[Chem. Pharm. Bull. 33(12)5369—5374(1985)]

Quantitative Analysis of Tertiary and Quaternary Alkaloids in Corydalis Tuber by Ion-Pair High-Performance Liquid Chromatography and Its Application to an Oriental Pharmaceutical Preparation

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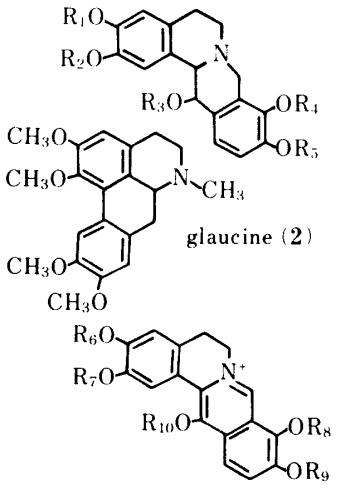
(Received January 17, 1985)

A new, simple and precise method using ion-pair high-performance liquid chromatography was developed for the determination of alkaloids in Corydalis Tuber and Anchusan-to dry extract, *i.e.*, tetrahydrocolumbamine, glaucine, tetrahydropalmatine, corydaline, columbamine, coptisine, palmatine, berberine and dehydrocorydaline. Reversed-phase systems with an octadecylsilane chemically bonded silica gel column using a mixture of sodium dodecyl sulfate, 0.05 M tartaric acid and acetonitrile (0.5 : 62 : 38) and a mixture of sodium dodecyl sulfate, Britton–Robinson buffer (pH 3.0) and acetonitrile (0.5 : 56 : 34) as the mobile phases were suitable for the separation of tertiary alkaloids and quaternary alkaloids, respectively, in Corydalis Tuber.

Keywords——ion-pair high-performance liquid chromatography; alkaloid; determination; Corydalis Tuber; Anchusan-to dry extract

Corydalis Tuber (*Corydalis yanhusuo*), which is called Engosaku in Japan, is well known as an analgic crude drug in Chinese traditional medicine. The abundant alkaloids contained in Engosaku can be divided into two groups; tetrahydrocolumbamine (**1**), glaucine (**2**), tetrahydropalmatine (**3**) and corydaline (**4**), which are tertiary alkaloids, and columbamine (**5**), coptisine (**6**), palmatine (**7**), berberine (**8**) and dehydrocorydaline (**9**),[1] which are quaternary alkaloids (Chart 1). In addition to these compounds, other minor alkaloids are known.[1]



glaucine (**2**)

tetrahydrocolumbamine (**1**)
$R_2=R_5=H$, $R_1=R_3=R_4=CH_3$
tetrahydropalmatine (**3**)
$R_5=H$, $R_1=R_2=R_3=R_4=CH_3$
corydaline (**4**)
$R_1=R_2=R_3=R_4=R_5=CH_3$
dehydrocorydaline (**9**)
$R_6=R_7=R_8=R_9=R_{10}=CH_3$
columbamine (**5**)
$R_7=R_{10}=H$, $R_6=R_8=R_9=CH_3$
berberine (**8**)
$R_{10}=H$, $R_6=R_7=-CH_2-$, $R_8=R_9=CH_3$
palmatine (**7**)
$R_{10}=H$, $R_6=R_7=R_8=R_9=CH_3$
coptisine (**6**)
$R_6, R_7=-CH_2-$, $R_8, R_9=-CH_2-$, $R_{10}=H$

Chart 1

In order to determine the amounts of individual alkaloids, analytical methods based on gas chromatography,[2,3] high-performance liquid chromatography (HPLC)[3] and thin layer chromatography-densitometry[2,3] have been reported to data. However, the gas chromatography requires a complicated pretreatment which involves the reduction of quaternary alkaloids to tertiary ones using sodium borohydride for the analysis of the quaternary alkaloids, and the separation of each reduced alkaloid appears to be incomplete. In the case of the thin layer chromatography-densitometry, good separation of the tertiary alkaloids has not been obtained. In 1976, Kohno and Matsukura[3] reported analytical methods for tertiary and quaternary alkaloids in Corydalis Tuber by HPLC based on a normal-phase system. However, the separation of each alkaloid again appears to be incomplete. Accordingly, these methods are not suitable for routine use. However, ion-pair high-performance liquid chromatography (IP-HPLC) has recently become available for the analysis of various components in crude drugs.[4]

In this report, the application of IP-HPLC to the determination of tertiary and quaternary alkaloids in Corydalis Tuber and an oriental pharmaceutical preparation is described. Each alkaloid in both groups was simply, rapidly and accurately determined by the use of sodium dodecyl sulfate (SDS) as a counter-ion in the mobile phase.

