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A Simultaneous Determination of Norephedrine, Pseudoephedrine, Ephedrine and Methylephedrine in Ephedrae Herba and Oriental Pharmaceutical Preparations by Ion-Pair High-Performance Liquid Chromatography

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A new, simple and rapid analytical method using ion-pair high-performance liquid chromatography was developed for simultaneous determination of norephedrine, pseudoephedrine, ephedrine and methylephedrine in Ephedrae Herba and oriental pharmaceutical preparations. A reversed-phase system consisting of an ODS chemically bonded silica gel column and a mixture of water, acetonitrile, sodium dodecyl sulfate and phosphoric acid (65 : 35 : 0.5 : 0.1) as the mobile phase was used. The four components extracted with the mobile phase could be completely separated within 15 min.

The detection limits for the four components were 20 ng at a signal-to-noise ratio of 3 : 1.

Keywords——ion-pair high-performance liquid chromatography; Ephedrae Herba; ephedrine; pseudoephedrine; norephedrine; methylephedrine; crude drugs

The oriental crude drug, Ephedrae Herba (Mao), is very well known and is contained in various oriental pharmaceutical preparations. The sympathomimetic amines in this crude drug, such as ephedrine, pseudoephedrine and homologous compounds, are usually called ephedrine alkaloids. Since the content ratio of the ephedrine alkaloids differs according to the species, and their pharmacological activities also differ,[1] the quantitative analysis of individual ephedrine alkaloids is important in order to evaluate the quality of Ephedrae Herba.

Although a number of methods for the quantitative analysis of these alkaloids have been reported, including the copper complex method,[2] thin-layer chromatographic (TLC) methods,[3] gas liquid chromatographic (GLC) method,[4] ^{13}C-nuclear magnetic resonance (NMR) method,[5] high-performance liquid chromatographic (HPLC) methods using silica gel as the stationary phase[6] and isotachophoresis,[7] most of these methods seem to be unsuitable for routine and detailed analysis because of the need for time-consuming pretreatments such as extraction and/or derivation, and the resolution is limited to three analogues at most. Therefore, a simplified and accurate method is required.

In recent years, the ion-pair reversed-phase chromatographic method has become a useful technique for the analysis of berberine alkaloids[8] and aconitine alkaloids[9] in crude drugs. In this paper, we report the development of a rapid and simple method for simultaneous determination of norephedrine, pseudoephedrine, ephedrine and methylephedrine by utilizing the ion-pair technique on an ODS chemically bonded silica gel column. It was possible to apply this method not only to the crude drug but also to some oriental pharmaceutical preparations containing various crude drugs.

Experimental

Materials and Reagents——Ephedrine hydrochloride, norephedrine hydrochloride and methylephedrine hydrochloride purchased from Fuji Chemical Industries Ltd. were used. Pseudoephedrine isolated from Ephedrae Herba by a conventional method was purified by repeated recrystallization from ether before use. Dry extracts of various oriental pharmaceutical preparations (Kakkon-to 葛根湯, Mao-to 麻黄湯, Shoseiryu-to 小青龍湯, Daiseiryu-to 大青龍湯) and Ephedrae Herba-lacking preparations were prepared in our laboratory.

High-Performance Liquid Chromatography——A Hitachi model 200-10 spectrophotometer (wavelength 210 nm) was used as a detector. A stainless-steel column (15 cm × 4 mm i.d.) was packed with ODS chemically bonded silica gel (TSK gel LS-410, 5 μm, Toyo Soda Co., Ltd.). The mobile phase was a mixture of water, acetonitrile, sodium dodecyl sulfate (SDS) and phosphoric acid (65 : 35 : 0.5 : 0.1). The eluant flow rate was 1 ml/min at 50 °C.

Assay Procedure——About 1.0 g of the dry powder of each crude drug was weighed accurately, added to 30 ml of the mobile phase and refluxed on a water bath at 85 °C for 15 min. This solution was then centrifuged and decanted. The residue was washed with two 10 ml portions of the mobile phase. The extract and washings were placed in a 50 ml volumetric flask and diluted to 50 ml with the mobile phase. Ten μl of this solution was injected into the HPLC. The norephedrine, pseudoephedrine, ephedrine and methylephedrine contents in Ephedrae Herba were calculated from the peak height ratio.

