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Short Communication

Determination of synephrine from Chinese medicinal drugs originating from *Citrus* species by ion-pair high-performance liquid chromatography

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ABSTRACT

A simple and precise method was established for the determnation of synephrine in Chinese crude drugs from *Citrus* plants using high-performance liquid chromatography with sodium dodecyl sulphate (SDS) as ion-pair reagent. Synephrine is known as a sympathomimetic drug contained in Chinese crude drugs from *Citrus* plants, namely Aurantii nobilis Pericarpium (Japanese name "Chinpi"), Aurantii fructus Immaturus ("Kijitsu"), "Kikoku" and "Seihi". The optimum conditions for extracting synephrine from these Chinese crude drugs was a 15-min reflux with water–acetonitrile–SDS–H_3PO_1 (65:35:0.5:0.1) as the mobile phase. Synephrine was eluted within 13 min without interference from co-existing components using an ODS column and SDS as an ion-pair reagent. The results revealed that synephrine was present at levels of 0.174–0.566% in the Chinese crude drugs, which were 1.3–2.2 times higher than those reported previously.

INTRODUCTION

Synephrine was developed originally as an orally applicable synthetic synpathomimetic drug and has been known to show actions such as vasoconstriction, raising blood pressure and bronchial muscle relaxation [1]. Since then, Stewart *et al.* [2] isolated synephrine as a hypertensive compound from the leaves and juice of tangerine. Subsequently, Kinoshita *et al.* [3] isolated it from Aurantii nobilis Pericarpium (Japanese name, "Chinpi"), Aurantii fructus Immaturus ("Kijitsu"), "Kikoku" and "Seihi" using bioassay for hot aqueous extracts of Chinese medicinal drugs with excised smooth muscle as a guideline and pointed out that synephrine is a common component found in Chinese Medicinal drugs originating from *Citrus* species. Quantitative analysis for synephrine revealed that the daily doses of 3–10 g of "Chinpi", "Kijitsu", "Kikoku" and "Seihi" contained 5–20 mg of synephrine, which would be a sufficient amount for exhibiting pharmacological effects. Further, Miyamoto and Furukawa [4] showed that "Chinpi" and synephrine had adrenergic β_2 activity. Hence the synephrine content is extremely important when considering pharmacological effects of these Chinese medicinal drugs and it is also assigned as one of standard components for quality evaluation [5].

Reports on the determination of synephrine include thin-layer chromatography densitometric

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analysis of hot aqueous extracts of Chinese crude drugs from *Citrus* species after treatment with an Amberlite column [3,6], high-performance liquid chromatography (HPLC) for 50% methanol extracts of "Kijitsu" after treatment with a cellulose ion-exchange column [5], HPLC with coulometric detection [7,8] and gas chromatography–negative-ion chemical ionization mass spectrometry [9]. However, these methods all employ complicated sample pretreatment and require long times.

In this study, we employed ion-pair HPLC and examined optimum conditions for extracting synephrine from "Chinpi". We also developed a simple, rapid and precise method for the determination of synephrine in Chinese crude drugs originating from *Citrus* species.

EXPERIMENTAL

Plant materials

"Chinpi" was derived from *Citrus unshiu* Markovich produced in Shikoku and "Kijitsu" from *C. natsudaidai* Hayata produced in China. "Kikoku" and "Seihi" were products from China but their original plants were unknown, as in a previous report [3]. We also conducted experiments on Aurantii Pericarpium ("Tohi"), derived from *C. aurantium* Linné var. *daidai* Makino, produced in China as "Tohi", which is an important crude drug although not a Chinese medicinal drug.

"Chinpi", "Kikoku", "Seihi" and "Tohi" were purchased from Matsuura Kanpo (Nagoya, Japan) and "Kijitsu" from Uchida Wakanyaku (Tokyo, Japan).

Apparatus and HPLC conditions

The HPLC system consisted of a CCPD pump, UV-8011 UV detector, CO-8010 column oven (Tosoh, Tokyo, Japan) and SIC Chromatocorder 12 integrator (System Instrument, Tokyo, Japan). A TSK gel ODS-120 T (particle diameter 5 μm) column (250 × 4.6 mm I.D.) (Tosoh) was used with a guard column (10 × 4.0 mm I.D.) (GL Sciences, Tokyo, Japan) packed with the same material. A Model M990 photodiode-array detector (Waters–Millipore, Milford, MA, USA) was used. Water–acetonitrile–sodium dodecyl sulphate–H_3PO_4 (65:45:0.5:0.1) adjusted to pH 2.6 was used as the mobile phase. The column temperature was maintained at 40°C and the flow-rate was 1.0 ml/min. The substances eluted were detected at a wavelength of 220 nm.

