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ARTICLES

Determination of Tamarixetin and Kaempferide in Rat Plasma and Urine by High-Performance Liquid Chromatography[1]

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Abstract—Tamarixetin and kaempferide, the major bioactive constituents of Xiheliu extract, have been simultaneously/quantitatively determined in rat plasma and urine by a sensitive high performance liquid chromatography method after oral administration the total flavonoids from Xiheliu. In this study, the biological samples were prepared by solid-phase extraction, then simultaneously detected at 254 nm and successfully separated and quantified using a reversed-phase C_{18} column with methanol-formic acid aqueous gradient solution, at a flow rate of 1 mL/min. Good linearity ($r > 0.989$) of tamarixetin was observed in plasma and urine with the calibration ranges both at 1.6–80 μg/mL. For kaempferide, the correlation coefficient reached 0.994 in plasma at 1.4–70 μg/mL. The RSD of intra- and inter-day were 1.9–6.5% for tamarixetin and 1.3–9.0% for kaempferide in plasma; in urine, the intra- and inter-day RSD for not only tamarixetin but also kaempferide was no more than 7.4 and 5.8%, respectively. The lowest extraction recovery was 87.6% for kaempferide and 93.2% for tamarixetin in plasma and urine for both low and high concentrations. Due to the high sensitivity (the LOQ for tamarixetin was 1.2 μg/mL and for kaempferide 1.4 μg/mL), accuracy, precision, and good selectivity, the assay was successfully applied to pharmacokinetic studies of both flavonols in rats. The half-lives of tamarixetin and kaempferide were 17.8 ± 1.4 and 92.5 ± 1.6 min, and the c_{max} were 3.1 ± 0.2 and 2.5 ± 0.4 μg/mL, respectively.

Keywords: tamarixetin, kaempferide, liquid chromatography

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Tamarix chinensis L. (Chinese name Chengliu) was widely distributed on the beach of Jiangsu province, P. R. China. The twig of Chengliu named Xiheliu, used as a traditional Chinese medicine (**TCM**) for the treatment of cough with dyspnea, wind chill cold, rheumatic bone pain and pruritus in Chinese folk [1]. Besides its good antihepatotoxic activity [2], Xiheliu extract could kill pneumococcus, alpha-streptococcus, *Monilia albicans*, *Haemophilus influenzae* in vitro [3] and inhibit the growth of various pest's larvae [4]. Clinical tests revealed the extract not only was good for treating rheumatoid arthritis [5], but also had significant analgetic and antipyretic effects [6].

In previous phytochemical investigations of TCM extract [7, 8], tamarixetin and kaempferide were testified to be the major flavonoids, which were considered to be the active constituents of Xiheliu. Tamarixetin was found to have antibacterial [9], superoxide anion scavenging [10], free radical scavenging [11, 12], hepatic protective [13] and antioxidant activity [14, 15]. Kaempferide was reported to have the peroxynitrite free radical scavenging [16], antitrypanosomal and antileishmanial [17], antioxidant and antiradical activities [18]. The above description indicates that the efficacy of Xiheliu is closely related to the activities of tamarixetin and kaempferide. To conclude, tamarixetin and kaempferide were the most important active constituents of Xiheliu.

Among variety of bioactive flavonoids [19], which were taked with the daily diet, tamarixetin and kaempferide were widely found in many plants [20], but no simultaneous determination reported in crude plant's extract, let alone in biological fluids. Like dietary flavonoids, the effective basis of most TCM could have pharmacological activity after oral administration. And no literature data were found concerning the simultaneous determination of bioactive compounds of Xiheliu in plasma or urine. Therefore, it is essential to determine them quantitatively in biological fluids after oral administration of the total flavonoids from Xiheliu (**TFX**).

[1] The article is published in the original.

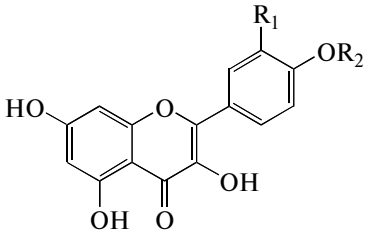
Table 1. Suitability parameters of HPLC system

Compound	k	α	N	T	R
Tamarixetin (in plasma)	0.403	1.54	2956	1.03	2.43
Kaempferide (in plasma)	0.949	1.73	3025	1.02	2.87
Tamarixetin (in urine)	0.126	1.53	2796	1.03	2.26
Kaempferide (in urine)	0.738	1.71	2944	1.03	2.75

The present study is aimed to develop a simple and reliable method for the simultaneous determination of tamarixetin and kaempferide in rat biological fluids after oral administration of TFX by using a solid-phase extraction (**SPE**) technique and high performance liquid chromatography (**HPLC**) with Diode Array Detection. Furthermore, pharmacokinetic study of tamarixetin and kaempferide in rat was carried out based on the good analysis results by this method. To our knowledge, this is the first investigation on simultaneous determination of tamarixetin and kaempferide in rat biological fluids after oral administration of TFX.