Experimental

Plant Material——Commercial Corydalis Tuber used in this study was purchased from Alps Pharmaceutical Ind. Co., Ltd. (Gifu) and was used as a dry powder.

Reagents——Berberine chloride purchased from Alps Pharmaceutical Ind. Co., Ltd. was used. The purified palmatine chloride and coptisine iodide were kindly supplied by Dr. A. Ikuta (Tokyo College of Pharmacy) and Dr. T. Hayashi (Koshirochuji Shoten Co., Ltd.), respectively. Tetrahydrocolumbamine, tetrahydropalmatine, corydaline, glaucine, columbamine and dehydrocorydaline were isolated and purified in our laboratory from Corydalis Tuber according to the previously reported techniques.[1] An oriental pharmaceutical preparation (Anchusan-to dry extract) was also prepared in our laboratory.

Apparatus——A Hitachi LC 635A pump system, a Hitachi 200-10 spectrophotometer (Hitachi, Ltd.) as a detector and a stainless-steel column (150 mm × 4 mm i.d.) packed with ODS chemically bonded silica gel (TSK gel LS-410, 5 μm, Toyo Soda Co., Ltd.) were used in this study.

Assay Procedure——1) Determination of Tertiary Alkaloids (Tetrahydrocolumbamine, Glaucine, Tetrahydropalmatine and Corydaline): To about 1.0 g of dry powder of Corydalis Tuber, previously weighed accurately, 15 ml of 7.5% NH_4OH solution, 6.0 g of NaCl and 20 ml of ethyl ether were added, and the mixture was shaken for 5 min in a centrifuge tube. The upper layer was removed, and the lower one was further extracted with two 20-ml portions of ethyl ether. The ethyl ether extracts were combined and evaporated. The residue was dissolved and diluted to 10 ml with a mixture of water and methanol (1 : 1) and used as the sample solution. Twenty microliters of this sample solution was injected into the column and chromatographed under the conditions described below. The contents of tertiary alkaloids in crude drugs were calculated by comparing the peak heights of samples with those of authentic standards.

2) Conditions for Quaternary Alkaloids (Columbamine, Coptisine, Palmatine, Berberine and Dehydrocorydaline): To about 1.0 g of the dry powder of Corydalis Tuber, previously weighed accurately, 30 ml of the mobile phase (see chromatographic conditions 2) for HPLC was added and the whole was refluxed on a water bath for 15 min at 85 °C. After cooling, the mixture was centrifuged and decanted. The residue was washed with two 30-ml portions of the mobile phase. The extract and the washings were combined and diluted to 100 ml with the mobile phase. Fifty microliters of this solution was injected into the column and chromatographed under the conditions described below. The contents of quaternary alkaloids were calculated from the peak heights.

Chromatographic Conditions——1) Conditions for Tertiary Alkaloids: A mixture of SDS, 0.05 M tartaric acid (pH 2.3) and acetonitrile (0.5 : 62 : 38) was used as the mobile phase. The chromatography was carried out at 30 °C at a flow rate of 1.5 ml/min. The substances eluted were detected by the ultraviolet (UV) detector at a wavelength of 280 nm.

2) Conditions for Quaternary Alkaloids: A mixture of SDS, Britton–Robinson buffer (pH 3.0) and acetonitrile (0.5 : 56 : 34) was used as the mobile phase. The chromatography was carried out at 40 °C at a flow rate of 1.0 ml/min. The substances eluted were detected by the UV detector at a wavelength of 345 nm.

Results and Discussion

We had hoped to separate tertiary and quaternary alkaloids under just one set of chromatographic conditions, but this was not achieved. In the case of tertiary alkaloids, some pretreatment of the sample was required prior to IP-HPLC.

Kohno and Matsukura[3] have reported an extraction method for the quantitative analysis of tertiary alkaloids in Corydalis Tuber. Accordingly, we tried to apply their method to our assay procedure as the pretreatment, and this was successful. It was also found that the pretreatment was not required for the determination of quaternary alkaloids, because the UV absorption of the tertiary alkaloids did not interfere.

