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Simultaneous Determination of Berberine, Palmatine and Coptisine in Crude Drugs and Oriental Pharmaceutical Preparations by Ion-Pair High-Performance Liquid Chromatography[1]

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A new, simple method using ion-pair high-performance liquid chromatography was developed for simultaneous determination of berberine, palmatine and coptisine in Coptidis Rhizoma, Phellodendri Cortex and oriental pharmaceutical preparations containing them. A reversed-phase system of ODS-silica gel with a mixture of sodium lauryl sulfate, 0.1 N tartaric acid, methanol and acetonitrile (0.5: 49.5: 10: 40) as the mobile phase was used. The three components extracted with the mobile phase could be completely separated within 15 min.

The detection limits for the three components were 15 ng at a signal-to-noise ratio of 5: 1. The results obtained with an ultraviolet detector were in good accord with those obtained with a fluorescence detector.

Keywords——ion-pair high-performance liquid chromatography; Coptidis Rhizoma; Phellodendri Cortex; berberine; palmatine; coptisine; quantitative analysis of crude drugs

Coptidis Rhizoma and Phellodendri Cortex are present as constituents in a number of oriental pharmaceutical preparations. The active components in these crude drugs, such as berber ine (Ber), palmatine (Pal) and coptisine (Cop), are usually called berberine-type alkaloids. Although various analytical methods[2] for them have been published, most of these methods are restricted to the analysis of only total berberine-type alkaloids. In recent years, several methods for determination of the amounts of individual components have been reported; thin-layer chromatography densitometry[3] and high-performance liquid chromatography (HPLC) on a starch gel column (TSK gel LS-170).[4] These methods, however, seemed to be unsuitable for the routine analysis of berberine-type alkaloids because the former method is inaccurate and the latter method gives inadequate separation between Cop and Pal. In 1980, Akada *et al.*[5] applied ion-pair HPLC to separate and quantify berberine and acrinol in pharmaceutical preparations. However, this ion-pair HPLC could not separate individual berberine-type alkaloids. Therefore, we hoped to find a method to separate completely individual berberine-type alkaloids in crude drugs and oriental pharmaceutical preparations, and we established a rapid and simple ion-pair HPLC procedure using ODS-silica gel. The results obtained by this method with a UV detector were in good accord with those obtained with a fluorescence detector.

Experimental

Materials and Reagents——Berberine hydrochloride purchased from Alps Pharm. Co. (Gifu-ken) was used. The purified palmatine hydrochloride and coptisine iodide were kindly provided by Dr. H. Itokawa (Tokyo College of Pharm.) and Dr. T. Hayashi (Koshiro Chuji-Shoten Co., Ltd.), respectively. Dry extracts of various oriental pharmaceutical preparations (Ohrento 黄連湯, Sanohshashinto 三黄瀉心湯, Shichimotsukokato 七物降下湯, Seishoekkito 清暑益気湯) were prepared in our laboratories.

High-Performance Liquid Chromatography——A Hitachi LC 633A equipped with a Hitachi 200-10 spectrophotometer (wavelength 345 nm) as a detector was used. A stainless-steel column (15 cm × 4 mm I.D.) was packed with ODS-silica gel (TSK gel LS-410, 5 μm, Toyo Soda Co., Ltd.). The mobile phase was

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a mixture of sodium lauryl sulfate (SLS), 0.1 N tartaric acid, methanol and acetonitrile (0.5: 49.5: 10: 40). The flow rate of the mobile phase was 1.5 ml/min at room temperature.

Assay Procedure——About 0.5 g of dry powder of each crude drug was weighed accurately, placed in 30 ml of the mobile phase and refluxed on a water bath at 85°C for 15 min. This solution was centrifuged and decanted. The residue was washed twice with 20 ml of the mobile phase. The extract and washings were put in a 100 ml measuring flask and made up to 100 ml with the mobile phase. The solution (20 μl) was injected into the HPLC. The contents of Ber, Pal and Cop in crude drugs were calculated by comparison of the peak heights of the samples with those of authentic standards.