Results and Discussion

Conditions for the Determination of Ephedrine Alkaloids

The elution parameters such as the organic component concentration and counter-ion concentration were varied to find the optimum elution conditions on an ODS chemically bonded silica gel column (TSK gel LS-410).

When methanol was used as an organic component of the mobile phase, norephedrine, ephedrine and methylephedrine were eluted with the same capacity factor. Using acetonitrile as an organic component, good separation of the four ephedrine alkaloids was obtained, and was best at 35% acetonitrile (Fig. 1). Fig. 2 shows the effect of the counter-ion (SDS) concentration on the capacity factor for each ephedrine alkaloid. As the concentration of SDS

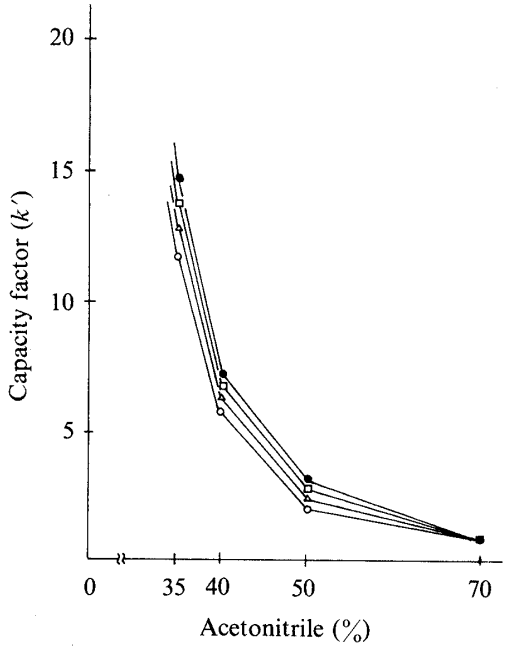

Fig. 1. Effect of Acetonitrile Concentration on Capacity Factor (k')

Solutes: ●, methylephedrine; △, ephedrine; □, pseudoephedrine; ○, norephedrine.
Column: TSK GEL LS-410, 150 mm × 4 mm i.d.
Flow rate: 1 ml/min. Temperature: 50 °C.
The mobile phase: Mixture of water and acetonitrile containing 0.5% SDS and 0.1% phosphoric acid.

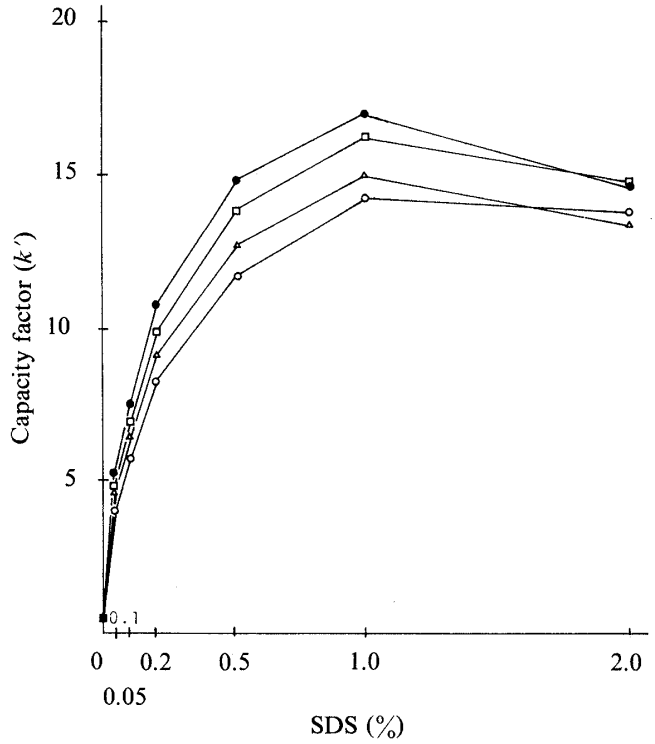

Fig. 2. Effect of SDS Concentration on Capacity Factor (k')

Solutes: ●, methylephedrine; △, ephedrine; □, pseudoephedrine; ○, norephedrine.
Column: TSK GEL LS-410, 150 mm × 4 mm i.d.
Flow rate: 1 ml/min. Temperature: 50 °C.
The mobile phase: Mixture of water and acetonitrile containing 0.1% phosphoric acid.