EXPERIMENTAL

Material. Twigs of Chengliu (5 kg) were collected to the north of Lianyungang, Jiangsu province, P. R. China, identified as Xiheliu by associate professor Hongshan Wan (Lianyungang Technical College), and extracted with 75% aqueous ethanol (3 × 2 h) under reflux. After evaporation of ethanol in vacuum, the concentrated extract was suspended in water, and the supernatant obtained by centrifugation was passed through a polyamide column and eluted by aqueous ethanol gradient solution series. The TFX was obtained from the 60% aqueous ethanol fraction. Tamarixetin and kaempferide standards were isolated from TFX by semi-preparative HPLC, and their structures were identified on the basis of spectral data (^{1}H NMR, ^{13}C NMR and MS) compared with literature values [21, 22]:



Tamarixetin: $R_1 = OH$, $R_2 = CH_3$
Kaempferide: $R_1 = H$, $R_2 = CH_3$
Quercetin: $R_1 = OH$, $R_2 = H$

HPLC analysis proved that the purity of tamarixetin and kaempferide was more than 99 and 98%, respectively. Quercetin used as internal standard (**IS**) was provided by professor Huimin Zhong (Qingdao University of Science and Technology), and has purity of more than 99%. Pure solutions of tamarixetin and kaempferide were prepared separately with HPLC grade methanol at concentrations of 800 and 1400 μg/mL, respectively. Then the two solutions were serially diluted with methanol to obtain the working solutions corresponding to 800, 400, 160, 80, 16, 8, 3.2 and 1.6 μg/mL for tamarixetin, 1400, 700, 280, 140, 70, 14, 7, 2.8 and 1.4 μg/mL for kaempferide. A stock solution containing tamarixetin and kaempferide was prepared by mixing and diluting the pure solutions with methanol to yield concentrations of 160 and 140 μg/mL, respectively. The IS solution at a final concentration of 275 μg/mL was prepared in methanol. All solutions were air-tight stored at −20°C and were testified to be stable after 30 days.

HPLC-grade methanol was purchased from Honeywell International Inc. (Burdick & Jackson, Muskegon, MI, USA). Analytical and semi-preparative SunFire™ reversed-phase C_{18} columns, oasis HLB C_{18} cartridge columns were purchased from Waters (Milford, MA, USA). Deionised water was prepared by using a Millipore academic water-purification system (Milford, MA, USA). The system suitability parameters are shown in Table 1.

Wistar rats (male, 170–190 g) were purchased from Qingdao Institute of Drug Control (Qingdao, Shandong province, P. R. China, SCXK2003020) and kept in regulated environment condition (relative humidity 65%, temperature 24 ± 2°C, 12 h dark/light cycle) for three days before the test, fed with food and water *ad arbitrium*. In the pharmacokinetics study all the rats were deprived of food but had free access to water for 12 h being housed in stainless cages for urine collection. All animal studies were performed according to the requirement of the National Act on the Use of Experimental Animal (China) that was approved by the Committee of Ethics of Animal Experimentation of Lianyungang City.

Instrumentation and chromatographic conditions. Chromatographic analysis was performed on an Agilent Technologies Series 1100 HPLC equipped with quaternary pump (G1311A), auto-injector (G1313A), column compartment (G1316A), Diode Array Detector (G1315B) and Chemstations software. The analytical column (5 μm, 250 mm × 4.6 mm) maintained at 30°C through the flavonols separation and quantitation in plasma and urine.