Results and Discussion

ODS-silica gel (TSK gel LS-410) was used for ion-pair HPLC. Various mobile phase systems were evaluated to achieve satisfactory separation of Ber, Pal and Cop in crude drugs and oriental pharmaceutical preparations. A mixture of SLS, 0.1 N tartaric acid, methanol and acetonitrile (0.5: 49.5: 10: 40) was found to be the best. The chromatograms of Ber, Pal and Cop in crude drugs and oriental pharmaceutical preparations are shown in Fig. 1 and Fig. 2, respectively.

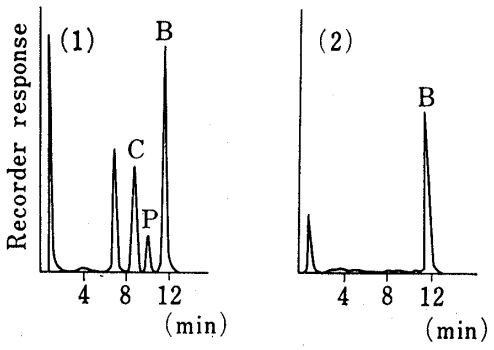

Fig. 1. Chromatograms of Berberine-Type Alkaloids in Coptidis Rhizoma and Phellodendri Cortex

Chromatograms: (1)=Coptidis Rhizoma, (2)=Phellodendri Cortex peaks: C=coptisine, P=palmatine, B=berberine.

Calibration curves for each berberine-type alkaloid was obtained from 5.0 to 50.0 μg/ml. The regression equations were as follows: $y=2.988x+1.702$ ($r=0.999$) for Ber, $y=2.333x+1.121$ ($r=0.999$) for Pal and $y=3.016x+0.788$ ($r=0.999$) for Cop, where y is the peak height (mm) of each compound and x is the concentration (μg/ml) of each compound. The detection limits for the three components were 15 ng at a signal-to-noise ratio of 5: 1. For the recovery tests, known amounts of Ber, Pal and Cop was added to Coptidis Rhizoma (Table II), in which the content of each berberine-type alkaloid had already been determined by this HPLC ptocedure. After extraction as described above, the sample was assayed by HPLC procedure. After extraction as described above, the sample was assayed by HPLC as well. The recovery tests were repeated five times and the results are shown in Table I.

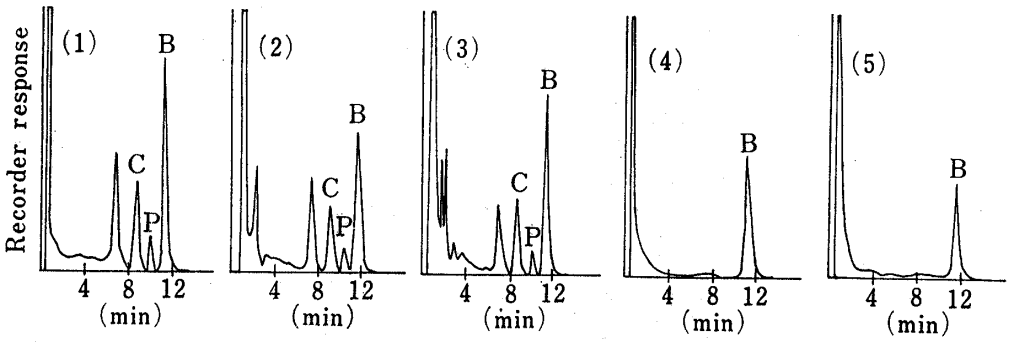

Fig. 2. Chromatograms of Berberine-Type Alkaloids in Various Oriental Pharmaceutical Preparations

Chromatograms: (1)=Ohrento, (2)=Sanohshashinto, (3)=Hangeshashinto, (4)=Shichimotsukokato, (5)=Seishoekkito.
Peaks: C=coptisine, P=palmatine, B=berberine.