TABLE I. Effect of Extraction Time and Extraction Solvent

Compounds	Time (min)	Extraction solvent		
		Mobile phase (%)	Methanol (%)	Water (%)
Ephedrine	15	0.660	0.578	0.577
	30	0.658	0.578	0.561
	60	0.674	0.581	0.558
	120	0.647	0.577	0.552
Pseudo-ephedrine	15	0.571	0.484	0.490
	30	0.567	0.485	0.474
	60	0.580	0.487	0.479
	120	0.559	0.472	0.471

was increased, the ephedrine alkaloids started to be retained on the column, and they were separated clearly at 0.5% SDS. The selectivity was, however, reduced at 2.0% SDS. Phosphoric acid was added to the mobile phase to avoid tailing of peaks caused by the influence of the silanol groups.

As the extraction solvent, the mobile phase was also examined besides water and methanol, which were conventionally employed. The results are shown in Table I. Whichever solvent was used, the amount extracted remained constant after 15 min, but the mobile phase was more efficient in the extraction of the ephedrine alkaloids from Ephedrae Herba than either water or methanol. This is considered to be a result of suppression of the adsorption of

Table II. Comparison of Analytical Values for Ephedrine Alkaloids in Ephedrae Herba using Two Different Extraction Methods

Compounds	Mobile phase (%)	Ether (%)
Norephedrine	0.021	0.021
Pseudoephedrine	0.556	0.551
Ephedrine	0.623	0.615
Methylephedrine	0.101	0.099
Total	1.301	1.286

Table III. Recovery of Added Ephedrine Alkaloids

Compounds	Added mg	Recovery ($n=5$)		
		mg	%	cv (%)
Norephedrine	0.149	0.151	101.3	2.31
	0.298	0.297	99.7	1.54
	0.446	0.453	101.6	0.96
Pseudoephedrine	3.276	3.286	100.3	2.04
	6.552	6.611	100.9	1.20
	10.733	10.765	100.3	0.38
Ephedrine	2.945	2.945	100.0	1.29
	5.894	5.855	99.4	0.34
	8.836	8.863	100.3	0.24
Methylephedrine	0.386	0.385	99.7	3.92
	0.773	1.777	100.5	3.08
	1.159	1.156	99.7	2.47

the ephedrine alkaloids onto the plant tissue because of the formation of ion-pairs between these alkaloids and the counter-ion.

Since the extract with the mobile phase was injected into HPLC directly in this method, other components in Ephedrae Herba may interfere with the chromatogram. To confirm the reliability of this method, only the alkaloid fraction which had been repeatedly extracted from Ephedrae Herba by 0.1 N hydrochloric acid, made alkaline and partitioned with ether was injected into the HPLC. As shown in Table II, the analytical values for each alkaloid were in good accordance with those obtained by this method. It was therefore proved that the quantitation of ephedrine alkaloids by this method was not affected by other components from Ephedrae Herba.

The calibration curve for each ephedrine alkaloid was obtained from 20 to 400 μg/ml. The regression equations were as follows: $y=0.836\,x+0.001$ ($r=0.999$) for norephedrine, $y=0.820\,x+0.003$ ($r=0.999$) for pseudoephedrine, $y=0.661\,x+0.002$ ($r=0.999$) for ephedrine and $y=0.601\,x+0.001$ ($r=0.999$) for methylephedrine, where y is the peak height (mm) for each compound and x is the concentration (μg/ml) of each compound. The detection limits for the four components were 20 ng at a signal-to-noise ratio of 3 : 1. To determine the recovery of the extraction procedure, known amounts of norephedrine, pseudoephedrine, ephedrine and methylephedrine were added to Ephedrae Herba. The results are shown in Table III.

