

Development of a UPLC-MS/MS Method for Pharmacokinetic and Tissue Distribution of Isoeleutherin, Eleutherin, and Eleutherol in *Bulbus eleutherinis* in Rats

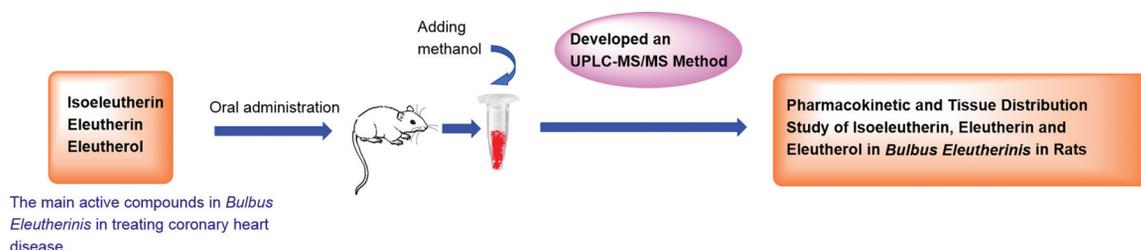
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Abstract

Bulbus eleutherinis is a classical traditional *Dai* medicine, and has been widely used in clinical treatment of coronary heart disease (CHD) in Yunnan, China. Naphthoquinone, as the main active compound in *Bulbus eleutherinis* in treating CHD, mainly contain isoeleutherin, eleutherin, and eleutherol. This study aimed to investigate the in vivo parameters of isoeleutherin, eleutherin, and eleutherol. In this work, male Sprague Dawley (SD) rats were treated with the three compounds by oral administration, and then blood and tissue samples were collected. A novel UPLC-MS/MS (ultra-performance liquid chromatography-tandem mass spectrometry) method has been developed to determine the absolute oral bioavailability, and the tissue distribution profile of the compounds. Acetonitrile and 0.1% (v/v) solution of formic acid were selected as the mobile phase of the chromatogram. C18 column was employed. Betamethasone was used as an internal standard in the method. The detection was performed with a multireaction monitor of scan type in positive ion mode by MS/MS. Our data showed linearity of the method with r over 0.9983. Lower limits of quantification of isoeleutherin, eleutherin, and eleutherol were 1.00, 3.84, and 0.498 ng/mL, respectively. The

Keywords

- ▶ *Bulbus eleutherinis*
- ▶ UPLC-MS/MS
- ▶ naphthoquinone
- ▶ pharmacokinetics
- ▶ tissue distribution

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overall precision of the compounds was less than 12.68%, recoveries ranged from 85.44 to 103.83%, and the accuracy of the compounds in plasma was between 91.56 and 110.75%. The stability assay showed that they were stable (87.83–114.62%) under different conditions in plasma. For oral administration, the half-lives of isoeleutherin, eleutherin, and eleutherol was 6.11, 7.30, and 3.07 hours, respectively. The absolute oral bioavailabilities were 5.38, 4.64, and 2.47%, respectively. Moreover, the three components had the highest distribution in small intestine. In conclusion, the established method was successfully applied to the determination of the *in vivo* parameters of the three components in SD rats. This work provides a reference for the development of new drugs of *Bulbus eleutherinis* in the future.

Introduction

According to the relevant statistics, the incidence of coronary heart disease (CHD) and the associated pathogenic factors has increased every year worldwide.^{1,2} Lipid-lowering drugs such as niacin drugs and antithrombotic drugs are currently used for CHD in clinics.³ Despite their effectiveness, these drugs show certain side effects.⁴ It is well known that natural product is an excellent candidate for alternative medicine for disease management. *Bulbus eleutherinis* is a famous traditional *Dai* medicine with good therapeutic effect and low toxicity. It is a whole herb of *Eleutherine plicata* originated from tropical America,^{5,6} and its multiple conventional functions, such as promoting blood circulation, relieving swelling and pain, detoxifying, as well as dehumidifying, make it suitable for treating chest tightness, shortness of breath, and CHD.^{7–9} Currently, *Bulbus eleutherinis* is widely used in the treatment of CHD in the clinic, especially for the *Dai* nationality living in Yunnan Province, China.^{10,11}

Bulbus eleutherinis contains naphthoquinone derivatives, glucosides, and a small amount of hydrazine.^{12–14} Previous studies showed that naphthoquinone in the ethanol extract of *Bulbus eleutherinis* can significantly improve blood viscosity and enhance hypoxia tolerance in myocardial ischemic animals,^{15–18} and thereby plays a key role in anti-CHD effect of the herb. Several naphthoquinone derivatives, such as isoeleutherin, eleutherin, and eleutherol, have been investigated as the main active fractions for CHD therapy.^{19–21} *Danshen* Injection is a commonly used drug in clinical treatment of CHD. Evidence suggested that in comparison with *Danshen* Injection, the active compounds mentioned above (isoeleutherin, eleutherin, and eleutherol) significantly increase coronary blood flow without side effects and toxicities,¹⁰ and have the value of developing new drugs for CHD therapy.⁷ At present, the research on the active ingredients of *Bulbus eleutherinis* has a certain foundation. It is necessary to study the *in vivo* metabolism of the active ingredients in *Bulbus eleutherinis* to investigate its druggability more comprehensively.

