ng/mL) for HYD in plasma. The final concentrations of QC samples were 3, 120, 1500 ng/mL for tadehaginoside in plasma samples, and 12, 120, 1500 ng/mL for tissue samples. In the same manner, three QC samples were set at 30, 120, 1500 ng/mL for HYD.

Preparation of sample solutions

The solution of ascorbic acid (5 μL) was transferred to 50 μL of rat plasma and then vortex-mixed for 15 s. Next, the sample was mixed with IS solution (5 μL , 10 $\mu\text{g/mL}$ in methanol) and methanol (150 μL) and mixed for 1 min. After centrifugation ($13,000 \times g$, 10 min), 5 μL of the supernatant was injected into the apparatus.

To investigate its tissue-distribution, each weighed tissue was homogenized in 0.9% NaCl (1:2, *w/v*) after thawing. Thereafter, 100 μL of the tissue homogenate and 10 μL of the ascorbic acid-saturated solution were added to a glass tube, and mixed for 15 s. The IS working solution (5 μL , 10 $\mu\text{g/mL}$ in methanol) and methanol (300 μL) were added to it in turn. After vortex-mixing for 1 min, then it was centrifuged at $13,000 \times g$ for 10 min under 4°C . The subsequent steps were conducted according to the procedure applied above.

Method validation

The method in the present study was validated according to the FDA and other related guidelines [13, 14]. The rat plasma and all of the target tissues or organs were analyzed to assess the specificity. Plasma and blank homogenates of the livers and kidneys as representative samples were screened for linearity, precision, accuracy, recovery, matrix effect, and stability.

Specificity

Specificity was determined by testing blank rat plasma and tissue homogenates from different rats, tadehaginoside, HYD and IS mixed with biological samples, and biological samples collected after treatment with tadehaginoside, respectively.

Linearity and LLOQ

The plasma samples and homogenates were handled as depicted in section 2.4.3, separately. 5 μL of working solutions were added into 45 μL of blank rat plasma to prepare the standard plasma samples. After spiking different concentrations of working solution (10 μL) to blank tissue homogenates (90 μL), the tissue standard solutions were obtained.

Calibration curves were constructed according to the previous report. Briefly, the least-squares linear regression method with $1/x^2$ weighting was used to generate the slope, intercept, and correlation coefficient of each linear regression equation. The lowest concentrations (LLOQ) of tadehaginoside and HYD in the calibration curve were detected with an acceptable precision $\leq 20\%$ and accuracy within $\pm 20\%$.

Accuracy and Precision

The accuracy and precision of the method in within-run and between-run conditions were evaluated using three consecutive batches and on more than two days at low, medium, and high QC levels ($n = 6$). The relative error (RE%) was applied to express the accuracy and the relative standard deviation (RSD%) was applied to express the precision.

Stability

Stability was assayed by quintuplicate determinations of QC samples for each concentration. The following conditions were applied to test the stability of tadehaginoside and its metabolite: (i) after 4 h at room temperature (samples which had undergone a protein-precipitation procedure); (ii) after 2 h at room temperature (samples which had not undergone a protein-precipitation procedure); (iii) after 6 h in the autosampler (15°C); (iv) after 24 h at 2-8°C; (v) after three freeze-thaw cycles; (vi) after 7 days of storage at -20°C.

Matrix effect

Blank plasma and tissues were processed, the QC samples were added, and the matrix effect in samples was analyzed in three levels (low, medium, and high). Next, the mean peak area of the analyte or IS in post-extracted spiked plasma/tissue homogenates was compared against the neat sample at the corresponding concentration.

