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Development and validation of an accurate HPLC method for the quantitative determination of picroside II in tablets

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Received July 9, 2006, accepted July 17, 2006

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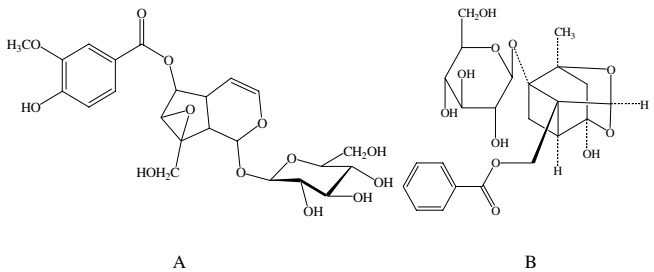
Pharmazie 62: 577–579 (2007) *doi: 10.1691/ph.2007.8.6133*

A simple, sensitive and accurate high performance liquid chromatographic method (HPLC) with UV detection was developed and validated to determine picroside II in a new tablet formulation with paeoniflorin as internal standard. Chromatographic separation was achieved on an Agilent XDB C_{18} column (250 × 4.6 mm I.D., 5 μm) using a mobile phase consisting of acetonitrile–water–acetic acid (18 : 82 : 0.4, v/v/v) at a flow rate of 1.0 ml/min. The UV detection wavelength was set at 265 nm. Linear calibration curves were obtained in the concentration range of 0.10–100 μg/ml with the limit of quantification (LOQ) of 0.10 μg/ml. The within- and between-run precisions in terms of % relative standard deviation (RSD) were lower than 5.7% and 6.3%, respectively. The accuracy in terms of % relative error (RE) ranged from −2.3% to 5.0%. This validated method was successfully applied to the determination of the content of picroside II in a new tablet formulation.

1. Introduction

Picroside II, one of the iridoid glucosides, is the major active constituent extracted from the dried root and rhizomes of *Picrorhiza scrophulariflora* Pennell which belongs to the *Scrophularia* plants (Weinges et al. 1972; Jia et al. 1999; Li et al. 2000a). It has been traditionally used to treat disorders of liver, upper respiratory diseases, fevers, dyspepsia, chronic diarrhea and scorpion sting (Gao et al. 2005a). Recent researches on picroside II are focused on its hepatoprotective, anticholestatic, antioxidant and immune-modulating activities (Li et al. 2000b, 2002; Gao et al. 2005a, 2005b).



A B

Picroside II (A) and paeoniflorin (B, internal standard)

A new tablet formulation of picroside II has been developed by our laboratory. For the new product, it was necessary to find an analytical method to determine picroside II in the tablets. Since picroside II and its pharmaceutical formulations have not been listed in any of the Pharmacopoeias yet and no references describing the determination of picroside II in pharmaceutical preparations have been found in publications and even related reports are quite limited (Wang et al. 2000; Yang et al. 2005), an analytical method needed to be developed for the quality control of the new pharmaceutical products. HPLC has been widely used in the analysis of pharmaceutical compounds and formulations due to its high efficiency of separation and quantification. The purpose of this present study was to develop and validate an HPLC method for the determination of picroside II in a new tablet formulation, which can be used for the quality control of the new product.

2. Investigations and results

During method development, top priority was given for complete separation of picroside II from the intermediates such as the excipients in the tablets. Selectivity was assessed by comparing the chromatograms of the blank solution containing typical tablet excipients (starch, microcrystalline cellulose, magnesium stearate, carboxymethyl starch) with the corresponding spiked tablet sample. The Fig. shows the typical chromatograms of a blank solution containing tablet excipients without picroside II, a blank sample spiked with picroside II and paeoniflorin, and a real tablet sample. As a result, the typical tablet excipients included in the drug formulation (starch, microcrystalline cellulose, magnesium stearate, carboxymethyl starch) do not interfere with the determination of picroside II and paeoniflorin. Typical retention times for picroside II and paeoniflorin were 13.0 and 8.1 min, respectively.

