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Determination of Four Alkaloids from *Sophora flavescens* Root by HPLC

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Four alkaloids (matrine, sophocarpine, oxymatrine and sophoridine) were simultaneous separated by high performance liquid chromatography. The analysis was carried out on a C_{18} column (250 × 4.6 mm, 5 μm) with the mobile phase (methanol/water/diethylamine (45:55:0.07, v/v/v)) at a flow rate of 0.6 mL/min. Results showed that there was a good linear relationship when the content of four alkaloids were in the range of 5.0 mg/L ~ 1000 mg/L (n = 5) and the detection limits were 1.0 mg/L, respectively. This method can be used to determine the four alkaloids in sophora flavescens root and it also can be provided a rapid method for calculating recovery and extraction rate in extraction process.

Key Words: Matrine, Sophoridine, Sophocarpine, Oxymatrine, HPLC.

INTRODUCTION

Matrine (MT), sophocarpine (SC), oxymatrine (OMT) and sophoridine (SRI) are the important chemical components in sophora flavescence root[1-3]. The thin layer chromatography-fluorescence quenching[4], HPLC[5], ESI-ITMS[6] and capillary electrophoresis[7] were reported to separate and analyze the alkaloids from sophora flavescens root in recent years. But there are less reports on separations of these four compounds at the same time. Therefore, a simple and effective analytical method needs to be established for their simultaneous determination. According to the similar chemical structures of these four alkaloids (Fig. 1), it is difficult to simultaneity separate them[8-11]. In this work, the determination of these four alkaloids on C_{18} column with HPLC was established. This method is simple, reproducible and sensitive which can be used for quality control of sophora flavescence root.

EXPERIMENTAL

Matrine, oxymatrine and sophoridine were provided by the National Institute for The Control of Pharmaceutical and Biological Products of China, sophocarpine was provided by Chengdou Mansite Biological Technology Co. Ltd., methanol, acetonitrile, *n*-propanol, diethylamine and trifluoroacetic acid (TFA) were all purchased from Duksan Ltd., Korea.

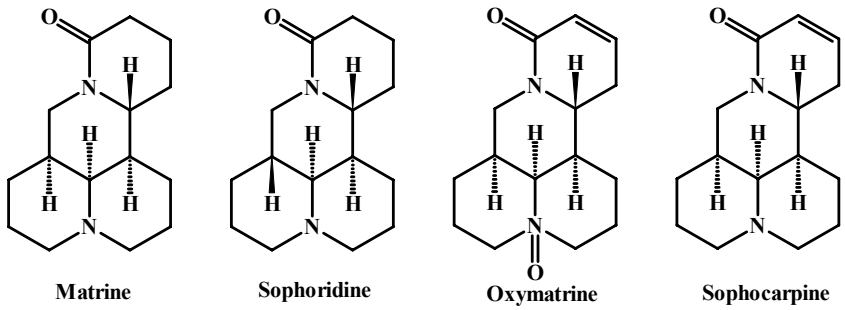

Fig. 1. Structure of matrine, sophoridine, oxymatrine and sophocarpine

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) and a Rheodyne injector (20 µL sample loop). A C_{18} column (250 × 4.6 mm, 5 µm) was from RStech Corporation (Daejeon, Korea). Distilled water was filtered with a vacuum pump (Division of Millipore, Waters, USA) and filter (HA-0.45, Division of Millipore, Waters, USA) before use. All the samples were filtered by using a filter (MFS-25, 0.2 µm TF, Whatman, USA) before injection into the HPLC system. The flow rate was 0.6 mL/min and UV wavelength was 220 nm.

Preparation of standard solutions: Stock solutions were prepared by dissolving 10 mg of drugs in 10 mL of methanol, respectively. Reference materials of matrine, sophoridine, sophocarpine and oxymatrine was weighed precisely separately, adding methanol solution, each made of 1.0 mg/mL single kind of solution.