In 2009, a high-performance liquid chromatography (HPLC) detection method for the three active compounds (isoeleutherin, eleutherin, and eleutherol) in *Bulbus eleutherinis* was reported by Liu et al.²² However, the determination

of these three active compounds *in vivo* after oral administration has not yet been reported. In this study a reliable and useful ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been developed to obtain pharmacokinetics (PK) and tissue distribution data of the three active compounds in rat plasma simultaneously after po (oral) and iv (intravenous) administration. A series of conditions and validation projects were verified. By obtaining the PK and tissue distribution parameters of the drug in the body, the action site and time of the drug in the body can be analyzed, which can provide a reference and basis for the subsequent research on the metabolism of active ingredients, mechanism of action, and new drug development.

Experimental

Chemicals and Reagents

Isoeleutherin (> 96%, HPLC), eleutherin (> 98%, HPLC), and eleutherol (> 98%, HPLC) were purchased from Chengdu Herbpurify Co., Ltd (Chengdu, China). Betamethasone (HPLC purity > 98%, internal standard [IS]) was purchased from Innochem Technology Co., Ltd (Peking, China). The structures of four compounds are provided in ►Fig. 1. The methanol and acetonitrile of chromatographic grade were purchased from ANPEL (Shanghai, China). UPLC grade formic acid was purchased from Merck (Darmstadt, Germany). *Bulbus eleutherinis* (Yunnan, China) were purchased from Yunnan Jinfa Pharmaceutical Co., Ltd. (Yunnan, China). Millipore Alpha-Q system (Billerica, Massachusetts, United States) was used to prepare ultra-pure water. Medicinal materials were stored in Xishuangbanna Prefecture *Dai* Medicine Research Center in School of Pharmacy, Shanghai Jiao Tong University.

UPLC-MS/MS Condition

Chromatography separation was operated by an ACQUITY UPLC system (Waters, Milford, Massachusetts, United States). Waters Cortecs C18 column (1.7 μ m, 2.1 mm \times 50 mm) was used in chromatography with a flow rate at 0.40 mL/min. The column temperature and the autosampler temperature were set as 45°C and 4°C, respectively. The gradient elution of 0.1% aqueous formic acid (A) and acetonitrile (B) were selected as

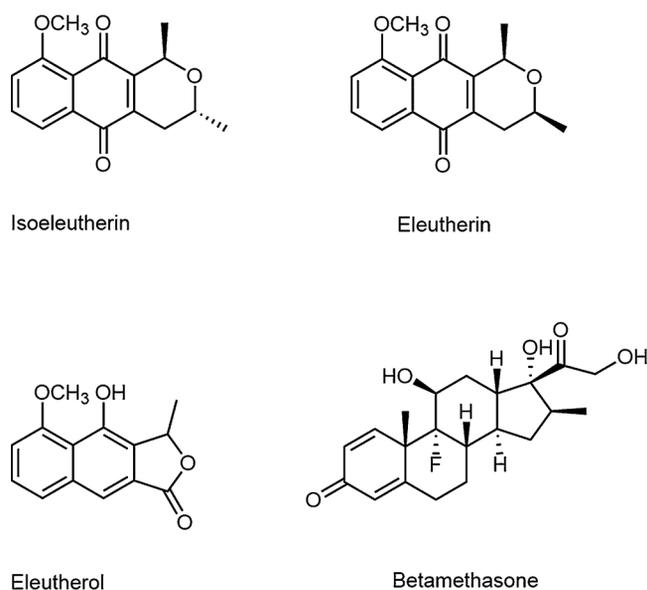


Fig. 1 Chemical structures of analytes.

mobile phases. The process is listed as follows: 30–39% B at 0–3.3 minutes, 39–95% B at 3.3–3.4 minutes, 95–95% B at 3.4–4.4 minutes, 95–30% B at 4.4–4.5 minutes, 30% B at 4.5–6.0 minutes. The injection volume was 3 μ L. The AB SCIEX Qtrap 5500 LC/MS/MS system (SCIEX, United States) with an electron spray ionization source in multiple reaction monitoring (MRM) mode was used as a MS analysis tool. The detection was performed under the following conditions: s = source temperature (at set point): 550.0°C, positive ion mode, GS1: 40 psi, GS2: 40 psi, CUR: 45 psi, CXP:15V, DP: 100V, EP: 10V. Quantitative analysis of analytes in MRM mode to determine the transition of precursor ions to specific product ions (m/z): isoeleutherin, m/z 273.1 $[M + H]^+ \rightarrow m/z$ 229.0 (collision energy, CE: 17V), eleutherin, m/z 273.1 $[M + H]^+ \rightarrow m/z$ 229.0 (CE: 17V), eleutherol, m/z 244.8 $[M + H]^+ \rightarrow m/z$ 227.1 (CE: 23V), IS, m/z 393.2 $[M + H]^+ \rightarrow m/z$ 373.2 (CE: 17V). The data were processed with MultiQuant 2.1 Software (SCIEX, California, United States).

Determination of Isoeleutherin, Eleutherin, and Eleutherol

Naphthoquinone in *Bulbus eleutherinis* was obtained by purifying the ethanol extract of the medicinal material using macroporous adsorption resin, and 0.03 g of which was weighed and dissolved ultrasonically in 25 mL methanol. The solution was filtered with 0.22 μ m microporous membrane, and the content of isoeleutherin, eleutherin, and eleutherol was determined using UPLC-MS/MS conditions mentioned above. The contents of isoeleutherin, eleutherin, and eleutherol in naphthoquinone were 6.37, 23.13, and 9.71%, respectively.