The mobile phase for HPLC analysis consisted of methanol (A) and 0.15% formic acid solution (pH 2.7)

(B) with a flow rate of 1.0 mL/min, and the change of gradient was different for plasma and urine samples. For plasma samples the initial elution condition was A–B 10 : 90 (v/v) and held for 5 min, linearly changed to A–B 20 : 80 (v/v) at 10 min, to A–B 30 : 70 (v/v) at 15 min, to A–B 55 : 45 (v/v) at 23 min, to A–B 65 : 45 (v/v) at 30 min, and to A–B 70 : 30 (v/v) at 35 min, then held A–B 70 : 30 (v/v) until 40 min. For urine samples the initial elution condition was the same as for plasma samples but holding for 10 min, linearly changed to A–B 25 : 75 (v/v) at 14 min, to A–B 40 : 60 (v/v) at 18 min, to A–B 60 : 40 (v/v) at 25 min, to A–B 65 : 45 (v/v) at 30 min, and further changed as for plasma samples. For plasma 20 μL of the sample was injected into HPLC system for analysis, and 15 μL was the volume of urine.

Sample preparation. The TFX was distributed in 0.5% sodium carboxymethyl cellulose solution, and the finial concentration was 20 mg/mL. Each rat was dosed 450 mg/kg of TFX solution orally, which contained 95 mg/kg tamarixetin and 77.4 mg/kg kaempferide.

In order to effectively remove interferences from biological samples with a high recovery, optimization of extraction methods for tamarixetin and kaempferide in plasma and urine were conducted, including protein precipitation, SPE and liquid–liquid extraction method [23–25]. SPE was testified to be the most efficient method because proteins and interfering compounds could be removed by water, while the marker analytes, which could be completely eluted by methanol in the following step, retained on the C_{18} cartridge. The result was consistent with literature [26–28].

Blood was collected from the retrobulbar capillary plexus at 5, 10, 15, 20, 25, 30, 40, 60, 120, 180 and 240 min after oral administration. Plasma was separated from blood samples by centrifugation at 3500 rpm (10 min), and stored at –20°C until analysis. A 200 μL plasma sample was removed to a 1.5 mL eppendorf (**EP**) tube and spiked with 5 μL of IS with adjusting pH to 3.0 using 20 μL 1% phosphoric acid solution. The mixed solution was vortexed about 60 s and removed to SPE cartridge, which was eluted by water (0.4 mL) and methanol (0.4 mL) successively. The methanol fraction was evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted in 200 μL of methanol and stored at 4°C for 30 min, then centrifuged (12000 rpm) for 10 min.

The urine samples were collected during the following time ranges: 0–1, 1–3, 3–5, 5–7, 7–9, 9–11, 11–15, 15–24, 24–30, 30–36, 36–48 and 48–60 h after oral administration, and the actual volume of each urine sample was recorded. After pretreated by polyamide (100–200 mesh) column chromatography the urine samples were also stored at –20°C until analysis. 2 mL of urine samples supernatant was extracted by C_{18} cartridge and eluted by water (2 mL) and methanol (2 mL) successively. The methanolic fraction was also evaporated to dryness under the same condition as plasma sample, and the residue was reconstituted in 300 μL of methanol, treated as plasma sample.

Calibration. 15 μL of working solutions were added to EP tubes and evaporated to dryness under a gentle stream of nitrogen at 40°C. For plasma samples, 200 μL of blank plasma, 10 μL of IS, and 20 μL of 1% phosphoric acid solution were added to the residues, while 2 mL of blank urine and 10 μL of IS were added for urine samples. All the mixed solutions were vortexed about 60 s in order to complete uniform at each step, and extraction proceeded as described in sample preparation section.

Method validation. As described in the section of calibration, 15 μL of high, middle and low concentrations working solutions were evaporated to dryness under nitrogen stream in EP tubes, and the residues were used for method validation.

The same volume working solutions were added to the biological samples and mobile phase, and then the recoveries were calculated by comparing the average peak areas obtained from plasma or urine with those from the mobile phase. The above residues were added to 200 μL of blank plasma and 20 μL of 1% phosphoric acid solutions for plasma sample. The following extraction proceeded as described in plasma sample preparation section. For urine sample the residues were spiked to 2 mL blank urine, and the extraction was performed as described in urine sample preparation section.

The short-term, long-term and freeze-thaw stability of tamarixetin and kaempferide in biological fluids was demonstrated. 5 μL of IS were added to the residues, followed by 200 μL of blank plasma for plasma sample and 2 mL of blank urine for urine sample. For freeze-thaw stability the solutions were treated as under "Sample preparation" after one, two, and three cycles; the storage periods were 1, 6, and 30 days for long-time stability; and the short-time stability was assessed at 4, 12, and 24 h after mixing the solutions. Stability evaluation was conducted by comparing the results at each interval to the initial concentration (samples treated immediately after being freshly prepared).