Linearity was evaluated by determining six standard working solutions of picroside II ranging from 0.10 to 100 μg/ml, which were prepared by appropriate dilution of 100 μg/

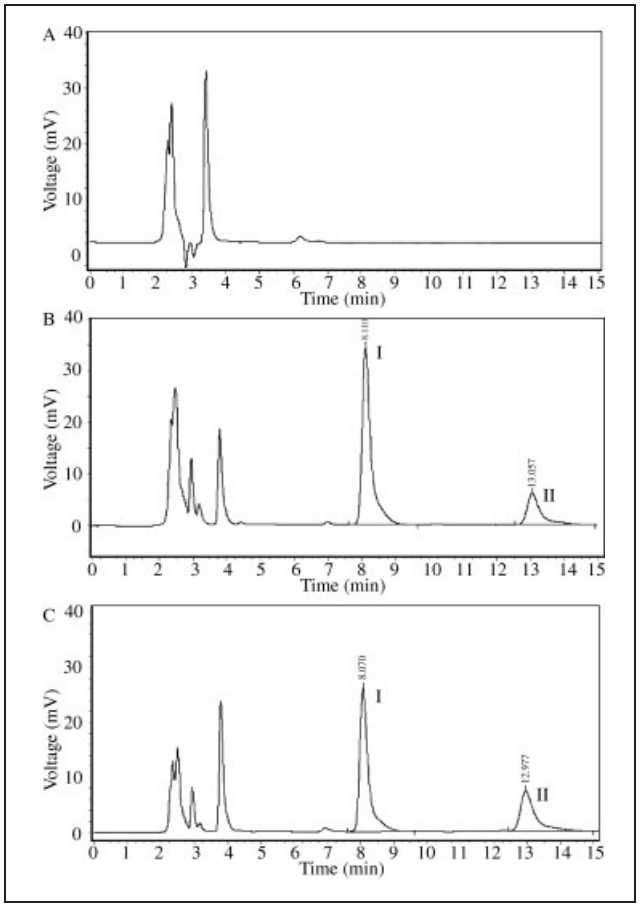


Fig.: Representative chromatograms of picroside II and paeoniflorin determined by HPLC-UV method. (A) blank solution containing the tablet excipients; (B) blank sample spiked with picroside II and paeoniflorin (I.S., 50 μg/ml); (C) real tablet sample. Peak I, paeoniflorin; peak II, picroside II

ml standard solution of picroside II with the mobile phase. The peak area ratio of picroside II and paeoniflorin (Y) and concentration of picroside II (X) were subjected to a weighted ($1/X^2$) least squares linear regression analysis to calculate calibration equation and correlation coefficients. A typical equation of the calibration curve was as follows: $Y = 3.54310^{-4} + 1.936 \times 10^{-2}$ X ($R = 0.9998$, $n = 5$), the linear range was from 0.10 to 100 μg/ml. The results show that an excellent correlation existed between the peak area ratio of picroside II and paeoniflorin and concentration of picroside II.

The limit of detection (LOD) and the limit of quantification (LOQ) were separately determined at a signal-to-noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of picroside II until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations. The LOD and the LOQ of picroside II were found to be 0.03 and 0.1 μg/ml, respectively.

Accuracy and precision were assessed by determining QC samples at three concentration levels (0.20, 5.00 and 90.0 μg/ml) on the 3 different validation days. The accuracy was expressed by (mean observed concentration − spiked concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (RSD). The Table summarizes the within- and between-run precision and accuracy for picroside II from QC samples. In this assay, the within- and between-run precisions ranged from 2.7% to 5.7% and from 3.4% to 6.3% for each QC level, respectively. The accuracy ranged from −2.3% to 5.0%. The results, calculated with a one-way ANOVA, indicated that the values were within the acceptable range; thus the method is accurate and precise.

Table: Accuracy and precision of the HPLC-UV method to determine picroside II in a new tablet formulation

Concentration (μg/ml)		RSD (%)		RE (%)
Added	Found	Within-run	Between-run	
0.20	0.21	3.6	4.2	5.0
5.00	5.18	5.7	3.4	3.6
90.0	87.9	2.7	6.3	−2.3

The stability of picroside II in the mobile phase solution at different times was investigated at room temperature. Results show that picroside II is stable for at least 24 h at room temperature in the mobile phase solution of acetonitrile-water-acetic acid (18 : 82 : 0.4, v/v/v).

Experimental results of the content of picroside II in the tablets of three lots, expressed as the percentage of the label claim, were separately 100.8, 100.1 and 99.9% ($n = 3$). The results show that the content of picroside II in the tablets meets the requirements (90–110% of the label claim).

