Determination of Mangiferin, Jateorrhizine, Palmatine, Berberine, Cinnamic Acid, and Cinnamaldehyde in the Traditional Chinese Medicinal Preparation Zi-Shen Pill by High-Performance Liquid Chromatography

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Abstract

High-performance liquid chromatography is employed to determine the contents of six marker components such as mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde in the traditional Chinese medicinal preparation Zi-Shen pill. The separation is performed on a C_{18} column by stepwise gradient elution with water (0.2%, v/v, triethylamine adjusted to pH 4 with phosphoric acid)–methanol–acetonitrile (0.01 min, 98:0:2; 20 min, 80:5:15; 30 min, 65:13:22; and 55 min, 65:13:22) as the mobile phase at a flow rate of 0.9 mL/min, with UV detection at 280 nm. Six regression equations show good linear relationships between the peak area of each marker and concentration. The recoveries of the markers listed are 95.5%, 98.3%, 96.8%, 99.5%, 101.7%, and 102.1%, respectively. The repeatability and reproducibility (relative standard deviation) of the method are less than 2.5% and 3.3%, respectively.

Introduction

Traditional Chinese medicinal (TCM) prescriptions have been used for over 1000 years. Most of them are composed of many herbs, which contain complicated chemical constituents. Because of the complexity and interference, the effectiveness and safety of TCMs still remain to be established. Thus, proper methods for quality control are needed.

The Zi-Shen pill (1), which was recorded originally in Secret Record of the Chamber of Orchids in the years of Yuan Dynasty in China (1279–1368 A.D.), consists of three kinds of common crude drugs. The formula is used for treating prostatitis, prostatomegaly, and infection of the urinary system diseases and has produced a quite good effect (2). Although many high-performance liquid chromatographic (HPLC) methods have been developed for the determination of one or two constituents in crude drugs or preparations (3,4), there have been few reports on the simultaneous determination of multiple constituents in preparations. In order to promote the Good Manufacture Practice of Chinese medicinal prescriptions and establish rapid and simple HPLC methods for routine quantitative analysis, we have tried to develop a method to assay multiple constituents in this preparation simultaneously. Among them, the six marker components mangiferin (present in *Anemarrhenae rhizoma*); jateorrhizine, palmatine, and berberine (in *Phellodendri cortex*); and cinnamic acid and cinnamaldehyde (in *Cinnamomi cortex*) were selected for analysis (5,6) (Figure 1). An HPLC method was developed for the simultaneous determination of the contents of the six markers by using aqueous acid–methanol–acetonitrile as the elution, and the method was validated.

Experimental

Materials and reagents

Anemarrhenae rhizoma, *Phellodendri cortex*, and *Cinnamomi cortex* all were purchased at Tianyitang TCM shop (Shenyang, China). The Zi-Shen pill was prepared according to the conven-

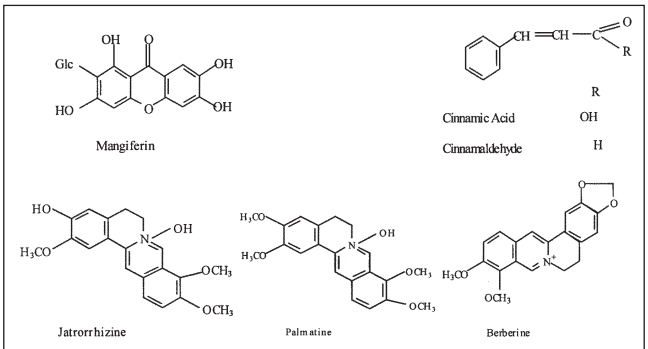

Figure 1. Structures of marker components.

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tional method; mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde were all ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Triethylamine and phosphoric acid were analytical grade, and methanol and acetonitrile were chromatographic grade.

