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Research paper

Determination of eleutheroside E and eleutheroside B in rat plasma and tissue by high-performance liquid chromatography using solid-phase extraction and photodiode array detection

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Abstract

A HPLC method with photodiode array detection (PDA) was developed for the determination and a pharmacokinetic study of eleutheroside E (ELU E) and eleutheroside B (ELU B) in rat plasma and tissue following an eleutherococcus injection. The analysis was performed on a Kromasil C_{18} column, using water-acetonitrile as the gradient mobile phase and 0.8 mL/min flow rate. Detection wavelengths of ELU E and ELU B were 220 and 206 nm, respectively. Protein from the biological sample was deposited using acetonitrile. ELU E and ELU B were extracted from the biological samples using acetonitrile, separated by solid-phase extraction, and eluted from the cartridge using 60% methanol. The extraction recovery of ELU E and ELU B was 91.2 and 88.8%, respectively. The limit of detection was 37.6 ng/mL for ELU E and 37.0 ng/mL for ELU B ($S/N=3$) in plasma. Blood drug level-time cuvers of ELU E and ELU B in Wister rats following administration of an eleutherococcus injection into femoral vein were shown to fit a three-compartment model. The half-life ($t_{1/2}$) was 4.662 h for ELU E and 2.494 h for ELU B. Following administration of a single eleutherococcus injection, the concentration of ELU E and ELU B in the tissue was $C_{liver} > C_{kidney} > C_{spleen} > C_{heart}$ and $C_{kidney} > C_{liver} > C_{heart}$. We believe the method described in the present paper is accurate and reliable and can be used for pharmacokinetic studies of ELU E and ELU B in rats. In addition, the method for sample preparation, using solid phase extraction, is precise, simple and rapid.

Keywords: Eleutherococcus injection; Eleutheroside E; Eleutheroside B; Pharmacokinetics; Tissue distribution; High-performance liquid chromatography

1. Introduction

Acanthopanacis is a well-recognised bush and is widely distributed in the northeastern region of China, Korea, and Japan, and the far-eastern region of Russia. It contains eleutheroside A, B, B_1, C, D, E, F, G, flavonoids, and polysaccharides [1,2]. ELU B, also known as syringin, has an anti-fatigue function [3]. ELU E has the most pronounced anti-stress effect [4]. The fluorescence spectra of ELU B and ELU E were determined by Barenboim et al. [5]. Todorov et al. [6] studied the effect of ELU B on protein biosynthesis. In 1985, ELU B and ELU E were acknowledged as major active components of the acanthopanax species [7]. In 1989, eight eleutherosides were determined from eleutherococcus roots, rhizomes and extraction using a spectrophotometric method [8]. In 2001, the contents of ELU B and ELU E from the acanthopanax species were determined by high performance liquid chromatography [9,10].

The eleutherococcus injection was prepared using acanthopanacis as the raw material. The process of production involved extraction and sterilization of acanthopanacis. Currently, it remains one of the traditional Chinese medicine preparations in the Pharmacopoeia of the Peoples Republic of China. It is efficient in invigorating the liver and kidney, replenishing the vital essence, and strengthening bone, and can be used to relieve symptoms such as transient cerebral ischemia attack, cerebral arteriosclerosis, cerebral thrombosis and cerebral embolism caused by a deficiency in the liver and kidney. In addition, it is used to treat coronary heart disease, angina pectoris, combined neurosis and the menopausal syndrome [11]. The content of ELU E and ELU B in the eleutherococcus injection can be determined simultaneously by high-performance liquid chromatography [12]. There has been

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no previous report published to-date regarding the pharmacokinetic study of ELU E and ELU B in the plasma and tissue of rats.

Owing to the fact that the chemical ingredients of traditional Chinese medicine are rather complex, pharmacokinetic studies are in great demand. The aim of this study was to develop a simple and rapid analytical method, based on HPLC, for the quantification of ELU E and ELU B in the plasma and tissue of the rat. Following validation, the method was used to perform a preliminary pharmacokinetic study in the rat.