Intra-day precisions were assessed injecting standard solution five times during a day of each analyte at three different concentrations. Inter-day precision experiments were done after treatment of the standard solution in the same method, and the analysis was conducted every day over 5 days.

RESULTS AND DISCUSSION

Validation. HPLC is an economic and convenient method for biological samples analysis with good selectivity provided by difference in the retention times of analyte. To actualize perfect chromatographic separation, various mobile phases and analytical columns were tested and validated. It was found that the objective peaks were separated from endogenous substance peaks completely, which was necessary for the determination

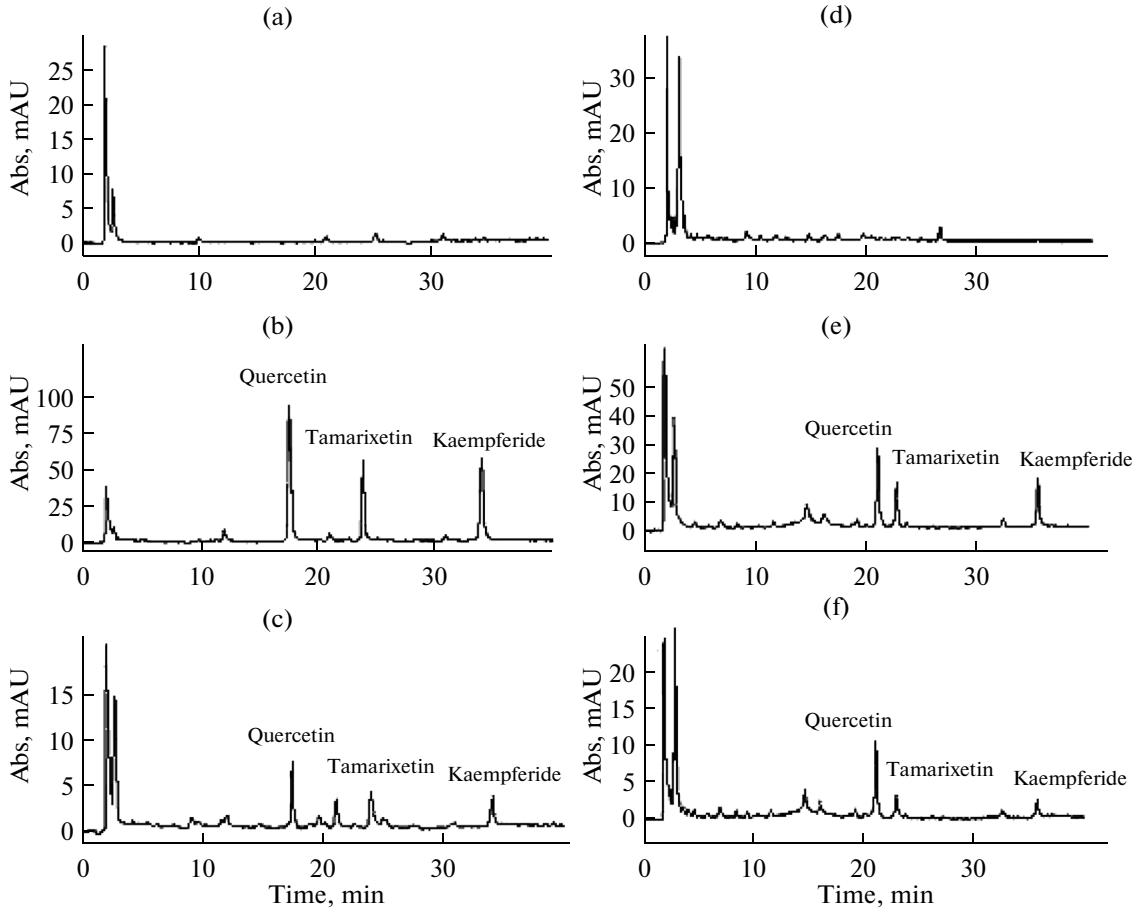


Fig. 1. Chromatographic profiles of plasma and urine samples: blank plasma (a); blank plasma spiked with tamarixetin, kaempferide and quercetin (b); plasma sample 25 min after oral administration of TFX (c); blank urine (d); blank urine spiked with tamarixetin, kaempferide and quercetin (e); urine sample 7 h after oral adminstion of TFX (f).

of analytes quantitatively, when the Waters reversed-phase C_{18} column was used in the combination with a mobile phase consisting of methanol-formic acid aqueous gradient solution. Figure 1 shows the chromatographic profiles of blank plasma (a), blank plasma spiked with tamarixetin, kaempferide and IS (b), plasma obtained 25 min after oral administration of TFX (c), blank urine (d), blank urine spiked with tamarixetin, kaempferide and IS (e), and urine collected 7 h after dosing (f). It was observed that none of the peaks appear at the same retention time as the analytes and IS peaks.