Animals

Male Sprague Dawley (SD) rats (180–220 g) were used for the study. The rats were housed in a professional animal breeding room (relative humidity: 55–60%, temperature:

25°C) for 1 week. Experimental operation of rats was in strict accordance to the animal experiment's ethical requirements. The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University. Rats were given free access to drinking water throughout the experiment and kept fasting for 24 hours before the experiment.

Preparation of Standard Working Solution and Quality Control Samples

Isoeleutherin, eleutherin, and eleutherol were dissolved in methanol to obtain a mixed standard stock solution (isoeleutherin: 1 μ g/mL, eleutherin: 3.84 μ g/mL, eleutherol: 0.498 μ g/mL). The mixed stock solution was diluted with methanol to prepare a series of different concentration gradient mixed standard solutions. A 2.0 ng/mL IS solution was prepared from betamethasone solution (500 μ g/mL) with methanol.

Rats were sacrificed in a professional animal breeding room (relative humidity: 55–60%, temperature: 25°C), and then blood and tissues (heart, liver, kidney, small intestine) were collected. The blood was centrifuged (5,000 rpm, 4°C) to obtain plasma. Tissues were flushed with saline to remove blood or other contents, and blotted dry with filter paper. The accurately weighed tissue was then homogenized using a tissue homogenizer in saline (four times the tissue weight).

A 20.0 μ L of the mixed standard solution was added to 40.0 μ L of blank plasma or 20 μ L of blank tissue homogenate. The supernatant was obtained by centrifugation after mixing evenly with calibration standard. Calibration standards included a series of concentrations of isoeleutherin (1–200 ng/mL), eleutherin (3.84–768 ng/mL), and eleutherol (0.498–99.6 ng/mL). Four concentrations of analytes were used to prepare lower limit of quantification (LLOQ) and quality control (QC) samples of plasma as follows: 1, 2, 50, and 150 ng/mL of isoeleutherin; 3.84, 7.68, 192, and 576 ng/mL of eleutherin; 0.498, 0.996, 24.9, and 74.7 ng/mL of eleutherol. Three concentrations of analytes were used to prepare QC samples of tissue as follows: 2, 5, and 15 ng/mL of isoeleutherin; 7.68, 19.2, and 38.4 ng/mL of eleutherin; 0.996, 2.49, and 4.98 ng/mL of eleutherol.

Preparation of Plasma and Tissue Sample Containing IS

For plasma sample preparation, 180 μ L of IS solution was mixed with 40 μ L of plasma sample. The protein precipitate was removed by centrifugation at 13,000 rpm for 10 minutes. For tissue sample preparation, 180 μ L of IS solution was mixed with 20 μ L of tissue homogenate and the protein precipitate was removed by centrifugation at 13,000 rpm for 10 minutes. The supernatant was transferred to the injection vial, and then detected by the UPLC-MS/MS condition mentioned above.

Selectivity

The blank biological samples (plasma samples, tissue samples) from six different rats were evaluated to determine the

selectivity of UPLC-MS/MS method. The chromatograms of blank biological matrix samples and the three compounds (isoeleutherin, eleutherin, and eleutherol) were compared to confirm the presence of any interference.

Linearity and the Lower Limit of Quantification

Calibration curves of isoeleutherin, eleutherin, and eleutherol were obtained by measuring the ratio of the peak response of different compound concentrations in the rat plasma and tissue samples to the IS peak area (weighting factor was $1/x^2$). The LLOQ was determined by a signal to noise ratio of 10:1 as the lowest concentration in the standard curve.

Recovery and Matrix Effect

The extraction recoveries and matrix effects were determined by evaluating QC samples prepared according to the method in the "Preparation of Standard Working Solution and Quality Control Samples" part mentioned above ($n=6$). The extraction recoveries of isoeleutherin, eleutherin, and eleutherol were determined by calculating the ratio of extracted samples versus extracts of blanks spiked with the analyte postextraction. The peak areas of the three compounds from the postprocessed sample and the standard solutions were compared to calculate the matrix effects.

Precision and Accuracy

The precision and accuracies of compounds were determined by evaluating QC samples prepared according to the method in the "Preparation of Standard Working Solution and Quality Control Samples" part mentioned above ($n=6$). The intra-accuracies and inter-accuracies were obtained by comparing the spiked concentrations and the calculated concentration of QC samples for 1 or 3 days. Intra-precision and inter-precision were validated by calculating the repeatability of the concentration measured by the QC samples for 1 or 3 days.

Stability Tests of Isoeleutherin, Eleutherin, and Eleutherol in Rat Plasma

The stability tests of isoeleutherin, eleutherin, and eleutherol in rat plasma samples were performed by determining six replicate QC samples in three storage environments. The QC samples were stored at 25°C for 6 hours. The contents of the three compounds were determined to evaluate the short-term stability. Their freeze-thaw stability was evaluated by determining the concentration in the QC samples after three freeze and thaw cycles. Autosampler stability was conducted by evaluating changes in the active compounds of the QC samples placed at 4°C for 8 hours in the autosampler.

