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Determination of baicalin and puerarin in traditional Chinese medicinal preparations by high-performance liquid chromatography

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ABSTRACT

HPLC methods for the determination of baicalin in Scutellariae Radix and puerarin in Puerariae Radix were established for the quality control of Chinese medicinal preparations containing these drugs. Ethyl paraben and methyl paraben were used as the internal standards for baicalin and puerarin, respectively. The samples were separated on a Cosmosil $5C_{18}$ column with 0.03% phosphoric acid–acetonitrile (79:21), 0.03% phosphoric acid–acetonitrile (87:13) and 2% acetic acid–methanol (79:21) as mobile phases at flow-rates of 1.0 ml/min. Very satisfactory and reproducible results were obtained within 25 min for baicalin and 50 min for puerarin. These methods were also applicable to other prescriptions containing Scutellariae Radix and Puerariae Radix such as Chair-Ger-Jie-Ji-Tang, Dang-Guei-Nian-Tong-Tang and San-Jong-Kuey-Jian-Tang.

INTRODUCTION

Traditional Chinese medicines were used for thousands of years in China because of the advantages of low toxicity and rare complications. Nowadays, the concentrated herbal preparations are very popular in Taiwan for their convenience of use, but whether there is the same efficacy between the traditional preparation and the concentrated type is still very difficult to establish. There are many variants among Chinese herbs. Therefore, it is very important to establish simple, convenient and efficient methods for the qualification of Chinese herbs and quality control of concentrated herbal preparations.

In Japan, since 1985 the Ministry of Health and Welfare has required that all concentrated herbal preparations submitted for inspection and registration should include a content analysis with at least two chemical components as markers [1]. In this study, we selected the preparations Chair-Ger-Jie-Ji-Tang, Dang-Guei-Nian-Tong-Tang and San-Jong-Kuey-Jian-Tang, which all contain Scutellariae Radix and Puerariae Radix in their prescriptions, and searched for the optimum conditions for the determination of baicalin (present in Scutellariae Radix) and puerarin (present in Puerariae Radix) in both standard decoction and commercially available concentrated herbal preparations. Scutellariae Radix is the root of *Scutellaria baicalensis* Georgi (Labiatae) and Puerariae Radix is the root of *Pueraria pseudohirsuta* Tang. et Wang. (Leguminosae). The structures of the two flavonoids are shown in Fig. 1.

Although a number of high-performance liquid chromatographic (HPLC) methods for the determination of Scutellariae Radix [2–7] and Puerariae Radix [8–11] have been reported, only two reports concerning the determination of baicalin or puerarin in preparations have been published [12–13]. In this study, we have developed rapid and simple HPLC methods for the determination of baicalin

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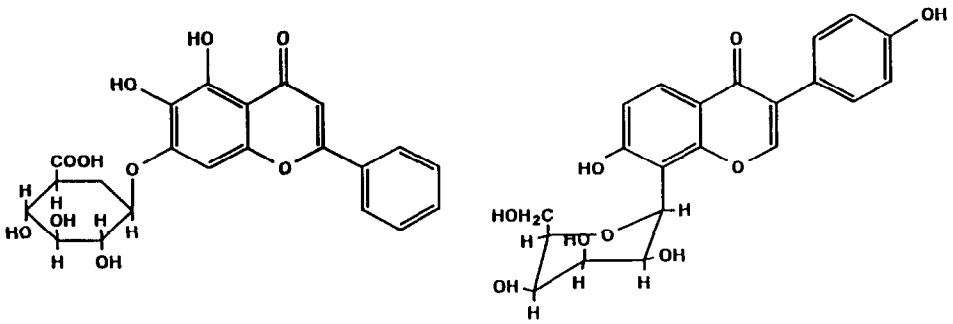

Fig. 1. Structures of the two marker components.

and puerarin that should be suitable for the routine quantitative analysis of concentrated herbal preparations and might also be applicable to some other preparations containing Scutellariae Radix and Puerariae Radix.