Investigation of the Separation Systems of Alkaloids

1) Tertiary Alkaloids——Various parameters such as ratio of organic solvent, concentration of counter-ion, pH and column temperature were examined to achieve a satisfactory separation of each compound.

When methanol was used as an organic component of the mobile phase, tertiary alkaloids extracted from Corydalis Tuber were eluted with the same capacity factor. Using acetonitrile as an organic component, good separation of the four tertiary alkaloids was obtained, and the best was at 38% acetonitrile.

As regards the counter-ion, the more the concentration of SDS was increased, the more the tertiary alkaloids were retained on the column. They were clearly separated when the mobile phase containing 0.5% (w/v) SDS was used (Fig. 1).

The effect of pH was also examined by varying the pH from 1.9 to 6.0. Good separation was obtained at pH 2.3 (Fig. 2). Accordingly, 0.05 M tartaric acid, which showed pH 2.3, was used as a polar component of the mobile phase.

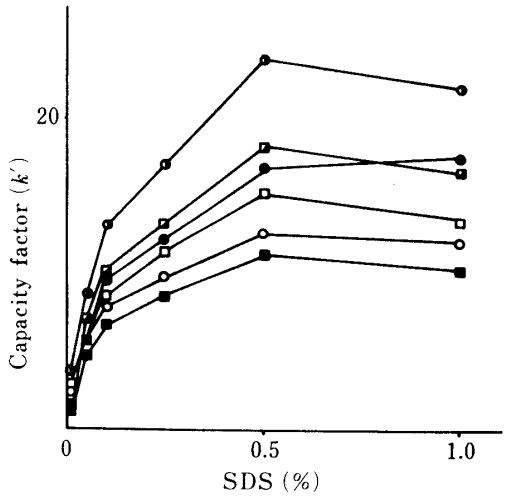


Fig. 1. Effect of SDS Concentration on Capacity Factor (k')

Solutes: ■, tetrahydrocolumbamine; ●, glaucine; ◪, tetrahydropalmatine; ◑, corydaline; □, unknown; ○, unknown.

Flow rate, 1.5 ml/min. Temperature, 30 °C. The mobile phase was a mixture of 0.05 M tartaric acid and acetonitrile (62 : 38).

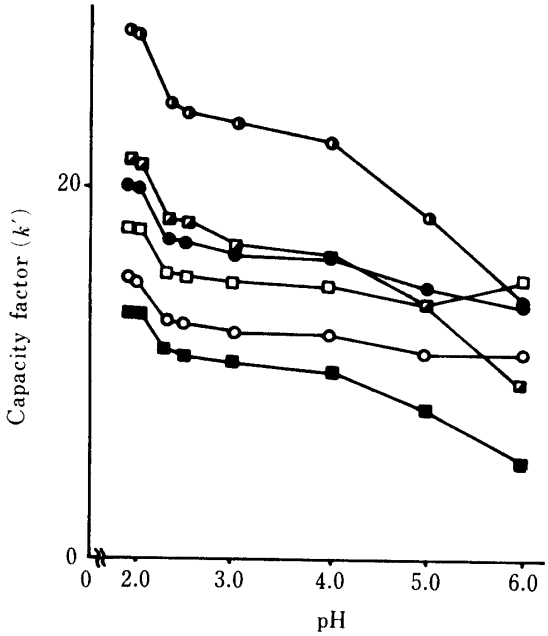


Fig. 2. Effect of pH on Capacity Factor (k')

Solutes: ■, tetrahydrocolumbamine; ●, glaucine; ◪, tetrahydropalmatine; ◑, corydaline; □, unknown; ○, unknown.

The mobile phase was a mixture of buffer and acetonitrile (62 : 38) containing 0.5% SDS. Other conditions, see Fig. 1.

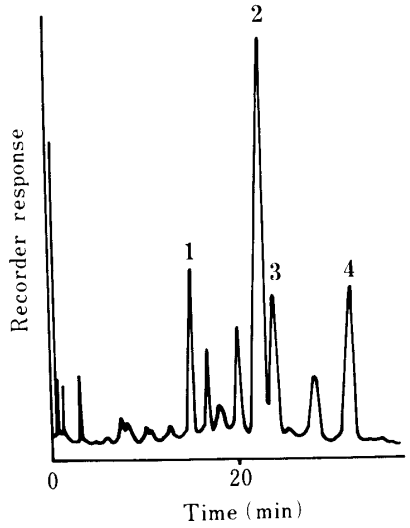


Fig. 3. Chromatograms of Tertiary Alkaloids in Corydalis Tuber

Solutes: **1**, tetrahydrocolumbamine; **2**, glaucine; **3**, tetrahydropalmatine; **4**, corydaline.