Pharmacokinetics Study

The active compounds were added to 0.5% sodium carboxymethylcellulose and mixed well. Oral administration of a single dose of 50 mg/kg (3.18 mg/kg of isoeleutherin, 11.57 mg/kg of eleutherin, 4.86 mg/kg of eleutherol) was selected based on effective dose results from previous laboratory pharmacodynamics experiments and the lowest dose that

could be quantified in plasma. Blood samples (0.10–0.15 mL) were obtained from the eyelids subsequently at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after oral administration. Similarly, blood samples were collected at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after iv (10 mg/kg in glycerin). Plasma samples were obtained by centrifuging the blood samples to take the supernatant. Plasma samples were prepared according to sample preparation, and detection was performed using the UPLC-MS/MS condition mentioned above. The PK results (including $t_{1/2}$, T_{max} , C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, $AUC_{0-t/0-\infty}$) were analyzed based on the noncompartmental method by PK solver software (version 2.0, China Pharmaceutical University).

Tissue Distribution

For tissue distribution study, the SD rats were randomly divided into three groups ($n=5$). Various tissue samples (heart, liver, small intestine, kidney) were collected at 10 minutes, 30 minutes, and 120 hours after po of 0.1 g/kg of effective parts, which is the lowest dose that could be quantified in the tissue. Subsequently, the tissues were immediately rinsed with normal saline to remove the blood or other content and blotted dry with filter paper. The accurately weighed tissues were homogenized using a tissue homogenizer in normal saline (four times the tissue weight) and stored at -80°C until analysis. The tissue samples were operated with the same procedure as described in section "Preparation of Plasma and Tissue Sample Containing IS".

Results and Discussion

UPLC and MS/MS Condition Optimization

To obtain suitable chromatographic results, a series of chromatographic conditions were tested and selected for the standards, including analysis time, peak shape, and response intensity. The results showed that acetonitrile had high efficiency as an organic phase. The best separation effect was achieved with 0.1% aqueous formic acid as the aqueous phase.

The standard solution was injected into the mass spectrometer only, for scan with positive ion mode and negative ion mode, respectively. The mass spectrum showed that the positive ion mode could give better results. The target was to select the precursor ion and the product ion after detection in positive ion mode. The UPLC-MS/MS method established in this study has better sensitivity than the previously reported HPLC, and is suitable for the detection of biological samples or samples with low content. At the same time, the new method greatly reduces the detection time. The MS/MS spectra of isoeleutherin, eleutherin, and eleutherol are shown in ►Fig. 2.

There were no reports of the method of internal standard yet. 2-Methoxy-1,4-naphthoquinone, betamethasone, and 5-hydroxy-1,4-naphthoquinone were selected as candidates. The experiment showed that the response of 5-hydroxy-1,4-naphthoquinone in QC samples was too low, as shown in ►Fig. 3A. The retention time of 2-methoxy-1,4-

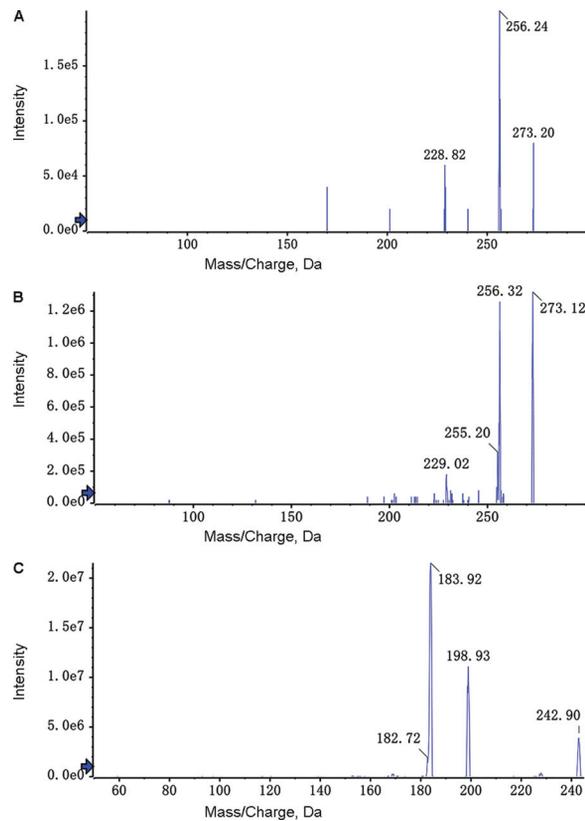


Fig. 2 Product ion mass spectra of (A) isoeleutherin, (B) eleutherin, and (C) eleutherol.

