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Short Communication

Determination of isoquinoline alkaloids in *Chelidonium majus* L. by ion-pair high-performance liquid chromatography

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ABSTRACT

A simple and precise method using ion-pair high-performance liquid chromatography was developed for the simultaneous determination of eight isoquinoline alkaloids, namely chelidonine, berberine, protopine, coptisine, tetrahydrocoptisine, 6-methoxydihydrochelerythrine, 6-methoxydihydrosanguinarine and dihydrosanguinarine, in *Chelidonium majus* L.. A reversed-phase system consisting of a chemically bonded ODS silica gel column and 0.05 *M* tartaric acid–methanol–acetonitrile (44:10:46) containing 0.5% sodium dodecyl sulphate as the mobile phase was used. The eight alkaloids were completely separated within 40 min. The analytical results for various samples are presented.

INTRODUCTION

Chelidonium majus L. is one of the most important medicinal plants in the Papaveraceae, owing to its chemically and pharmacologically interesting alkaloids. Its biologically active components are mainly isoquinoline alkaloids [1]. The clinical use of *C. majus* dates back to 1896 when Botkin reported two cases of carcinoma which responded to treatment with *C. majus* extracts [10]. Subsequent clinical reports include the use of chelidonine sulphate for gastric cancer and *C. majus* extracts for breast cancer, and in other clinical trials [2]. In China, *C. majus* has intensive clinical used. It is used to cure whooping cough and chronic bronchitis and as an analgesic, the effective rates being 94.5%, 95.3% and 96%, respectively [3]. Each individual alkaloid has its own medical uses. Therefore, the determination of the individual alkaloids is important in evaluating the quality and developing and utilizing the resources of *C. majus*.

To data, there have beeen only three reports on the separation and determination of isoquinoline alkaloids in *C. majus* by high-performance liquid chromatogra-

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phy (HPLC). Freytay [4] put forward two alternative methods and analysed only three alkaloids, chelidonine, sanguinarine and chelerythrine. For the determination of quaternary alkaloids in *C. majus*, Bugatti *et al.* [5] employed several methods, including reversed-phase and ion-pair chromatography, but the results were not satisfactory. Finally they chose normal-phase HPLC to determine berberine, sanguinarine and chelerythrine. Dzido [6] used di(2-ethylhexyl) orthophosphate as the ion-pair reagent in reversed-phase HPLC and separated allocryptopine, protopine and chelidonine, but the resolution was not complete. Other methods have also been employed to determine analyse *C. majus*, such as thin-layer chromatography (TLC) [7], high-performance, TLC [8] and capillary isotachophoresis [9]. All these methods can determine only three or four alkaloids. In order to determine the content of more alkaloids rapidly and precisely, it is necessary to establish a new method.

EXPERIMENTAL

Plant materials

C. majus was collected in the Botanical Garden of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, and other locations in July, 1989.

Apparatus

A Shimadzu 4A liquid chromatograph equipped with an SPD-2AS UV spectrophotometric detector and linked to a C-R_2AX data processor was used. A stainless-steel column (250 mm × 4 mm I.D.) packed with chemically bonded ODS silica gel (YWG-C_{18}, 10 μm) (Tianjin Second Chemical Reagent Factory, Tianjin, China) was employed.

Reagents

Eight alkaloid standards were provided by Professor Qi-cheng Fang of this Institute. The ion-pair reagents, namely sodium decyl sulphate, sodium dodecyl sulphate (SDS) and tetradecyl sulphate, were supplied by Wako (Osaka, Japan) and stilbene (internal standard) by Beijing Chemical Factor (Beijing, China). Acetonitrile of chromatographic grade was used.

HPLC conditions

Acetonitrile–methanol–0.05 *M* tartaric acid (46:10:44) containing 0.5% SDS was used as the mobile phase. The temperature was ambient (20–25°C) and the flow-rate was 1.0 ml/min. The substances eluted were detected with a UV detector operated at 290 nm.

Assay procedure

C. majus dry powder (0.5 g) was immersed in 5 ml of chloroform–ethanol (1:1), then macerated for 12 h and ultrasonicated for 30 min. After clarification (lay aside), the upper solution was retained and internal standard (2 ng/μl) was added. A 1-μl volume of this solution was injected into the HPLC system. The content of each alkaloid was calculated by the internal standard method.