The extraction recoveries of tamarixetin and kaempferide from rat plasma determined by five replicates of rat plasma spiked with low, medium and high concentrations are shown in Table 2. The recoveries for tamarixetin, kaempferide and IS were 96.2–99.5, 89.6–100 and 97.6–98.7%, respectively. These data indicate that the extraction recoveries of tamarixetin, kaempferide, and IS from the plasma are acceptable.

The extraction recoveries of tamarixetin and kaempferide from rat urine are shown in Table 3. The recoveries were determined by the same way as for the plasma sample. The recoveries were above 87.6%, and the average recovery of IS was not less than 95.7%, which also proves that the experiment result is desirable.

High, middle and low concentrations of analytes were compared against calibration standards to analyze precision and accuracy of this method in biological samples. Analytical precision and accuracy data expressed by the mean concentration and relative standard deviation (**RSD**) are shown in Table 2 (for plasma) and Table 3 (for urine). The RSD in biological samples were less than 7.5 and 7.2% in plasma and in urine, respectively, indicating that the accuracy and precision were within recommended limits.

The calibration curves were obtained by linear least-squares regression analysis, and the correlation coefficient (r) were larger than 0.989 and 0.994 for tamarixetin and kaempferide, respectively. The calibration range of kaempferide in plasma was at 1.4–70 μg/mL, in urine was at 2.8–70 μg/mL and tamarixetin was performed over 1.6–80 μg/mL in plasma and urine.

The limit of detection (**LOD**) and limit of quantitation (**LOQ**) were determined for tamarixetin and

Table 2. Intra- and inter-day accuracy, precision and recovery for kaempferide and tamarixetin in rat plasma

Spiked concentration, μg/mL	Intra-day ($n = 5$)		Inter-day ($n = 5$)		Recovery, % ($n = 5$)	RSD, %
	measured, mean ± SD, μg/mL	RSD, %	measured, mean ± SD, μg/mL	RSD, %		
Kaempferide						
70	70.6 ± 0.9	1.3	69.8 ± 1.4	2.0	100 ± 1	1.2
14	13.4 ± 0.7	5.1	13.4 ± 0.7	5.6	95 ± 2	2.3
1.4	1.29 ± 0.09	6.7	1.18 ± 0.11	9.0	90 ± 7	7.5
Tamarixetin						
80	80.1 ± 1.6	1.9	78.5 ± 2.0	2.5	99 ± 3	2.6
16	16.2 ± 1.1	6.6	15.7 ± 0.7	4.6	98 ± 4	4.1
1.6	1.56 ± 0.09	6.1	1.51 ± 0.08	5.5	96 ± 6	6.5
Quercetin						
110					98.7 ± 1.4	1.4
27.5					98.3 ± 1.8	1.8
11					97.6 ± 2.9	3.0

Table 3. Intra- and inter-day accuracy, precision and recovery for kaempferide and tamarixetin in rat urine

Spiked concentration, μg/mL	Inter-day ($n = 5$)		Intra-day ($n = 5$)		Recovery, % ($n = 5$)	RSD, %
	measured, mean ± SD, μg/mL	RSD, %	measured, mean ± SD, μg/mL	RSD, %		
Kaempferide						
70	70.9 ± 2.2	3.1	68.9 ± 2.0	2.9	99 ± 4	4.4
14	13.3 ± 0.5	3.9	12.7 ± 0.7	5.7	95 ± 7	7.2
1.4	1.23 ± 0.09	7.4	1.21 ± 0.07	5.8	88 ± 6	6.8
Tamarixetin						
80	79.5 ± 1.5	1.9	79.0 ± 1.1	1.5	99 ± 2	2.0
16	15.3 ± 0.8	5.4	15.3 ± 0.5	3.3	95 ± 3	3.3
1.6	1.55 ± 0.1	6.7	1.51 ± 0.07	4.5	93 ± 3	3.6
Quercetin						
110					98.5 ± 0.9	0.9
27.5					97.8 ± 1.8	1.9
11					95.7 ± 3.2	3.4

kaempferide (Table 4) in biological samples. In accordance with the FDA Guidance the response of the analyte should be at least five times that of the blank. A series of diluted urine and plasma standard samples were investigated and reproducibly analyzed with at least five replicates by this method. Table 4 shows that the sensitivity of this method for tamarixetin and kaempferide is satisfactory.