EXPERIMENTAL

Materials

According to the literature [14], the following materials listed are used to prepare each prescription: Chair-Ger-Jie-Ji-Tang: Bupleuri Radix 4.0 g, Puerariae Radix 4.0 g, Notopterygii Rhizoma 2.0 g, Angelicae Radix 2.0 g, Scutellariae Radix 3.0 g, Paeoniae Radix 3.0 g, Platycodi Radix 2.0 g, Gypsum Fibrosum 5.0 g, Glycyrrhizae Radix 2.0 g and Zingiberis Rhizoma 1.0 g; Dang-Guei-Nian-Tong-Tang: Angelicae Sinensis Radix 2.5 g, Anemarrhenae Rhizoma 2.5 g, Notopterygii Rhizoma 2.5 g, Artemisiae Capillaris Herba 2.5 g, Scutellariae Radix 2.5 g, Atractylodis Macrocephalae Rhizoma 2.5 g, Polyporus 2.5 g, Alismatis Rhizoma 2.5 g, Atractylodis Rhizoma 2.0 g, Ledebouriellae Radix 2.0 g, Puerariae Radix 2.0 g, Ginseng Radix 2.0 g, Sophorae Radix 1.0 g, Cimicifugae Rhizoma 1.0 g and Glycyrrhizae Radix 1.0 g; and San-Jong-Kuey-Jian-Tang: Angelicae Sinensis Radix 1.5 g, Paeoniae Radix 1.5 g, Bupleuri Radix 1.5 g, Scutellariae Radix 1.5 g, Coptidis Rhizoma 1.5, Forsythiae Fructus 1.5 g, Cortex Phellodendri 1.5 g, Anemarrhenae Rhizoma 1.5 g, Trichosanthis Radix 1.5 g, Platycodi Radix 1.5 g, Gentianae Radix 1.5 g, Puerariae Radix 1.5 g, Scirpi Rhizoma 1.5 g, Zedoariae Rhizoma 1.5 g, Laminariae Thallus 1.5 g, Sargassi Thallus 1.5 g, Cimicifugae Rhizoma 1.5 g, Zingiberis Rhizoma 1.5 g and Glycyrrhizae Radix 1.5 g.

For the concentrated herbal preparations, samples were obtained from three different manufacturers.

Chemicals and reagents

Reference standards of baicalin and puerarin were purchased from Nacalai Tesque (Kyoto, Japan). The internal standards ethyl paraben and methylparaben were obtained from Sigma (St. Louis, MO, USA).

All solvents used were of HPLC grade.

Liquid chromatography

The HPLC system was equipped with a Holochrome variable-wavelength UV detector (Gilson). Peak areas were calculated with a Shiunn Haw computing integrator. A stainless-steel column (150 mm × 4 mm I.D.) packed with ODS chemically bonded silica gel (Cosmosil $5C_{18}$, 5 μm), (Nalcalai Tesque) was used. The solvent systems were as follows: 0.03% phosphoric acid–acetonitrile (79:21) for baicalin in the three preparations; 0.03% phosphoric acid–acetonitrile (87:13) for puerarin in Chair-Ger-Jie-Ji-Tang and Dang-Guei-Nian-Tong-Tang and 2% acetic acid–methanol (79:21) for puerarin in San-Jong-Kuey-Jian-Tang. The analyses were carried out at a flow-rate of 1.0 ml/min, with UV detection at 270 nm for baicalin and 250 nm for puerarin. Pretreatment of the solvent with a solvent filter kit for degassing was carried out.

Sample preparation for HPLC

Calibration graphs. Baicalin and puerarin were accurately weighed and dissolved in 70% methanol to give various concentrations within the ranges 0.0625–0.1000 and 0.00625–0.05000 mg/ml, respectively. An appropriate amount of internal stadard was added to each solution to give concentrations of 1.0 mg/ml of ethyl paraben or 0.1 mg/ml of methyl paraben. Calibration graphs were plotted based on linear regression analysis of the peak-area ratios with concentration.