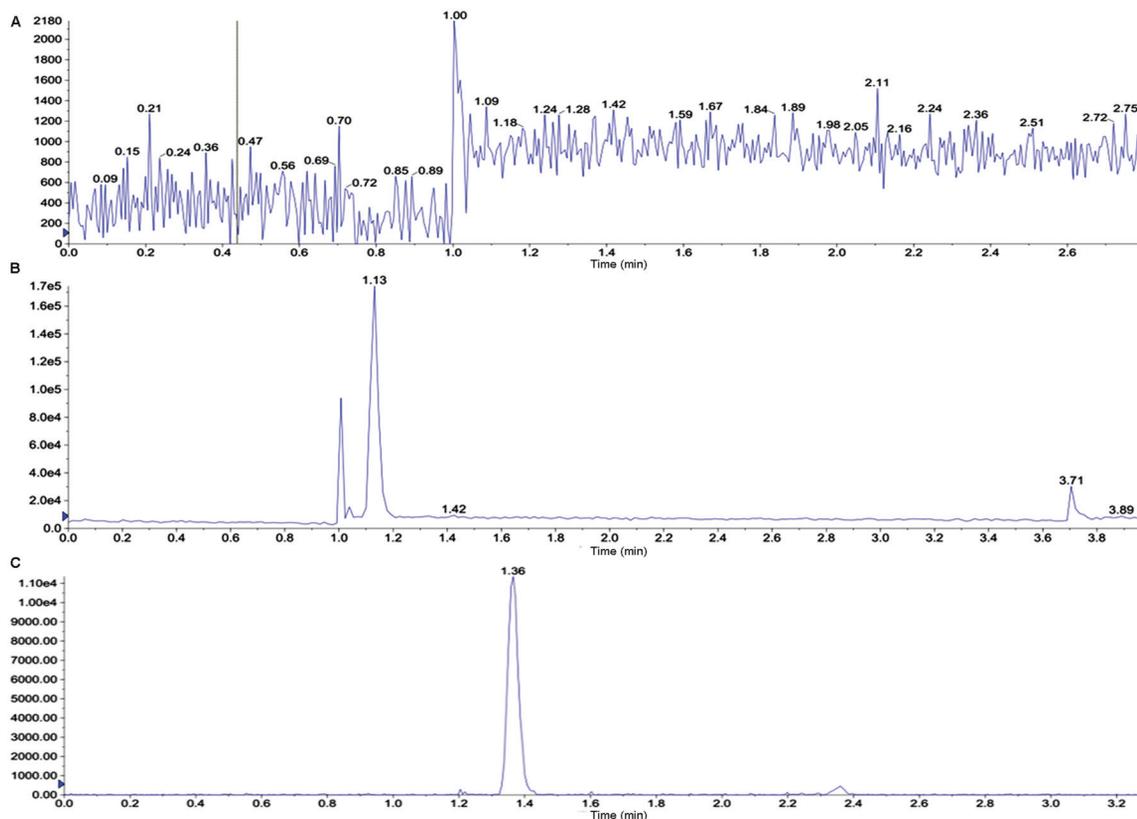


Fig. 3 Typical MRM chromatograms of (A) 5-hydroxy-1,4-naphthoquinone in blank plasma samples; (B) 2-methoxy-1,4-naphthoquinone in blank plasma samples; and (C) betamethasone in blank plasma samples. MRM, multiple reaction monitoring.

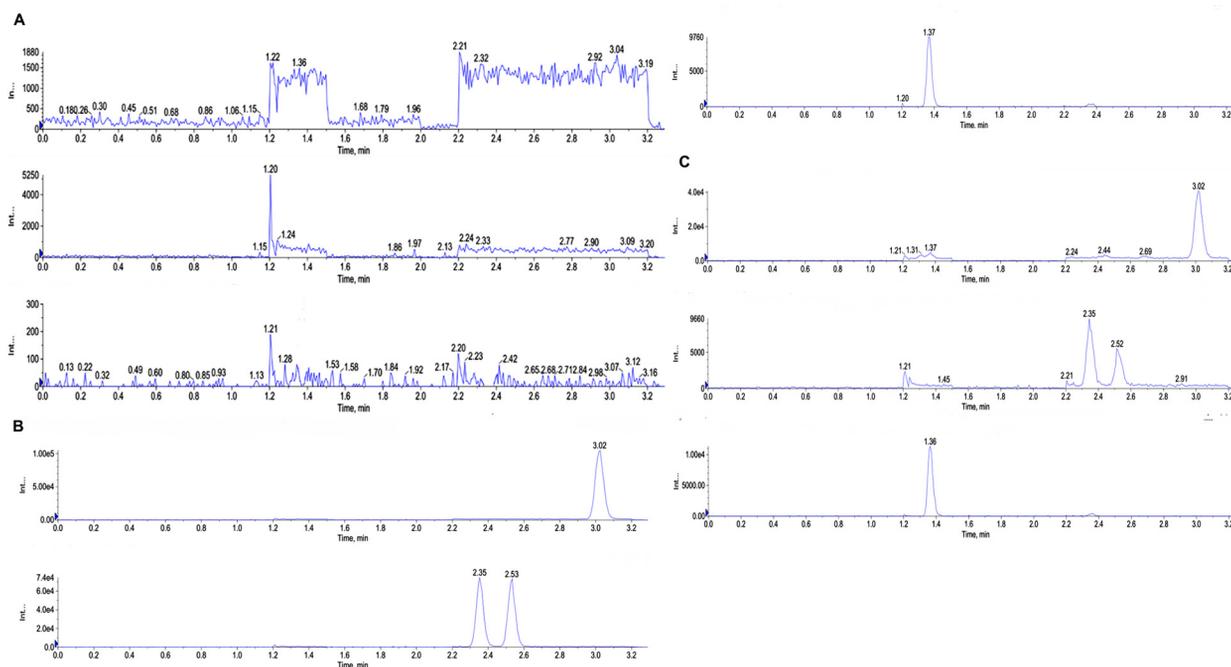


Fig. 4 Typical MRM chromatograms of isoeleutherin, eleutherin, and eleutherol and IS in rat plasma. (A) MRM chromatograms of blank plasma; (B) MRM chromatograms of blank plasma spiked with the three analytes at 24.9, 50, 192 ng/mL and IS; and (C) MRM chromatograms of plasma sample collected at 4 hours after oral administration of naphthoquinone in *Bulbus eleutherinis* (50 mg/kg). IS, internal standard; MRM, multiple reaction monitoring.

naphthoquinone was far from the retention time of the target compound (►Fig. 3B). By comparing the retention time and intensity of the response of the three compounds, betamethasone, an anti-inflammatory drug, was selected as internal standard.