The short-term, freeze-thaw and long-term stability was adopted to determine the analytes stability by this method. After being stored 24 h at room temperature and 30 days at −20°C three freeze (12 h) − thaw (3 h) cycles were performed and the results of tamarixetin and kaempferide were determined. Taking the recoveries showed in Tables 5, 6, and 7 into account, samples were regarded as stable for the deviation from the initial condition within ±15%. In order to ensure better veracity, in pharmacokinetics studies all samples were stored at −20°C no more than two days.

Pharmacokinetics studies. 18 rats were used for plasma samples collection, the urine samples were obtained from six rats, and ten rats were free of TFX to collect blank plasma and urine. Blank samples were collected at the same time points, and five duplicates were obtained at every time interval. Figure 2 shows the concentration-time profiles of analytes in rat plas-

Table 4. Calibration curves for kaempferide and tamarixetin in plasma and urine*

Compound	Standard curve	r	SE (a)	SE (b)	Test range, μg/mL	LOD, μg/mL	LOQ, μg/mL
Kaempferide (in plasma)	$Y = 0.00283X - 0.0448$	0.994	0.00139	0.00235	1.4–70	0.28	1.4
Kaempferide (in urine)	$Y = 0.00257X - 0.0696$	0.985	0.00130	0.00199	2.8–70	0.56	1.4
Tamarixetin (in plasma)	$Y = 0.00264X - 0.0219$	0.993	0.00143	0.00243	1.6–80	0.32	1.2
Tamarixetin (in urine)	$Y = 0.00259X - 0.0396$	0.989	0.00121	0.00196	1.6–80	0.32	1.6

* Y—peak area ratio (analyte/IS); X—concentration of the tamarixetin and kaempferide, mg/mL; LOQ, $S/N = 10 : 1$; LOD, $S/N = 3 : 1$, SE(a)—standard error of slope; SE(b)—standard error of intercept.

Table 5. Short-term stability data for the tamarixetin and kaempferide in rat plasma and urine

Compound	Added, μg/mL	Found, mean ± SD, μg/mL		
		4 h	12 h	24 h
Tamarixetin	160 (in plasma)	104 ± 1.3	101 ± 1.8	97.1 ± 2.2
	160 (in urine)	102 ± 1.0	99.9 ± 1.2	96.0 ± 2.7
	80 (in plasma)	101 ± 3.2	98.8 ± 2.7	95.7 ± 3.5
	80 (in urine)	99.6 ± 4.0	97.2 ± 3.8	94.4 ± 4.2
	8 (in plasma)	95.9 ± 4.4	94.5 ± 4.9	91.6 ± 4.2
	8 (in urine)	96.0 ± 6.1	92.6 ± 5.5	90.1 ± 6.3
Kaempferide	140 (in plasma)	102 ± 1.4	101 ± 2.1	96.5 ± 2.0
	140 (in urine)	103 ± 2.4	97.9 ± 3.2	95.6 ± 3.6
	70 (in plasma)	98.6 ± 2.1	98.8 ± 2.3	95.3 ± 3.2
	70 (in urine)	97.7 ± 3.1	95.7 ± 2.6	94.9 ± 4.1
	7 (in plasma)	95.8 ± 2.3	94.1 ± 3.5	91.1 ± 3.9
	7 (in urine)	93.6 ± 3.4	89.1 ± 4.1	89.7 ± 3.4

Table 6. Freeze-thaw stability data for the tamarixetin and kaempferide in rat plasma and urine