2. Materials and methods

2.1. Materials and reagents

ELU E and ELU B were obtained from Sigma (chemical company, USA). Internal standard salicin was obtained from Bai Ling Wei Company (Beijing, China). The chemical structures are shown in Fig. 1. The acetonitrile used was of chromatographic grade. All other reagents used were of analytical grade. Eleutherococcus injection (batch number: 020614, 20 mL) was produced by (Wu Su Li Jiang Pharmaceutical Factory, Heilongjiang province, China). The content of ELU E and ELU B in the injection was 273.79 and 150.67 μg/mL, respectively, using high-performance liquid chromatography. The solid-phase column was a Waters Oasis HLB cartridge (1 mL, 30 mg). Experimental Wister rats were provided by the laboratory animal center, Lanzhou University.

2.2. Chromatographic conditions

Analysis was performed using a Waters Alliance 2487 HPLC with a 2996 photodiode array detection and a Waters 717 automatic injector, M32 ADD-ON single system. The detection wavelength for ELU E was 220 nm and for ELU B 206 nm. ELU E and ELU B were separated using a reverse-phase Kromasil ODS column (250×4.6 mm, 5 μm particle) coupled to a 10×4.6 mm C_{18} guard column (Zirchrom Science instrument Co., Ltd, Tian Jing, China) operating at 25 °C. The mobile phase was water and acetonitrile with a water step gradient from 90 to 80% for 15 min, then from 80 to 50% for 25 min at a flow rate of 0.8 mL/min. The column was restored to the initial conditions and equilibrated over 20 min. All standards were identified and quantified using photodiode array detection. The injection volume used was 10 μL.

2.3. Preparation of standard solution

Stock solutions of standard ELU E (50.42 μg/mL), ELU B (46.72 μg/mL) and internal standard salicin (16.68 μg/mL) were prepared by dissolving in methanol.

2.4. Plasma and tissue sample collection

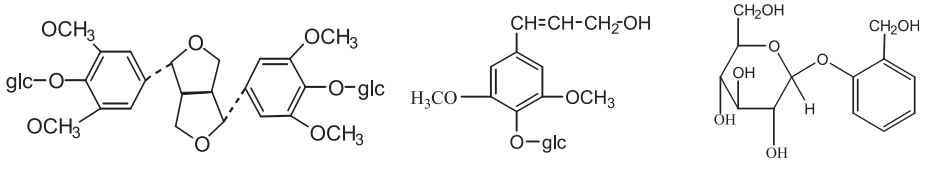
Twenty-four Wister rats (half of which were female, weight 240 ± 20 g) were anaesthetized and an eleutherococcus injection was administered into the femoral vein (8.0 mL/kg). At different time periods, post-injection, a sample of blood was obtained from the femoral artery. The rats were then sacrificed and the tissue isolated. Blood and tissue collection time ranged from 5 min to 12 h, respectively.

2.5. Sample preparation

The tissue collected was ground, and the protein from the collected plasma and ground tissue was deposited using acetonitrile. ELU B and ELU E were extracted, using acetonitrile as the solvent, with an ultrasonic device. The mixture was separated by centrifugation at 10,000 rpm for 20 min. The upper-stratum was evaporated to dry. The residue was dissolved in 5% methanol (1.0 mL).

2.6. Solid-phase extraction

Waters Oasis HLB C_{18} cartridges (1 mL, 30 mg), previously activated using methanol (1 mL) and equilibrated with double-deionized water (1 mL), were used for the solid-phase extraction. The previously prepared sample was then loaded on to the cartridge followed by elution with solvents. When a spiked sample had passed through the cartridge, slight pressure was applied to allow complete passage of the materials through the cartridge. Two methods of elution were employed. In the first method, the cartridge was rinsed successively with water, 5% MeOH, 10% MeOH, 20% MeOH, 30% MeOH, 40% MeOH, 50% MeOH and 60% MeOH (1.0 mL), respectively. ELU B and ELU E were eluted when 40% MeOH and 50% MeOH (2.0 mL in total) were added, a slight pressure being applied each time. The second method involved another loaded pre-conditioned cartridge which was rinsed successively with water, 10% MeOH, 20% MeOH, 60% MeOH, 70% MeOH (1.0 mL). ELU B and ELU E were eluted specifically and completely at 60% MeOH. A slight pressure was applied each time to maintain the volume at 1.0 mL



Eleutheroside E Eleutheroside B Salicin

Fig. 1. Chemical structure of eleutheroside B, eleutheroside E and salicin.

