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Short communication

Determination and pharmacokinetics of ascaridole in rat plasma by gas chromatography–mass spectrometry

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ABSTRACT

A rapid and sensitive method for determination of ascaridole in rat plasma was developed based on gas chromatography–mass spectrometry (GC/MS). The analyte and internal standard (IS), naphthalene, were extracted from plasma with ethyl acetate and then separated by GC on a HP-5MS capillary analytical column (30 m × 0.25 mm, 0.25 μm) and determined by a quadrupole mass spectrometer detector operated under selected ion monitoring mode (SIM). Excellent linearity was found to be from 10 to 1000 ng/mL with a lower limit of quantitation (LLOQ) of 10 ng/mL. The accuracy was between 85.3% and 114.0%, and the precision was less than 14.5% (intra- and inter-day). The method was successfully applied to investigate the pharmacokinetic study of ascaridole in rats after a single oral dose of 30, 60 and 120 mg/kg, respectively.

1. Introduction

Ascaridole, 1-methyl-4-(1-methylethyl)-2,3-dioxabicyclo[2.2.2]oct-5-ene (Fig. 1), the major component of the volatile oil of *Chenopodium ambrosioides* L., which is a potent anthelmintic and used as a popular remedy against intestinal worm infections, stomach cramps, measles and fungicidal infections [1–3]. Furthermore, the results from the *vitro* studies showed that ascaridole exerts antineoplastic activity [4].

Although the chemical components and pharmacodynamics of the volatile oil of *Chenopodiu ambrosioides* L. have been researched a great deal [5–8], there is little report on the determination of ascaridole in biosamples and pharmacokinetic studies because of its low concentration in plasma and the thermal isomerization. In the present study, a rapid and sensitive gas chromatography–mass spectrometry (GC/MS) method was developed and validated to quantify ascaridole in rat plasma and the main pharmacokinetic parameters of ascaridole in rat following a single oral gavage dose were evaluated, the study could be helpful the reasonable usage of *Chenopodium ambrosioides* L. and its preparations.

2. Experimental

2.1. Materials and animals

Ascaridole (purity >96.5%) was separated and purified by Tasly R&D Institute (Tianjin, China). Naphthalene (purity >98.8%) was purchased from Sigma–Aldrich (St. Louis, USA). Ethyl acetate (HPLC grade) was obtained from Fisher Scientific (Pittsburgh, PA, USA), while all other chemicals were analytical grade and used without further purification. The distilled water, prepared from demineralized water, was used throughout the study. Male wistar rats, weighing 200 ± 20 g, were provided by Vital River Lab Animal Technology Co., Ltd. (Beijing, China).

2.2. GC/MS instrument and conditions

The analysis was performed by a GC/MS system consisted of an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector, Agilent 7683 series autosampler and injector. A HP-5MS capillary column (30 m × 0.25 mm i.d.) was used coated with a 0.25 μm thick film of 5% phenyl methyl siloxane (Agilent, USA) as the stationary phase. The conditions for gas chromatographic separation were as follows: the oven temperature was set at 120 °C, and the temperature at the injection port was 150 °C. Helium was used as the carrier gas and set at the flow rate of 1.0 mL/min, and the injection was in the split mode with a split ratio of 1:10.

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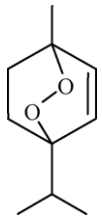

Fig. 1. Chemical structure of ascaridole.

The temperatures of the transfer line, ion source and quadrupoles were 280, 230 and 150 °C, respectively. The ionization mode was electron impact (EI) with a 70 eV electron beam, multiplier voltage was 2047 V. The mass detector was operated in the selected ion monitoring mode (SIM) mode using ions *m*/*z* 121 and *m*/*z* 128 as quantitative ions for ascaridole and naphthalene, respectively (Fig. 2).

2.3. Extraction procedure

Thawed samples were vortexed thoroughly at room temperature and employed as follows: to 100 μL plasma sample in a 1.5 mL Eppendorf tube, 50 μL IS solution (250 ng/mL, prepared in ethyl acetate) and 50 μL ethyl acetate were added. After vortexing for 30 s, the sample was centrifuged at 6500 × *g* for 3 min and the supernatant (about 80 μL) was transferred in a vial and 1 μL was injected into the GC/MS system for analysis.

2.4. Method validation

2.4.1. Specificity

The specificity of the method for the analyte and IS versus endogenous substances in the matrix was assessed by comparing the lowest concentration in the calibration curves with reconstitutions prepared with blank plasma.