Standard decoction. Amounts of crude drug equivalent to a daily dose of each preparation were weighed and pulverized, a twentyfold mass of water was added and the mixture was boiled for more than 30 min to halve the original volume. After filtration, the filtrate was diluted with methanol to give a 70% methanol solution and a suitable amount of internal standard was then added to the solution to give concentrations of 1.0 mg/ml of ethyl paraben or 0.1 mg/ml of methyl paraben.

Concentrated herbal preparations. Samples of about 1.0 g of each concentrated herbal preparation obtained from three different factories were weighed accurately and extracted with 70% methanol (25 ml) for 30 min by using an ultrasonic bath. After extraction, the samples was filtered and diluted to 25 ml with the addition of internal standard to give concentrations of 1.0 mg/ml of ethyl paraben or 0.1 mg/ml of methyl paraben.

RESULTS AND DISCUSSION

HPLC conditions

Baicalin and puerarin are bioactive components and have been determined previously using reversed-phase systems [2–10, 12–13], so we chose an ODS column with 0.03% phosphoric acid–acetonitrile (79:21), 0.03% phosphoric acid–acetonitrile (87:13) or 2% acetic acid–methanol (79–21) as mobile phases for baicalin or puerarin in three preparations.

The peak purities of these two components were tested at two or three wavelengths. Baicalin was detected at 254, 270 and 280 nm and puerarin at 242 nm and 250 nm; no interferences were found.

For the selection of internal standards, we tried many comounds with structures similar to that of the marker, but many overlapped with other components in the preparations or the retention times were too long. Finally, methyl paraben was used as the internal standard for puerarin and ethyl paraben for baicalin, because these two compounds could be well resolved from other components in the complex preparations and their retention times are reasonable for routine analysis.

Calibration graphs for baiccalin and puerarin were obtained for the ranges of 0.0625–0.1000 and 0.00625–0.05000 mg/ml, respectively. The regression equations were $y = 1.234 - 0.161\ x$ ($r = 0.9994$) for baicalin, $y = 0.336 - 0.0046\ x$ ($r = 0.9998$) for puerarin in Chair-Ger-Jie-Ji-Tang and Dang-Guei-Nian-Tong-Tang and $y = 0.443 - 0.00025\ x$ ($r = 0.9997$) for puerarin in San-Jong-Kuey-Jian-Tang, where y is the peak-area ratio of the marker to the internal standard and x is the concentration of the marker.

Sample preparation

Traditional Chinese medicines are usually prepared by boiling with water. However, extraction of concentrated preparations with water may lead to difficult filtration, so 70% methanol was employed as the extraction solvent for concentrated herbal preparations in routine work.

The components of Chinese herbal preparation are very complex. In this study, blank solutions without Scutellariae Radix or Puerariae Radix were prepared and analysed, to monitor the absence of interference of other crude drugs with the marker component. The comparison of the chromatograms of baicalin and its blank solutions of three preparations (Figs. 2–4) shows that satisfactory separations were obtained. Figs. 5–7 show the analysis of puerarin and its blank solutions of three preparations. Two different mobile phase systems were used for the determination of puerarin; indeed, these preparations cannot be measured with the same mobile phase because some interferences in three blank solutions at the retention time of puerarin were detected.

Determination of baicalin and puerarin

The markers used for the quality control of concentrated herbal preparations from crude drugs to the concentrated type should be satisfy some characteristics: (1) soluble in water; (2) stable; (3) not destroyed by boiling; and (4) non-volatile. In this