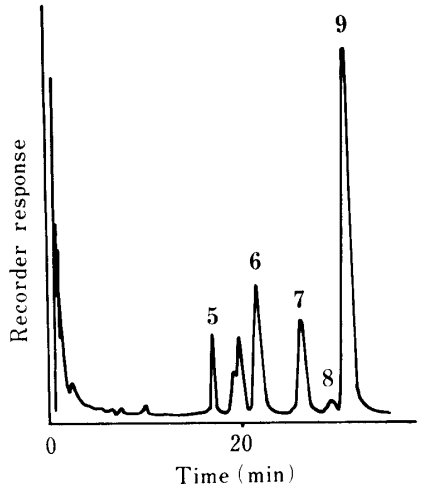


Fig. 4. Chromatograms of Quaternary Alkaloids in Corydalis Tuber

Solutes: **5**, columbamine; **6**, coptisine; **7**, palmatine; **8**, berberine; **9**, dehydrocorydaline.

The effect of column temperature between 25 and 40 °C was examined under the standard conditions, and the best chromatogram was obtained at 30 °C. A typical chromatogram of tertiary alkaloids extracted from Corydalis Tuber is shown in Fig. 3.

An identification study of the unknown peaks in Fig. 3 is in progress.

To confirm the homogeneity of those four peaks obtained by this method, each peak was collected and analyzed by thin layer chromatography (TLC) according to Kohno and Matsukura.[3] Each peak showed just one spot on TLC at the *Rf* value of the corresponding authentic standards.

2) Quaternary Alkaloids——The separation system for quaternary alkaloids was also examined. The details are not given here, because the behaviors of quaternary alkaloids were similar to those of tertiary alkaloids.

The fixed system was as follows: mobile phase, a mixture of SDS, Britton–Robinson buffer (pH 3.0) and acetonitrile (0.5 : 56 : 34); column temperature, 40 °C; detection, UV at 345 nm.

A chromatogram of quaternary alkaloids in Corydalis Tuber is shown in Fig. 4. An unknown peak having a shoulder appeared at about 20 min. An identification study of it is in progress.

To confirm the reliability of the data obtained by this method, the results were compared with those obtained using a fluorescence detector (Hitachi 650-10 LC, Ex. 350 nm, Em. 520 nm) under the same HPLC conditions.

Calibration Curves

Calibration curves for tertiary alkaloids were obtained from 40 to 250 μg/ml. The regression equations were as follows: $y=0.525x-2.400$ ($r=0.999$) for corydaline, $y=0.584x-3.969$ ($r=0.999$) for tetrahydropalmatine, $y=0.748x-4.039$ ($r=0.999$) for tetrahydrocolumbamine and $y=0.636x-6.309$ ($r=0.999$) for glaucine. Calibration curves for quaternary alkaloids were obtained from 3 to 20 μg/ml. The regression equations were as follows: $y=6.630x+2.185$ ($r=0.999$) for columbamine. $y=1.947x-1.089$ ($r=0.999$) for palmatine, $y=3.462x-1.729$ ($r=0.999$) for dehydrocorydaline. $y=5.388x-2.199$ ($r=0.999$) for berberine and $y=6.715x+7.982$ ($r=0.999$) for coptisine, where y is the peak height (mm) of each compound and x is the concentration (μg/ml) of each compound.

Recovery Test

To determine the recovery rates in the extraction procedure, corydaline (**4**) and dehydrocorydaline (**9**) were selected as representatives of tertiary and quaternary alkaloids. Known amounts were added to the dry powder of crude drug and the content of each alkaloid was determined by the present IP-HPLC methods.

After the extraction, the samples were assayed by the IP-HPLC methods. The recovery test was repeated five times. The results (Table I) show that IP-HPLC methods for both tertiary and quaternary alkaloids can be satisfactorily used for the quantitative determination of the alkaloids in crude drugs.

Determination of Individual Alkaloids

Each alkaloid in three samples of Chinese Corydalis Tuber was determined by the present methods (Tables II, III).