Selectivity

The typical MRM chromatograms of the blank sample, isoeleutherin, eleutherin, eleutherol, and IS in blank plasma samples and plasma samples are shown in ►Fig. 4. There was no overlap or interaction at the retention time of compounds and IS. Isoeleutherin, eleutherin, eleutherol, and IS are approximate at 2.35, 2.53, 3.02, and 1.37 minutes, respectively. The UPLC-MS/MS method was shown to have acceptable selectivity.

Calibration, Linearity, LLOQ, and Detection

The calibration curves of isoeleutherin, eleutherin, and eleutherol in plasma and various tissue homogenates are displayed in ►Table 1. The calibration curves of the above three compounds showed qualified linearity ($r = 0.9983$ – 0.9997). The LLOQ for all analytes were 1.00, 3.84, and 0.498 ng/mL. The results confirmed that their linear ranges met the requirements of PK.²³

Recovery and Matrix Effect

As shown in ►Table 2, LLOQ samples and three concentrations of QC samples in plasma were selected to determine the recoveries and matrix effects. The mean recoveries of isoeleutherin, eleutherin, and eleutherol were 87.41–103.39, 97.90–

99.79, and 89.55–103.33%, respectively. The matrix effects of the three compounds in plasma ranged from 89.62 to 113.08%. The extraction recoveries and matrix effect for the three analytes (at three different QC concentrations) and IS in various tissues are shown in the Supporting Information (►Tables S1 and S2 [online only]). The results showed that the recoveries and matrix effect of the three analytes in the tissue homogenate ranged from 85 to 115%. It indicated that the matrix effect and recovery was insignificant under current processing methods.²³

Precision and Accuracy

The results of precision and accuracy in plasma were obtained by measuring four concentrations of QC samples. The results are shown in ►Table 3. The precision of each analyte was below 13.04%. At the same time, the accuracy of each analyte was acceptable, ranged from 91.56 to 110.75%. The results of precision in various tissues are presented in the Supporting Information (►Table S3, online only). The results show that the precision of the three analytes in the tissue homogenate is within $\pm 15\%$, indicating the stability and high reliability of the method.²³

Stability

Stabilities of the three compounds in plasma were determined under different conditions. As shown in ►Table 4, after three freeze–thaw cycles, the analytes displayed excellent stability in plasma (104.28–114.61%). Simultaneously, insignificant changes were found at 4°C for 8 hours (98.18–110.42%) and at 25°C for 6 hours (87.83–114.62%), suggesting

Table 1 Calibration curves and linear ranges of the analytes ($n = 6$)

	Analyte	Calibration curve	r	Linear range (ng/mL)	LLOQ (ng/mL)
Plasma	Isoeleutherin	$y = 0.1989x + 0.0753$	0.9983	1–200	1
	Eleutherin	$y = 0.0478x + 0.0022$	0.9987	3.84–768	3.84
	Eleutherol	$y = 0.6355x - 0.0942$	0.9997	0.498–99.6	0.498
Heart	Isoeleutherin	$y = 0.1689x + 0.0409$	0.9989	1–20	1
	Eleutherin	$y = 0.0362x + 0.0015$	0.9991	3.84–76.8	3.84
	Eleutherol	$y = 0.7075x + 0.0758$	0.9995	0.498–9.96	0.498
Liver	Isoeleutherin	$y = 0.1819x + 0.0533$	0.9993	1–20	1
	Eleutherin	$y = 0.0412x + 0.0023$	0.9994	3.84–76.8	3.84
	Eleutherol	$y = 0.6012x + 0.0104$	0.9997	0.498–9.96	0.498
Small intestine	Isoeleutherin	$y = 0.1605x + 0.0592$	0.9999	1–20	1
	Eleutherin	$y = 0.0325x + 0.0051$	0.9990	3.84–76.8	3.84
	Eleutherol	$y = 0.5202x + 0.0405$	0.9988	0.498–9.96	0.498
Renal	Isoeleutherin	$y = 0.1701x - 0.01537$	0.9991	1–20	1
	Eleutherin	$y = 0.0352x + 0.0041$	0.9990	3.84–76.8	3.84
	Eleutherol	$y = 0.5212x - 0.0321$	0.9999	0.498–9.96	0.498

Abbreviation: LLOQ, lower limit of quantification.