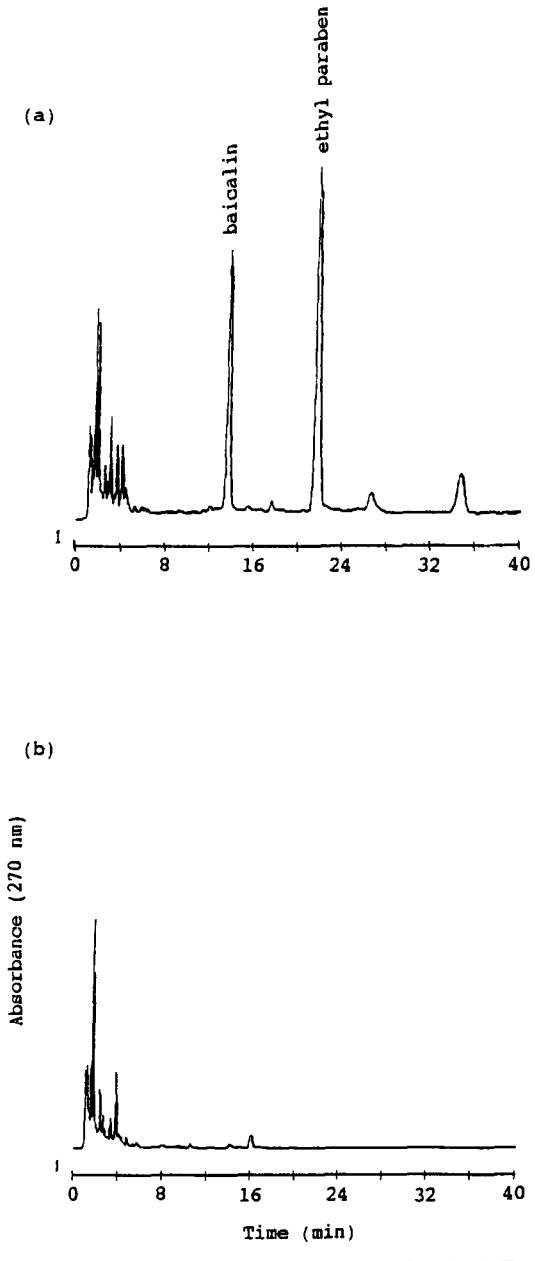

Fig. 2. Chromatogram of baicalin in Chair-Ger-Jie-Ji- Tang and its blank solution. Column, Cosmosil $5C_{18}$ (5 μm) (150 × 4 mm I.D.); mobile phase, 0.03% phosphoric acid–acetonitrile (79:21); flow-rate, 1.0 ml/min. (a) Standard decoction; (b) standard decoction without Scutellariae Radix.