Chromatographic system

The chromatographic system used consisted of: (*i*) a Shimadzu LC-2010A (Kyoto, Japan) composed of a 4 quaternary gradient system, high-speed autosampler, column oven, and UV detector; (*ii*) a Class-VP v. 6.0 chromatography data system (Shimadzu); and (*iii*) a 150- × 4.6-mm i.d. column with a Shimadzu ODS C_{18} (5-µm particle size) stationary phase. The mobile phase was a stepwise gradient of water (0.2%, v/v, triethylamine adjusted to a pH of 4 with phosphoric acid)–methanol–acetonitrile (0.01 min, 98:0:2; 20 min, 80:5:15; 30 min, 65:13:22; and 55 min, 65:13:22). The analyses were carried out at a flow rate of 0.9 mL/min with UV detection at 280 nm. The operation temperature was at 23ºC.

Preparation of standard solutions

To prepare a standard solution containing mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde, accurately weighed amounts of each compound were dissolved in methanol to give serial concentrations with the ranges 21.12–105.60, 0.64–3.20, 6.40–32.00, 8.16–40.80, 0.768–3.840, and 0.672-3.360 µg/mL, respectively. Calibration graphs were plotted after linear regression of the peak area with concentrations.

Preparation of sample solutions

An appropriate amount of Zi-Shen pill was extracted with a 10-fold mass of 50% ethanol solution by refluxing on a waterbath for 1 h and then filtered. The extraction was repeated twice. The extraction solvent was combined and diluted with ethanol to give a 70% ethanol solution, filtered after the ethanol precipitated, and the ethanol was then removed with reduced-pressure evaporation. The residue was dissolved in methanol. All samples were filtered through a 0.45-µm Millipore (Kaide, Tianjin, China) filter and injected for HPLC analysis.

Interference test

An appropriate amount of crude drugs of Zi-Shen pill, without *Anemarrhenae rhizoma*, *Phellodendri cortex*, or *Cinnamomi cortex*, were weighed one at a time; a tenfold mass of 50% ethanol solution was added, and each was refluxed on a waterbath for 1 h and then filtered. The extraction was repeated twice. The extraction solvent was combined and diluted with ethanol to give a 70% ethanol solution, filtered after the ethanol precipitated, and the ethanol was then removed with reduced-pressure evaporation. The residue was dissolved in methanol. All samples were filtered through a 0.45-µm Millipore filter and used for analysis blank samples.

Recovery tests

An appropriate amount of Zi-Shen pill was weighed accurately and extracted as previously stated. The filtrate was divided into four portions (one as a control group), and each portion was spiked with different concentrations of standard solution to add various concentrations of mangiferin (10.56, 21.12, and 31.68 µg/mL), jateorrhizine (0.40, 0.80, and 1.20 µg/mL), palmatine (4.00, 8.00, and 12.00 µg/mL), berberine (5.10, 10.20, and 15.30 µg/mL), cinnamic acid (0.384, 0.768, and 1.152 µg/mL), and cinnamaldehyde (0.420, 0.840, and 1.260 µg/mL). All samples were filtered through a 0.45-µm Millipore filter and injected for HPLC analysis to calculate the recoveries.

Table I. Linear Regression Results

Marker component	Regression analysis equation	Correlation coefficient
Mangiferin	$y = 1.4E + 10^4x + 141{,}425$	0.9981
Jateorrhizine	$y = 3.0E + 10^5x - 133{,}764$	0.9996
Palmatine	$y = 1.0E + 10^5x + 106{,}151$	0.9990
Berberine	$y = 1.0E + 10^5x - 105{,}649$	0.9996
Cinnamic acid	$y = 1.4E + 10^5x - 12{,}585$	0.9991
Cinnamaldehyde	$y = 7.6E + 10^4x - 8601$	0.9986

Table II. Intra- and Interday RSDs (n = 5)

Marker component	Concentration (µg/mL)	%RSD	
		Intraday	Interday
Mangiferin	42.24	1.4	2.3
Jateorrhizine	1.60	0.4	1.4
Palmatine	16.00	1.7	1.9
Berberine	20.40	2.0	2.3
Cinnamic acid	1.536	1.8	1.4
Cinnamaldehyde	1.680	2.5	3.3