Table 2 Recovery and matrix effect of isoeleutherin, eleutherin, and eleutherol in rat plasma ($n = 6$)

Analyte	Concentration (ng/mL)	Recovery (%)		Matrix effect (%)	
		Mean	RSD	Mean	RSD
Isoeleutherin	1	103.39	9.99	98.11	6.20
	2	96.98	7.13	99.15	4.18
	50	87.41	7.60	97.92	4.25
	150	98.60	5.30	102.68	3.59
Eleutherin	3.84	99.79	9.36	110.69	6.83
	7.68	98.27	6.39	106.62	3.49
	192	97.90	9.46	113.08	4.30
	576	98.14	6.21	108.40	6.09
Eleutherol	0.498	103.33	8.06	89.62	10.87
	0.996	99.84	6.82	98.67	2.91
	24.9	89.55	7.81	95.19	5.19
	74.7	102.04	7.89	97.87	4.59

Abbreviation: RSD, relative standard deviation.

that the three compounds have good stability under the tested conditions.²³

Pharmacokinetic Study

As shown in ►Fig. 5, the plasma concentration curve of the three active compounds in *Bulbus eleutherinis* was obtained using a verified detection method after oral and intravenous administration. The mean PK parameters are displayed in ►Table 5. The half-lives ($t_{1/2}$) of isoeleutherin, eleutherin, and eleutherol were 6.11, 7.30, and 3.07 hours, respectively. Their absolute oral bioavailabilities were 5.38, 4.64, and 2.47%, respectively. The results showed that they reached the maxi-

mum concentration in a short time. However, their bioavailability is low. According to the results, there is a possibility that most of the active ingredients do not enter the body circulation in the intestinal tract but are directly excreted, so follow-up research on the absorption mechanism and in vivo metabolism of the three active ingredients is necessary.

Tissue Distribution

The measurement results in the tissue are all within the standard curve range. The overview of the tissue distribution of the three components is shown in ►Fig. 6. The content of isoeleutherin in the liver and intestine is relatively high, and

Table 3 Precision and accuracy of isoeleutherin, eleutherin, and eleutherol in rat plasma

Analyte	Spiked concentration (ng/mL)	Intra-batch ($n = 6$)			Inter-batch ($n = 6 \times 3$)		
		Measured concentration (mean \pm SD, ng/mL)	Precision (RSD, %)	Accuracy (RE, %)	Measured concentration (mean \pm SD, ng/mL)	Precision (RSD, %)	Accuracy (RE, %)
Isoeleutherin	1	0.98 \pm 0.04	4.58	97.92	1.10 \pm 0.11	10.28	110.75
	2	2.017 \pm 0.13	6.52	100.29	2.03 \pm 0.29	5.88	100.50
	50	50.30 \pm 1.16	2.30	100.60	52.37 \pm 2.80	5.34	104.75
	150	148.08 \pm 3.97	2.68	98.72	151.67 \pm 14.73	9.71	101.11
Eleutherin	3.84	3.61 \pm 0.06	1.53	94.10	3.52 \pm 0.40	11.25	91.56
	7.68	7.35 \pm 0.15	2.06	95.73	7.86 \pm 0.70	8.88	102.28
	192	211.5 \pm 20.17	9.54	110.17	199.63 \pm 26.03	13.04	103.97
	576	596.33 \pm 20.45	3.43	103.53	585.79 \pm 68.95	11.77	101.70
Eleutherol	0.498	0.52 \pm 0.05	9.27	105.07	0.51 \pm 0.05	9.52	102.03
	0.996	1.02 \pm 0.05	4.82	102.59	0.97 \pm 0.06	6.03	97.23
	24.9	25.85 \pm 0.42	1.64	103.83	25.55 \pm 1.32	5.16	102.62
	74.7	79.04 \pm 3.85	4.87	105.81	74.42 \pm 4.67	6.28	99.63

Abbreviations: RSD, relative standard deviation; RE, relative error; SD, standard deviation.

Table 4 Stability of isoeleutherin, eleutherin, and eleutherol in rat plasma ($n = 5$)

Analyte	Concentration (ng/mL)	Content Change (%) ^a	Content Change (%) ^b	Content Change (%) ^c
Isoeleutherin	2	95.51	104.28	107.06
	50	101.39	111.96	110.42
	150	99.28	110.81	108.16
Eleutherin	7.68	87.83	113.86	98.18
	192	105.41	104.47	109.05
	576	103.61	109.12	104.50
Eleutherol	0.996	94.39	106.87	107.55
	24.9	114.62	114.61	105.66
	74.7	99.26	113.73	104.34

^aQC samples were stored at 25°C for 6 hours.

^bQC samples were stored at freeze-thaw cycles.

^cQC samples were placed at 4°C for 8 hours in the autosampler.

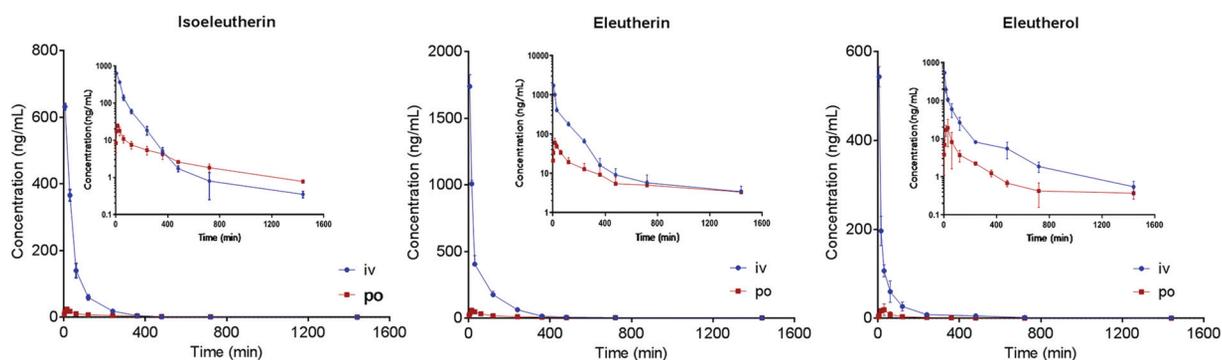
**Fig. 5** Mean plasma concentration–time curves of three analytes after intravenous administration (10 mg/kg) and oral administration (50 mg/kg) to rats. Data are presented as mean \pm standard deviation ($n = 6$).