Based on these experiments, this new procedure was applied to the analysis of crude drugs and oriental pharmaceutical preparations. The results are shown in Table II and Table III.

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Table I. Recovery of Added Berberine-Type Alkaloids

Berberine-type alkaloids	Added mg	Recovery ($n=5$)		
		mg	%	CV(%)
Coptisine	0.065	0.066	101.5	3.01
	0.126	0.126	100.0	2.25
	0.189	0.190	100.5	1.62
Palmatine	0.011	0.010	90.9	3.54
	0.022	0.023	104.5	3.32
	0.033	0.034	103.0	2.97
Berberine	0.315	0.314	99.7	2.23
	0.630	0.630	100.0	1.45
	0.945	0.946	100.1	1.14

Table II. Determination of Berberine-Type Alkaloids in Crude Drugs (UV and Fluorescence Detectors)

Sample	UV (%)				Fluorescence (%)			
	Cop	Pal	Ber	Total	Cop	Pal	Ber	Total
Coptidis Rhizoma	1.21	0.16	5.77	7.14	1.20	0.16	5.70	7.06
	1.20	0.15	5.75	7.10	1.20	0.16	5.75	7.11
	1.23	0.15	5.82	7.20	1.21	0.15	5.80	7.16
	1.25	0.16	5.88	7.29	1.23	0.15	5.85	7.23
	1.23	0.16	5.80	7.19	1.22	0.16	5.80	7.18
$\bar{X}_5$	1.22	0.16	5.80	7.18	1.21	0.16	5.78	7.15
Phellodendri Cortex	0	Trace	2.77	2.77	0	Trace	2.66	2.66
	0	Trace	2.76	2.76	0	Trace	2.60	2.60
	0	Trace	2.76	2.76	0	Trace	2.61	2.61
	0	Trace	2.74	2.74	0	Trace	2.63	2.63
	0	Trace	2.79	2.79	0	Trace	2.70	2.70
$\bar{X}_5$	0	Trace	2.76	2.76	0	Trace	2.64	2.64

Table III. Determination of Berberine-Type Alkaloids in Various Oriental Pharmaceutical Preparations (UV and Fluorescence Detectors)

Sample	UV (%)				Fluorescence (%)			
	Cop	Pal	Ber	Total	Cop	Pal	Ber	Total
1	0.096	0.020	0.475	0.591	0.095	Trace	0.472	0.567
2	0.432	0.083	1.982	2.497	0.422	Trace	1.986	2.408
3	0.067	0.009	0.291	0.367	0.066	Trace	0.294	0.360
4	0	Trace	0.039	0.039	0	Trace	0.040	0.040
5	0	Trace	0.099	0.099	0	Trace	0.100	0.100

Samples: 1=Ohrento, 2=Sanohshashinto, 3=Hangeshashinto, 4=Shichimotsukokato, 5=Seishoekkito.

To confirm the reliability of the data obtained with the UV detector, 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 (Table II and Table III). Calibration curves of each compound with the fluorescence detector were obtained from 5.0 to 50.0 μg/ml. The regression equations were as follows: $y=0.790x+1.174$ ($r=0.999$) for Ber, $y=0.458x+0.709$ ($r=0.999$) for Pal and $y=4.196x+0.640$ ($r=0.999$) for Cop. The analytical results for each compound in crude drugs and oriental pharmaceutical preparations obtained with the

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fluorescence detector are shown in Table II and Table III. The data obtained with the UV detector were in good accord with those obtained using the fluorescence detector.

From these results, it was found that the other crude drugs did not interfere with the determination of each berberine-type alkaloid in five oriental pharmaceutical preparations. This ion-pair HPLC method is simpler and more accurate than previous methods, and is suitable for the simultaneous determination of Ber, Pal and Cop in crude drugs and oriental pharmaceutical preparations.

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References and Notes

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