2.4.2. Linearity and sensitivity

The working solutions of ascaridole were prepared in ethyl acetate. Calibration curves of ascaridole were prepared by adding 50 μL IS solution and 50 μL working solution into 100 μL blank plasma, the concentrations of ascaridole in plasma were 10, 25, 50, 100, 250, 500, 1000 ng/mL. The samples were dealt with the Section 2.3 and injected into GC/MS. Linearity was assessed by a weighed ($1/x^2$) least squares regression analysis.

2.4.3. Precision and accuracy

Precision and accuracy were evaluated through determining replicate QC samples (25, 100, and 800 ng/mL) on three different days. Precision was measured by inter- and intra-day R.S.D. (%). The accuracy was evaluated by the deviation or bias (%) of the observed concentration from the actual concentration.

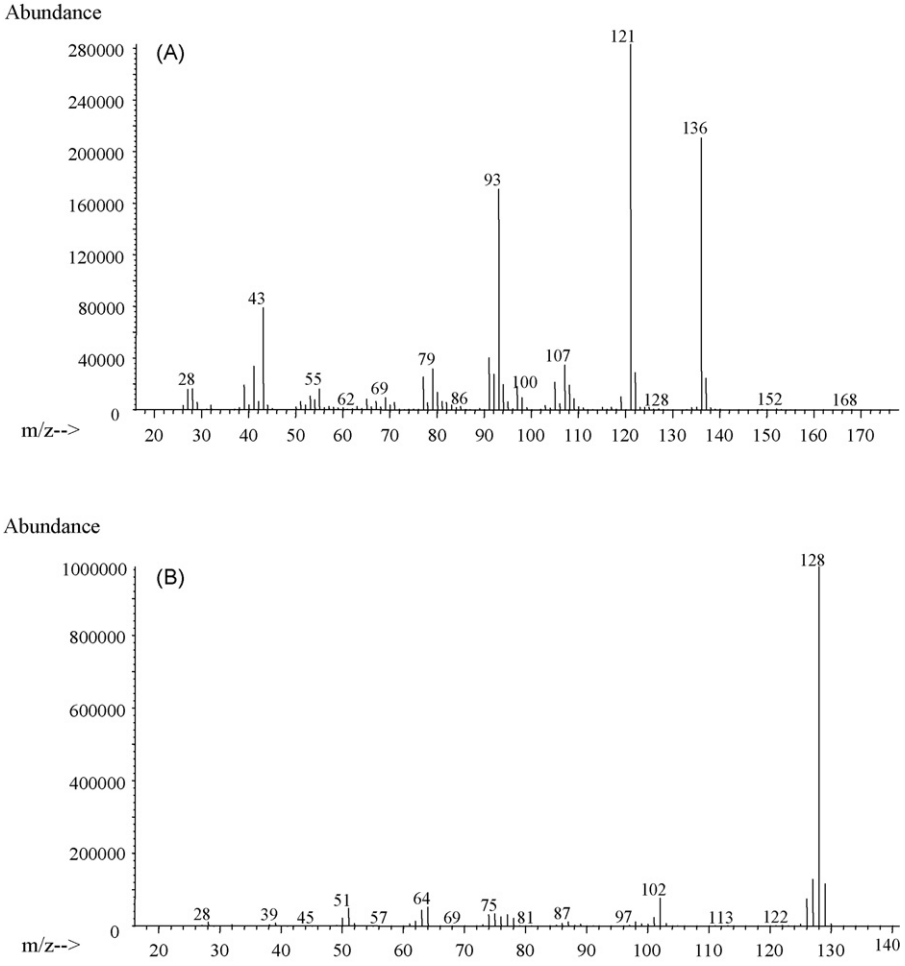

Fig. 2. Full scan mass spectrum of ascaridole (A) and naphthalene (B).

2.4.4. Recovery

The recovery of the method was calculated by comparing the peak areas of ascaridole (25, 100, and 800 ng/mL) in QC samples with those of the standard solutions, which were prepared in the same way as QC samples except water substituted for blank plasma.

2.4.5. Stability

The stability of ascaridole was evaluated in three successive freeze/thaw cycles, and processed samples in plastic autosampler vials at 25 °C for 2 h. QC samples were to be concluded stable if the average deviation was within ±15% of the actual value.