Table 5 Pharmacokinetic parameters of naphthoquinone in *Bulbus eleutherinis* in SD rats

PK parameters	Isoeleutherin		Eleutherin		Eleutherol	
	iv	Po	iv	po	iv	po
$t_{1/2}$ (min)	279.686 ± 99.05	366.55 ± 59.43	330.09 ± 70.64	489.11 ± 110.76	126.86 ± 13.83	184.03 ± 39.77
T_{max} (min)	–	15 ± 0	–	25 ± 8.90	–	20 ± 7.07
C_{max} (ng/mL)	631.96 ± 9.49	24.50 ± 3.26	1,739.13 ± 89.01	76.87 ± 12.83	543.39 ± 22.47	28.32 ± 7.10
AUC_{0-t} (ng/mL × min)	18,649.22 ± 1,229.65	4,525.88 ± 901.29	60,089.26 ± 2,218.14	13,789.47 ± 4,764.36	17,629.90 ± 3,727.02	2,047.63 ± 684.88
$AUC_{0-\infty}$ (ng/mL × min)	18,799.83 ± 1,307.42	5,059.32 ± 835.31	62,948.35 ± 4,078.59	15,189.65 ± 2,579.49	17,925.43 ± 3,744.45	2,209.46 ± 655.16
$AUC_{0-t}/0-\infty$	0.992 ± 0.003	0.900 ± 0.02	0.963 ± 0.03	0.907 ± 0.017	0.984 ± 0.003	0.920 ± 0.033

Abbreviations: AUC_{0-t} , the area under the curve (0– t); $AUC_{0-\infty}$, the area under the curve (0– ∞); $AUC_{0-t}/0-\infty$, the area under the curve (0– t)/(0– ∞); C_{max} , maximum concentration; iv, intravenous; PK, pharmacokinetic; po, oral; $t_{1/2}$, half-life; T_{max} , peak time.

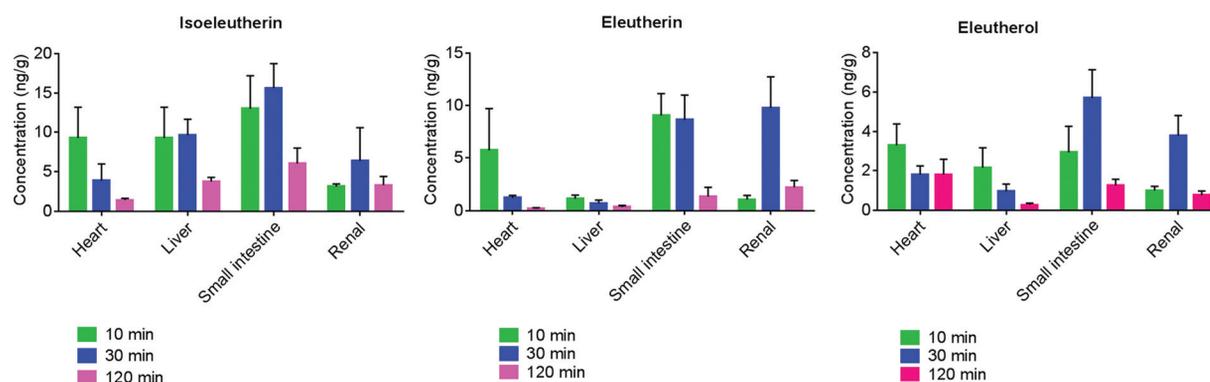


Fig. 6 The distributed amount of three analytes at 10, 30, and 120 minutes after oral administration to rats. Data are presented as mean ± standard deviation ($n = 6$).

the content begins to decrease after 30 minutes. The content of eleutherin is less in the kidney, the content of eleutherin is higher in the small intestine, and the content reaches the highest in the kidney at 30 minutes; the distribution of eleutherol in the tissues of eleutherin is similar, and the content of both the small intestine and the kidney is similar. The highest is reached at 30 minutes. All the three components reached the highest concentration in the heart at 10 minutes, indicating that the drug can take effect in a short time.

Conclusion

An accurate and reliable method of UPLC-MS/MS for the isoeleutherin, eleutherin, and eleutherol in *Bulbus eleutherinis* was developed. The results proved that the method was simple, efficient, and useful. The PK and tissue distribution data of the compounds in SD rats have been successfully obtained that lays a foundation for its follow-up studies. Simultaneously, the IS in the method was also selected and analyzed. This method can also be used to study the metabolites of the active components in *Bulbus eleutherinis*, and provide a method reference for the *in vivo* study of new drug preparations. Overall, the research will provide a scientific reference for the druggability of total naphthoquinone in *Bulbus eleutherinis*.

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Conflict of Interest

The authors declare no conflicts of interest.

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