Determination of Shionone in Rat Plasma by HPLC and Its Pharmacokinetic study

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Abstract: **Objective** To develop a sensitive, simple, and accurate method for the determination of shionone in rat plasma after ig administration of *Asteris Radix* petroleum ether extract (RAPE). **Methods** The separation was achieved by HPLC on a RP_{18} column (150 mm × 3.9 mm, 5 μm) with a mobile phase composed of acetonitrile-0.05% phosphoric acid water (98∶2) at a flow rate of 1.0 mL/min. UV Detector was set at 200 nm and friedelin was chosen as an internal standard. **Results** The linear range of the standard curves was (0.3443–22.0) μg/mL with the correlation coefficient of 0.9968. The intra- and inter-day precisions were all below 10% and the relative error was −3.5%–1.1%. **Conclusion** The developed method can be successfully applied to the pharmacokinetic study. After ig administration of RAPE, $T_{1/2(ka)}$ is (33.09 ± 7.32) min and $T_{1/2(ke)}$ is (84.95 ± 22.34) min.

Key words: *Asteris Radix*; pharmacokinetic study; shionone

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Introduction

Asteris Radix (root of *Aster tataricus* L. f; *Ziwan* in Chinese) is a famous traditional Chinese medicine (TCM), which has been used for more than one thousand years as an antitussive remedy. It has been reported that *Asteris Radix* petroleum ether extract (RAPE) exhibited effects of nourishing lung, dispersing phlegm, and relieving cough (Lu *et al*, 1999). Triterpenes are usually considered as its main pharmacological effective compounds. Shionone is one of the bioactive components (Ng *et al*, 2003) and has been used as a phytochemical marker for the quality control (QC) of *Asteris Radix* in *Chinese Pharmacopoeia* (Pharmacopoeia Committee of P. R. China, 2005). Analytical techniques including high-performance liquid chromatography (HPLC) with ultraviolet-visible (UV) detector (Gao *et al*, 2003; Xiu *et al*, 2006; Li, 2005) or evaporative light scattering detection (ELSD) (Zhang *et al*, 2003) and thin-layer chromatography (TLC) (Qin *et al*, 2004) were applied for the quantification of shionone from various herbal medicines and complex prescription.

It is plausible that an elucidation of the pharmacokinetic study of shionone would lead to a better understanding of action mechanism and facilitate further research and development of shionone. However, to our knowledge, there has been no report on the pharmacokinetic study of shionone in rats. We developed and validated a rapid and sensitive RP-HPLC method to determine shionone in rat plasma and successfully applied to the pharmacokinetic study of shionone after ig administration of RAPE at a single dose of 270 mg/kg.

Materials and methods

Materials and reagents

The reference standards of shionone and internal standard (IS) friedelin (Fig. 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile was of HPLC grade and obtained from Tedia (Tedia, Fairfield, USA). The distilled water was prepared from de-mineralized water and used throughout the study and other chemicals used were of analytical grade.

Preparation of RAPE

Asteris Radix (9 kg) was pulverized into pieces and extracted thrice with 95% ethanol for 3 h each time.

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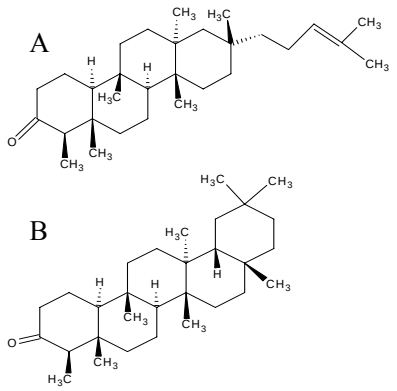

Fig. 1 Chemical structures of shionone (A) and IS friedelin (B)

After ethanol was removed from the extract solution under a reduced pressure, the residue was dissolved in water and then extracted thrice with petroleum ether followed by purification with silica gel chromatography. The eluted fraction was concentrated under reduced pressure and lyophilized. The dried powder (63 g) was stored at −20 ˚C.

Chromatographic conditions and apparatus

The HPLC system consisted of a Waters 1525 pump coupled with a 2487 UV detector. Chromatographic separation of shionone was achieved by a symmetry shield TM RP_{18} column (150 mm × 3.9 mm, 5 μm) purchased from Waters Corporation (Ireland). The UV detection of analyte was set at 200 nm and the column temperature was kept at 30 ℃. The separation was carried out with the mobile phase consisting of acetonitrile-0.05% phosphoric acid water (98∶2) at a flow rate of 1.0 mL/min. Data acquisition and management were achieved with Empower chromatographic workstation.

Animals, drug administration, and sampling

Male Sprague-Dawley rats with body weight (250 ± 20) g were obtained from the Hebei Laboratory Animal Center (Shijiazhuang, China). They were kept in an environmentally controlled breeding room for 5 d before starting the experiments and fed with standard laboratory food and water ad libitum. All rats were dosed following an overnight fast (except for water).

