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Short communication

High-performance liquid chromatography spectrometric analysis of tripterin in rat plasma

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Abstract

A quick, precise and reliable HPLC method has been developed to determine tripterin in rat plasma. After liquid–liquid extraction, the analytes was analyzed on a Discovery ODS C_{18} column (5 μm, 4.6 mm × 250 mm) with an isocratic elution consisting of methanol–water–phosphoric acid (87:13:0.2, v/v/v). Ultraviolet detection was at 425 nm. Using trioxymethylanthraquinone as an internal standard, the assay was linear over the concentration range of 0.025–1.60 μg/mL ($r^2 = 0.9988$). The extraction recovery of tripterin in rat plasma was more than 62%. The intra- and inter-day precision was less than 13% (CV). This validated method was successfully applied to the pharmacokinetics of tripterin in rats.
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Keywords: Tripterin; HPLC; Rat; Plasma concentration

1. Introduction

Tripterin (a quinone methide triterpene, also called ‘Celastrol’, Fig. 1) is one of the major active components extracted from the root bark of the Chinese medicine “Thunder of God Vine” (*Tripterygium wilfordii* Hook F.) [1]. Tripterin has been reported to have beneficial protecting properties, such as antioxidant, anti-inflammatory effect [2–4], immunosuppressive effect [5]. The most important pharmacological action of tripterin is its cancer treatment activity as it is a natural proteasome inhibitor, and inhibits cancer cell proliferation both *in vitro* and *in vivo* [6–8].

At the same time, the toxicity of tripterin is fairly great [9]. To control the quantity of tripterin in medical material and correlated praeparatum, Xia et al. established a quantitative analysis method with high-performance liquid chromatography (HPLC) to determine the content of tripterin in *T. wilfordii* and its tablets [10]. However, there is no analytical method available for tripterin in plasma, and no report about its pharmacokinetics. In this paper, we presented a HPLC method to accurately measure the concentration of tripterin in rat plasma, which will enable its pharmacokinetics to be studied.

2. Experimental

2.1. Chemical reagents and animals

Tripterin and trioxymethylanthraquinone (used as the internal standard) were supplied by the Medicinal Chemistry Department of School of Pharmacy, Yantai University (China) (purity >97%). Methanol was HPLC grade (Merck, Germany); other reagents were of analytical grade. Water was of deionized distilled water.

Sprague–Dawley rats weighing 269 ± 12 g were provided by the Experimental Animal Center of Shandong Luye Pharmaceutical Co. Ltd. (Yantai, China). Animals were maintained and handled according to the recommendations of the Institutional Ethic Committee (INRA), in accordance to the degree no. 87-848.

2.2. Instrumentation and operating conditions

The analysis was carried out in Agilent 1100 Series (USA) liquid chromatograph equipped with online degasser (model G1379A), quarpump (model G1311A), autosampler (model G1313A), column oven (model G1316A) and a VWD detector (model G1314A).

The chromatographic separation was carried out with a Discovery ODS C_{18} analytical column (5 μm, 4.6 mm × 250 mm)

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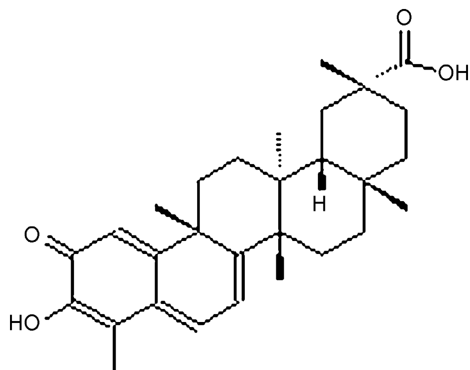

Fig. 1. Chemical structure of tripterin.

protected by a precolumn and kept at 35 °C. The mobile phase consisting of a mixture of 0.2% (v/v) phosphoric acid in methanol and water (87:13, v/v) was set at a flow rate of 1 mL/min. The detection wavelength was 425 nm, and the injection volume was 40 μL.