TABLE IV. Ephedrine Alkaloids Contents in Ephedrae Herba

Sample	Norephedrine (%)	Pseudoephedrine (%)	Ephedrine (%)	Methylephedrine (%)	Total (%)
China 1	0.021	0.556	0.623	0.101	1.301
2	0.142	0.361	1.396	0.069	1.968
3	0.040	0.963	0.620	0.057	1.680
4	0.039	0.295	0.838	0.093	1.265
5	0.032	0.352	0.845	0.086	1.492
6	0.033	0.654	0.719	0.086	1.492
7	0.034	0.384	0.937	0.134	1.489
8	0.023	0.298	0.725	0.087	1.126
Pakistan	0.027	0.187	0.526	0.031	0.771
Russia	0.018	0.897	0.917	0.032	1.864
Cultivated sample (Saitama)	0.071	0.074	0.695	0.041	0.881

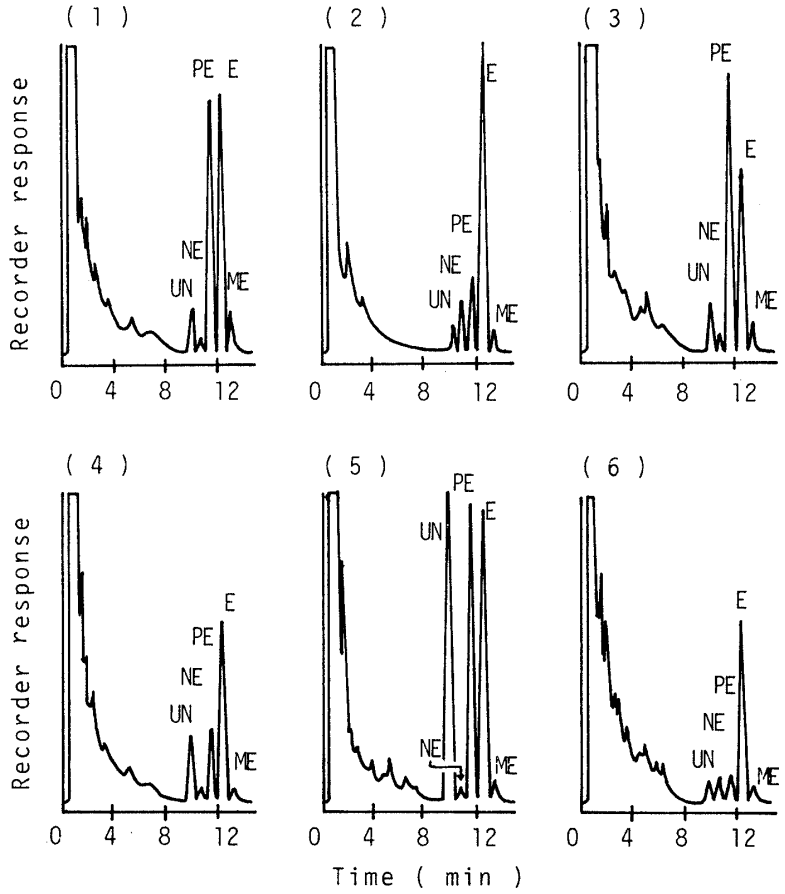

Fig. 3. Chromatograms of Ephedrine Alkaloids in Ephedrae Herba

Chromatograms: (1) China 1; (2) China 2; (3) China 3; (4) Pakistan; (5) Russia; (6) cultivated sample (Saitama).
Peaks: UN=unknown; NE=norephedrine; PE=pseudoephedrine; E=ephedrine; ME=methylephedrine.

Determination of Individual Ephedrine Alkaloids

The analytical results for Ephedrae Herba from various countries are shown in Table IV, and the chromatograms are shown in Fig. 3. Pseudoephedrine and ephedrine were the main components in these Ephedrae Herba samples and the ratio ranged from 1:9 to 3:2.