The matrix factor (MF) of analytes (or IS) and IS-normalized MF were evaluated using Eq. (1) and Eq. (2) [11, 15].

$$MF = \frac{\text{Peak area of analyte in the presence of biomatrix components}}{\text{Peak area of analyte in the mobile phase}} \quad (1)$$

$$\text{IS-normalized MF} = \frac{\text{Peak area ratio of analyte to IS in the presence of biomatrix components}}{\text{Peak area ratio of analyte to IS in the absence of biomatrix components}} \quad (2)$$

Recovery

Recovery experiments were calculated via the determination of six replicates from the QC samples. The extraction recoveries were obtained by comparing the response of analytes from the extracted samples with the response of the same concentration of analytes spiked into the solution extracted from blank biological samples.

Pharmacokinetic study

Two groups (n = 5 per group) were set up by randomly dividing the ten male Sprague-Dawley rats. One group was given tadehaginoside intravenously at a dose of 5 mg/kg. The other group was orally administered tadehaginoside at doses of 25 mg/kg. Blood samples (0.2 mL) were gathered immediately from the suborbital vein and placed in heparinized 1.5-mL polythene tubes at 0, 5.0, 10.0, 15.0, 20.0, 30.0, 45.0, 60.0, 90.0, 120.0, 240.0, and 360.0 min following intravenous and oral administration. Then, each blood sample was immediately centrifuged immediately at $2000 \times g$ for 10 min at 4°C, and plasma was harvested and stored at -20°C until further treatment.

Tissue-distribution study

The tissue-distribution investigation was conducted on twenty Sprague-Dawley rats which were divided randomly into five groups. Rats were intravenous administration at a dose of 5 mg/kg and sacrificed by overdose of pentobarbital sodium (100 mg/kg) intraperitoneally for each time point (30.0, 60.0, 120.0, and 240.0 min). To remove superficial blood and contents, tissues (brain, heart, liver, spleen, lungs, kidneys, stomach, small intestine, skeletal muscle, body fat, and testes) were harvested and rinsed with ice-cold physiologic (0.9%) NaCl. Next, tissues were blotted with filter paper, weighed accurately, and homogenized in 0.9% NaCl (1:2, m/v). The obtained tissue homogenates were immediately stored at -20°C until analysis.

Statistical analysis

To calculate pharmacokinetic parameters, DAS 3.2.8 (Mathematical Pharmacology Professional Committee of China, Shanghai, China) was applied as a non-compartmental model. The half-life, area under the curve, clearance rate and mean residual time were calculated. Results are all expressed as the mean \pm standard deviation (SD).

Results

Method conditions

An HPLC–MS/MS was established to investigate and optimize the separation of tadehaginoside, HYD, and IS. Chromatographic separation was conducted on a Synergi™ Fusion -RP 80 Å C18 column (4 µm, 2.10 mm i.d × 50 mm) with a mixture of the aqueous solution with formic acid (0.1%, v/v) and methanol containing formic acid (0.1%, v/v) as the mobile phase. The MRM with an electronic spray ionization source was performed to measure the response of tadehaginoside (m/z 433.3→125.2), HYD (m/z 162.8→119.0), and quercetin (m/z 301.1→151.0).

Accuracy And Precision

Data regarding precision and accuracy were showed in Table 4. The within-run and between-run precision of tadehaginoside and HYD were less than 11.8% and 10.6%, respectively. Moreover, the within-run and the between-run accuracy of the method were determined lower than 109.2% and 107.2%.

Matrix Effects

Matrix effects were used to reflect the accuracy of the analysis results [19]. The mean IS-normalized MF was evaluated by using the same method in plasma and tissue homogenates with RSD% <15%. No significant suppression or enhancement of ions due to matrix components was found, indicating the method of extraction met the requirements and the analytical method was reliable.

Pharmacokinetic Study And Tissue-distribution Study

The LC-MS/MS method was used in investigating the pharmacokinetics of tadehaginoside and HYD, which followed a single dose (25 mg/kg for intragastric and 5 mg/kg for intravenous) administration. The major pharmacokinetic parameters of tadehaginoside and HYD were calculated by non-compartmental model and demonstrated in Table 5. The mean plasma concentration-time curves as depicted in Figure. 4. The concentrations of tadehaginoside in tissues determined at 0.5 and 1 h are shown in Figure. 5.