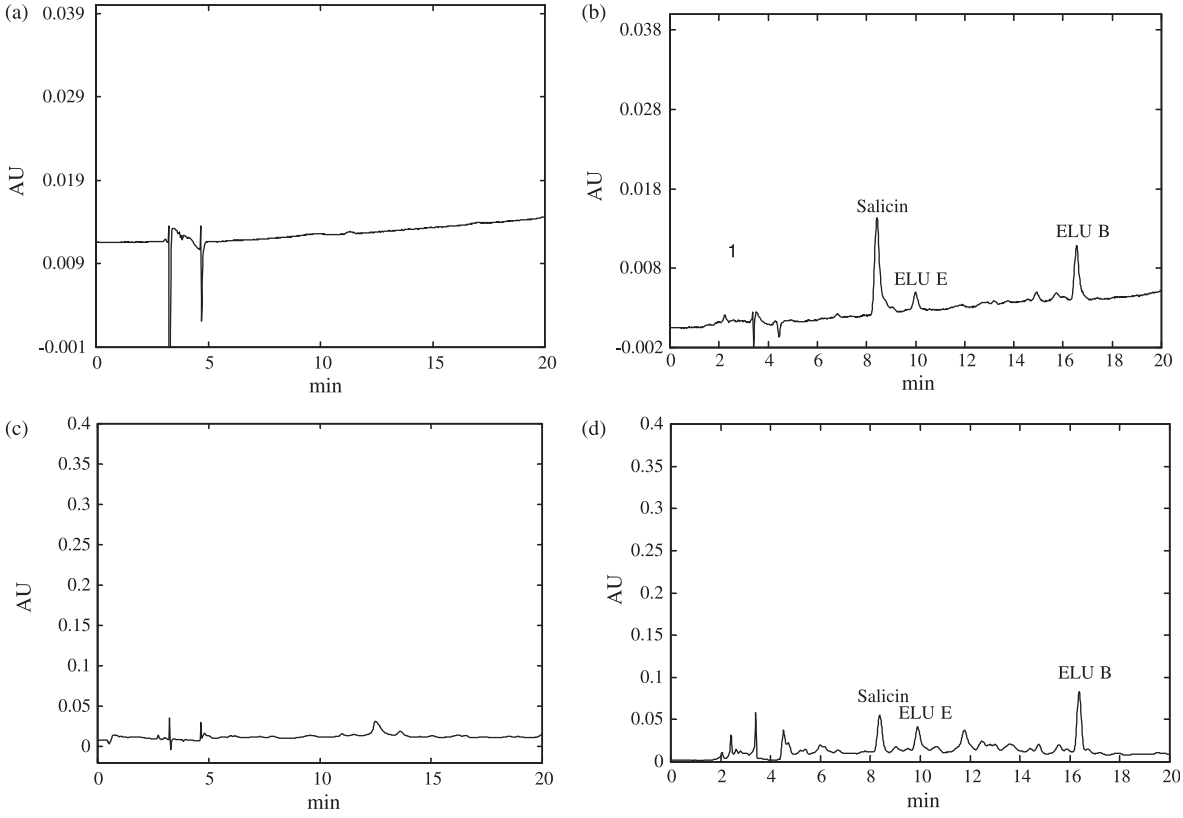


Fig. 2. Chromatograms of (a) control plasma; (b) rat plasma injected 15 min after acanthopanax injection; (c) control kidney; (d) rat kidney injected 30 min after acanthopanax injection, Chromatograms were recorded at 220 nm.

Using the first method, ELU B and ELU E could not be eluted with 1.0 mL MeOH. However, using the second method, ELU B and ELU E were eluted with 1.0 mL 60% MeOH and the solid-phase extraction recovery of ELU B and ELU E was 99.8 and 98.5%, respectively. Thus, the procedure for second solid phase extraction was adapted for analysis of the acanthopanax injection and selected for usage. As a slight pressure was applied to each solution-loaded cartridge to allow the complete passage of materials, there was no change in the volume of solution passing through the cartridge [13]. Finally, following the application of 1.0 mL 60% MeOH, a slight pressure was once again applied to allow the complete passage of ELU E and ELU B, in the plasma and tissue, through the cartridge. Therefore, the procedure whereby the solution was dried and subsequently dissolved in methanol as described previously [14] was omitted, and no more than 10 min was needed for sample treatment. A fixed amount (1.668 ng) of salicin was added to the 60% MeOH solution as the internal standard, and was then analyzed by HPLC.

