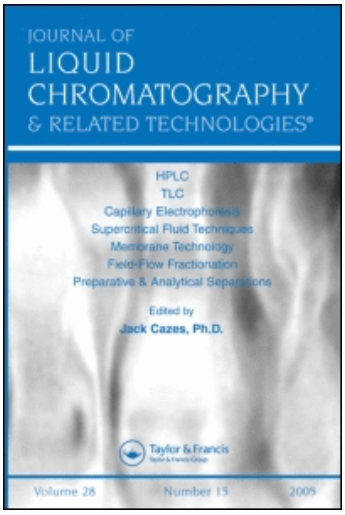

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SIMULTANEOUS EXTRACTION AND SEPARATION OF MARTRINE, SOPHORIDINE AND SOPHOCARPINE FROM *SOPHORA FLAVESCENS AIT* BY RP-HPLC WITH ANALYTICAL AND PREPARATIVE CHROMATOGRAPHY

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□ *The simultaneous extraction and separation of martrine, sophoridine and sophocarpine from* Sophora flavescens Ait *by RP-HPLC was developed by liquid-liquid extraction. The optimum extraction conditions were established using different extraction solvents, procedures and time. The extracts were separated and determined using a* C_{18} *column with methanol/water/diethylamine (55/45/0.10, v/v) as the mobile phase. Different preparative columns with different packing sizes were used to isolate the three compounds from the extracts, and the column with a 12 μm particle size showed better separation. The amounts and recovery of sophocarpine, sophoridine and martrine obtained were 0.081 mg/g, 0.031 mg/g and 0.051 mg/g, and 85.38%, 87.14% and 76.5%, respectively.*

Keywords extraction and separation, martrine, RP-HPLC, sophocarpine, sophoridine

INTRODUCTION

Sophora flavescens Ait (SFA) has been used as a traditional Chinese herb to treat many diseases since ancient times. The sophora flavescens Ait has recently attracted a great deal of attention in natural medication research on account of its high pharmacological activity. Sophora alkaloids are its chief active components, including matrine (MT), sophocarpine (SC), sophoridine (SRI) (Figure 1) and others.[1]

Studies of pharmacological effect have shown that SRI has wide range of pharmacological effects including anti-arrhythmic, anti-tumor,[2] immunological enhancement,[3] immunosuppressant, antiseptic and central nervous system excitation effects.[4] MT and SC were reported to show sedative, depressant, anti-tumor, antipyretic, cardio tonic activity[5] and

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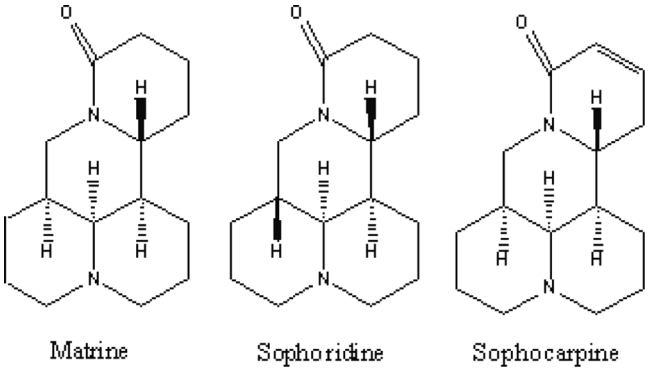

FIGURE 1 Molecular structure of matrine, sophoridine and sophocarpine.

anti-hepatitis B virus (HBV) activity.[6–8] Several papers have been published concerning the separation and quantification of the alkaloids in SFA, and several methods such as high performance liquid chromatography (HPLC),[9,10] high performance capillary electrophoresis (HPCE),[11,12] and gas chromatography (GC)[13] have been applied to the separation and determination of matrine type alkaloids in the SFA root. HPLC is the most widely used separation technique for this application owing to its simplicity and general applicability to matrine type alkaloids.[14] HPCE techniques can be used when HPLC is not suitable or efficient for the samples of interest. Sample preparation is the most important aspect in the application of HPLC or HPCE. Extraction procedures including liquid–liquid extraction, solid-phase extraction, and other methods, can be selected according to requirements of precision, accuracy, and reproducibility.[15,16] With a similar chemical structure, it is hard to separate the three compounds (SRI, SC and MT) by HPLC. Moreover, there are a few reports showing how to extract and purify the three compounds simultaneously from SFA by preparative columns.