Discussion

Optimization of method conditions

The stable-isotope labeled analogs of the analytes are usually used as IS. However, the isotope labeled analogs of tadehaginoside are challenging to synthesize and the cost is too expensive. In this study, different possible internal standards were tested including chlorogenic acid and quercetin as their chromatographic behaviors and extraction efficiencies were similar to those of tadehaginoside. Chlorogenic acid was found to be unsuitable owing to poor peak shape and strong interference in the MRM channels. To control matrix effect, we used quercetin, which is believed to be the most appropriate IS for quantitative LC–MS/MS. Chromatography conditions such as the constitution of mobile phase have vital role in attaining a good result (for instance, appropriate ionization) [16, 17]. In order to optimize the analytical performance, two different mobile phases which are methanol-water, acetonitrile-water used. The result displayed that acetonitrile fell short of the target as the organic phase, but methanol could perform a perfect peak shape and a better resolution during the experiment. Moreover, the different concentrations of the organic reagent (50%, 60%, 70%, 80%, 90%, and 100%, respectively) that improved the speed of sample analyses and peak shape were investigated. The results showed that mobile phase of water (containing 0.1% acetic acid)-methanol

(containing 0.1% acetic acid) was a more suitable combination to achieve the optimal retention time and ionization of analytes.

The MS conditions were optimized to achieve high recovery, sensitivity, and selectivity. In Q1 scan mode, singly charged protonated precursor ion $[M-H]^-$ of tadehaginoside was found to be m/z 433.3. In product ion scan, abundant product ion was observed at m/z 125.0 or 125.2 for tadehaginoside. Therefore, the MRM transition of m/z 433.3 \rightarrow 125.0 was used for the quantification of tadehaginoside. Next, the transition ion of m/z 162.9 \rightarrow 119.0 for HYD was determined at the same way (Figure. 2). Finally, the MS conditions of quercetin initially were based on the literature for IS [18]. In order to obtain the highest relative abundance of precursor and product ions from the MS/MS product ions of analytes, the parameters such as fragment energy and collision energy were optimized respectively.

Sample Preparation

In the selection of treatment conditions for plasma samples, the peaks of tadehaginoside and quercetin of those samples without ascorbic acid solution in protein precipitation approach were degraded to different degrees after being placed at room temperature or in the automatic sampler for a long time. This phenomenon was especially shown in quercetin. Therefore, a suitable amount of ascorbic acid solution was added during plasma protein treatment to make the sample more stable.

Conclusions

To our knowledge, we present the first comprehensive pharmacokinetics and tissue-distributions study of tadehaginoside in rats after intragastric and intravenous administration. A specific, sensitive, and reliable LC-MS/MS method was established to determine tadehaginoside and its metabolite in the plasma and tissues of rats. Our method has significant advantages in terms of simple preparation of samples and short analysis times. We conclude that tadehaginoside could be distributed widely and eliminated rapidly after administration. One of the important targets in our next research could relate to taking some reasonable and effective methods to improve the oral bioavailability of tadehaginoside. The present study will contribute tadehaginoside to be a promising, natural health product, which also provides insights into the further pharmacological investigation of tadehaginoside.

Abbreviations

HPLC-MS/MS

High-performance liquid chromatography-tandem mass spectrometry

HYD

p-hydroxycinnamic acid

MRM

Multiple reactions monitoring

SD

Sprague–Dawley

ESI

Electrospray ionization source

IS

Internal standard

QC

Quality control

LLOQ

Lowest concentrations

RE%

The relative error

RSD%

The relative standard deviation

MF

Matrix factor

C_{max}

The peak drug concentration

T_{max}

Peak time

T_{1/2}

Half-life values

MRT_(0-t)

The mean residence time from the time of dosing to the time of the last measurable concentration

Declarations

Ethics declarations

Ethics approval and consent to participate

Experimental procedures on animals were undertaken following the National Guidelines and were approved by the animal ethics committee of Hainan Medical University (reg. no. 201506017/HMU).