2.7. Pharmacokinetic study

Following administration of 8.0 mL/kg eleutherococcus injection, plasma and tissue samples from Wister rats were collected at different time points (plasma 0–8 h, tissue 0–12 h) and the sample solutions prepared as previously described in Sections 2.5 and 2.6. The drug level in both blood and tissue was determined by HPLC.

3. Results and discussion

3.1. Chromatography

Typical chromatograms of control plasma, rat plasma and tissue, injected using an acanthopanax injection, are shown in Fig. 2. The retention time of ELU E and ELU B was 9.996 and 16.546 min, respectively. The retention factor [K′] of ELU E and ELU B were 1.63 and 3.35, respectively. Chromatograms of plasma and tissue samples ($n=10$) were compared with control samples of plasma and tissue, respectively. The control sample showed no peaks at the retention time corresponding to the ELU E and ELU B samples. Evidently, there was no interference between endogenous components of plasma, tissue and ELU; therefore, the chromatographic condition could be used for quantification of ELU E and ELU B.

Table 1
Recovery of ELU from plasma

Compound	Spiked concentration (μg/mL)	Observed concentration (μg/mL)	Recovery (%) $n=5$	RSD (%)
ELU B	4.672	4.041	86.5	4.31
	14.016	12.558	89.6	2.17
	23.360	21.094	90.3	3.24
ELU E	5.042	4.719	93.6	4.40
	15.126	13.734	90.8	2.69
	25.210	22.487	89.2	2.81

Table 2
intra-day and inter-day precision and accuracy of ELU in spiked plasma samples

Compound	Spiked concentration (μg/mL)	Observed concentration (μg/mL)	Accuracy (%) ($n=3$)	(RSD%)	Precision (RSD%)	
					Intra-day ($n=5$)	Inter-day (5d, $n=5$)
	0.467	0.478	102.4	3.06	4.46	4.21
ELU B	1.402	1.310	93.4	5.21	3.98	2.88
	2.336	2.136	91.4	4.62	2.75	4.61
	0.504	0.478	94.8	2.08	2.98	3.10
ELU E	1.513	1.623	107.3	3.26	4.64	2.90
	2.521	2.600	103.1	4.81	3.72	4.80

3.2. Calibration curve procedure

The standard calibration curves of plasma and tissue were prepared by spiking the control rat plasma (100 μL) or tissue homogenate (100 μL) with the appropriate stock solutions of ELU. These solutions were extracted using solid-phase extraction, as previously described in Section 2.6. Calibration curves were constructed by plotting the peak-area ratios of the added ELU of a certain concentration to the internal standard vs. the added concentration of ELU in the plasma and tissue. Calibration curves of ELU E and ELU B in plasma were $A=1.12\times10^5$ C -1.20×10^5 and $A=1.15\times10^5$ C -1.12×10^5 with the concentration ranging from 2.521 to 25.210 and 2.336 to 23.360 (μg/mL), in the liver $A=5.91\times10^4$ C -3.14×10^4 and $A=1.02\times10^5$ C -2.81×10^4 with the linearity ranging from 0.300 to 2.700 and 0.280 to 2.502 (μg/mL), in the spleen $A=6.04\times10^4$ C -3.73×10^4 and $A=6.11\times10^4$ C -3.56×10^4 ranging from 0.300 to 2.700 and 0.280 to 2.502 (μg/mL), in the kidney $A=6.16\times10^4$C -4.66×10^4 and $A=1.13\times10^5$ C -4.79×10^4 ranging from 0.550 to 5.400 and 0.510 to 5.005 (μg/mL), in the heart $A=6.09\times10^4$ C -3.80×10^4 and $A=1.04\times10^5$ C -3.26×10^4 ranging from 0.300 to 2.700 and 0.280 to 2.502 (μg/mL), respectively. The correlation coefficients obtained were higher than 0.9992 ($n=5$).

The limit of detection was defined as the lowest concentration of the sample resulting in a signal-to-noise ratio of 3:1 (ELU E u37.6 ng/mL; ELU B 37.0 ng/mL $n=6$; RSD = 2.60%).

3.3. Recovery, precision and accuracy

The recovery test was carried out by the added various known amount of standard solution into each sample for five times (Table 1).