Table III. Recoveries of Mangiferin, Jateorrhizine, Palmatine, Berberine, Cinnamic Acid, and Cinnamaldehyde in the Zi-Shen Pill

Marker component	Added (µg/mL)	Found (µg/mL)	Relative recovery (%)	Mean ± SD (%)	%RSD
Mangiferin	10.56	10.11	95.7	95.5 ± 1.6	2.5
	21.12	20.47	96.9		
	31.68	29.72	93.8		
Jateorrhizine	0.40	0.39	97.5	98.3 ± 1.4	1.5
	0.80	0.78	97.5		
	1.20	1.20	100.0		
Palmatine	4.00	3.83	95.8	96.8 ± 0.9	1.0
	8.00	7.81	97.6		
	12.00	11.65	97.1		
Berberine	5.10	5.12	100.4	99.5 ± 1.3	1.0
	10.20	10.22	100.2		
	15.30	14.99	98.0		
Cinnamic acid	0.384	0.383	99.7	101.7 ± 3.0	2.9
	0.768	0.770	100.3		
	1.152	1.211	105.1		
Cinnamaldehyde	0.420	0.435	103.5	102.1 ± 2.6	2.5
	0.840	0.832	99.1		
	1.260	1.307	103.7		

Results and Discussion

Calibration graphs for mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde were obtained over the ranges 21.12–105.60, 0.64–3.20, 6.40–32.00, 8.16–40.80, 0.768–3.840, and 0.672–3.360 µg/mL, respectively. The regression equations are given in Table I, where y is the peak area of the marker and x is the concentration (µg/mL) of the marker. These results showed good linear relationships between peak area and concentration.

To check the precision of this method, we injected standard solutions of mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde at the concentrations of 42.24, 1.60, 16.00, 20.40, 1.536, and 1.680 µg/mL, respectively, five times on the same day. The intraday relative standard deviations (RSDs) were 1.4%, 0.4%, 1.7%, 2.0%, 1.8%, and 2.5%, respectively. The interday RSDs obtained for a 5-day period were 2.3%, 1.4%, 1.9%, 2.3%, 1.4%, and 3.3%, respectively (Table II). The recoveries of mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde were 95.5%, 98.3%, 96.8%, 99.5%, 101.7%, and 102.1%, respectively (Table III). For herbal analysis, the values mentioned indicated acceptable precision and accuracy.

To ensure the specificity and selectivity of the method, we prepared three blank samples for comparison. They were combined one at a time, excluding *Anemarrhenae rhizoma*, *Phellodendri cortex*, and *Cinnamomi cortex*. The chromatograms are shown in Figures 2 and 3. The retention times of the marker components (i.e., mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde) were 21.0, 31.0, 33.8, 34.3, 39.6, and 41.0 min, respectively. On inspection of the chromatograms, these constituents all showed good purity. There was no peak found at their retention times in the blank sample.