Consent for publication

Not applicable.

Availability of data and materials

All data and materials in the current study are included in this published article.

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Contributions

ZHS and XPZ conceived and organized the review. CYZ and YTL performed the experiments and drafted the manuscript. YFT and DL revised the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Parameters of standard curves of tadehaginoside and HYD in the rats as determined by LC-MS/MS during method validation

Sample	Matrix	Run	Slope (10^{-4})	Intercept (10^{-3})	r
		1	8.78	2.51	0.9967
	plasma	2	8.65	5.25	0.9985
		3	8.8	4.36	0.9984
		1	7.68	-1.22	0.9984
Tadehaginoside	liver	2	7.37	7.03	0.997
		3	7.58	4.36	0.9988
		1	7.11	4.18	0.9985
	kidney	2	7.17	1.42	0.9974
		3	7.06	6.36	0.9962
		1	16.1	1.69	0.9983
HYD	plasma	2	17.1	2.85	0.9978
		3	17.9	2.99	0.9992

Table 2. Accuracy and precision of the LC-MS/MS method to determined tadehaginoside and HYD in rat plasma and various tissues (n = 6)

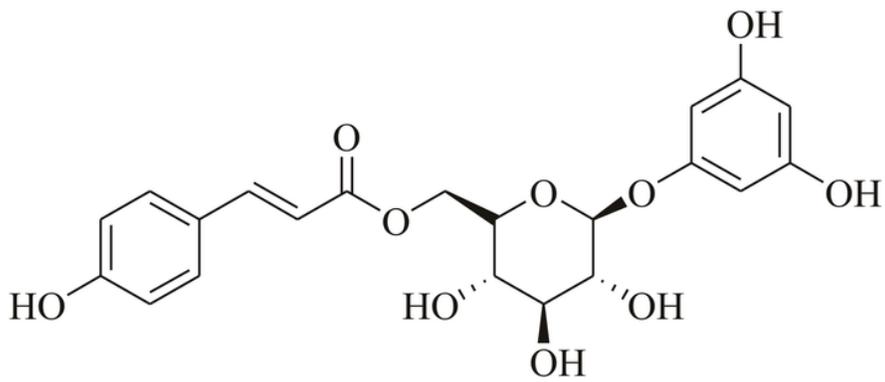
Sample	Matrix	concentration [ng/mL]	Batch	Within-run		Between-run	
				Precision (RSD %)	Accuracy (% nominal)	Precision (RSD %)	Accuracy (% nominal)
	plasma		1	7.7	98		
		1500	2	7.3	93.5	7.1	95.5
			3	6.7	94.9		
			1	5.7	97.1		
		120	2	6.9	97.3	5.9	96.9
			3	6.1	96.3		
			1	8.2	96		
		3	2	9.7	97.4	9.1	98
			3	10.1	100.7		
	liver		1	7.4	97		
		1500	2	9.8	98.2	8.8	99.8
			3	8.7	104.3		
			1	8.3	100.6		
Tadehaginoside		120	2	8.7	98.2	7.7	100.1
			3	7.2	101.5		
			1	9.5	109.2		
		12	2	7.4	107.8	8.5	107.2
			3	9.6	104.7		
	kidney		1	9.5	98.3		
		1500	2	9.1	91.6	9.6	95.4
			3	10.3	96.5		
			1	6.4	98.2		
		120	2	7.5	100.8	6.6	98.2
			3	6	95.6		
			1	7.6	105.9		
		12	2	8.6	103.5	7.4	105.1
			3	7.3	105.7		
		1	10.7	98.4			
	1500	2	11.0	100.6	10.6	99.4	
		3	11.8	99.1			

			1	7.4	98.6		
HYD	plasma	120	2	9.4	99.0	8.1	98.94
			3	8.9	99.2		
			1	10.5	95.2		
		30	2	9.0	98.9	9.1	98.1
			3	8.7	100.1		