This study examined the feasibility of extracting three major alkaloids from SFA using water by dipping method. Reverse-phase high-performance liquid chromatography (HPLC) with preparative column was applied to the purification of SRI, SC and MT. The procedure has the highly desired properties, such as no toxicity, low cost, and high extraction yield.

EXPERIMENTAL

Chemicals

SFA was purchased from Anguo, Hebei, China (Approximately 2008, August). Matrine and sophoridine were provided by the National Institute

for the Control of Pharmaceutical and Biological Products of China, sophocarpine was provided by Chengdu Mansite Biological Technology Co. Ltd. Methanol, acetonitrile, n-propanol, diethylamine were all purchased from Duksan Ltd., Korea.

HPLC Analysis

The chromatography system consisted of M930 multi solvent delivery system, a variable wavelength M720 UV detector, the data processing was carried out with Autochrowin Ver. 1.42 (Young Lin Co. Korea). A C_{18} commercial column (250 mm × 4.6 mm, 5 μm) was from RStech Corporation (Daejeon, Korea). All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before inject into the HPLC system.

Samples and Columns Preparation

SFA had been dried at 30°C, sliced and crushed into powder for further extraction experiments. SRI, SC and MT were dissolved in methanol to yield a final concentration of 0.0625 mg/mL. Different HPLC columns were packed by using different diameters of C_{18} particles as stationary phase. The uniform C_{18} particles (12 μm and 40/63 μm) purchased from YMC Co. (Kyoto, Japan) were suspended in methanol and degassed by helium. The slurries were pressed into the hollow HPLC columns (250 mm × 4.6 mm) using a pump, respectively. After then, the packed columns were washing by methanol until a stabile baseline was observed. All experiments were carried out at ambient room temperature.

Determination of SRI, SC and MT

Mixture of standard solvents of SRI, SC and MT were separated by a commercial C_{18} column with a mobile phase (methonal/water/diethylamine, 55/45/0.1, v/v). The flow rate was 0.6 mL/min and UV wavelength was 220 nm.

Choose the Optimum Extraction Solvent

The different extraction solvents which used in the experiment were water, ethanol, methanol, acetone n-hexane and chloroform. 25 mL solvent were used to extract 1.0 g powder of SFA by using the same dipping time (48 hr) under room temperature respectively (Table 1).

TABLE 1 Extract Amounts of Different Extraction Solvents

Extract Amounts (μg/g) / Solvents	SC	MT	SRI	Total
Water	71.3	25	42.1	138.4
Ethanol	68.9	71.9	20.2	161
Methanol	67.4	18.1	11.3	96.8
Acetone	*	*	*	*
Chloroform	*	*	*	*
n-Hexane	*	*	*	*

*not be detected.

Extraction with Different Methods

1.0 g powder of SFA mixed with 25.0 mL water by using the dipping time for 0.5, 1, 6, 24, 48 and 54 hr under room temperature. The same samples were then prepared again with 10, 20, 30, 45, 60 and 80 min ultrasonic, respectively.

Effect of Different Extraction Temperatures

25.0 mL water was used to extract 1.0 g *Sophora Flavescens Ait* under the different temperature. The dipping temperature is 20, 35, 45, 60, 75, 85, 94 and 100°C, respectively.

Separation of Extracts Using Different Particle Size Columns

In order to determine the maximum injection volume in the analytical column, injection volumes of 200 μL, 350 μL and 500 μL extracts were assessed.

