High-performance liquid chromatography is employed to determine the contents of five marker components, safflor yellow A, puerarin, ferulic acid, ginsenoside Rg1, and Rb1, in the Traditional Chinese Medicinal preparation Naodesheng injection. The separation is performed on a C18 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.

Experimental

Materials and reagents

Pueraria lobata, Carthamus tinctorius, Panax notoginseng, Ligusticum chuanxiong Hort, and Crataegus pinnatifida Bge all were purchased at Tianyi TCM shop (Shenyang, China). The Naodesheng injection was prepared according to the conventional method. The standard safflor yellow A (present in Carthamus tinctorius), puerarin (present in Pueraria lobata), ferulic acid (present in Ligusticum chuanxiong Hort), ginsenoside Rg1, and Rb1 (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.
Preparation of standard solution

To prepare a standard solution containing safflor yellow A, puerarin, ferulic acid, ginsenoside Rg1, and Rb1, 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 through 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 tinctorius, Pueraria lobata (Wild.) 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.

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 concentrations 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 Rg1 (0.186, 0.371, and 0.557 mg/mL), and Rb1 (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 Rg1, and Rb1 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 Rg1, and Rb1, 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.
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 tinctorius, 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 Rg1, and Rb1) 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 Rg1, and Rb1 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.
References


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Manuscript received October 15, 2005; revision received December 14, 2005.