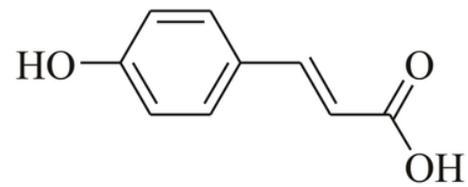
Table 3. Pharmacokinetic parameters of tadehaginoside and HYD after intragastric and intravenous administrations

PK parameters	i.g.(25 mg/kg)		i.v. (5 mg/kg)	
	Tadehaginoside	HYD	Tadehaginoside	HYD
$t_{1/2}$ (h)	2.51 ± 2.21	1.24 ± 0.038	1.27 ± 1.19	0.86 ± 0.58
C_{max} (ng/mL)	6.01 ± 2.15	837.75± 446.66	109.77 ± 4.29	1536.45 ± 193.93
T_{max} (h)	0.25 ± 0.08	1.25 ± 0.43	0.08 ± 0.00	0.08 ± 0.00
AUC_{0-t} (h•ng/mL)	7.92 ± 5.43	2027.58 ± 1091.20	52.85 ± 5.11	597.24 ± 103.90
$AUC_{0-\infty}$ (h•ng/mL)	14.12 ± 4.60	2099.87 ± 1103.33	59.22 ± 10.79	608.43 ± 98.42
$MRT_{(0-t)}$ (h)	0.52 ± 0.44	1.84 ± 0.11	0.54 ± 0.07	0.39 ± 0.07
V_z (L/kg)	6089.77 ± 4011.02	30.63 ± 28.50	143.27 ± 102.37	10.96± 8.95
CL_z (L/h•kg)	1937.91 ± 770.51	15.19 ± 9.74	86.44 ± 13.88	8.38 ± 1.28

Figures



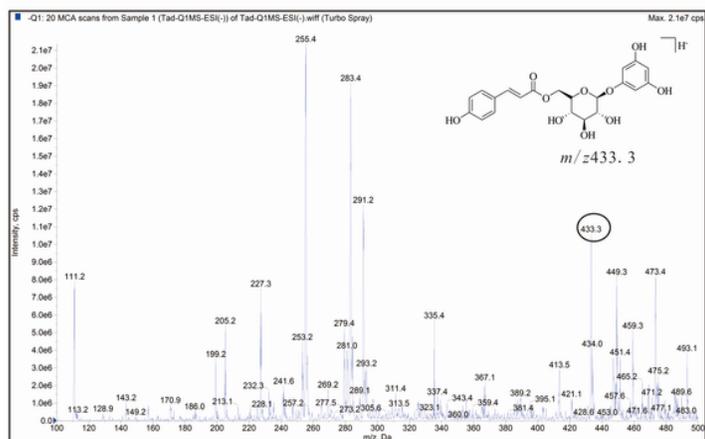
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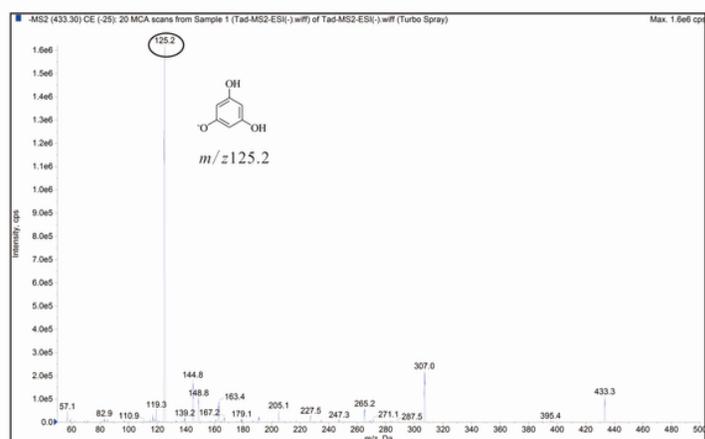
B