RESULT AND DISCUSSION

Effect of Different Extraction Solvents

Table 1 shows the amounts of the three compounds extracted by different solvents under room temperature. SRI, SC and MT were obtained the largest amounts by water which is a polar solvent. Therefore, water was used in subsequent experiments.

Effect of Different Extraction Methods

The dipping method was used first. The amounts of the three compounds increased with the dipping time increasing from 20 min to 48 hr,

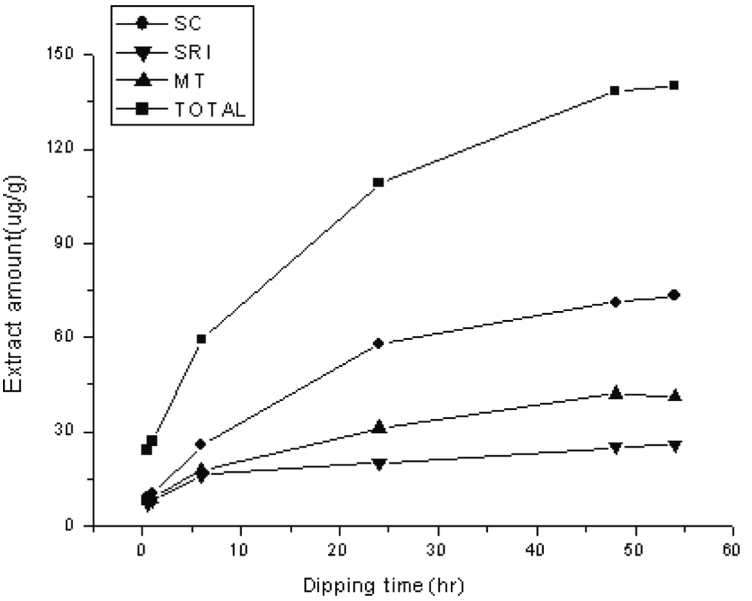

FIGURE 2 Effect of different dipping times on the extracted amounts.

but there was no obvious increase after 48 hr (Figure 2). Therefore, 48 hr was used as the optimum dipping time.

Equivalent samples were then prepared by ultrasonic method. Figure 3 shows that the amounts of the three compounds in extract increased with the ultrasonic time increasing. However, comparing the results of the two methods, it was found that although the amounts extracted of the three

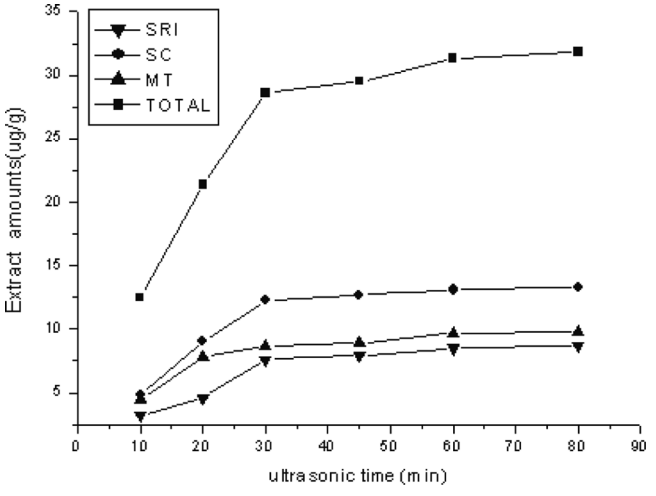

FIGURE 3 Extract of different ultrasonic times on the extracted amounts.

compounds via the ultrasonic method increased in 30 min, the extracted amounts were lower while much more energy was required in the experiments. Thus, it was determined that the ultrasonic method was not appropriate for this approach.

Effect of Different Temperature on the Extracted Concentrations of SC, SRI and MT

Figure 4 shows that the amounts of SRI, SC and MT increased with the temperature of extraction solvent increasing. However, the amounts extracted were similar when the solvent temperature was 93°C or higher (Table 2). Therefore, 93°C was considered the suitable extraction solvent temperature.