Compound	Added, μg/mL	Found, mean ± SD, μg/mL		
		one cycle	two cycles	three cycles
Tamarixetin	160 (in plasma)	103 ± 1.8	102 ± 2.0	99.1 ± 1.8
	160 (in urine)	103 ± 1.5	100 ± 1.5	98.6± 1.3
	80 (in plasma)	100 ± 2.8	97.8 ± 2.3	96.3 ± 3.8
	80 (in urine)	98.7 ± 1.1	96.6 ± 5.7	94.1 ± 4.8
	8 (in plasma)	97.4 ± 2.1	95.1 ± 2.8	92.2 ± 5.2
	8 (in urine)	97.0 ± 4.3	96.2 ± 6.4	88.7 ± 4.3
Kaempferide	140 (in plasma)	105 ± 2.5	100 ± 1.6	99.0 ± 2.5
	140 (in urine)	103 ± 1.9	99.0 ± 2.6	94.7 ± 2.0
	70 (in plasma)	96.7 ± 6.4	94.5 ± 4.4	92.1 ± 4.3
	70 (in urine)	97.5 ± 8.2	93.4 ± 7.1	90.6 ± 5.1
	7 (in plasma)	93.6 ± 3.0	90.7 ± 6.3	88.5 ± 4.3
	7 (in urine)	94.4 ± 2.6	88.7 ± 6.5	89.6 ± 5.4

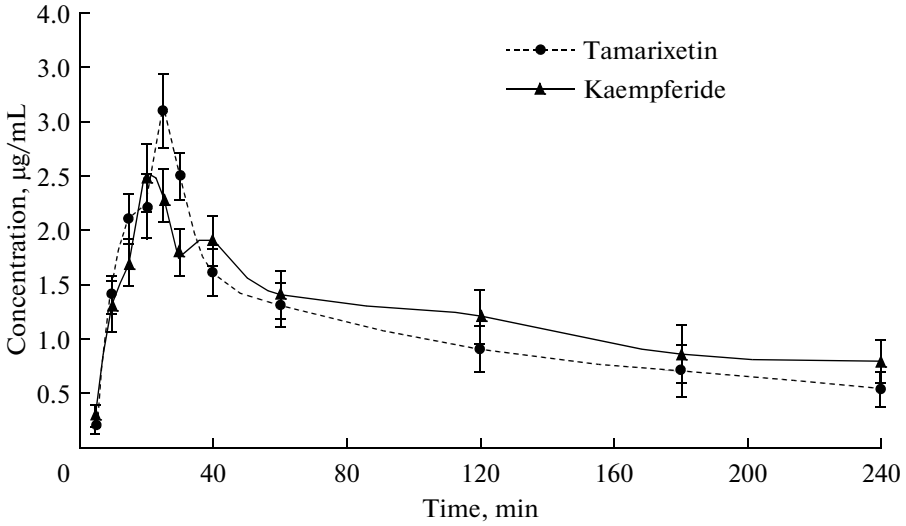


Fig. 2. Concentration-time profile of tamarixetin and kaempferide in rat plasma after oral adminstion of TFX ($n = 6$).

ma ($n = 6$) after oral administration of TFX. The concentrations of tamarixetin and kaempferide at 5 min were both less than 0.5 μg/mL and more than 1.5 μg/mL at the second time point. This phenomenon suggested that the analytes were absorbed very quickly after oral administration and the concentration (especially tamarixetin) can hold at relatively high value (more than 1 μg/mL) in 160 min, which maybe useful to explain why TCM have lasting characteristic. The pharmacokinetic studies were established by 3P87 software, and the results showed that the half-lives of tamarixetin and kaempferide were 17.9 ± 1.4 and 92.6 ± 1.6 min after oral administration (Table 8).

Figure 3 presents the cumulative excretion of tamarixetin and kaempferide in urine ($n = 6$) after oral administration. The amount of tamarixetin and kaempferide increased quickly at 7 h after administration, between 9 and 11 h the concentration reached the maximum.

Table 7. Long-term stability data for the tamarixetin and kaempferide in rat plasma and urine

Compound	Added, μg/mL	Found, mean ± SD, μg/mL		
		1 day	6 days	30 days
Tamarixetin	160 (in plasma)	103 ± 0.9	102 ± 1.2	101 ± 2.3
	160 (in urine)	103 ± 1.0	101 ± 1.4	100 ± 1.1
	80 (in plasma)	100 ± 0.7	99.8 ± 2.4	98.6 ± 2.7
	80 (in urine)	99.3 ± 1.1	97.2 ± 6.1	96.4 ± 6.4
	8 (in plasma)	98.7 ± 1.6	98.1 ± 9.1	96.7 ± 3.3
	8 (in urine)	98.2 ± 2.1	98.1 ± 5.7	95.2 ± 6.2
Kaempferide	140 (in plasma)	103 ± 1.1	102 ± 1.6	102 ± 1.4
	140 (in urine)	103 ± 2.4	101 ± 2.2	98.5 ± 2.4
	70 (in plasma)	99.3 ± 5.2	98.7 ± 4.5	92.6 ± 5.8
	70 (in urine)	101 ± 4.9	97.4 ± 5.5	94.1 ± 6.4
	7 (in plasma)	96.2 ± 7.3	92.9 ± 6.8	88.2 ± 4.7
	7 (in urine)	96.1 ± 4.6	93.2 ± 5.6	89.6 ± 5.4