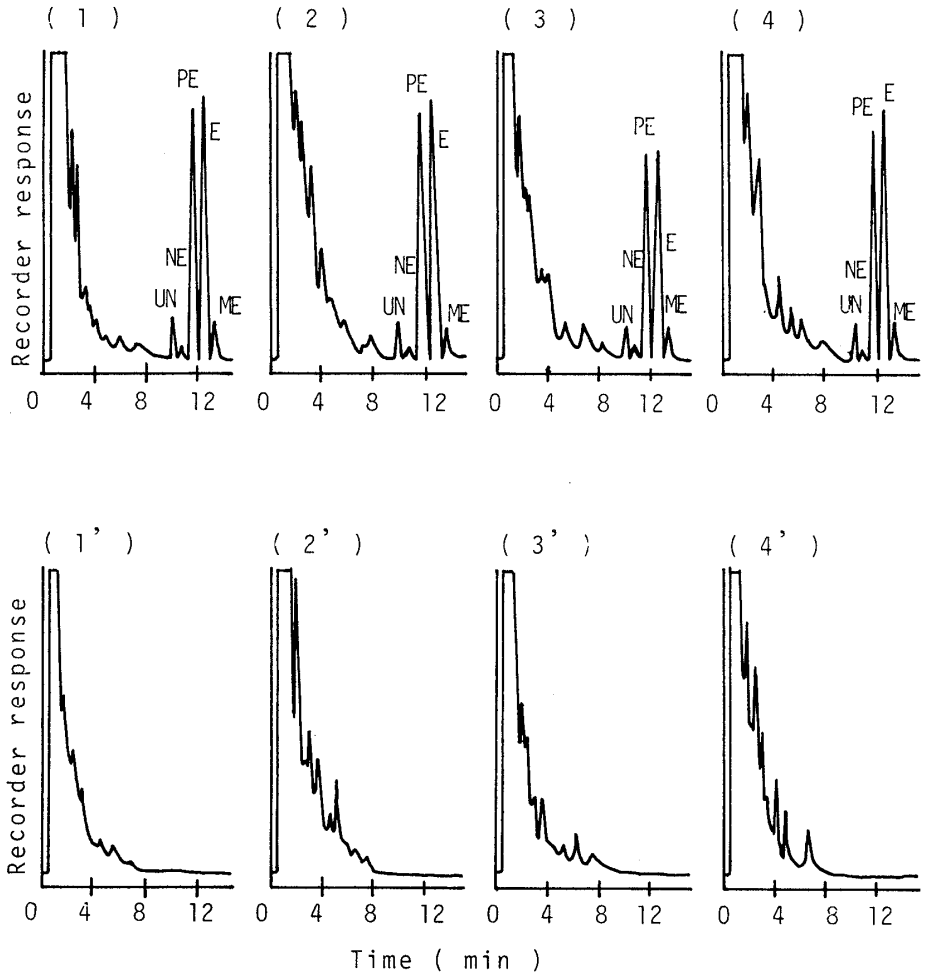

Fig. 4. Chromatograms of Oriental Pharmaceutical Preparations and the Ephedrae Herba-lacking Preparations

Chromatograms: (1) Kakkon-to; (1′) Ephedrae Herba-lacking Kakkon-to; (2) Mao-to; (2′) Ephedrae Herba-lacking Mao-to; (3) Shoseiryu-to; (3′) Ephedrae Herba-lacking Shoseiryu-to; (4) Daiseiryu-to; (4′) Ephedrae Herba-lacking Daiseiryu-to.
Peaks: UN=unknown; ME=norephedrine; PE=pseudoephedrine; E=ephedrine; ME=methylephedrine.

Although China 6 and China 7 showed the same analytical value for total alkaloids, the quality of these samples was dissimilar judging from the analytical value for each ephedrine alkaloid. It is of interest that Ephedrae Herba cultivated at our laboratory showed a low pseudoephedrine content compared with those from other countries.

This method was also applied to the oriental pharmaceutical preparations Kakkon-to, Mao-to, Shoseiryu-to and Daiseiryu-to (Fig. 4). As shown in Fig. 4, there was no interference at the retention times of the alkaloids on the chromatogram.

This new ion-pair technique developed for the simultaneous determination of the ephedrine alkaloids has several advantages. 1) The extraction is completed within 15 min using the mobile phase. 2) The extract from Ephedrae Herba with the mobile phase is injectable into the chromatograph without any pretreatment. 3) Only this method among the analytical methods reported so far can determine four alkaloids simultaneously. 4) It is possible to apply this technique to various oriental pharmaceutical preparations containing a number of crude drugs.

This rapid and simple method should be valuable for evaluating the quality of Ephedrae Herba, and appears to be suitable for routine quantitative analysis of oriental pharmaceutical

preparations.

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