Method Validation

To ensure the specificity and selectivity of the method, concentrations of 0.125, 0.0313, 0.025 and 0.005 mg/mL were applied for standard solutions of the three compounds. Each concentration was injected 3 times. The analyze peak area values were plotted against the corresponding concentrations of the analyzed and the calibration curves constructed by means of the least-square method. The calibration curves of each alkaloid were constructed by regressing peak areas against the concentration with liner regression analysis: $Y = aX + b$. Here Y is the peak area of alkaloid, while X is the concentration of alkaloid, a is the slope and b is the intercept

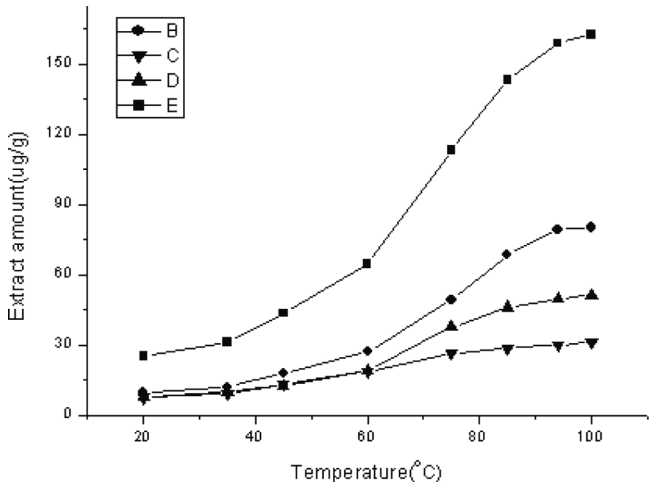

FIGURE 4 Extract in different temperatures on the extracted amounts.

TABLE 2 Effect of Different Temperatures on the Extracted Amounts

Extract Amount (μg/g)					
Heating for1hr	Temperature (°C)	SC	SRI	MT	Total
	20	9.8	7.5	7.8	25.1
	35	12.1	9.2	9.8	31.1
	45	17.6	13.1	12.7	43.4
	60	26.9	18.7	18.9	64.5
	75	49.2	26.4	37.6	113.2
	85	68.3	28.7	46.1	143.1
	94	79.4	29.8	49.6	158.8
	100	80.1	31.1	51.3	162.5
Heating for 1.5 hr	100	80.7	31.0	51.4	163.1

of regression line. Calibration curves of 3 compounds showed good linearity ($r^2 > 0.992$), the regression equations of SRI, SC and MT were $Y = 15866X + 80.29$, $Y = 8083X + 61.04$, $Y = 11403X + 136.86$ (x from 5.0 to 1000.0 mg/L), respectively.

Three concentrations of standards SRI (8.0, 80.0, 800.0 mg/mL), SC (3.0, 30.0 and 300.0 mg/mL) and MT (5.0, 50.0 and 500.0 mg/mL) were added to each 3.0 mL of real sample and filled up to 6.0 mL. The measured concentration was compared with the theoretical concentration to calculate the recovery rate. Table 3 shows the RSD of the precision tests for 5 times everyday, the limit of detections (LOD) on standard solutions and the recovery rates. For real sample analysis, the above mentioned values shows acceptable precision and accuracy.

Preparative Separation on Different Particles Sized Columns

Figure 5 shows the chromatogram with 15 μL injection volume. Larger injection volumes were used to determine the effect of the injection

TABLE 3 Recovery Studies of SRI, SC and MT in SMB (n = 5)

	RSD (%)		Recovery Rate			
Compounds	Intra-day	Inter-day	Amount Added (μg/mL)	Average Recovery (%)	RSD (%)	LOD (μg/mL)
SRI	1.6	1.5	8.0	84.87	1.3	35
	1.7	1.9	80.0	86.02	1.6	
	1.2	1.1	800.0	85.25	1.9	
SC	1.6	1.8	3.0	86.93	1.8	20
	1.5	1.6	30.0	86.87	1.5	
	1.0	1.9	300.0	87.62	1.9	
MT	1.1	1.0	5.0	75.86	1.9	32
	1.2	1.3	50.0	76.23	2.1	
	1.0	1.2	500.0	77.41	1.8	