Calibration graphs, precision and detection limits

A set of eight standard solutions containing between 0.01 and 0.28 mg/ml of each alkaloid were prepared. These were injected into the HPLC system to obtain data for calibration graphs, precision and detection limits.

RESULTS AND DISCUSSION

HPLC conditions

Elution parameters such as the organic content of the mobile phase, the kind and concentration of the counter ion and pH were varied to establish the optimum elution conditions on chemically bonded ODS silica gel.

When only buffer solution and acetonitrile were used as mobile phase components, complete resolution could not be achieved. It was found that a certain amount of methanol was necessary. As acetonitrile exerted the main role in the mobile phase, its concentration was examined (see Fig. 1). An increase in acetonitrile concentration caused a decrease in the capacity factors of eight alkaloids. The optimum separation was obtained at 46% acetonitrile.

As regards the kind of counter ion, alkyl sulphates were examined. Sodium decyl sulphate could not separate two alkaloids (coptisine and tetracoptisine), and sodium tetradecyl sulphate required too long a time and gave unsatisfactory peak shapes. The optimum separation was obtained with SDS (Fig. 2). The SDS concentration in the mobile phase was varied from 0.3 to 0.6%. As the SDS concentration

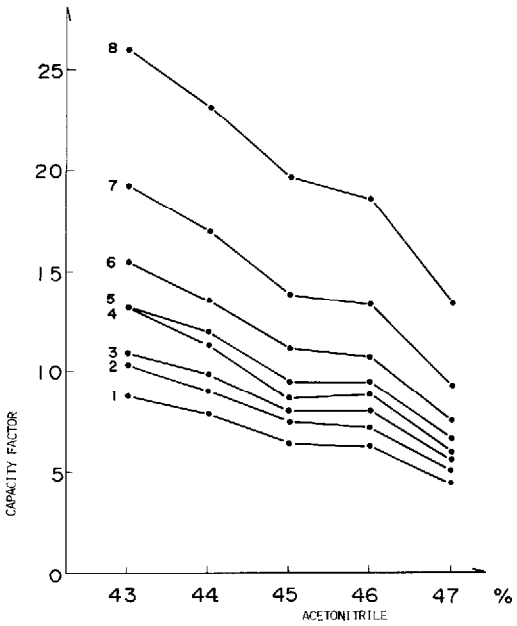

Fig. 1. Effect of acetonitrile concentration on capacity factor. 1 = Protopine; 2 = chelidonine; 3 = coptisine; 4 = tetrahydrocoptisine; 5 = 6-methoxydihydrosanguinarine; 6 = berberine; 7 = 6-methoxydihydrochelerythrine; 8 = dihydrosanguinarine.

