Determination of Safflor Yellow A, Puerarin, Ferulic Acid, Ginsenoside Rg_1, and Rb_1 in the Traditional Chinese Medicinal Preparation Naodesheng Injection by High-Performance Liquid Chromatography

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Abstract

High-performance liquid chromatography is employed to determine the contents of five mark components, safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1, in the Traditional Chinese Medicinal preparation Naodesheng injection. The separation is performed on a C_{18} column by stepwise gradient elution with water (0.1%, v/v, phosphoric acid)–acetonitrile (0 min, 86:14; 48 min, 75:25; and 68 min, 50:50) as the mobile phase at a flow rate of 1.0 mL/min, with UV detection at 203 nm. Five regression equations show a good linear relationship between the peak area of each marker and concentration. The recoveries of the markers listed are 99.6%, 100.2%, 99.7%, 100.0%, and 99.7%, respectively. The repeatability and reproducibility (relative standard deviation) of the method are less than 1.4% and 1.8%, respectively.

Introduction

The ever-increasing worldwide attention to the pharmaceutical research of Traditional Chinese Medicinal (TCM) prescriptions has made it essential to carry out stringent quality control measures (1,2). Most of the TCMs are composed of many crude herbs, which contain complicated chemical constituents. Because of the complexity and interference, it is widely accepted that multiple constituents are responsible for the therapeutic effects of TCM. To ensure the effectiveness and safety of TCMs, therefore, it is necessary to quantitatively determine the multiple bioactive components of TCM (3,4).

Naodesheng injection was prepared in the laboratory according to the conventional formula of Naodesheng pill (tablet), which consists of five kinds of common crude drugs. The formula is used for treating cerebral arteriosclerosis, ischemic cerebral stroke, and apoplexy linger effect (5). Although many methods have been developed for the determination of one or two constituents in crude drugs or preparations with thin-layer chromatography, high-performance liquid chromatography (HPLC)–UV, or liquid chromatography–mass spectrometry (6–13), there have been few reports on the simultaneous determination of multiple constituents in preparations. In order to promote the good manufacturing practice of Chinese medicinal prescriptions and establish rapid and simple HPLC methods for routine quantitative analysis, a method has been developed to assay multiple constituents in this preparation simultaneously. Among them, the five mark components, safflor yellow A (present in Carthamus tinctrius), puerarin (present in Pueraria lobata), ferulic acid (present in Ligusticum chuanxiong Hort), ginsenoside Rg_1, and Rb_1 (present in Panax notoginseng), were selected for analysis (Figure 1). An HPLC method was developed for the simultaneous determination of the contents of the five markers by using aqueous acid–acetonitrile as the elution, and the method was validated.

Experimental

Materials and reagents

Pueraria lobata, Carthamus tinctrius, Panax notoginseng, Ligusticum chuanxiong Hort, and Crataegus pinnatifida Bge all were purchased at Tianyitang TCM shop (Shenyang, China). The Naodesheng injection was prepared according to the conventional method. The standard safflor yellow A was separated in our laboratory. The standard puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1 were ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile were of chromatographic grade. Phosphoric acid was of analytical grade.

Chromatographic system

The HPLC system consisted of a Shimadzu LC-10ATvp series

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binary pump, a Shimadzu SPD-10Avp UV detection, and HT-130 column heater (Shimadzu, Kyoto, Japan). An ANASTAR chromatography data system (Tianjin Autoscience, Tianjin, China) was used for data acquisition and integration. Separations were carried out with a Kromasil C_{18} column (5-µm, 250 × 4.6 mm Science Instruments, Tianjin, China). The mobile phase was a stepwise gradient of water (0.1%, v/v, phosphoric acid)–acetonitrile (0 min, 86:14; 48 min, 75:25; 68 min, 50:50). Chromatography was performed at a flow rate of 1.0 mL/min with a wavelength of 203 nm and operated at 35°C.

Preparation of standard solution

To prepare a standard solution containing safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1, accurately weighed amounts of each compound were dissolved in a solution of methanol–water (1:1, v/v) to give serial concentrations within the ranges of 0.00972–0.1944, 0.14–2.8, 0.0152–0.304, 0.0742–1.484, and 0.0862–1.724 mg/mL, respectively. Calibration graphs were plotted after linear regression of the peak area with concentrations.

Preparation of sample solution

An appropriate amount of Naodesheng injection sample was filtered though a 0.45-µm Millipore filter (Kaide, Tianjin, China) and injected for HPLC analysis.