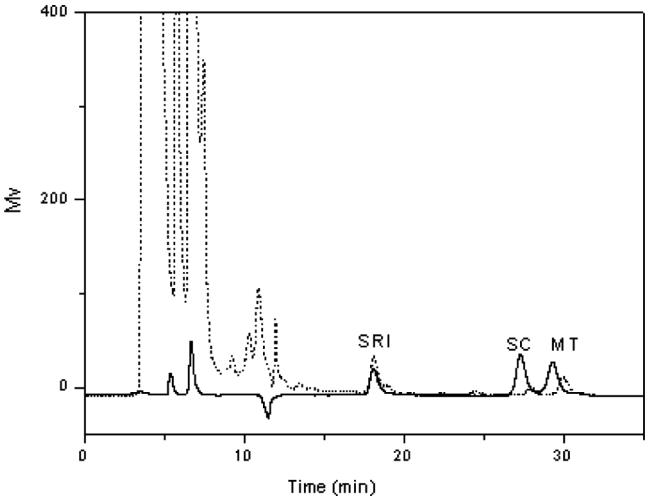

FIGURE 5 Chromatograms of the standard sample and the extracts from SFA. (Mobile phase: methanol/water/diethylamine (55/45/0.1, v/v), column: C_{18} (5 μm, 250 mm × 4.6 mm), flow rate: 0.6 mL/min, UV: 220 nm, injection volume: 15 μL, 0.125 mg/mL).

volumes in the analytical column. The peak area increased with increasing injection volume. On the commercial C_{18} column, SRI, SC and MT could be separated well when the injection volumes were 350 μL, as shown in Figure 6. When the injection volume was increased to 400 μL, SC and MT could not be discerned from the baseline. MT was not purely separated when the injection volume was 400 μL. When the injection volume was

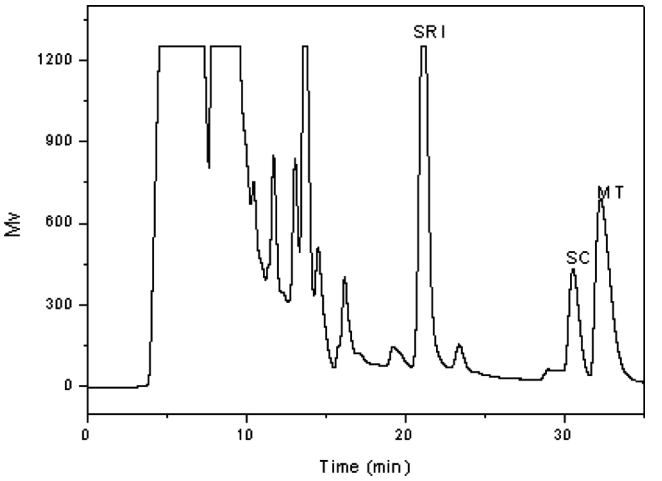

FIGURE 6 Chromatogram of the extract from SFA. (Mobile phase: methanol/water/diethylamine (55/45/0.1, v/v), column: C_{18} (5 μm, 250 mm × 4.6 mm), flow rate: 0.6 mL/min, UV: 220 nm, injection volume: 350 μL, 0.125 mg/mL).