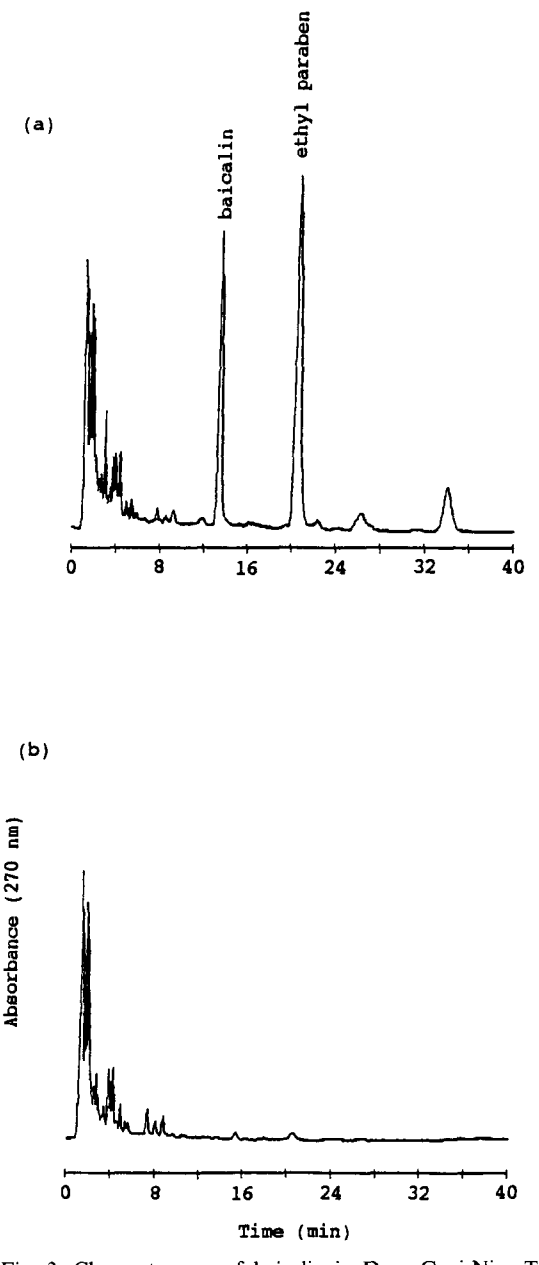

Fig. 3. Chromatogram of baicalin in Dang-Guei-Nian-Tong-Tang and its blank solution. Conditions as in Fig. 2. (a) Standard decoction; (b) standard decoction without Scutellariae Radix.

work we chose baicalin and puerarin as markers they both satisfy these characteristics and both have been used previously as marker components [1,15].

The quantitative results for the standard decoctions prepared in our laboratory and the purchased concentrated herbal preparations are given in Ta-

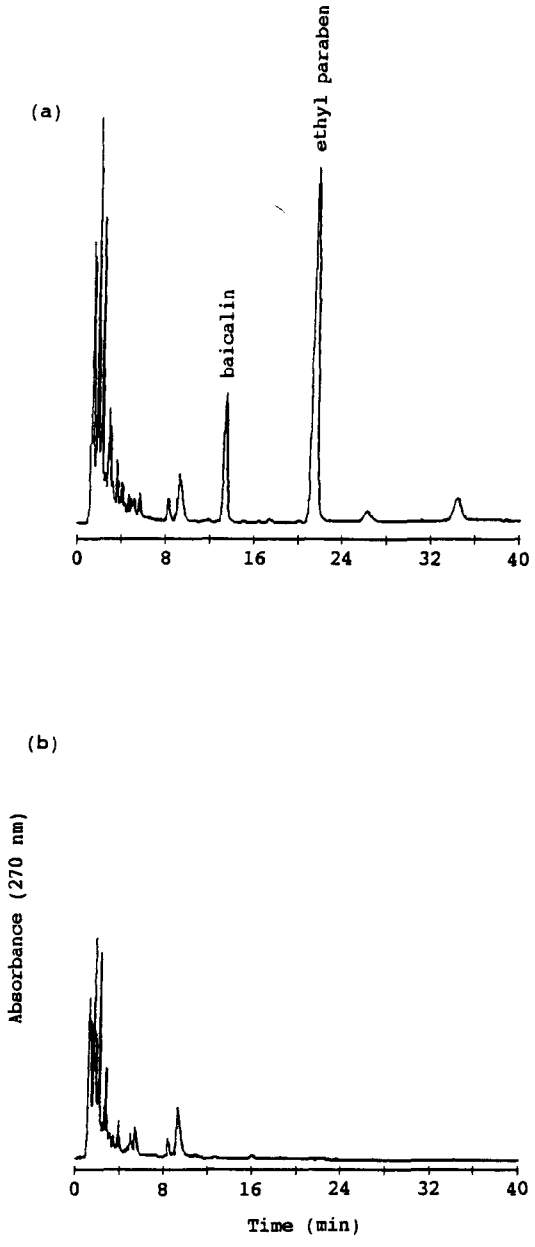

Fig. 4. Chromatogram of baicalin in San-Jong-Kuey-Jian-Tang and its blank solution. Conditions as in Fig. 2. (a) Standard decoction; (b) standard decoction without Scutellariae Radix.