For pharmacokinetic study, the RAPE suspended with 0.5% carboxymethylcellulose sodium was ig administered to rats (n = 6), and then 0.5 mL blood samples were obtained from fossa orbitalis vein according to the specific schedule 0, 10, 20, 30, 60, 90, 120, 180, 240, 360, and 580 min. The blood samples were put into heparinized micro-centrifuge tubes and followed by centrifuging at approximately 3000 × g for 10 min. The resulting plasma layers were separated and stored in micro-centrifuge tubes at −20 ˚C.

Preparation of standard solutions and QC samples

The shionone was dissolved in acetonitrile to yield a concentration of 220.0 μg/mL. A series of standard solution were obtained by further dilution of the above stock solution with acetonitrile to obtain calibrations of shionone. To prepare the standard calibration samples, 200 μL of blank plasma was added to the tube with 50 μL shionone standard solution and 5 μL IS solution (acetonitrile was evaporated to dryness under a gentle stream of nitrogen in advance). The mixture was then treated as described below.

QC samples were prepared to obtain 0.5000, 10.00, and 20.00 μg/mL. These samples were used to evaluate the inter- and intra-day precision and accuracy of the assay.

Biosample processing

Plasma sample (200 μL) together with IS (5 μL, 356 μg/mL) was added to a tube and extracted with ethyl ether (1 mL) by voltax-mixing for 2 min. The organic phases were collected and then evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 50 μL acetonitrile and the resulting solution was thoroughly vortex-mixed for 3 min. After centrifugation at 5000 × g for 10 min, the supernatant (20 μL) was injected into the HPLC system for analysis. All the plasma samples were prepared in the same manner.

Method validation

Specificity The degree of interference by endogenous substances was assessed by comparing chromatograms of blank plasma obtained from six rats with those of corresponding standard plasma sample spiked with shionone and IS and plasma sample after oral dose.

Linearity of calibration curve and lower limit of quantification Calibration curves of shionone had been established. A series of standard plasma were injected into the HPLC system and the peak area ratios (Y) of shionone over the IS were regressed against the concentration (X) of shionone to establish calibration curves. The data were fitted by the least squares linear regression method with a weighting factor, $1/\chi^2$.

Precision and accuracy The accuracy and precision were investigated by determining QC samples at three concentration levels of shionone for three consecutive days. Precision was expressed as relative standard deviation (RSD) and accuracy as relative error (RE).

Extraction recovery and stability The recoveries of shionone were tested at three QC levels by comparing the peak areas from extracted plasma with those found by direct injection of standard solution at the same concentration.

The stability of shionone in biosamples was investigated under a variety of storage and process conditions: for storage stability, samples (five replicates at each QC concentration) were prepared and stored at −20 ˚C for 10 d. On day 10, all samples were thawed and analyzed along with the freshly prepared set of QC samples; for freeze-thaw stability testing, the QC samples were determined after three freeze-thaw cycles and the concentration was compared to their nominal concentration.

Application of analytical method in pharmacokinetic studies

The pharmacokinetic model and the parameters were calculated by the practical pharmacokinetic program version 3P97 (Committee Society of Mathematic Pharmacology, Beijing, China). The compartment model was established by the methods of the residual square sum, the Akaike's information criterion (AIC) and the fitted degree (r^2).

Results

Method validation

Specificity Fig. 2 presented typical chromatograms of blank plasma spiked with shionone and rat plasma after ig administration of RAPE. It was indicated shionone was well separated and no interference was detected from endogenous substances. The typical t_R for shionone was approximately 11.4 min.

Linearity of calibration curve and lower limit of quantification The linear regression equation analyte was Y = 2.7549 X + 0.0837 (r = 0.9968, n = 6). The linear range for shionone was (0.3443–22.00) μg/mL, which was adequate for this method to be used in the current pharmacokinetic studies.

The lower limit of quantification (LLQ) and the limit of detection (LD) of the assay were determined to be 35 and 11 ng/mL, respecitively. The LD and LLQ under the present chromatographic conditions were defined as the analyte mass resulting in a signal-to-noise (S/N) ratio of 3 and 5, respectively.

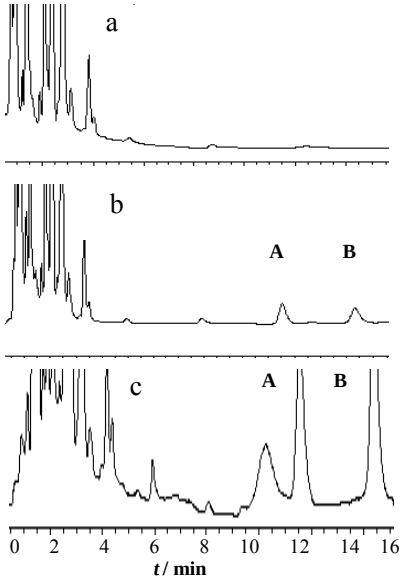

Fig. 2 Representative chromatograms of blank plasma (a), plasma sample spiked with shionone and IS (b) and a plasma sample collected at 30 min from rat after oral administration of RAPE at a single dose of 270 mg/kg (c)
A: shionone B: friedelin

Precision and accuracy Results are summarized in Table 1. For each QC level, the intra- and inter-day precisions were all below 10%, while the RE was −3.5%–1.1 %, indicating an acceptable precision and accuracy of the present method for determination of shionone in rat plasma.