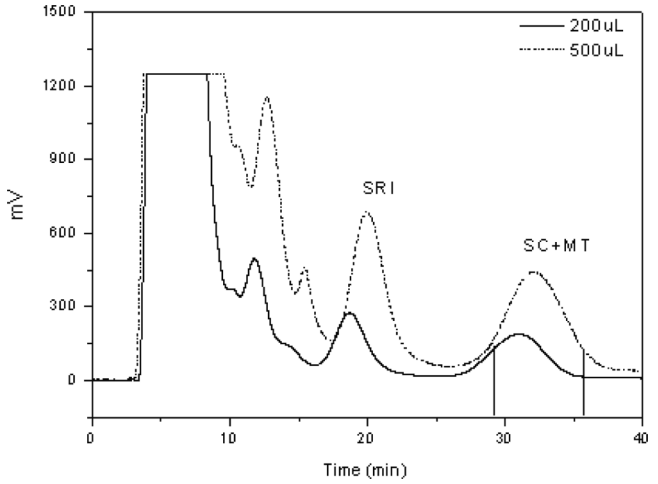

FIGURE 7 Chromatogram of the standard sample of SFA by different injection volumes. (Mobile phase: methanol/water/diethylamine (55/45/0.1, v/v), column: C_{18} (12 μm, 250 mm × 4.6 mm), flow rate: 0.6 mL/min, UV: 220 nm, injection volume: 350 μL, 0.125 mg/mL).

increased to 500 μL, the peaks for SRI and SC combined into a large peak. Therefore, the maximum injection volume of the analytical column was determined to be 350 μL.

Figure 7 shows the chromatograms at a particle size of 12 μm. Compared with the results at an injection volume of 200 μL, MT can be separated well in both the preparative and commercial columns. However,

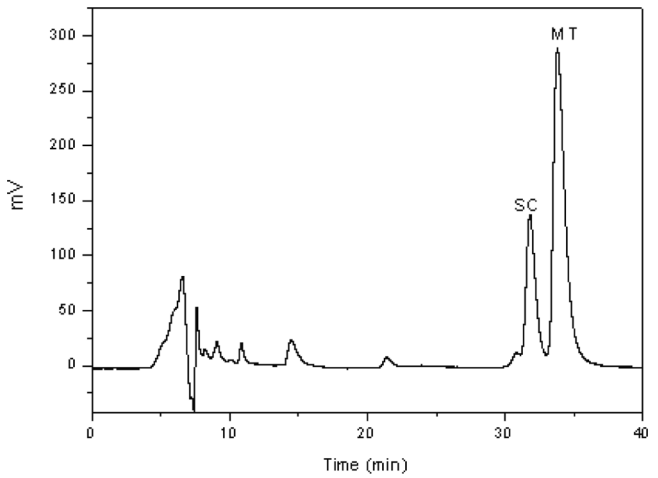

FIGURE 8 Chromatogram of the collected solvents which contained MT and SC. (Mobile phase: methanol/water/diethylamine (55/45/0.1, v/v), column: C_{18} (5 μm, 250 mm × 4.6 mm), flow rate: 0.6 mL/min, UV: 220 nm, injection volume: 200 μL, 0.125 mg/mL).

the peaks for SC and MT overlapped with interference in the 12 μm column, and the other 2 compounds were barely separated. As the particle size was increased, the column efficiency and resolution deteriorated as a result of the smaller contact area of the sample with the surface of the solid packing, larger diffusivity, and longer flow path. SC and MT from SFA were purified by the preparation column and separated completely on the commercial column (Figure 8). Therefore, from the results, it was determine that a particle size of 12 μm can be used in the preparative column.

CONCLUSION

In this study, a simple and convenient method for extracting SRI, SC and MT was examined, the amounts of SRI, SC and MT extracted were 0.08, 0.05 and 0.03 mg/g, respectively, and the recovery was 85.38%, 87.14% and 76.50%, respectively. The extracts from *Sophora flavescens Ait* were separated successfully by the preparative columns with different packing sizes. From the results, the 12 μm can separate the SRI well and can be used to purify the three compounds from the extracts of SFA.