2.3. Preparation of standard and quality controls

The stock solutions of tripterin and internal standard were prepared in methanol at concentration of 5.0 and 0.20 mg/mL, respectively. These solutions were stored at 4 °C within 2 months. The tripterin stock solution was serially 10-fold diluted with methanol to obtain the working standard solutions of tripterin. The calibration curve in rat plasma was prepared by spiking known amounts of working standard solutions of tripterin into drug-free rat plasma (150 μL). Plasma concentration of tripterin was 0.025, 0.050, 0.10, 0.20, 0.40, 0.80 and 1.60 μg/mL, respectively. The calibration curve was constructed by plotting the peak area ratio of tripterin to internal standard against the concentration of tripterin with a linear regression. Internal standard stock solution was diluted to 2.0 μg/mL in methanol as working solution.

Quality control samples were prepared daily at low (0.050 μg/mL), middle (0.20 μg/mL), and high (0.80 μg/mL) concentrations to evaluate the inter- and intra-day precision and accuracy of this assay method, and to be used during the pharmacokinetic study.

2.4. Sample preparation

Blood samples (about 300 μL) were collected into heparinized tubes, and then centrifuged at 2000 × *g* for 10 min. The plasma samples obtained were stored at −20 °C until analysis.

A 10 μL of internal standard working solution (2.0 μg/mL) was added to 150 μL of plasma, followed by 30 s mixture and 3 min liquid–liquid extraction with 1 mL of ethyl acetate. The organic layer was separated and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted into 80 μL of mobile phase by vortex mixing for 1 min, and then a 40 μL of this solution was injected into the HPLC system for analysis.

2.5. Validation procedure

The validation parameters were specificity, linearity, extraction recovery, precision, and accuracy. Five blank rat plasmas were screened to determine the specificity. Linearity was tested on five different sets of calibration curves. The lowest concentration of the linear regression defined the lower limit of quantification (LLOQ).

The extraction recovery of tripterin was assessed ($n = 5$) at different concentrations of quality controls, and calculated by comparing the peak area of extracted quality control samples to the peak area obtained when the extracted blank plasma spiked with analyte which was added at the same concentration as the sample extracted.

Intra-day assay accuracy and precision of quality controls were measured using five determinations per concentration at the same day. Inter-day assay accuracy and precision of quality controls were measured using five determinations per concentration on three consecutive days.

2.6. Application

The method was used to determine the concentration of tripterin in rat plasma after intravenous administration of a single dosage of tripterin (1.0 mg/kg) dissolved in slight propylene glycol then diluted with physiologic saline. Five rats were employed for this study. Blood samples (about 300 μL) were collected into heparinized tubes at the time points of 0, 0.033, 0.083, 0.25, 0.5, 0.75, 1.0, 2.0, 4.0 and 6.0 h. The plasma samples were separated by centrifugation at 2000 × *g* for 10 min and stored at −20 °C until analysis.

3. Results and discussion

3.1. Method validation

3.1.1. Chromatography

The greatest absorbed wavelength of tripterin is at 425 nm. The longer wavelength resulted in lower background and better specificity. To match maximal absorbed wavelength of tripterin, trioxymethylanthraquinone was used as the internal standard. By adding 0.2% phosphoric acid to the mobile phase, the symmetrical chromatographic peaks were obtained, and the sensitivity of the method was improved. Fig. 2 shows some representative chromatography of rat plasma corresponding to the blank plasma extract (a), drug-free plasma sample spiked with tripterin and internal standard (b and c), and a plasma sample collected at 15 min after intravenous administration of tripterin (1 mg/kg) to a rat (d). There were no interfering components at the elution times for either tripterin or the internal standard.

3.1.2. Linearity and detection limit

The calibration curve provided a reliable response from 0.025 to 1.60 μg/mL. The mean regression equation weighted by 1/conc. was $y = (3.550 \pm 0.721)x - (0.0748 \pm 0.0339)$ ($r^2 = 0.9988$). The LLOQ for tripterin was 0.025 ng/mL in rat plasma at a signal-to-noise (S/N) ratio of 5.