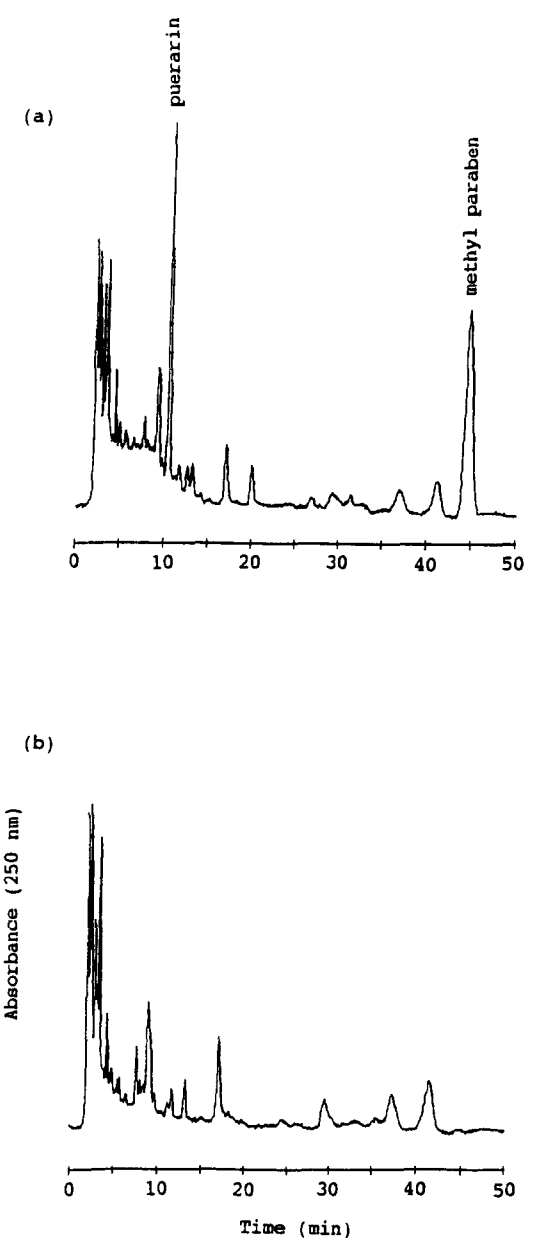

Fig. 5. Chromatogram of puerarin in Chair-Ger-Jie-Ji-Tang and its blank solution. Mobile phase, 0.03% phosphoric acid–acetonitrile (87:13); other conditions as in Fig. 2. (a) Standard decoction; (b) standard decoction without Puerariae Radix.

bles I–III. The daily dose was used for calculating the contents of the marker components in order to compare the products from different manufacturers.

The contents of the marker components in the standard decoctions were found to be much higher than those in concentrated herbal preparations, and there were differences among the products from dif-

TABLE I

CONTENTS OF MARKER COMPONENTS IN STANDARD DECOCTION AND CONCENTRATED HERBAL PREPARATIONS OF CHAIR-GER-JIE-JI-TANG

Sample[a]	Crude drug	Content of crude drug in a daily dose in preparation (g/g)	Marker component	Content of marker component in prescription		Marker component in crude drug (mg/g)
				mg/g	mg/day	
Standard decoction	Scutellariae Radix	3.0/30	Baicalin	3.21	96.24	30.08
	Puerariae Radix	4.0/30	Puerarin	0.18	5.32	1.33
Concentrated herbal preparation A	Scutellariae Radix	2.4/6	Baicalin	3.02	18.11	7.55
	Puerariae Radix	2.4/6	Puerarin	1.20	7.22	0.46
Concentrated herbal preparation B	Scutellariae Radix	2.5/6	Baicalin	5.14	30.81	12.32
	Puerariae Radix	2.0/6	Puerarin	0.31	1.84	0.77
Concentrated herbal preparation C	Scutellariae Radix	2.0/6	Baicalin	2.13	12.79	6.39
	Puerariae Radix	2.0/6	Puerarin	0.09	0.54	0.27

[a] A, B, C = concentrated herbal preparations from three different manufactures.