3. Discussion

The most widely used solvents for HPLC are methanol, acetonitrile, water, and different kinds of buffers such as phosphate buffer, acetate buffer, etc. The mobile phase was chosen after several trials with acetonitrile, methanol and water in various proportions and in different pH values. When the proportions of acetonitrile and water were adopted separately in 18 : 82 (v/v), it was found that good separation was achieved between the analytes of interest and the intermediates. But under this conditious the peak shapes of the analytes of interest were not satisfactory. Because both compounds of picroside II and paeoniflorin are weak acids, symmetrical peak shapes could be obtained by addition of 0.4% acetic acid in the mobile phase. All things considered, a mobile phase consisting of acetonitrile–water–acetic acid (18 : 82 : 0.4, v/v/v) was finally selected in order to achieve optimal separation, high sensitivity and good peak shape.

Furthermore, flow rates from 0.8 to 1.5 ml/min were studied. Finally, a flow rate of 1.0 ml/min was employed since it produced a reasonable retention time of picroside II and paeoniflorin as well as acceptable resolution from its related compounds.

Maximum UV absorption of picroside II in the mobile phase was found to be at 220, 265 and 292 nm, respectively. Since the absorbance at 265 nm was higher than that at 292 nm, and the interferences at 265 nm was less than that at 220 nm in the chromatograms, the wavelength 265 nm was chosen as the detection wavelength.

Under the proposed chromatographic conditions, picroside II could be well separated from its related compounds. The retention times of picroside II and paeoniflorin were 13.0 and 8.1 min, respectively. Total analysis time of one sample was less than 15 min.

In order to rectify the probable error in sample processing and determination, an internal standard was used. In the present study, several kinds of internal standards such as icariin, picroside I and paeoniflorin were investigated. But unsuitable retention time of icariin and instability of picro-

side I made them be given up. At last paeoniflorin was chosen as the internal standard because of suitable retention behavior and good stability.

In conclusion, the developed HPLC method for the determination of picroside II in the tablet formulation offers sufficient selectivity, accuracy, precision and a short running time. The developed method can be used for the determination of picroside II in pharmaceutical formulations.

4. Experimental

4.1. Chemicals and reagents

Standards of picroside II and paeoniflorin (internal standard) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Tablets of picroside II were produced by our laboratory. Acetonitrile of HPLC grade was purchased from Yuwang Chemical (Shandong, China). Distilled water, prepared by using a Milli-Q water purification system from Millipore (Molsheim, France), was used throughout the study. All other chemicals used were of analytical grade.

4.2. Instrumentation

Chromatographic separation was performed on a Shimadzu LC-10AD pump equipped with a Shimadzu SPD-10Avp UV-Vis detector (Shimadzu Co., Kyoto, Japan). Data integration was done using CK Chrom data software (Scientific System Inc.). Injections were carried out using a 20 μL loop.

4.3. Chromatographic conditions

The LC separation was performed using an Agilent XDB C_{18} column (250×4.6 mm I.D., 5 (μm) and a Shim-pack GVP-ODS C_{18} guard column (10×4.6 mm I.D.). The isocratic mobile phase, consisting of acetonitrile–water–acetic acid (18 : 82 : 0.4, v/v/v), was delivered at a flow-rate of 1.0 ml/min. Prior to use, the mobile phase was filtered through 0.45 μm Millipore membrane filters and degrassed by sonication in an ultrasonic bath. Detection was set at 265 nm and the column temperature was maintained at 20 °C.

4.4. Assay of tablets

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of picroside II was transferred to a 50 ml volumetric flask. After 20 ml of the mobile phase was added into the flask, the solution was sonicated for 5 min to make picroside II dissolved. Then the solution was diluted to volume with the mobile phase, mixed and filtered. After discarding the first 10 ml of filtrate, 1 ml of the following filtrate was quantitatively transferred to a 10 ml volumetric flask. After addition of 1 ml of the paeoniflorin solution (internal standard, 50 μg/ml), the solution was diluted to volume with the mobile phase. The sample solution was filtered through 0.45 μm Millipore membrane filters and injected into the chromatographic system and the chromatograms and peak areas were recorded.

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