Analytical accuracy and precision (intra day and inter day) data are shown in Table 2. Precision was calculated at three concentrations. Accuracy was determined by comparing the observed concentration with the spiked concentration.

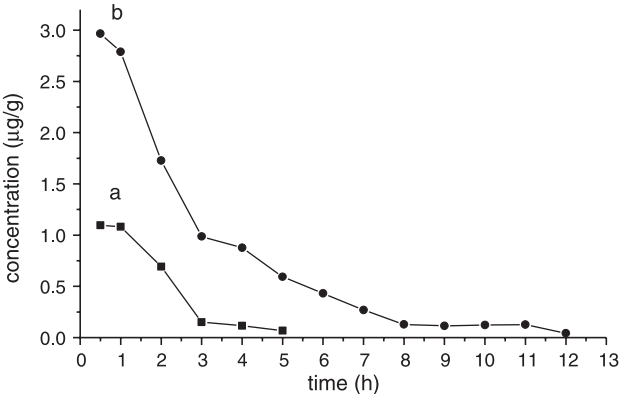


Fig. 4. The concentration-time curve of ELU B (b) and ELU E (a) in rat plasma.

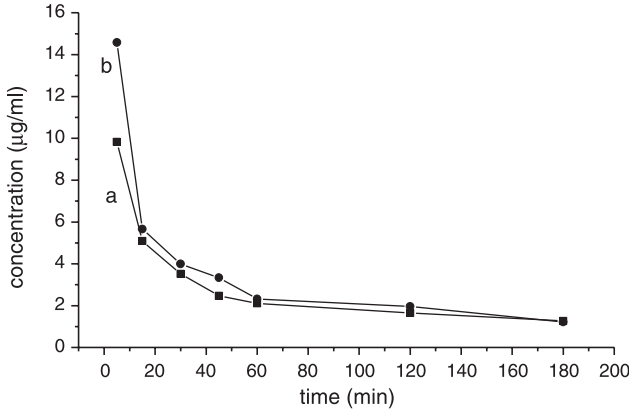


Fig. 3. The concentration-time (a) curve of ELU B (b) and ELU E (a) in rat plasma, $P<0.05$.

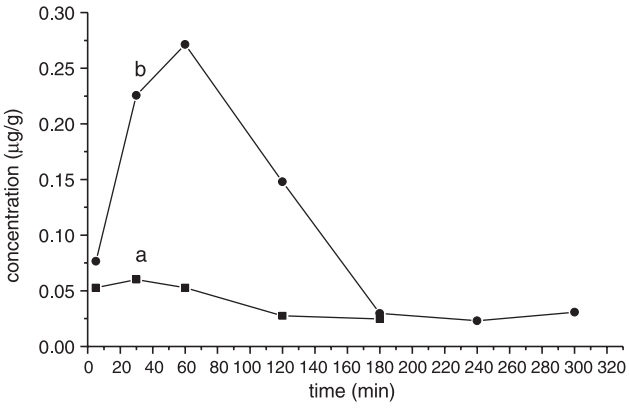


Fig. 5. The concentration-time curve of ELU B (b) and ELU E (a) in rat heart.

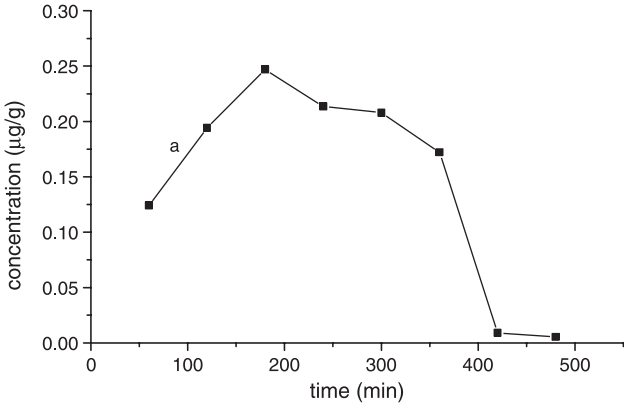


Fig. 6. The concentration-time (b) curve of ELU E (a) in rat spleen.