Figure 1

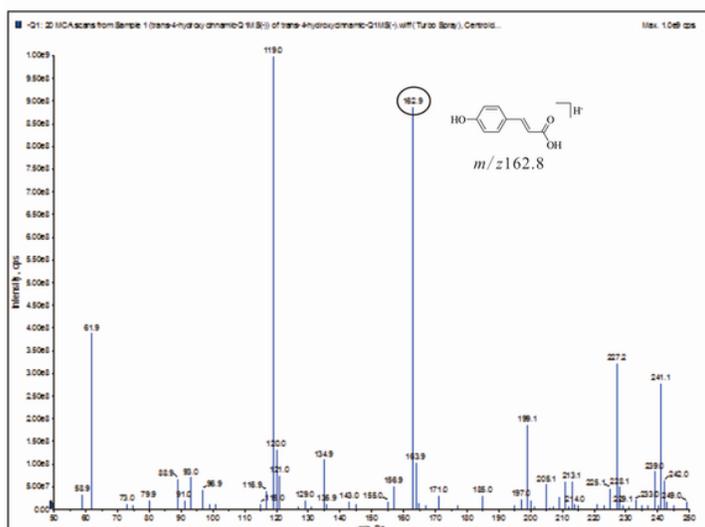
Recent studies have revealed that tadehaginose displayed a broad spectrum of biological activities and deemed as a lead compound for further drug development.



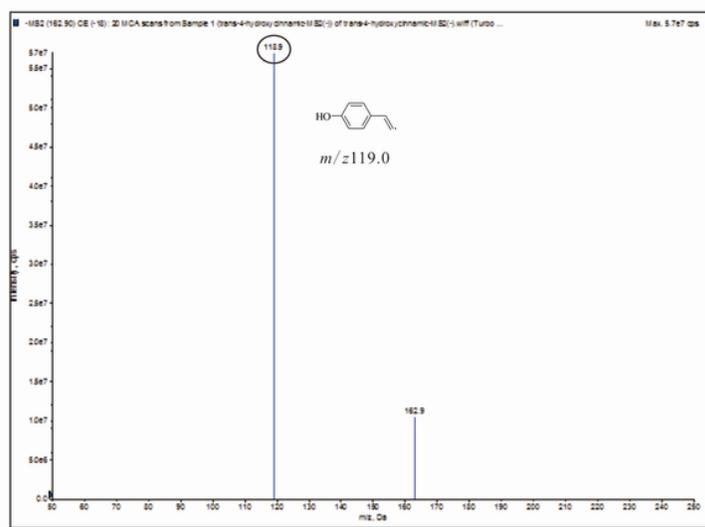
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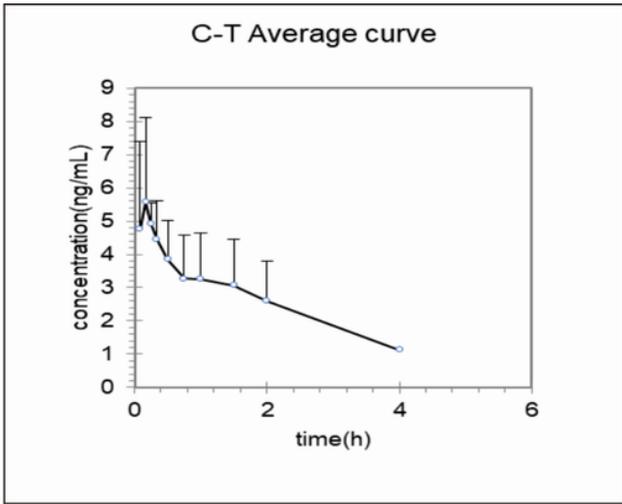
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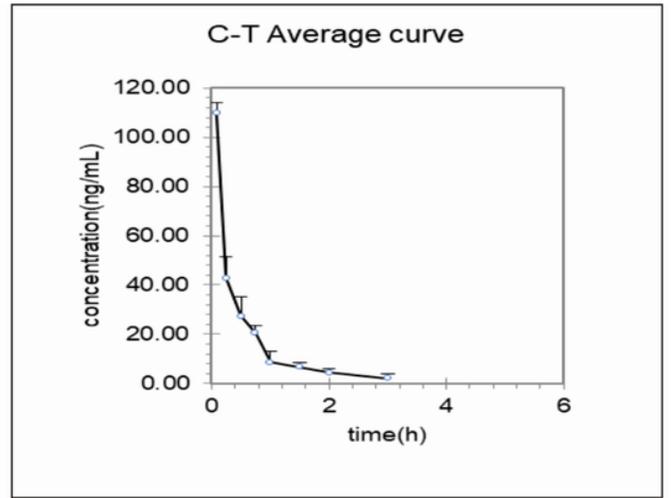
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Figure 2