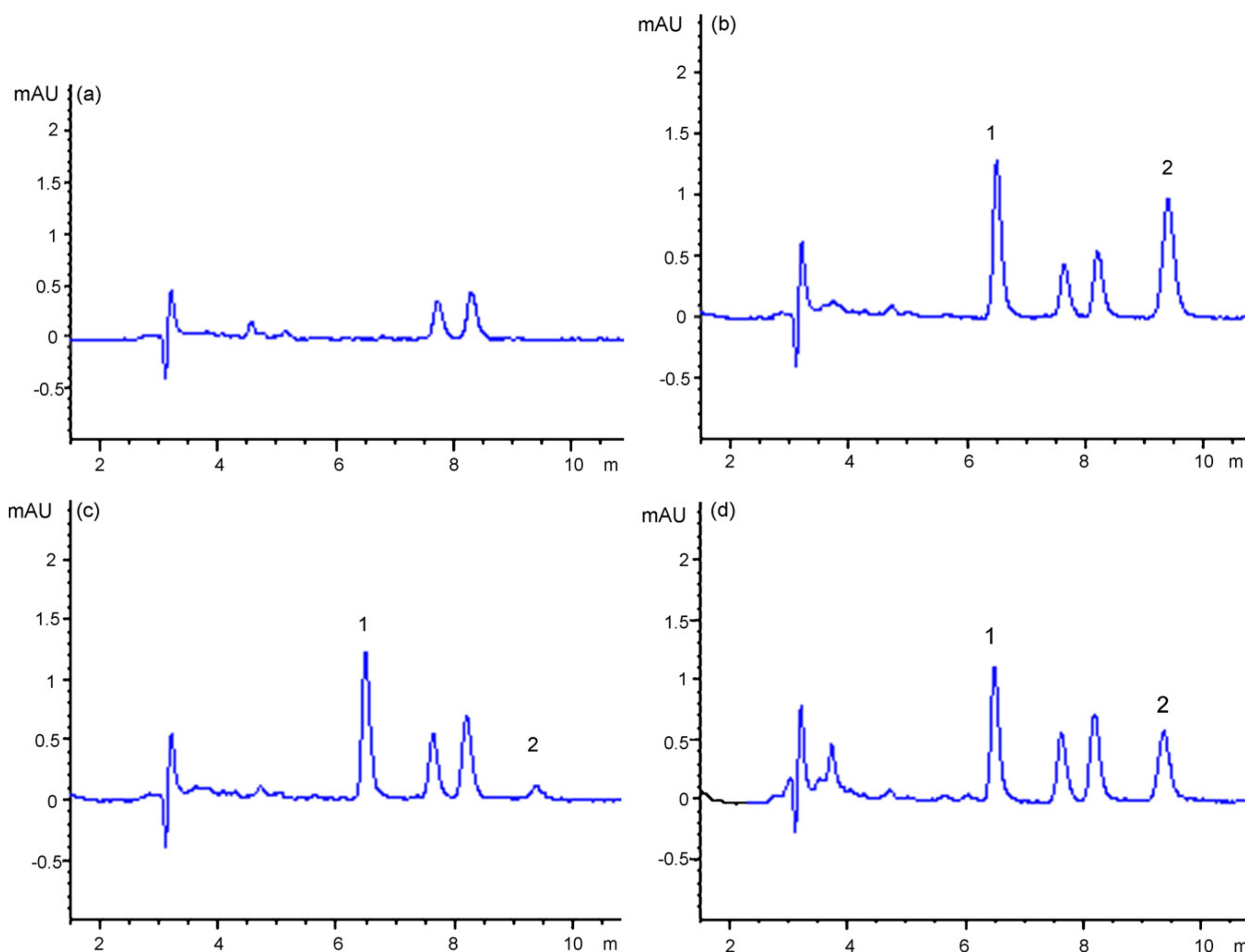

Fig. 2. Typical HPLC-UV chromatogram of tripterin at 425 nm in rat plasma: (a) blank plasma, (b) blank plasma spiked with I.S. (2.0 μg/mL) and tripterin (0.20 μg/mL), (c) blank plasma spiked with I.S. (2.0 μg/mL) and tripterin (0.025 μg/mL, LLOQ), and (d) a rat plasma 15 min after intravenous administration of tripterin (1.0 mg/kg). Peak 1: I.S.; peak 2: tripterin.