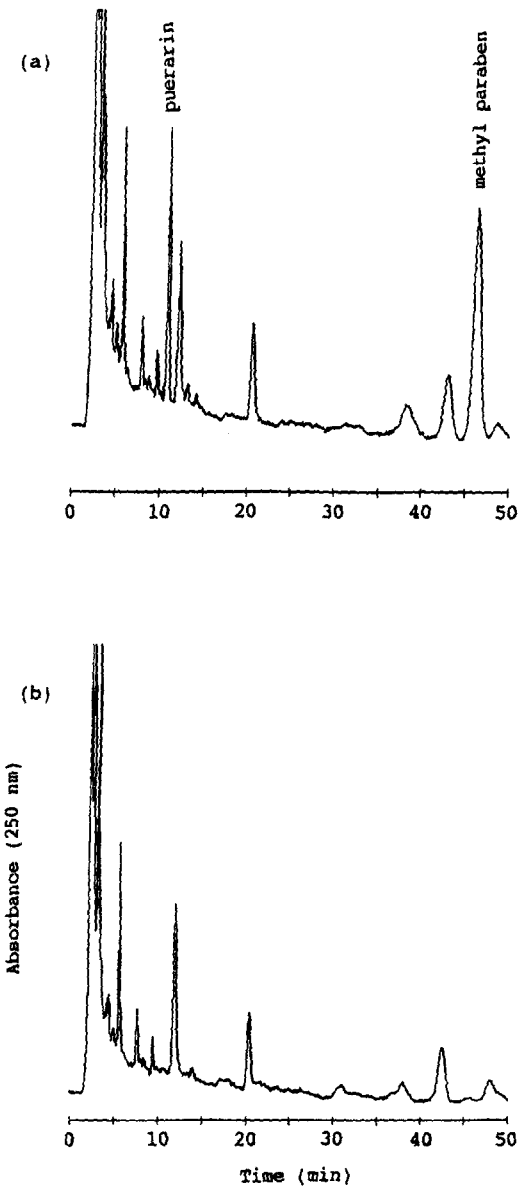

Fig. 6. Chromatogram of puerarin in Dang-Guei-Nian-Tong-Tang and its blank solution. Conditions as in Fig. 5. (a) Standard decoction; (b) standard decoction without Puerariae Radix.