Extraction recovery and stability The results showed that the mean extraction recoveries of analyte were 76.23%, 83.64%, and 80.80% (n = 6) and RSD were 4.7%, 5.5%, and 10.5% at concentration of 0.5000, 10.00, and 20.0 μg/mL, respectively.

The stock solutions of shionone and IS were found to be stable for 6 h at room temperature and for 10 d at −20 ˚C. Analysis of samples consistently afforded values that were nearly identical to those of freshly prepared QC samples (RSD < 5.0%), thus confirming the overall stability of shionone in plasma under frozen storage.

Pharmacokinetic studies

The plasma concentration-time curve of shionone is shown in Fig. 3, from Table 2, we can find that $T_{1/2(ka)}$ is (33.09 ± 7.32) min, $T_{1/2(ke)}$ is (84.95 ± 22.34) min (n = 6). A one-compartment open model gave the best fit to the plasma concentration *vs* time curve obtained in rats, and the weight was $1/c^2$. The main pharmacokinetic parameters in rats are summarized in Table 2.

Table 1 Precision and accuracy data for shionone in plasma

Added / (μg·mL^{-1})	Found / (μg·mL^{-1})	RE / %	Precision / %	
			Inter-day	Intra-day
0.50	0.49 ± 0.04	−1.9	4.31	3.61
10.00	9.65 ± 0.07	−3.5	6.66	4.62
20.00	20.31 ± 0.12	1.1	9.38	3.54

intra-day: n = 6; inter-day: 6 replicates per day, 3 d

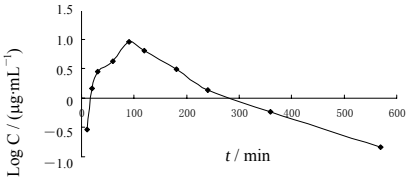

Fig. 3 Log C-t plot of shionone in rats after ig administration of RAPE

Table 2 Pharmacokinetic parameters of shionone after ig administration of RAPE ($\bar{X}$ ± s, n = 6)

Parameters	Units	Values
$T_{1/2(ka)}$	min	33.09 ± 7.32
$T_{1/2(ke)}$	min	84.95 ± 22.34
C_{max}	μg·mL^{-1}	4.27 ± 0.53
T_{max}	min	71.71 ± 7.13
$AUC_{(0-\infty)}$	μg·min·mL^{-1}	1308.52 ± 286.04
$MRT_{(0-t)}$	min	155.96 ± 10.59
$AUC_{(0-t)}$	μg·min·mL^{-1}	1271.37 ± 246.84

$T_{1/2(ka)}$: half-life of absorotion; $T_{1/2(ke)}$: half-life of elimination; C_{max}: maximum plasma concentration; T_{max}: time to reach C_{max}; AUC: area under the plasma concentration *vs* time curve; MRT: mean residence time

Discussion

Various mobile phase systems such as acetonitrile-water and acetonitrile-aqueous acid were tested. Acid was used to improve the peak shape and different acids were tested for optimization. During the test, it was found that the use of formic acid or acetic acid as modifier in mobile phase resulted in baseline drift, which would cause strong impairment for peak identity. The use of acetonitrile had relatively short analysis time. So acetonitrile and phosphoric acid were employed for mobile phase system. The present method had a relatively simple elution system and a relatively short chromatography run time.

Several substances (rutin, β-amyrin, and epi-friedelanol) were tested as IS. Among them, friedelin has been chosen to be the most appropriate in the present analysis because it was stable and did not exist endogenously in plasma. Moreover, in the present study, the structure and t_R of friedelin was near that of shionone.

The identity of shionone was confirmed by comparison of t_R and spectrum peak with that of reference compound. In this study, HPLC-DAD analyse of shionone was also performed. The ability of the detector scanning the wavelength range (190–400) nm allowed us to obtain three-dimensional chromatograms showing the variation of the absorbance and wavelength with time. The peak of shionone corresponds to a unique compound (i.e. it is a pure peak) as the spectra in all of its points coincide with the spectrum for the compound concerned.

In our previous work, the pharmacokinetics of shionone in rats was studied after ig administration of pure shionone. Compared to shionone preparations, the RAPE administration had a relatively high concentration in plasma (4.27 *vs* 0.39 μg/mL) and a about 10-fold enhancement of AUC_{0-t} and $AUC_{0-\infty}$ (1308.52 *vs* 93.32 μg·min/mL; 1271.37 *vs* 86.29 μg·min/mL) at the same dosage. These results indicated that some ingredients in RAPE might increase the dissolution of shionone and enhance its absorption. It was also probably caused by the transformation of other constituents. The reason needs further study.

A rapid and specific RP-HPLC assay was presented and validated for the determination of shionone in rat plasma. The developed method included a liquid-liquid extraction procedure and RP-HPLC analysis with UV detection, and demonstrated adequate specificity and sensitivity.

Acknowledgements

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