3.1.3. Extraction efficiency

The mean extraction recovery of tripterin at the low, middle and high concentration (of 0.050, 0.20 and 0.80 μg/mL) was greater than 60%, ranging from 62.69% to 65.78%. The data are shown in Table 1. Although the recovery was low it was reproducible at this level. The extraction recovery of the internal standard was 83.07%. Even though it was more than that of tripterin, it was reproducible, and the R.S.D. of it was 4.76%. That ensured the method pass through the validation. Moreover, a number of methods for the extraction of tripterin from plasma were tested, such as perchloric acid precipitation, acetonitrile precipitation and liquid–liquid extraction with mixed solvents dichloromethane ether. By comparison, the liquid–liquid extraction procedure with ethyl acetate was much better than others.

3.1.4. Accuracy and precision

The data of accuracy and precision are shown in Table 2. The precision of the intra- and inter-day assay of quality controls, defined by the coefficient of variation, ranged from 3.67% to 12.81%. The accuracy of the intra- and inter-day assay of quality controls, expressed as percent relative error (RE%), ranged from −3.68% to 10.3%.

Table 1
Recovery of tripterin and the internal standard in rat plasma (%) ($n = 5$)

Concentration (μg/mL)	Recovery (mean ± S.D.)	R.S.D. (%)
0.05	64.84 ± 6.63	10.23
0.20	62.69 ± 7.29	11.64
0.80	65.78 ± 7.48	11.36
2.0 (I.S.)	83.07 ± 3.96	4.76

Table 2
Intra- and inter-day precision and accuracy of tripterin in rat plasma

Concentration (μg/mL)	Inter-day precision ($n = 15$)		Intra-day precision ($n = 5$)	
	R.S.D. (%)	RE (%)	R.S.D. (%)	RE (%)
0.05	3.67	10.3	11.17	7.5
0.20	9.98	−1.52	12.81	0.7
0.80	8.50	−3.68	11.22	0.5

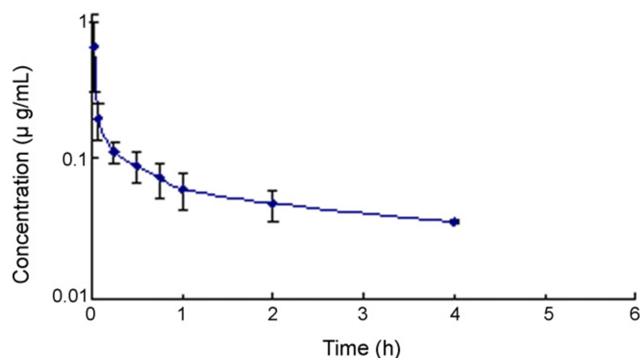

Fig. 3. Mean plasma concentration–time profile of tripterin in rat plasma after intravenous administration of single dosage of 1.0 mg/kg ($n = 5$).

3.2. Application of the method

The assay method was applied to study the pharmacokinetic characteristics of tripterin in rats. The average plasma concentration–time curve of tripterin after intravenous administration (1 mg/kg) in five rats is shown in Fig. 3.

4. Conclusion

In conclusion, a simple, rapid and reliable HPLC-UV assay method was developed and validated for the determination of tripterin in rat plasma. The specificity, sensitivity, accuracy and linearity of the method were acceptable for the determination of tripterin in rat plasma. The method was successfully applied to study the pharmacokinetics of tripterin in rats after intravenous administration.

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