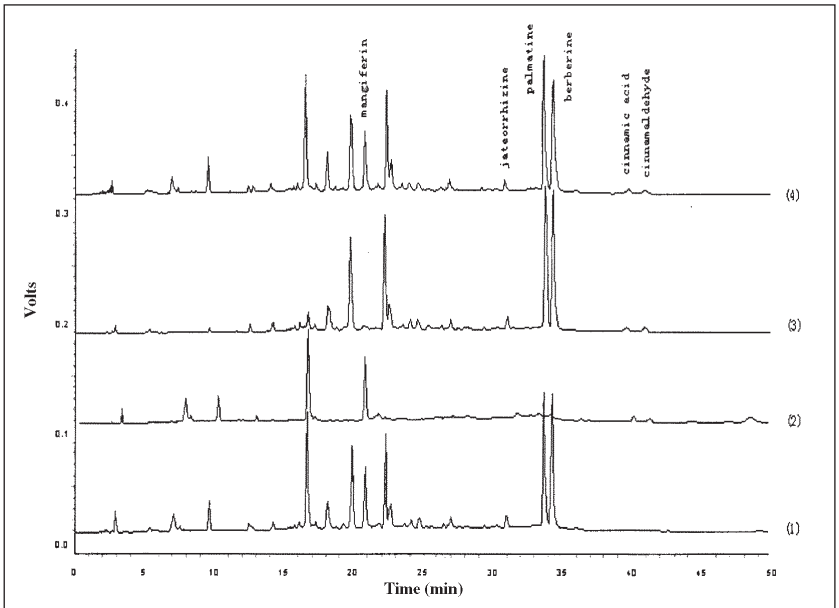

Figure 2. Chromatograms of mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde in the Zi-Shen pill: (1) test sample without *Cinnamomi cortex*, (2) test sample without *Phellodendri cortex*, (3) test sample without *Anemarrhenae rhizoma*, and (4) test sample of Zi-Shen pill.

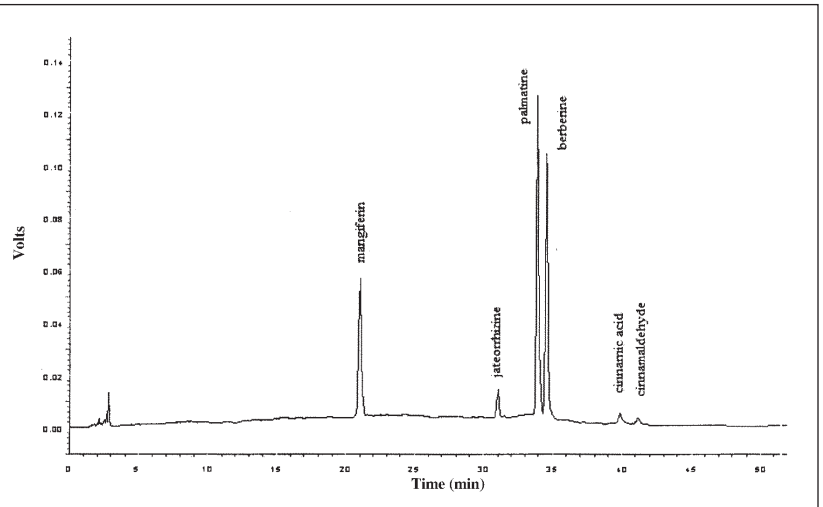

Figure 3. Chromatograms of the standard mixture.

In this study, the six marker components of the Zi-Shen pill could not be separated effectively by using the isocratic mobile solvents. In order to find an easy way to analyze the specimen, we employed a gradient solvent system (methanol–acetonitrile–phosphoric acid solution), which can effectively separate six makers simultaneously. Different combinations of the three-solvent gradient were investigated. An increase of aqueous solved the problem of less retention time of mangiferin, whereas a slight amount of triethylamine provided sufficient resolution and good peak shape of alkaloids in *Phellodendri cortex*.

The UV absorption maxima of mangiferin, jateorrhizine, palmatine, and berberine were approximatley 320–340 nm, and cinnamic acid and cinnamaldehyde were approximately 280 nm. A monitoring wavelength for quantitative determination at 280 nm was used for the determination of cinnamic acid and cinnamaldehyde with selectivity because the dose ratio of *Anemarrhenae rhizoma, Phellodendri cortex*, and *Cinnamomi cortex* in this preparation was 1:1:0.1.

TCMs are usually prepared by boiling with water. However, extraction of constituents from a pill with water tends to be a difficult procedure. For this reason, four solvents; water; and 95%, 75%, and 50% ethanol solution were tried for extraction. The chromatographic results indicate that the best extraction was obtained with 50% ethanol solution.

Conclusion

The described method is found to be rapid, linear, accurate, reproducible, and capable of simultaneously quantitating six marker compo-

nents in the Zi-Shen pill, thus it can be used for the routine analysis of stability samples and the quality control of products.

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