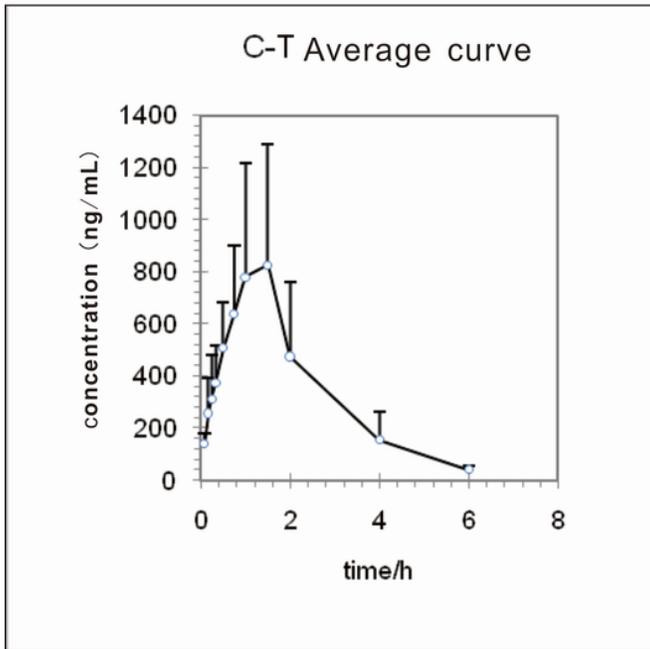
Finally, the MS conditions of quercetin initially were based on the literature for IS [18]. In order to obtain the highest relative abundance of precursor and product ions from the MS/MS product ions of analytes, the parameters such as fragment energy and collision energy were optimized respectively.



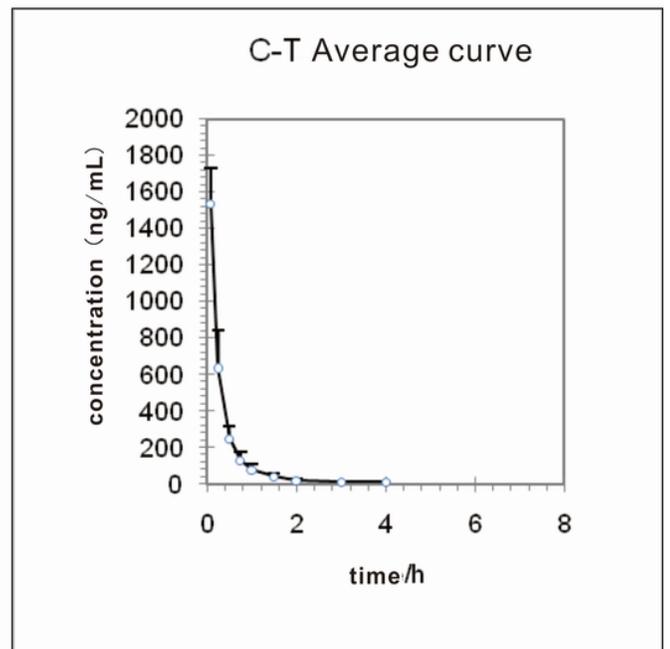
A



B



C



D

Figure 4

The LC-MS/MS method was used in investigating the pharmacokinetics of tadehaginoside and HYD, which followed a single dose (25 mg/kg for intragastric and 5 mg/kg for intravenous) administration. The major pharmacokinetic parameters of tadehaginoside and HYD were calculated by non-compartmental model and demonstrated in Table 1. The mean plasma concentration-time curves as depicted in