Interference test

An appropriate amount of crude drug extracts of Naodesheng injection, without Carthamus tinctrius, Pueraria lobata (Willd.) Ohwi, Ligusticum chuanxiong, or Panax notoginseng, was weighed with the same proportion as the formula and extracted according to the conventional method. These extracts were dissolved in appropriate solvent, and all samples were filtered through a 0.45-µm Millipore filter and then used as blank samples.

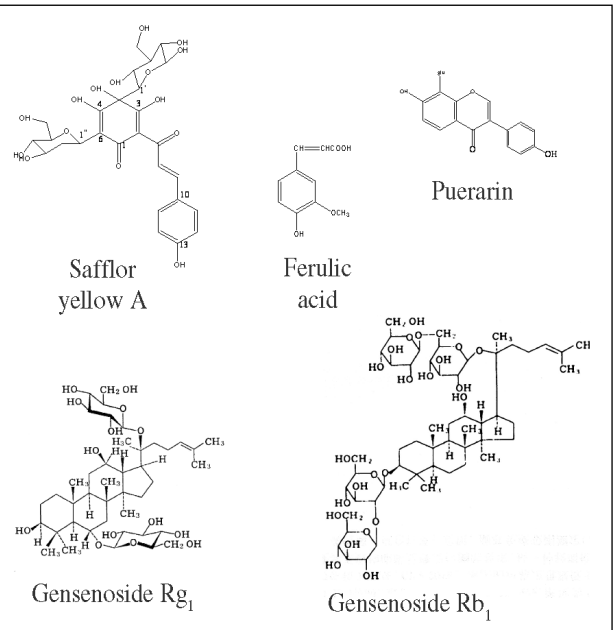

Figure 1. Structure of safflor yellow A, puerarin, ferulic acid, gensenoside Rg_1, and Rb_1.

Recovery tests

Four portions of the appropriate amount of Naodesheng injection were transferred accurately (one as a control group), and each portion was spiked with different concentration of safflor yellow A (0.0243, 0.0486, and 0.0729 mg/mL), puerarin (0.35, 0.7, and 1.05 mg/mL), ferulic acid (0.038, 0.076, and 0.114 mg/mL), ginsenoside Rg_1 (0.186, 0.371, and 0.557 mg/mL), and Rb_1 (0.216, 0.431, and 0.647 mg/mL). All samples were filtered through a 0.45-µm Millipore filter and injected for HPLC analysis to calculate the recoveries.

Results and Discussion

Calibration graphs for safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1 were obtained over the ranges of 0.00972–0.1944, 0.14–2.8, 0.0152–0.304, 0.0742–1.484, and 0.0862–1.724 mg/mL, respectively. The regression equations are given in Table I, where y is the peak area of the marker, and x is the concentration (mg/mL) of the marker. These results showed good linear relationships between peak area and concentration.

To check the precision of this method, a sample of Naodesheng injection, which consists safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1, was injected at concentrations of 0.0475, 0.724, 0.071, 0.388, and 0.450 mg/mL, respectively, five times on the same day and on five different days, respectively. The intraday relative standard deviations (RSDs) were 1.1%, 0.5%, 1.2%, 0.9%, and 1.4%, respectively. The interday RSDs obtained for a 5-day period were 1.3%, 1.0%, 1.8%, 1.3%, and 1.7%, respectively (Table II). The recoveries were 99.6%, 100.2%, 99.7%, 100.0%, and 99.7%, respectively (Table III). For herbal analysis, the values mentioned indicated acceptable precision and accuracy.

Table I. Linear Regression Results

Mark component	Regression analysis equation	Correlation coefficient
Safflor yellow A	$y = 8E + 10^6x + 1266$	0.9997
Puerarin	$y = 2E + 10^7x - 7234.1$	0.9999
Ferulic acid	$y = 2E + 10^7x - 16120$	0.9997
Ginsenoside Rg_1	$y = 2E + 10^6x - 11226$	0.9997
Ginsenoside Rb_1	$y = 334762x + 1455.6$	0.9995

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

Mark component	Concentration (mg/mL)	%RSD	
		Intraday	Interday
Safflor yellow A	0.0475	1.1	1.3
Puerarin	0.724	0.5	1.0
Ferulic acid	0.071	1.2	1.8
Ginsenoside Rg_1	0.388	0.9	1.3
Ginsenoside Rb_1	0.450	1.4	1.7

Table III. Recoveries of Safflor Yellow A, Puerarin, Ferulic Acid, Gensenoside Rg_1, and Rb_1 in the Naodesheng Injection