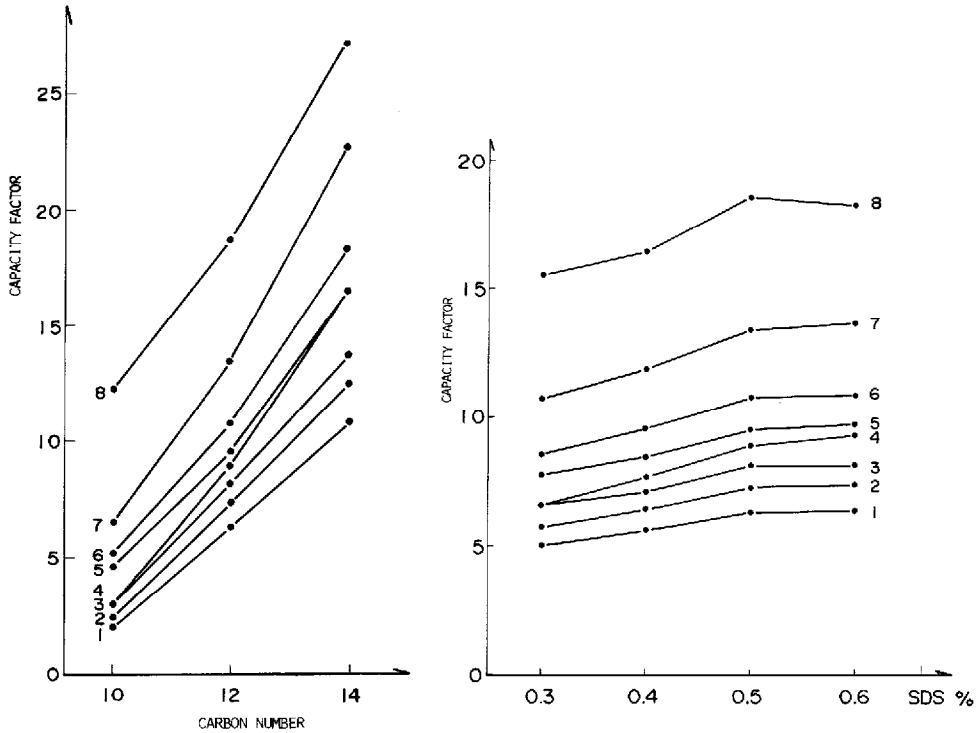

Fig. 2. Effect of the carbon number of alkyl sulphate on capacity factor. 1 = Protopine; 2 = chelidonine; 3 = coptisine; 4 = tetrahydrocoptisine; 5 = 6-methoxydihydrosanguinarine; 6 = berberine; 7 = 6-methoxydihydrochelerythrine; 8 = dihydrosanguinarine.

Fig. 3. Effect of SDS concentration on capacity factor. 1 = Protopine; 2 = chelidonine; 3 = coptisine; 4 = tetrahydrocoptisine; 5 = 6-methoxydihydrosanguinarine; 6 = berberine; 7 = 6-methoxydihydrochelerythrine; 8 = dihydrosanguinarine.

increased, the capacity factors became larger. The most suitable concentration was 0.5% SDS (Fig. 3).

The pH of the mobile phase strongly affected the retention times. When the concentration of tartaric acid changed from 0.05 to 0.04 *M*, the capacity factors became too large and caused incomplete separation of several alkaloids. It was proved that a decrease in acid concentration was not favourable for the separation, but a suitable pH of the chemically bonded ODS column is 2–8. When 0.05 *M* tartaric acid was added to the mobile phase in the proportion of 44%, the pH of the mobile phase was 2.1, so we selected 0.05 *M* tartaric acid for use in the mobile phase.

Finally, a mobile phase consisting of 0.05 *M* tartaric acid–methanol–acetonitrile (44:10:46) containing 0.5% SDS was selected optimum for the separation of these alkaloids.

Extraction conditions

The extraction efficiency for the eight isoquinoline alkaloids was examined us-

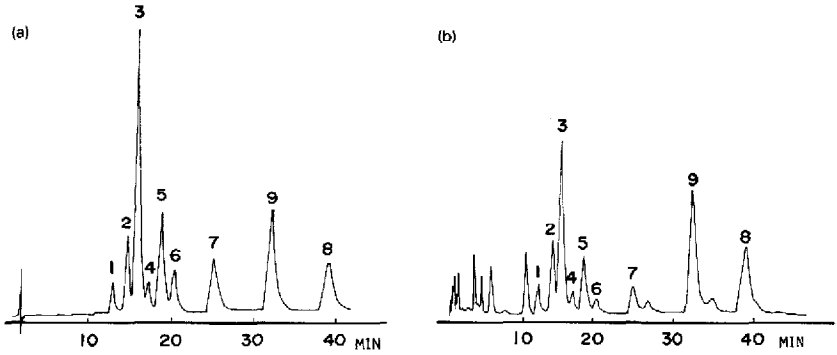

Fig. 4. Chromatograms of (a) standards and (b) alkaloid extract of subterrean parts from *C. majus*. 1 = Protopine; 2 = chelidonine; 3 = coptisine; 4 = tetrahydrocoptisine; 5 = 6-methoxydihydrosanguinarine; 6 = berberine; 7 = 6-methoxydihydrochelerythrine; 8 = dihydrosanguinarine. Mobile phase: 0.05 *M* tartaric acid–methanol–acetonitrile (44:10:46) containing 0.5% SDS. Flow-rate: 1 ml/min. Injection volume: 1 μl.

ing different solvents, *viz.*, chloroform, ethanol and chloroform–ethanol (1:1, v/v). The most efficient solvent was found to be chloroform–ethanol (1:1, v/v). Using this extraction solvent, we then compared the heating extraction method with the macerating extraction method. The two methods gave the same extraction efficiency for four isoquinoline alkaloids, but not for tetrahydrocoptisine, 6-methoxydihydrochelerythrine, 6-methoxydihydrosanguinarine and dihydrosanguinarine. As these alkaloids are readily oxidized to the quaternary analogues, the heating extraction efficiency was lower than that of the macerating method.