RESULTS AND DISCUSSION

Effect of different mobile phase: According to the different characters of organic phases, methanol/water, acetonitrile/water, *n*-propanol/water and methanol/*n*-propanol/water were used as the mobile phase. According to the retention time and resolution of the four compounds, methanol was selected as the optimum organic solution in mobile phase.

In this experiment, methanol/water of different ratios (10:90, 45:55, 70:30, v/v) were carried on the experiment as the mobile phase to separate the four compounds. When methanol/water (10:90, v/v) as the mobile phase,the four compounds can reach the baseline separation, but the retention time of the matrine was 110 min, the retention time is too long, this condition is not suitable to separate. When methanol/water (70:30, v/v) as the mobile phase, the four compounds can not reach the baseline separation,the resolution is smaller than 0.5, this condition is also not suit to separate. So, methanol/water (45:55, v/v) as the mobile phase was used for the next experiment.

Effect of mobile phase additives: Sophocarpine, sophoridine, matrine and oxymatrine are all alkaloids. It is important to find a suitable additive for separation. Three mobile phase additives (trifluoro acetic acid, diethylamine and triethylamine) were

selected in this experiment. When the mobile phase was methanol/water/trifluoro acetic acid (45:55:0.1, v/v/v), part of the peak shape was improved, but the resolution of the peak is only about 0.7, it can not get the baseline separation in the final. The reason is that the trifluoro acetic acid in the mobile phase interact with the hydrophobic bond and the polar residues in a variety of modes, which can only improve the peak shape, reduce the peak broadening and overcome tailing problems. Diethylamine and triethylamine are the common additives for separation. Triethylamine has low solubility in methanol/water and had UV absorption on 220 nm, so it is avoided to be used. Diethylamine is a weaker alkaline, which can solute in water, mellow and ether and has steady chemical character. So it is often as the additive in alkaline material analysis. Under the condition of methanol/water/diethylamine (45/55/0.07), the four alkaloids got the baseline separation (Fig. 2). Compare the retention factor (k) and the resolution (R) of the four mobile phases (Table-1), the methanol/water/diethylamine (45:55:0.07, v/v/v) was selected as the optimum mobile phase.

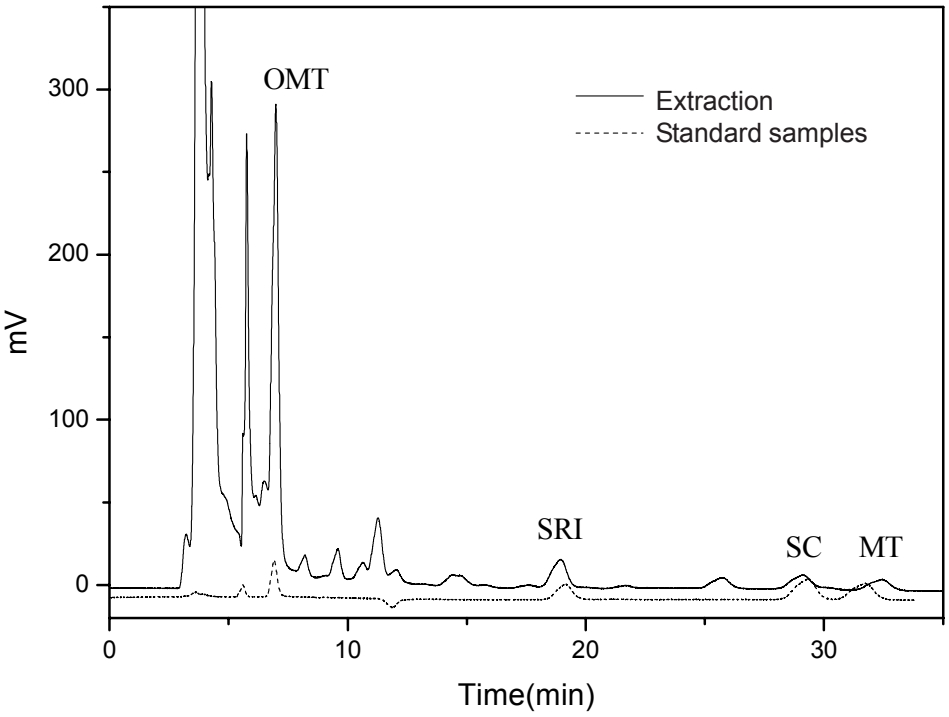

Fig. 2. Chromatograms of standard samples and the extract from natural plant

Effect of different temperatures: The column temperatures were decreased from 20 to 50 ºC. Based on the retention time and the resolution (Table-2), the 30 ºC was the suitable temperature for the experiment.