Reagents

DL-Synephrine [1-(4-hydroxyphenyl)-2-methylaminoethanol] (Sigma, St. Louis, MO, USA) was recrystallized from methanol. Sodium dodecyl sulphate (SDS) was purchased from Nacalai Tesque (Kyoto, Japan) and acetonitrile of special grade from Kanto Chemical (Tokyo, Japan).

Assay procedure

"Chinpi", "Kijitsu", "Kikoku", "Seihi" and "Tohi" were powdered and 500 mg of each drug were weighed accurately and subjected to a 15-min extraction by refluxing in 40 ml of the mobile phase on a water-bath. After cooling, the extract was centrifuged at 3000 *g* for 10 min and the supernatant was reserved while the residue was washed twice with 2 ml of the mobile phase. The supernatant together with the washings was made up to 50 ml with the mobile phase to serve as the test sample. After filtering this sample with a 0.45-μm membrane filter, a 10-μl volume was injected for HPLC. The amount present was calculated according to the calibration regression equation (see below).

Calibration

A calibration graph was prepared from peak areas obtained by injecting 10 μl of the compound for HPLC over the concentration range 20–50 μg/ml. The resulting calibration graph was linear. The regression equation was $y = 3.3160 \cdot 10^{-8}x + 2.61774 \cdot 10^{-3}$ ($r = 0.999$), where y is the concentration (mg/ml) and x is the peak area.

Solvent and time of extraction

Amounts of 500 mg of "Chinpi" were subjected to extraction with four solvents, namely methanol, methanol–water (50:50), water and the mobile phase, and the respective extracts were treated as described under *Assay procedure*. For methanol and methanol–water (50:50), each extract was made up to 50 ml, 10 ml were taken and concentrated under reduced pressure and a sample solution was prepared by addition of 10 ml of the mobile phase. Extraction was carried out for 15, 30, 45 and 60 min in order to compare synephrine contents quantitatively.

TABLE I

EFFECT OF EXTRACTION TIME AND EXTRACTION SOLVENT

Sample: "Chinpi".

Extraction time (min)	Synephrine content (%)			
	Mobile phase	Methanol	Methanol–water (50:50)	Water
15	0.408	0.350	0.363	0.345
30	0.398	0.365	0.376	0.354
45	0.388	0.373	0.370	0.352
60	0.382	0.375	0.363	0.356

RESULTS AND DISCUSSION

It was found that a 15-min extraction of "Chinpi" with the mobile phase showed the highest content of synephrine (Table I). The determination of synephrine in the various Chinese crude drugs was then carried out on the basis of the above extraction conditions.

Fig. 1 shows the chromatograms obtained. The peak for synephrine appeared at 13 min and there were no interfering peaks. For examination of a single synephrine peak in the respective chromatograms, we measured the three-dimensional chromatogram (contour chromatogram) using a photodiode-array detector. As shown in Fig. 2, synephrine in each Chinese crude drug exhibited maximum absorption at 222.5 and 274.5 nm, coinciding with those of the standard. No peaks due to impurities were present.

Table II gives the synephrine contents in the Chinese crude drugs as determined by the proposed method, together with values reported in the litera-