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REFERENCES

1. Liu, M.; Liu, X.Y.; Cheng, J.F. Pharmacological research of matrine. Zhongguo Zhong Yao Za Zhi **2003**, *28* (9), 801–804.
2. Liu, S.X.; Chiou, G.C. Effects of Chinese herbal products on mammalian retinal functions. J. Ocul. Pharmacol. Ther. **1996**, *12* (3), 377–386.
3. Jiang, H.; Hou, C.; Zhang, S.; Xie, H.; Zhou, W.; Jin, Q.; Cheng, X.; Qian, R.; Zhang, X. Matrine upregulates the cell cycle protein E2F-1 and triggers apoptosis via the mitochondrial pathway in K562 cells. Eur. J. Pharmacol. **2007**, *559* (2–3), 98–108.
4. Yamazaki, M. The pharmacological studies on matrine and oxymatrine. Yakugaku Zasshi **2000**, *120* (10), 1025–1033.
5. Zhang, M.J.; Huang, J. Recent research progress of anti-tumor mechnism matrine. Chin. J. Chin. Med. **2004**, *29* (2), 115–118.
6. Hu, Z.L.; Zhang, J.P.; Yu, X.B.; Lin, W.; Qian, D.H.; Wan, M.B. Effect of matrine on lipopolysaccharides/D-galactosamine-induced hepatitis and tumor necrosis factor release from macrophages in vitro. Acta Pharmacol. Sin. **1996**, *17* (4), 351–353.
7. Hu, Z.L.; Zhang, J.P.; Wan, M.B.; Yu, X.B.; Lin, W.; Qian, D.H. Effect of matrine on mouse hepatitis and tumor necrosis factor production induced by Propionibacterium acnes/lipopolysaccharides. Acta Pharmacol. Sin. **1996**, *31* (9), 662–665.
8. Hu, Z.L.; Zhang, J.P.; Qian, D.H.; Lin, W.; Xie, W.F.; Zhang, X.R.; Chen, W.Z. Induction of apoptosis in human leukemia K562 cells by alpha-anordrin. Acta Pharmacol. Sin. **1996**, *17* (3), 259–261.

9. Lin, W.; Zhang, J.P.; Hu, Z.L.; Qian, D.H. Inhibitory effect of matrine on lipopolysacchride-induced tumor necrosis factor and interleukin-6 production from rat Kupffer cells. Acta Pharmacol. Sin. **1997**, *32* (2), 93–96.
10. Chen, X.; Yi, C.; Yang, X.; Wang, X. Liquid chromatography of active principles in Sophora flavescens root. J. Chromatogr. B **2004**, *812* (1–2), 149–163.
11. Li, K.; Wang, H.J. Simultaneous determination of matrine, sophoridine and oxymatrine in Sophora Flavescens Ait by high performance liquid chromatography. Biomed. Chromatogr. **2004**, *18*, 178–182.
12. Song, J.; Xu, H.; Tian, S.; Butt, P.P.H. Determination of quinolizidine alkaloids in traditional Chinese herbal drugs by nonaqueous capillary electrophoresis. J. Chromatogr. A **1999**, *857*, 303–311.
13. Su, X.; Wang, X.; Chen, Y.; Liu, H. Determination of alkaloids in Sophora Flavescens Ait and Sophora viciifolia hance by capillary zone electrophoresis. J. Microcolumn Sep. **2001**, *13*, 221–226.
14. Sit, D.S.T.; Gao, G.; Law, F.C.P.; Li, P.C.H. Gas chromatography-mass spectrometry determination of matrine in human plasma. J. Chromatogr. B **2004**, *808* (2), 209–214.
15. Li, Y.; He, L.R. Pharmacological study of Sophora alkaloid actions on the cardiovascular system. Chinese Trad. Herbal Drugs **2000**, *31*, 227–229.
16. Wang, G.F.; Zhang, S.Y.; Guo, Z.G.; Liu, Y. Simultaneous determination of three alkaloids in Sophora flavescens Ait and in Yeyean Lotions by high performance capillary electrophoresis. Chin. J. Pharm. Anal. **2000**, *20*, 331–333.
17. Wang, H.Y.; Che, D.; Miao, M.G.F.; Luo, G.H. Quality standard of compound Muji'an Koufuye. Chin. Pharm. J. **1999**, *34*, 262–264.