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Research Article

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Quantitative evaluation of Loureirin A and Loureirin B in Dragon's blood capsules from different manufacturers by HPLC

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ABSTRACT

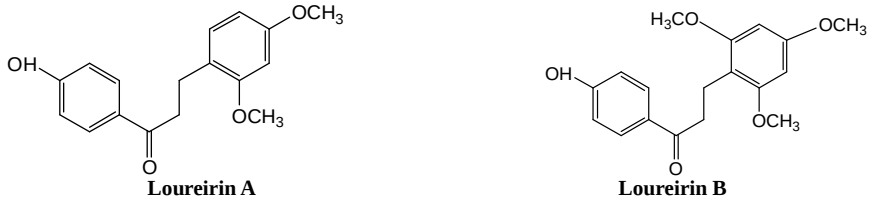
An analytical evaluation strategy, based on quantitatived determination of loureirin A and loureirin B in Dragon's blood capsules from five manufacturers by RP-high performance liquid chromatography. The samples were extracted with methanol by the ultrasonic extraction method and separated on a Kromasil C_{18} (5 μm, 4.6×200 mm) chromatographic column by using acetonitrile–0.1% acetic-acid (37: 63) as mobile phase with the detection wavelength at 280 nm. The effects of experimental conditions, such as extraction method, column temperature, and different type of chromatographic column, on separation efficiency were investigated. Under the optimal conditions, the main ingredients of loureirin A and loureirin B in dragon's blood capsules reached the baseline separation. The method showed good linearity with correlative coefficient of 1.0000. The recoveries were 98.07%~101.29%, and the relative standard deviations were 0.29~0.45%. The developed method can be adopted for the determination of loureirin A and loureirin B in dragon's blood capsules of five manufacturers. The results showed that there was remarkable difference in different origin of Dragon's blood.

Key words: Dragon's blood capsule, RP-HPLC, Loureirin A, Loureirin B

INTRODUCTION

In China, People describe Dragon's blood as a "dark red resinous substance derived from various plants: *Dracaena* spp. (Dracaenaceae), *Daemonorops* spp. (Arecaceae), *Croton* spp. (Euphorbiaceae) and *Pterocarpus* spp. (Fabaceae)" [1]. It has been used widely in many kinds of disease of blood system since ancient times, and it was called "effective medicine of promoting blood circulation" [2,3]. *Dracaena cochinchinensis* was first found in Yunnan Province, China in the 1970s [4]. Since then it has been used as the main source of Dragon's blood, substituting of other species. Dragon's blood capsules are made from the ethanol extract of stems of the *Dracaena cochinchinensis*, they are clinically used for the treatment of blood stasis syndrome, trauma, gynecopathy and allergic dermatitis[5-7]. It has been proven that the pharmacological effects resulted from its flavonoid components[8], of which Loureirin A and loureirin B (Fig. 1) are two active flavonoid compounds in Dragon's blood, used as markers to identify different sources or to control the quality of preparations containing Dragon's blood[9,10].

In this study, Dragon's blood capsules from five manufacturers were quantitative determined by RP- High Performance Liquid Chromatography. An analytical evaluation by comparing the content of loureirin A and loureirin B in Dragon's blood from different orgins was discussed.



Loureirin A **Loureirin B**

Fig. 1 Loureirin A and Loureirin B

EXPERIMENTAL SECTION

Instrumentation

The separation was carried out on HPLC system (Shimadzu LC-20A) and Kromasil 100-5C_{18} reversed-phase column (200 mm × 4.6 mm, 5 μm) at 280 nm using UV detector.

Chemicals and Reagents

Loureirin A(111660-200) and loureirin B(111558-200) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Dragon's blood capsules: (100160，100312，100402，100309，100624, Yunnan Yunhe Pharmaceutical Co., Ltd; 071119，090724，091027，100105，100122, XishuangbannaYulin Pharmaceutical Co., Ltd; 090202，090203, 090302, 100303, 100304, Xishuangbanna Jianlong Pharmaceutical Co., Ltd; 091101，091102，091103，100102，100203, Zhaoqing Xinghu Pharmaceutical Co., Ltd; 090701，090901，090903，100205，10030, Guilin Sanjin Pharmaceutical Co., Ltd). All solvents with HPLC grade quality were purchased from Merck (Darmstadt, Germany). Methanol, ethanol, acetonitrile and acetic acid were purchased from Fengchuan Chemical Co., Ltd (Tianjin, China).

Preparation of standard stock solution

The stock solutions of loureirin A and loureirin B were prepared by dissolving 0.8 mg of loureirin A and 0.6 mg of loureirin B in 10 mL of methanol and then diluted with methanol to give the final concentration of 80 and 60μg mL^{-1}. Working solutions were prepared by appropriate dilution of the stock solution with methanol. All the solutions were stored at 0°C before use.