Table 8. Pharmacokinetic parameters of tamarixetin and kaempferide after a single oral administration ($n = 5$)

Compound	c_{max}, mg/mL	T_{max}, min	$AUC_{0\to240}$, μg min/mL	$AUC_{0\to\infty}$, μg min/mL	K_{el}, min^{-1}	$T_{1/2}$, min
Tamarixetin	3.2 ± 0.2	26.1 ± 2.5	(2.5 ± 1.0) × 10^2	(3.2 ± 0.9) × 10^2	0.0398 ± 0.008	17.9 ± 1.4
Kaempferide	2.6 ± 0.4	21.4 ± 3.1	(2.8 ± 1.0) × 10^2	(3.8 ± 0.9) × 10^2	0.00773 ± 0.002	92.6 ± 1.6

Comparing the rats free of TFX, the urine amount obviously increased from 7 till 24 h after administration, the volume excreted in this period was 54.8 mL, and the total amount in this experiment was no more than 75.6 mL; after 30 h the urine amount began to decline gradually and reached the normal level. This indicates that the TFX may have a diuretic effect, mostly caused by tamarixetin and kaempferide. Unfortunately, no literature appeared on the examination of the treatment of urinary diseases for Xiheliu, although this TCM was recorded to have diuretic effect in Compendium of Materia Medica (Chinese name: Bencaogangmu, author: Shizhen Li, 1518–1593, Ming dynasty) for more than 400 years. The peaks of tamarixetin and kaempferide in plasma occurred less than 25 min after administration and last at a high level for long time, the urine excretion increased at about 7 h and returned to normal by 30 h. This result suggests that the major active constituents of Xiheliu were absorbed quickly and had a slow onset and long duration of potential diuretic effect.

* * *

The present results demonstrate a simple, reliable and reproducible reverse-phase HPLC method for simultaneous determination of tamarixetin and kaempferide in rat plasma and urine after oral administration of TFX. In this method biological sample pretreatment by a solid-phase extraction resulted in an excellent recovery. Due to the higher sensitivity and better selectivity the present method has been successfully utilized for the pharmacokinetic study of the analytes in rats, which would be helpful to ongoing studies for the development of TFX as new diuretic herbal lead. To our knowledge, this is the first report on simultaneous determination and pharmacokinetic study of tamarixetin and kaempferide in rat biological fluids after oral administration of TFX.

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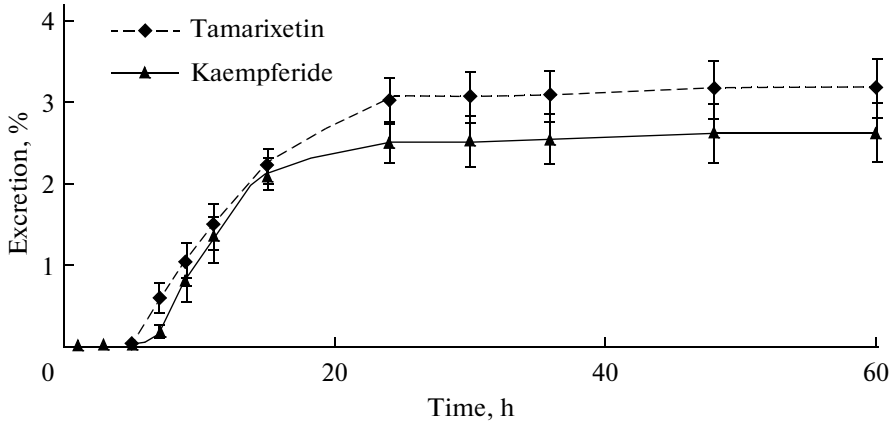

Fig. 3. Cumulative excretion of tamarixetin and kaempferide recorded after oral administration of TFX to rats ($n = 6$).

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