Mark component	Original (μg/mL)	Added (μg/mL)	Found (μg/mL)	Relative recovery (%)	Mean ± SD (%)	%RSD
Safflor yellow A	0.0475	0.0243	0.0241	99.1	99.6 ± 0.9	0.9
	0.0475	0.0486	0.0487	100.1		
	0.0475	0.0729	0.0726	99.6		
Puerarin	0.724	0.350	0.3508	100.2	100.2 ± 0.4	0.4
	0.724	0.700	0.7001	100.0		
	0.724	1.050	1.0501	100.0		
Ferulic acid	0.071	0.038	0.0380	99.8	99.7 ± 0.5	0.4
	0.071	0.076	0.0760	99.9		
	0.071	0.114	0.1140	99.4		
Ginsenoside Rg_1	0.388	0.1855	0.1857	100.1	100.0 ± 0.4	0.4
	0.388	0.3710	0.3708	100.0		
	0.388	0.5565	0.5566	100.0		
Ginsenoside Rg_1	0.450	0.2155	0.2153	99.5	99.7 ± 0.4	0.4
	0.450	0.4310	0.4317	99.8		
	0.450	0.6465	0.6467	99.9		

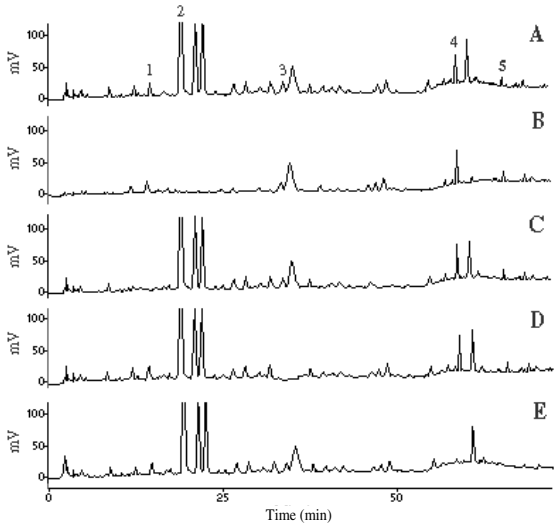

Figure 2. Chromatograms of safflor yellow A, puerarin, ferulic acid, gensenoside Rg_1, and Rb_1 in Naodesheng injection: test sample of Naodesheng injection (A), test sample without *Pueraria lobata* (*Willd.*) *Ohwi* (B), test sample without *Carthamus tinctrius* (C), test sample without *Ligusticum chuanxiong* (D), and test sample without *Panax notoginseng* (E). The peak numbers are: safflor yellow A, 1; puerarin, 2; ferulic acid, 3; ginsenoside Rg_1, 4, and Rb_1 5.

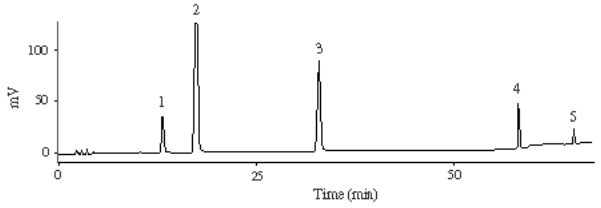

Figure 3. Chromatograms of the standard mixture. The peak numbers are: safflor yellow A, 1; puerarin, 2; ferulic acid, 3; ginsenoside Rg_1, 4, and Rb_1 5.

To ensure the specificity and selectivity of the method, four blank samples were prepared for comparison. They were combined one at a time, excluding Carthamus tinctrius, Pueraria lobata (Willd.) Ohwi, Ligusticum chuanxiong, or Panax notoginseng. The chromatograms are shown in Figures 2 and 3. The retention times of the mark components (i.e., safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1) were 14.5, 18.8, 34.5, 58.8, and 65.7 min, respectively. There was no coeluted peak at their retention time in the blank sample for each single crude drug.

In this study, the five mark components of Naodesheng injection could not be separated effectively using the isocratic mobile solvents. In order to find an easy way to analyze the specimen, a gradient solvent system [acetonitrile–water (phosphoric acid)] was employed, which can effectively separate the five markers simultaneously. Different combinations of three solvent gradients were investigated.

The UV absorption maxima of safflor yellow A, puerarin, ferulic acid, ginsenoside Rg_1, and Rb_1 were 403, 280, 320, 203, and 203 nm, respectively. A monitoring wavelength for quantitative determination was set at 203 nm because a wavelength of 203 nm was extensively applicable for simultaneous determination of these five compounds.

Conclusion

The described method is found to be linear, accurate, reproducible, and capable of simultaneously quantitating the five mark components in Naodesheng injection. Thus, it can be used for the routine analysis of stability samples and the quality control of products.

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