2.5. Application of the method to pharmacokinetics

After an overnight fasting period, 18 wistar rats were administered 30, 60, and 120 mg/kg ascaridole by oral gavage, respectively. Blood samples (200 μL) were collected into heparinized tubes from each rat by the puncture of the retro-orbital sinus prior to dosage and at 0.083, 0.167, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, and 2.5 h thereafter. Following centrifugation (3500 × *g* for 8 min), plasma samples were stored at −20 °C and analyzed within 1 week.

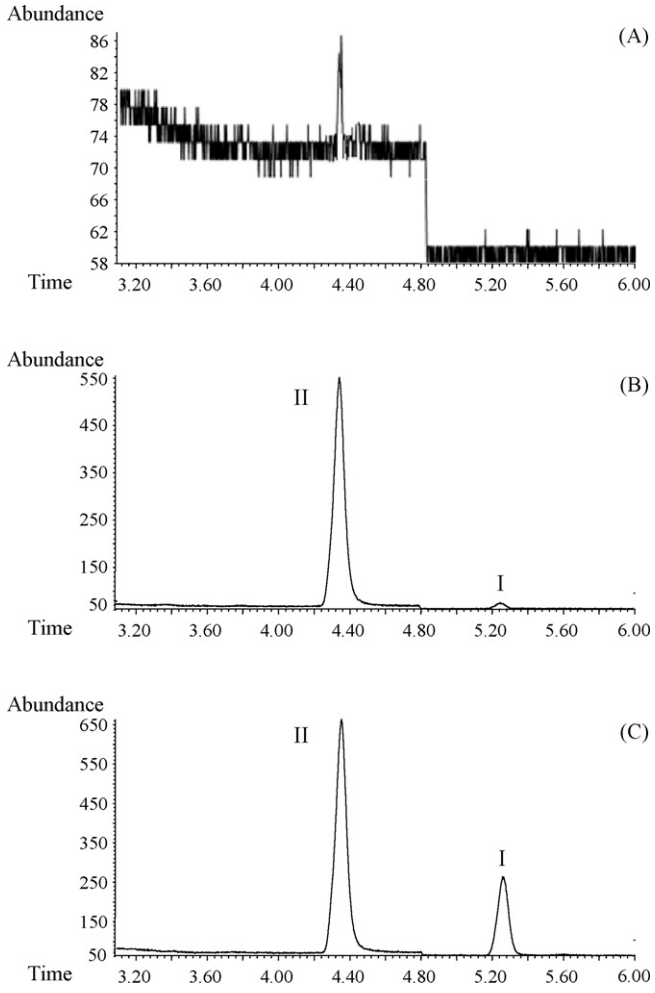

Fig. 3. Chromatograms of blank plasma (A), blank plasma spiked with ascaridole (at the limit of quantitation, 10 ng/mL) and naphthalene (B) and plasma sample 0.5 h after oral administration of ascaridole (30 mg/kg) (C). Peak I, ascaridole; Peak II, naphthalene.

Table 1
Precision and accuracy results of ascaridole in rat plasma (3 days, six replicates per day)

Spiked concentration (ng/mL)	Measured concentration (ng/mL)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	RE (%)
25	24.9 ± 2.1	1.62	8.90	−0.40
100	93.5 ± 7.6	14.5	6.90	−6.50
800	743.0 ± 42.7	11.9	4.30	−7.13

3. Results and discussion

3.1. GC/MS

Ascaridole is a heat-sensitive compound which rearranges to isoascaridole on treatment at 150 °C and above [3,9], and the isomerization can give rise to inaccurate quantification of ascaridole. After careful comparison of many temperatures, we finally adopted the temperatures of 150 °C at the injection port and 120 °C at the oven for optimal monitoring of the analyte. This condition minimized the formation of isoascaridole, in addition, it yielded suitable retention time and peak shape for ascaridole and offered relatively short analytical runtimes.

3.2. Method validation

3.2.1. Specificity

Representative chromatograms of blank plasma, blank plasma spiked with ascaridole at the limit of quantitation (10 ng/mL) and a study sample of ascaridole were shown in Fig. 3, and no interference was observed at the retention time of the analyte 5.26 min and IS 4.35 min due to endogenous substances in blank rat plasma.

3.2.2. Linearity and sensitivity

The calibration curves showed good linearity within the range 10–1000 ng/mL. Representative linear equation of calibration curve for the analyte was $Y = (4.26 \pm 0.71)E - 4X + (1.05 \pm 2.18)E - 3$ with a correlation coefficient of 0.9995, where Y was the peak area ratio of the analyte to the IS and the X was the concentration of the analyte. The LLOD and lower limit of quantitation (LLOQ) of this method were 2.5 ng/mL (S/N = 3) and 10 ng/mL (S/N = 10), respectively.