Preparation of sample

Weigh accurately a quantity content of Dragon's blood capsules 0.25g, extracted with 25 mL methanol and promoted by an ultrasonic bath under the frequency of 50 kHz for 30 min.

Chromatographic condition

The mobile phase consisting of 0.1% acetic acid -acetonitrile. They were filtered through 0.45 μm membrane filter before use and were pumped from the solvent reservoir in the ratio of 67:33v/v into the column at a flow rate of 1 mL/min, column temperature was maintained at 25 ℃. The volume of injection was 10 μL ; the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluent was detected at 280 nm.

RESULTS AND DISCUSSION

Optimization of extraction solvent and time

According to the properties of loureirin A and loureirin B, extraction methods were optimized. Four kinds of solvent were selected to extract the same sample (100624, Yunnan Yunhe Pharmaceutical Co., Ltd) at different time (Table 1).

Table1. Extraction results of different solvents

Compound	extraction conditions						
	chloroform (30 min)	chloroform (1 h)	ethyl acetate (30 min)	ethyl acetate (1 h)	methanol (30 min)	methanol (1 h)	acetonitrile-acetic acid (1→90)(39:61) (30min)
loureirinA (mg/g)	19.2771	20.6107	21.5678	21.7698	22.0892	22.0941	21.9126
loureirinB (mg/g)	7.5011	7.7712	7.5277	7.532	7.5942	7.6	7.66

The results showed that the ultrasonic extraction result of acetonitrile-acetic acid (1→90)(39:61) was as good as methanol, and better than chloroform and ethyl acetate. Sice methanol is easier to operate, so selected it to extract

the compounds. The extraction result for 30 minute is almost the same as for one hour, so selected 30 minute for the ultrasonic extraction time.

Optimization of chromatographic condition

Three chromatographic columns of different manufacturers, Elite Kromasil C_{18} (5 μm，4.6×200 mm), Hanbon C_{18} (5 μm，4.6×200 mm) and Agilent C_{18} (Zorbax Exlipse) (5 μm，4.6×200 mm) were optimized(Table 2). The results showed that the samples can reach the baseline separation of different brands of the same type C_{18} chromatographic column under different flow rates, column temperatures.

Table 2. Resolutions of two compounds under different flow rates, column temperatures, and different type of chromatographic columns

Resolution	chromatographic columns			flow rates (mL/min)			column temperatures (℃)		
	Kromasil C_{18}	Hanbon C_{18}	Agilent C_{18}	0.9	1	1.2	25	30	40
R	1.75	1.68	1.78	1.71	1.7	1.68	1.69	1.7	1.7

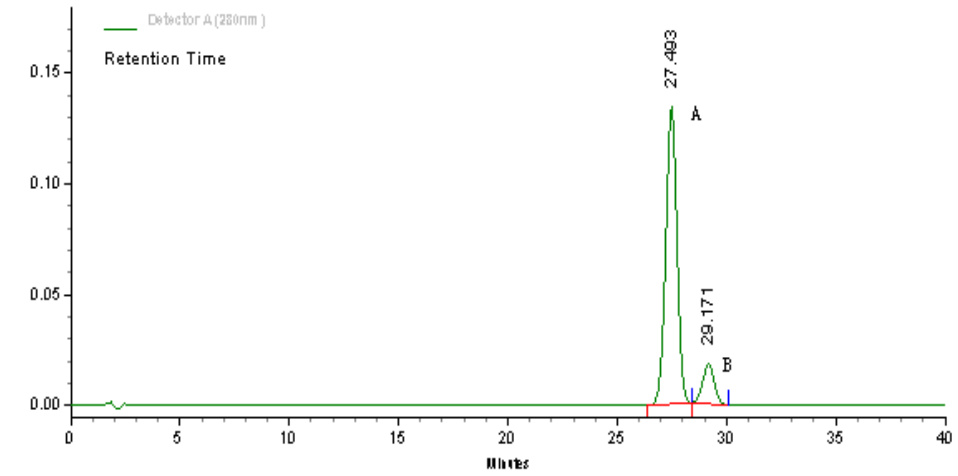
Validation of proposed method

The proposed method has been validated in terms of specificity, linearity, accuracy, precision, limit of detection and limit of quantification.

Specificity

Under the optimal conditions, representative chromatograms are shown in Fig. 2. The retention times of loureirin A and loureirin B were 27.4, 29.1 min, respectively. The peak corresponded to Dragon's blood capsules, and its standards were well resolved from instrumental noise and background interference.