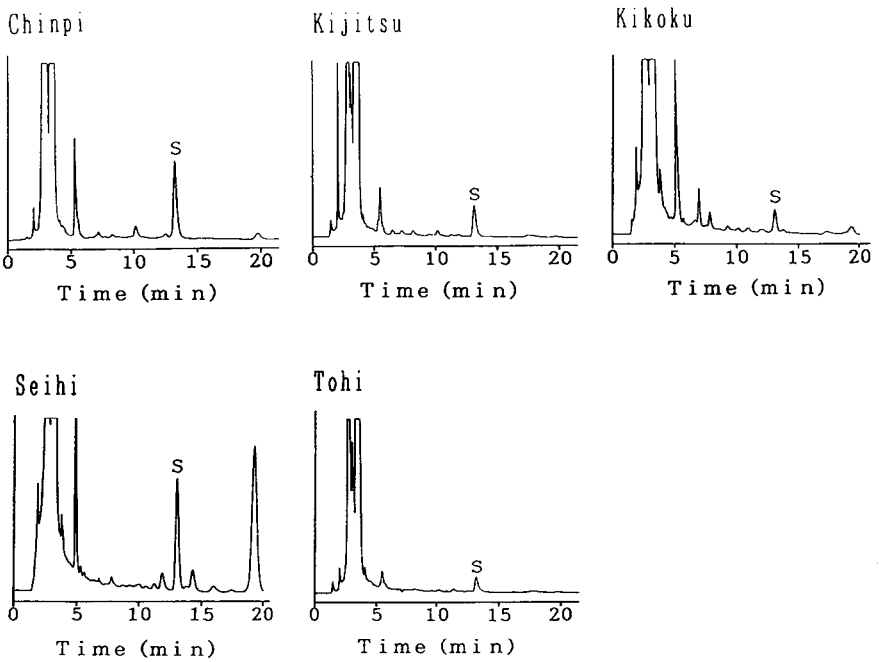

Fig. 1. Chromatograms of crude drugs. S = synephrine.

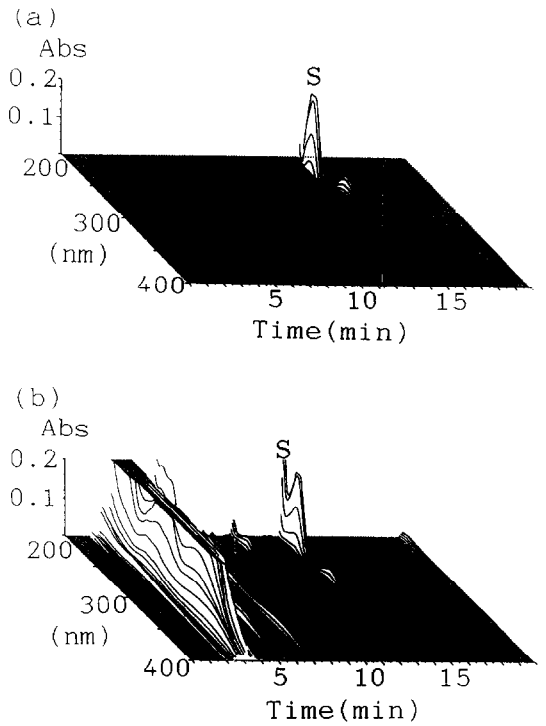

Fig. 2. Three-dimensional chromatograms: (a) standard synephrine; (b) "Chinpi". S = synephrine.

ture [3,5]. The highest content of synephrine was 0.566% in "Seihi", followed by 0.408% in "Chinpi", and the lowest value was 0.122% in "Tohi". The order of concentrations was the same as reported previously [3], but the individual concentrations were 1.3–2.5 times higher than those reported.

The daily consumption of synephrine calculated from the results of quantitative analysis amounted to 10–37 mg, the values at which pharmacological effects could be expected, as being pointed out by Kinoshita *et al.* [3], although the solvents used for extraction from Chinese crude drugs differed. The relative standard deviations were satisfactory (0.28–1.86%) and the method was very reliable. Further, the recovery rate of 93.0–107.0% after addition of a standard compound in a known amount was also satisfactory.

It is concluded that HPLC with SDS as the ion-pair reagent provides a simple, rapid and precise method for the determination of synephrine in Chinese medicinal drugs originating from *Citrus* species, giving higher quantitative values than those reported previously.

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TABLE II

SYNEPHRINE CONTENTS IN CHINESE CRUDE DRUGS

Crude drug	Synephrine content (%)	R.S.D. (%)[a]	Recovery (%)	Literature synephrine contents (%)
"Chinpi"	0.408	0.28	97.7	0.22 [3]
"Kijitsu"	0.335	0.90	93.0	0.21 [3]
				0.18 [5]
"Kikoku"	0.174	1.15	107.0	0.13 [3]
"Seihi"	0.566	0.37	104.7	0.26 [3]
"Tohi"	0.112	1.86	103.3	0.045 [3]

[a] Relative standard deviation (n = 5).