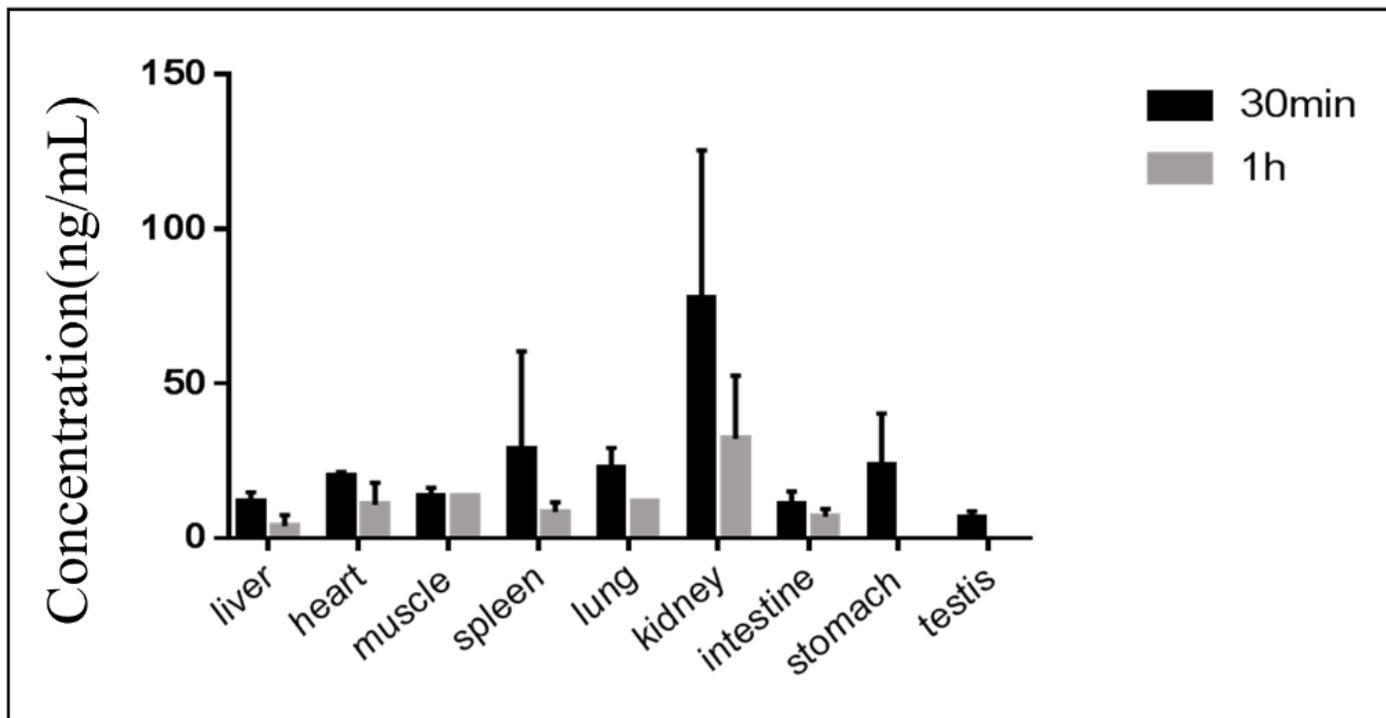


Figure 5

The concentrations of tadehaginoside in tissues determined at 0.5 and 1 h are

Supplementary Files

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- [abstract513.tif](#)