(a)



(b)

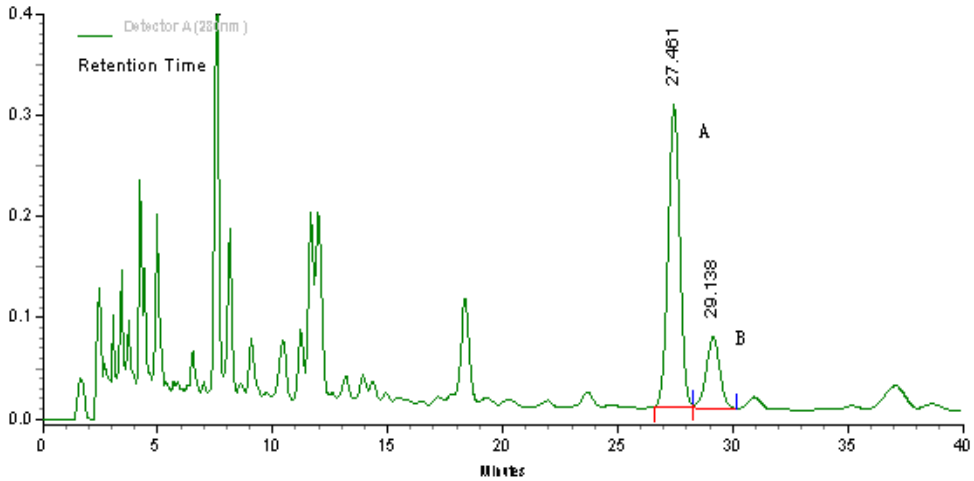


Fig. 2 Chromatograms of the reference solution (a) and the sample solution (b)

Precision, Linearity, LOD and LOQ

The linearity of this method was determined with six levels of loureirin A（0.1030 mg/mL）and loureirin B（0.0426 mg/mL)(1.0，5.0， 10.0， 15.0， 20.0，25.0μL). As a result, good linearity (r^2 =1) of the investigated concentration ranges was observed. All the detailed information of the calibration curves is listed in Table 3.

Table 3. Results of Precision Linearity of loureirin A and loureirin B, LOD and LOQ (n=6)

Compound	precision RSD(%)	linear range (μg)	calibration equation	correlation coefficient (r^2)	LOD (μg)	LOQ (μg)
loureirin A	0.295	0.1030 ~ 2.5750	y=3.8E+6x-9310.5	1.0000	0.0363	0.103
loureirin B	1.454	0.0426 ~ 1.0650	y=2.8E+6x-3598.9	1.0000	0.0142	0.0426

Repeatability and Stability

Injection repeatability was examined by the injection of six samples prepared with the same sample preparation procedure, taking the same sample (100624, Yunnan Yunhe Pharmaceutical Co., Ltd). According to the sample solution preparation method for the preparation of 6 for the test solution and calculate the content. The RSD is 0.47% for loureirin A, 1.30% for loureirin B, respectively.

Stability of loureirin A and loureirin B were evaluated at 0, 6, 12, 18, 24, 30, 36h. After the 36h storage time, loureirin A and loureirin B in all samples were found to be rather stable at the observed concentrations. RSD are 0.45% and 2.86% for loureirin A and loureirin B.

Table 4. Recovery of loureirin A and loureirin B (n = 6)

Analyte	No.	Sampling amount (g)	Content of the sample (mg)	Adding amount (mg)	Measure the amount (mg)	Recovery/%	RSD/%
loureirin A	1	0.1344	2.9449	3.1	6.0317	99.57	0.81
	2	0.1404	3.0764	3.1	6.1691	99.76	
	3	0.1325	2.9033	3.1	5.9454	98.13	
	4	0.1328	2.9098	3.1	5.9501	98.07	
	5	0.133	2.9142	3.1	6.0026	99.63	
	6	0.1329	2.912	3.1	6.0016	99.66	
loureirin B	1	0.1344	1.0174	1.144	2.1715	100.88	0.79
	2	0.1404	1.0628	1.144	2.2121	100.46	
	3	0.1325	1.003	1.144	2.1416	99.53	
	4	0.1328	1.0052	1.144	2.164	101.29	
	5	0.133	1.0068	1.144	2.142	99.23	
	6	0.1329	1.006	1.144	2.1513	100.11	

Table 5. The results of samples (n =3)