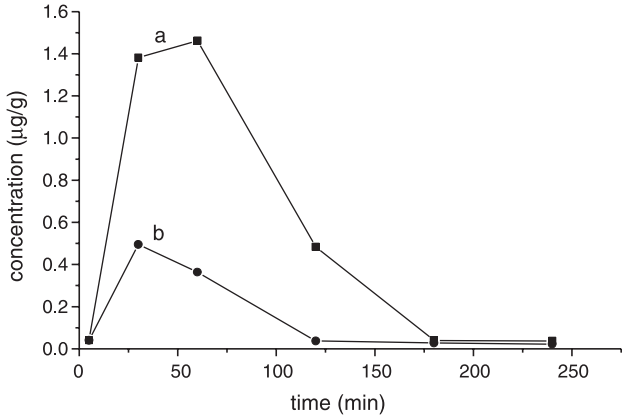


Fig. 7. The concentration-time curve of ELU B (b) and ELU E (a) in rat liver.

3.4. Stability

The stability of ELU in plasma and tissue was examined at 30 and −20 °C. ELU E (5.042, 0.600 μg/mL) and ELU B (4.672, 0.560 μg/mL) which were added to the plasma and tissue samples were observed as stable for 48 h at 30 °C. Storage stability at −20 °C was determined following storage of the samples (containing ELU E 5.042, 0.600 μg/mL and ELU B 4.672, 0.560 μg/mL) for a period of 2 months. Stability of the samples was maintained following the 2-month period.

3.5. Pharmacokinetics

The concentration-time curves of ELU E and ELU B in rat plasma and tissue ($n = 10$) are shown in Figs. 3–7. Blood drug level-time data was calculated using DAS Version 1.0 software. The blood drug level-time curves of ELU E and ELU B in rat plasma, following administration of the acanthopanax injection into a vein, were shown to fit a three-compartment model. Pharmacokinetic parameters are shown in Table 3. The $t_{1/2}\alpha$, $t_{1/2}\beta$, and $t_{1/2}\gamma$ for ELU E and ELU B were 0.027 and 0.043, 0.238 and 0.283, 4.662 and 2.494 h, respectively. The R^2 values for ELU E and ELU B were 0.999 and 0.998, respectively, and the pharmacokinetic results for ELU E and ELU B were reasonable.

The experimental results demonstrated that ELU E and ELU B were contained in the plasma, heart, kidney and liver of the rats. ELU E, and not ELU B, was present in the spleen. Following a single eleutherococcus injection, the concentrations of ELU E and ELU B were in the order $C_{liver} > C_{kidney} > C_{spleen} > C_{heart}$ and $C_{kidney} > C_{liver} > C_{heart}$, respectively. Concentrations of ELU E and ELU B were higher in the liver and kidney, which demonstrates that both ELU E and ELU B are metabolized and excreted primarily from the liver and kidney. The half-life ($t_{1/2}$) was 4.662 h for ELU E and 2.494 h for ELU B

4. Conclusion

The HPLC method described in the present paper is useful and reliable for the determination of ELU E and ELU B in rat plasma and tissue, following an eleutherococcus injection. The procedure for pre-treatment of the sample, which involves the deposition of protein with acetonitrile, extraction of ELU E and ELU B with acetonitrile, and a direct isolation with a cartridge is simple, rapid and complete (recoveries of solid-phase extraction were 98.5% for ELU E and 99.8% for ELU B). The method demonstrated reliable replication, accuracy and low limits of detection. We believe that the method is sufficiently sensitive to perform pharmacokinetic studies.

Table 3
Pharmacokinetic parameter of ELU E and ELU B in rat body

Parameter	Parameter value		Parameter	Parameter value	
(unit)	ELU E	ELU B	(Unit)	ELU E	ELU B
$t_{1/2}\alpha$(h)	0.027	0.043	k10 (1/h)	1.916	2.648
$t_{1/2}\beta$(h)	0.238	0.283	k12 (1/h)	12.397	6.441
$t_{1/2}\ \gamma$(h)	4.662	2.494	k21 (1/h)	8.564	4.684
Vd (L)	0.865	0.299	k13 (1/h)	5.617	4.344
V1 (L)	0.067	0.031	k31 (1/h)	0.688	0.892
CL (L/h)	0.129	0.083	Tmax (h)	0.083	0.083
AUC 0–3 (mg/Lh)	16.582	13.989	Cmax (mg/L)	9.830	14.577
AUC 0–∞ (mg/Lh)	17.022	14.490	R^2	0.999	0.998

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