3.2.3. Precision and accuracy

As is shown in Table 1, the method gave good precision and accuracy. Intra- and inter-day precisions were within 14.5% and 8.9%, respectively, while the accuracy was in the range 85.3–114.0%.

3.2.4. Recoveries and stability

The recoveries of ascaridole were 91.6 ± 5.2%, 97.4 ± 9.2%, and 90.4 ± 2.6% at concentrations of 25, 100, and 800 ng/mL, respectively. In processed samples, the analytes were stable for a period

Table 2
Stability results of ascaridole in rat plasma ($n = 3$)

Spiked concentration (ng/mL)	Measured concentration (ng/mL)	R.S.D. (%)
Room temperature storage for 2 h		
25	23.7 ± 0.8	3.30
100	91.4 ± 1.3	1.46
800	753.2 ± 11.8	1.56
Three freeze/thaw cycles		
25	23.3 ± 0.7	2.93
100	92.3 ± 1.3	1.37
800	749.6 ± 8.1	1.07

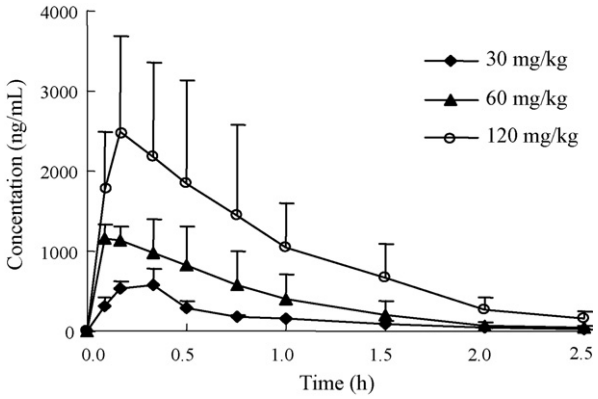

Fig. 4. Mean plasma concentration-time profile of ascaridole in wistar rats after a single oral dose of 30, 60 and 120 mg/kg.

Table 3
The main pharmacokinetic parameters of ascaridole in wistar rats after a single oral dose of 30, 60 and 120 mg/kg

Parameters	Dose (mg/kg)		
	30	60	120
k_e (1/h)	1.42 ± 0.14	1.43 ± 0.33	1.38 ± 0.20
$t_{1/2}$ (h)	0.49 ± 0.05	0.51 ± 0.11	0.51 ± 0.08
C_{max} (ng/mL)	648.2 ± 130.1	1244.4 ± 269.1	2701.4 ± 1282.6
T_{max} (h)	0.25 ± 0.09	0.26 ± 0.26	0.25 ± 0.09
AUC_{0-t} (ng h/mL)	422.2 ± 39.6	1011.7 ± 498.5	2450.8 ± 1338.3
$AUC_{0-\infty}$ (ng h/mL)	436.2 ± 38.7	1040.0 ± 506.8	2563.2 ± 1394.5
MRT (h)	0.68 ± 0.08	0.64 ± 0.09	0.77 ± 0.09
CL (L/min kg)	1.15 ± 0.10	1.14 ± 0.45	0.98 ± 0.48
V_d (L/kg)	49.0 ± 7.3	52.9 ± 28.3	43.4 ± 22.6

of 2 h at room temperature, and no instability of analytes in spiked samples was observed over three freeze/thaw cycles, the results were detailed in Table 2.

3.3. Application of the method to pharmacokinetics

The mean ascaridole plasma concentrations versus time profile in rat with the GC/MS method was shown in Fig. 4, and the main pharmacokinetic parameters of ascaridole using non-compartmental analysis with Topfit 2.0 software were summarized in Table 3. The mean C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values linearly related to dose ($r > 0.9985$, $P < 0.05$), while no significant differences in other pharmacokinetic parameters among the various dose groups were evaluated by ANOVA.

4. Conclusion

A simple, sensitive and rapid GC/MS method was developed and validated for quantitating ascaridole in rat plasma. The high sensitivity and selectivity, small sample volume requirement and relatively short analytical time of the method make it suitable for pre-clinical pharmacokinetic studies of ascaridole. So far it has been the first report of analytical method on the determination of ascaridole in *vivo* and the present study provided the necessary information for the further investigation of *Chenopodium ambrosioides* L.

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