No.	Manufacturer	Batch no.	Content determination of samples (mg/Capsule)		
			loureirin A	loureirin B	Loureirin A+B
1	Yunnan Yunhe	100161	5.2534	2.4682	7.7216
2	Yunnan Yunhe	100312	7.6160	2.5547	10.1707
3	Yunnan Yunhe	100402	6.5523	2.8548	9.4071
4	Yunnan Yunhe	100309	7.3480	2.7431	10.0911
5	Yunnan Yunhe	100624	6.1270	2.1990	8.3260
6	bannaYulin	71119	2.7330	1.8796	4.6126
7	bannaYulin	90724	2.0176	1.6818	3.6994
8	bannaYulin	91027	3.4268	1.6818	5.1086
9	bannaYulin	100105	2.5177	1.5823	4.1000
10	bannaYulin	100122	3.9101	1.8768	5.7869
11	banna Jianlong	90202	2.5450	1.9280	4.4730
12	banna Jianlong	90203	2.5149	2.0403	4.5552
13	banna Jianlong	100302	3.1046	2.4801	5.5847
14	banna Jianlong	100303	2.8714	1.9798	4.8512
15	banna Jianlong	100304	2.8763	2.0018	4.8781
16	Zhaoqing Xinghu	91101	2.1852	1.6257	3.8109
17	Zhaoqing Xinghu	91102	2.0607	1.6248	3.6855
18	Zhaoqing Xinghu	91103	2.3952	1.8141	4.2093
19	Zhaoqing Xinghu	100102	1.8447	1.7781	3.6228
20	Zhaoqing Xinghu	100203	2.2052	1.7294	3.9346
21	Guilin Sanjin	90901	5.1810	1.3500	6.5310
22	Guilin Sanjin	100205	1.2600	1.7100	2.9700
23	Guilin Sanjin	100301	1.3355	1.6239	2.9594
24	Guilin Sanjinl	100302	1.3281	1.6742	3.0023
25	Guilin Sanjin	100401	1.3015	1.7127	3.0142

Recovery

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. A known amount of standard solutions were added to the pre-analyzed sample solution and these mixtures were determined by the proposed method. Recovery (%) and RSD (%) were calculated for each concentration and results were given in Table 4.

Sample determination

According to the previous method, 25 batches samples for 5 manufacturers were determined. The results are shown in table 5.

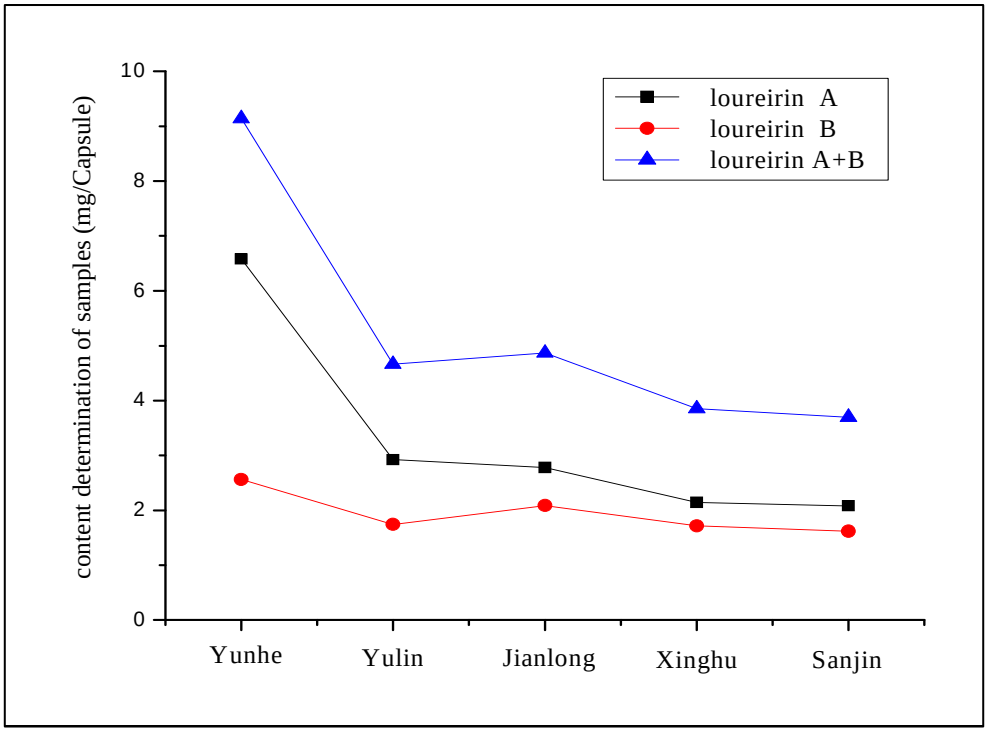


Fig. 3 The content of loureirin A and loureirin B of five manufacturers

The result shows that the contents of loureirin A in Dragon's blood capsule from different manufacturers vary greatly and the contents of loureirin B have little difference. The raw material sources from Yunnan areas have higher content of loureirin A than other area, and the source from Guanxi area has the lowerest level of loureirin A, almost near the content of loureirin B. Thus environmental factors have great influence on the loureirin A.

CONCLUSION

In the present investigation, we have optimized the method of extract loureirin A and loureirin B and studied the content of them in Dragon's blood capsules by RP- High Performance Liquid Chromatography. From the data it is clear that the effect on the content of loureirin A and loureirin B in Dragon's blood from different habitats is remarkable.

Acknowledgments

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