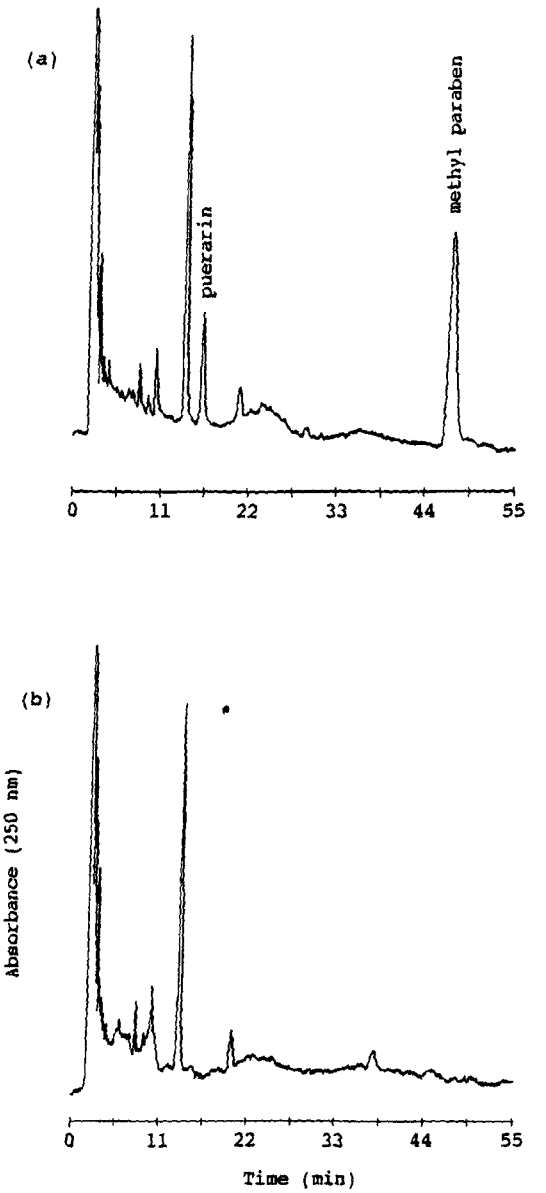

Fig. 7. Chromatogram of puerarin in San-Jong-Kuey-Jian-Tang and its blank solution. Mobile phase, 2% acetic acid–methanol (79:21); other conditions as in Fig. 1. (a) Standard decoction; (b) standard decoction without Puerariae Radix.

ferent companies. The standard decoctions were prepared by boiling with water and then filtering; the concentrated herbal preparations, in addition to boiling with water and filtering, were further concentrated and dried. Whether these latter processes affected the constitution of the products needs further investigation.

TABLE II

CONTENTS OF MARKER COMPONENTS IN STANDARD DECOCTION AND CONCENTRATED HERBAL PREPARATIONS OF DANG-GUEI-NIAN-TONG-TANG

Sample[a]	Crude drug	Content of crude drug in a daily dose in preparation (g/g)	Marker component	Content of marker component in prescription		Marker component in crude drug (mg/g)
				mg/g	mg/day	
Standard decoction	Scutellariae Radix	2.5/31	Baicalin	2.10	65.13	26.05
	Puerariae Radix	2.0/31	Puerarin	0.10	3.02	1.51
Concentrated herbal preparation A	Scutellariae Radix	1.0/6	Baicalin	0.23	1.37	1.37
	Puerariae Radix	0.5/6	Puerarin	0.08	0.49	0.97
Concentrated herbal preparation B	Scutellariae Radix	1.25/6	Baicalin	4.76	28.55	22.84
	Puerariae Radix	0.75/6	Puerarin	0.19	1.16	1.55
Concentrated herbal preparation C	Scutellariae Radix	2.5/6	Baicalin	3.86	23.15	9.26
	Puerariae Radix	1.0/6	Puerarin	0.07	0.39	0.39

[a] A, B, C = concentrated herbal preparations from three different manufacturers.

TABLE III

CONTENTS OF MARKER COMPONENTS IN STANDARD DECOCTION AND CONCENTRATED HERBAL PREPARATIONS OF SAN-JONG-KUEY-JIAN-TANG

Sample[a]	Crude drug	Content of crude drug in a daily dose in preparation (g/g)	Marker component	Content of marker component in prescription		Marker component in crude drug (mg/g)
				mg/g	mg/day	
Standard decoction	Scutellariae Radix	1.5/28.5	Baicalin	1.39	39.56	26.37
	Puerariae Radix	1.5/28.5	Puerarin	0.09	2.49	1.66
Concentrated herbal preparation A	Scutellariae Radix	1.5/6	Baicalin	0.92	5.53	3.68
	Puerariae Radix	0.5/6	Puerarin	0.11	0.66	1.32
Concentrated herbal preparation B	Scutellariae Radix	1.5/6	Baicalin	0.85	5.11	3.40
	Puerariae Radix	1.5/6	Puerarin	0.09	0.51	0.34
Concentrated herbal preparation C	Scutellariae Radix	2.4/6	Baicalin	0.56	3.34	1.39
	Puerariae Radix	0.6/6	Puerarin	0.03	0.20	0.33

[a] A, B, C = concentrated herbal preparations from three different manufacturers.

CONCLUSIONS

The proposed HPLC method is applicable to the quality control of concentrated herbal preparations containing Scutellariae Radix and Puerariae Radix. The advantages of the systems developed in this study are following: (1) the mobile phases are easy to prepare; (2) the isocratic elution requires simple equipment; (3) no pretreatment is required; and (4) quantification is effected with an internal standard. Therefore, these methods are simple, rapid and expedient for the routine quality control of Chinese herbal preparations.

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