The results indicate that glaucine and corydaline among tertiary alkaloids and dehydrocorydaline among quaternary ones are the main components. There was a considerable variation in the contents of alkaloids among the samples. The data for quaternary alkaloids obtained by this method were in good accord with those obtained using the fluorescence detector (Table III).

TABLE I. Recovery of Added Tertiary and Quaternary Alkaloids

Compound	Added (mg)	Recovery ($n=5$)		
		(mg)	(%)	C.V. (%)
Corydaline	0.640	0.639	99.8	2.78
Dehydrocorydaline	0.825	0.829	100.3	1.91

C.V., coefficient variation.

TABLE II. Contents of Tertiary Alkaloids in Corydalis Tuber

Sample	Determined by UV detector (%)			
	4H Col	Gla	4H Pal	Cor
1	0.049	0.058	0.039	0.033
2	0.072	0.128	0.077	0.083
3	0.097	0.193	0.130	0.148

4H Col, tetrahydrocolumbamine; Gla, glaucine; 4H, Pal, tetrahydropalmatine; Cor, corydaline.

TABLE III. Contents of Quaternary Alkaloids in Corydalis Tuber (UV and Fluorescence Detectors)

Sample	Determined by UV detector (%)					Determined by fluorescence detector (%)				
	Col	Cop	Pal	Ber	Deh	Col	Cop	Pal	Ber	Deh
1	0.033	0.087	0.108	0.006	0.160	—	0.089	0.107	0.006	0.156
2	0.030	0.121	0.111	0.008	0.194	—	0.119	0.114	0.008	0.196
3	0.022	0.152	0.135	0.007	0.172	—	0.148	0.133	0.008	0.170

Col, columbamine; Cop, coptisine; Pal, palmatine; Ber, berberine; Deh, dehydrocorydaline.

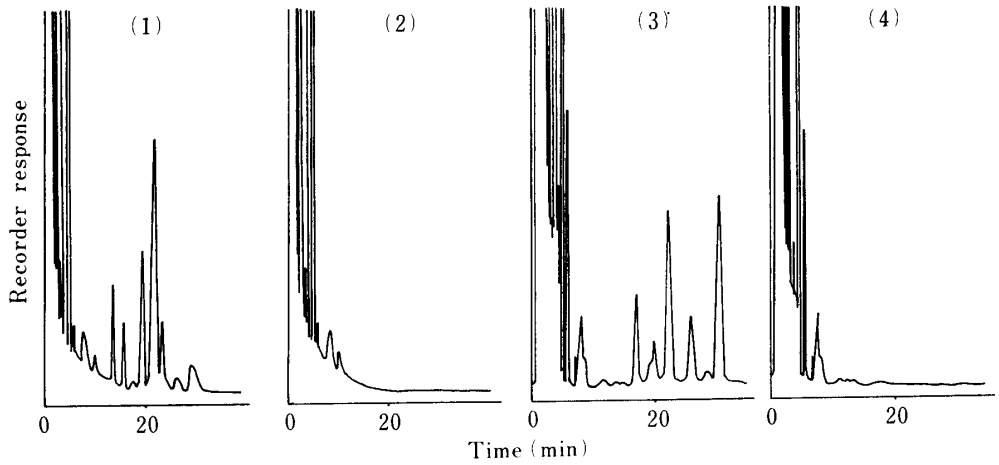


Fig. 5. Chromatograms of Anchusan-to Dry Extract and Corydalis Tuber-Deficient Anchusan-to Dry Extract

(1) Tertiary alkaloids in Anchusan-to dry extract.
(2) Tertiary alkaloids in Corydalis Tuber-deficient Anchusan-to dry extract.
(3) Quaternary alkaloids in Anchusan-to dry extract.
(4) Quaternary alkaloids in Corydalis Tuber-deficient Anchusan-to dry extract.

The present two methods were also employed to estimate the contents of tertiary and quaternary alkaloids in an oriental pharmaceutical preparation to assess their suitability for quality control of such preparations. Anchusan-to dry extract was selected and analyzed as an example of a prescription which contains Corydalis Tuber as a component. As shown in Fig. 5, it was found that other crude drugs did not interfere with the determination of tertiary and quaternary alkaloids from Corydalis Tuber in Anchusan-to dry extract on the chromatograms.

Thus, these IP-HPLC methods are simpler and more accurate than previous methods and appear to be suitable for the simultaneous determination of tertiary or quaternary alkaloids in Corydalis Tuber and an oriental pharmaceutical preparation.

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