TABLE-1
EFFECT OF k AND R BY DIFFERENT MOBILE PHASE ADDITIVES

	k	R
Methanol/water (45:55, v/v)		
Matrine	12.25	-
Sophoridine	12.25	0.2
Sophocarpine	12.49	0
Oxymatrine	12.49	0
Methanol/water/TFA (45:55:0.1, v/v/v)		
Matrine	7.12	-
Sophoridine	12.25	2.1
Sophocarpine	13.42	0.8
Oxymatrine	24.60	3.51
Methanol/water/diethylamine (45:55:0.07, v/v/v)		
Oxymatrine	0.87	-
Sophoridine	4.16	11.0
Sophocarpine	6.44	9.41
Matrine	7.17	1.15

TABLE-2
COMPARISON OF k AND R OF 4 ALKALOIDS BY DIFFERENT TEMPERATURES

T (ºC)	k				R		
	k_{OMT}	k_{SRI}	k_{SC}	k_{MT}	$R_{OMT, SRI}$	$R_{SRI, SC}$	$R_{SC, MT}$
20	0.89	4.18	6.49	7.33	12.34	10.26	1.15
30	0.87	4.16	6.44	7.17	11.00	9.41	1.15
40	0.81	4.00	6.43	7.12	10.68	8.00	1.10
50	0.76	3.97	6.14	7.03	8.30	0.91	0.92

Method validation: 1.0 mg/mL mixture of MT, SRI, SC and OMT were made and then diluted into the same concentration of 0.125, 0.0313, 0.025, 0.005 mg/mL. Filter in the 0.45 mm micro hole film, calculate the peak areas, 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 of regression line. The results were listed in Table-3.

It showed a good precision that the relative standard deviation (RSD) of the sophoridine, matrine, sophocarpine and oxymatrine were 0.3, 0.8, 0.3 and 0.4 %, respectively.

Determination of four alkaloids from the sophora alkaloids root: There are some methods such as ultrasonic, microwave and refluxing dipping to extract from the herbal medicine. This experiment followed with the extraction method. Extraction and separation the active substances with the water from the *Sophora flavescens* root, extraction is carried on according to the papper by Wan *et al.*[12], dipping with

TABLE-3
REGRESSION EQUATIONS AND LIMITS OF DETECTION OF OMT, SRI, SC AND MT

Sample	Regression equation	Linear range (mg/L)	r^2	Limit of detection (mg/L)
Oxymatrine	Y = 8658X + 70.32	5.0~1000.0	0.9950	1.0
Sophoridine	Y = 15866X + 80.29		0.9955	
Sophocarpine	Y = 8083X + 61.04		0.9922	
Matrine	Y = 11403X + 136.86		0.9926	

water, then separation and analysis, we can get the chromatogram (Fig. 2). Matrine, sophocarpine, oxymatrine and sophoridine all can reach the baseline separation. Under this method, the contents are 0.0644, 0.0633, 0.251 and 0.15%, respectively.

Conclusion

This study has established a method to separate the four alkaloids (matrine, sophocarpine, sophoridine and oxymatrine) by HPLC at the same time. It is successfully to separate the 4 alkaloids through changing the type, ration and the concentration of the organic mobile phase. The experiment results showed that the standard curve of the four alkaloids in the range of 5.0 mg/L -1000.0 mg/L had good linear relation, recoveries were from 94 to 104 % and RSD were from 0.3-0.8 %. It is a simple, rapid method and is efficient to calculate the recovery rate and the extraction rate of the sophora root. It can provide strong assurances to control the quality of medicine.

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