Finally, we compared different times of ultrasonic irradiation. The optimum extraction conditions were chosen, namely chloroform–ethanol (1:1, v/v) as extraction solvent, then macerating for 12 h and ultrasonicating for 30 min. This extraction method was applied under neutral conditions (pH 7). The recoveries of berberine, coptisine, protopine, chelidonine, 6-methoxidihydrocherythrine, tetrahydrocoptisine, 6-methoxydihydrosanguinarine and dihydrosanguinarine were 102, 101, 103, 102, 97, 95, 96 and 97%, respectively.

TABLE I

DATA FOR CALIBRATION GRAPHS

Alkaloid	Regression equation[a]	Correlation coefficient, r	Linear range (μg/ml)
Protopine	$y = 4.983x - 0.01596$	0.9997	15–150
Chelidonine	$y = 1.670x + 0.03617$	0.9997	28–256
Coptisine	$y = 9.111x - 0.00562$	0.9995	29–260
Tetrahydrocoptisine	$y = 4.925x - 0.00423$	0.9999	15–150
6-Methoxydihydrosanguinarine	$y = 17.20\ x - 0.3340$	0.9997	18–180
Berberine	$y = 9.216x - 0.2810$	0.9995	15–150
6-Methoxydihydrochelerythrine	$y = 14.40\ x - 0.3825$	0.9999	16–160
Dihydrosanguinarine	$y = 25.60\ x - 0.3750$	0.9998	13–130

[a] x = Content (μg/ml); y = peak area.

TABLE II

PRECISION AND DETECTION LIMITS FOR THE EIGHT ALKALOIDS

Alkaloid	Mean ± S.D. (μg) (n = 8)	Relative standard deviation (%)	Detection limit	
			Standard (ng)	Sample (μg)[a]
Protopine	0.304 ± 0.0056	1.8	10	102
Chelidonine	0.549 ± 0.0098	1.8	24	245
Coptisine	1.938 ± 0.0240	1.2	5	50
Tetrahydrocoptisine	2.215 + 0.0420	1.9	10	105
6-Methoxydihydro-sanguinarine	1.422 ± 0.0150	1.1	6	63
Berberine	0.791 ± 0.0260	3.3	5	50
6-Methoxydihydro-chelerythrine	0.956 ± 0.3490	3.7	5	52
Dihydrosanguinarine	1.053 ± 0.0190	1.8	4	42

[a] Per gram of plant material.

Analytical results

Fig. 4 illustrates the chromatograms of standards and extracts of subterranean parts of *C. majus*. The data for the calibration graphs and precision are shown in Tables I and II. The detection limits were determined at a signal-to-noise ratio of 3:1 for the peak heights (Table II). Table III gives the analytical results for samples collected in different locations. The results indicate that the individual contents of the alkaloids differ widely with plant location. The total amounts of alkaloids vary from 0.8 to 2.0%. Chelidonine and coptisine are the main components of the subterranean parts of *C. majus*, the others being minor components.

TABLE III

ALKALOID CONTENTS (%) OF SUBTERRANEAN PARTS OF *C. MAJUS* COLLECTED IN DIFFERENT LOCATIONS

Alkaloid	Jilin	Liaoning	Chengde	Juyongguan	Beijing
Protopine	0.022	0.020	Trace	0.029	0.114
Chetidonine	1.020	0.503	0.889	1.010	0.767
Coptisine	0.578	0.155	0.394	0.415	0.336
Tetrahydrocoptisine	Trace	Trace	0.064	Trace	Trace
6-Methoxydihydrosanguinarine	0.107	0.081	0.117	0.151	0.110
Berberine	0.035	Trace	Trace	0.034	0.038
6-Methoxydihydrochelerythrine	0.123	0.081	0.106	0.198	0.142
Dihydrosanguinarine	0.051	0.019	0.095	0.042	0.053
Total alkaloids	1.936	0.859	1.665	1.879	1.560

CONCLUSIONS

The reversed-phase ion-pair HPLC method developed is simpler, faster and more accurate than previous methods. It has several advantages over other methods: first, it is an isocratic HPLC system; second, no pretreatment is required except for extraction; and third, for the first time, eight alkaloids with very different polarities have been separated and determined. This method will be important and useful in developing and utilizing the resources of *C. majus*.

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