

## HPLC Determination of Glycyrrhizic and Glycyrrhetic acids in Weiyanning granule.

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### Abstract

To establish a method for determination of glycyrrhizic and glycyrrhetic acids in Weiyanning Granule. The contents of glycyrrhizic and glycyrrhetic acids are determined by HPLC under conditions as follows: Diamonsil C<sub>18</sub> (4.6 mm × 250 mm, 5 μm) column, mobile phase of acetonitrile-0.05 mol/L ammonium acetate solution (A: 0.05 mol/L ammonium acetate solution; B: acetonitrile, gradient elution: 0~30 min: 32% B; 30~60 min: 75% B); flow rate of 1.0 ml/min; and detection wavelength of 250 nm. The determined linear range of glycyrrhizic acid is 0.320~1.920 μg, and average recovery is 98.6%, with RSD = 1.98%; linear range of glycyrrhetic acid is 0.039~0.234 μg, and average recovery is 97.3%, with RSD = 3.20%. The method established in this paper is simple, accurate and reproducible, which provides a new way for the quality control of Weiyanning Granule.

**Keywords:** Weiyanning Granule; glycyrrhizic acid; glycyrrhetic acid; HPLC

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### Introduction

Weiyanning Granule is composed of ten Chinese medicinal materials including Lignum Santali albi, Fructus Crataegi, Fructus Mume and Radix Glycyrrhizae. It has the middle warming, spleen refreshing, stomach harmonizing, adverse qi descending, dampness eliminating and indigestion curing functions, which is used clinically for the treatment of atrophic gastritis, superficial gastritis, dyspepsia due to overeating and other digestive diseases [1-2].

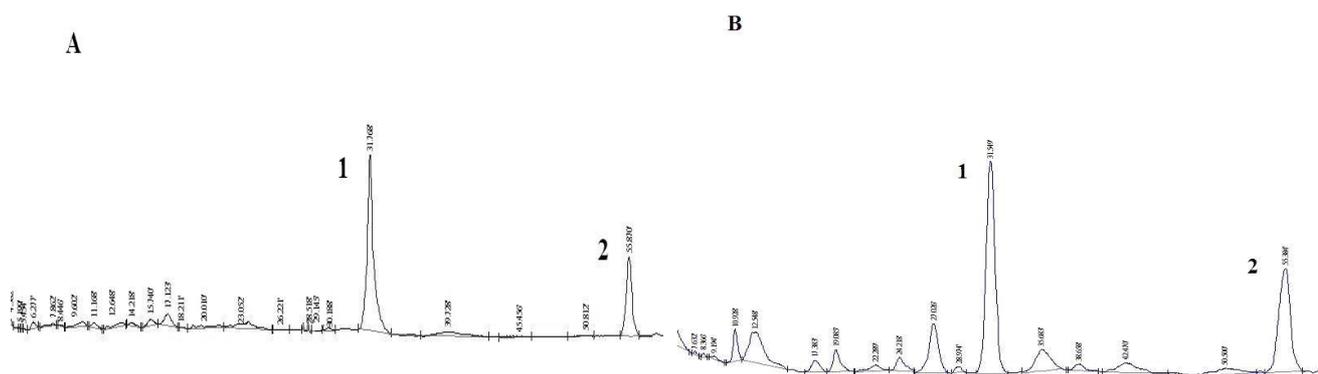
Radix Glycyrrhizae is the major component of Weiyanning Granule, it contains large amounts of flavonoids and phenolic acids [3-5], of which glycyrrhizic and glycyrrhetic acids are major active constituents [6-7]. Pilot study has found that Weiyanning Granule contains detectable glycyrrhizic and glycyrrhetic acids. The drug is not listed in the 2010 Edition of "Chinese Pharmacopoeia"; to effectively control the quality of the preparation, and provide more comprehensive and simpler approach for the research of extraction process and pharmacology of effective parts of Radix Glycyrrhizae, the determination of glycyrrhizic and glycyrrhetic acids in Weiyanning Granule is hereby reported as follows.

### Instruments, Reagents and Drug

Agilent 1200 HPLC; ultrasonic cleaner, purchased from Ningbo Ultrasonic Instrument Co., Ltd.; 0.01 mg accuracy electronic analytical balance, purchased from Tianjin Guangfa Precision Instrument Factory. Acetonitrile was purchased from Sigma (HPLC grade), water was redistilled water, and other reagents were all of analytical grade. Glycyrrhizic acid reference substance was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (batch No.: 965-200125), glycyrrhetic acid reference substance was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (batch No.: 111517-200632), Weiyanning Granule was purchased from Yikang Pharmaceutical Co., Ltd. (batch No.: 20140314, 20140315, 20140316).

### Methods and Results

Chromatographic conditions Column: Diamonsil C<sub>18</sub> column; mobile phase: A: 0.05 mol/L ammonium acetate solution, B: acetonitrile; gradient elution: 0~30 min: 32% B; 30~60 min: 75% B. Detection wavelength: 250 nm. Flow rate: 1.0 mL/min; column temperature: 30°C, injection volume: 20 μL. HPLC chromatograms are shown in Figure 1.



**Figure 1.** HPLC chromatograms of reference substance (A), sample (B)  
1. Glycyrrhizic acid 2. Glycyrrhetic acid

#### Preparation of reference solutions

10 mg of glycyrrhizic acid and 2 mg of glycyrrhetic acid reference substances were precisely weighed, and dissolved to 5 mL with methanol as the reference stock solutions, which were then diluted to obtain mixed reference solutions with glycyrrhizic acid concentration of 0.0320 mg/mL, and glycyrrhetic acid concentration of 0.0039mg/mL.

#### Preparation of test solution

0.2 g of Weiyanning Granule was precisely weighed, added with mobile phase (A:B of 1:1), and ultrasonicated at 80°C for 30 min, then diluted to 50 mL, filtered, and the resulting filtrate was used as the test solution.

#### Investigation of linear relations

2 mL of mixed reference solutions under item "2.2" were accurately weighed, and diluted to 25 mL with methanol, 1, 2, 3, 4, 5 and 6 mL of the resulting solutions were then taken, and diluted to 10 mL with methanol to obtain a total of six concentrations of reference solutions. 20  $\mu$ L of the solutions were precisely drawn, respectively, and determined under conditions prescribed in item "2.1", linear regression was carried out with injection volume X as the abscissa and peak area Y as the ordinate, and regression equation, correlation coefficients and linear range were obtained as follows:  $Y=1231.8X+0.9872$ ,  $r=0.9998$ , 0.320~1.920  $\mu$ g (glycyrrhizic acid);  $Y=1437X-4.296$ ,  $r=0.9997$ , 0.039~0.234  $\mu$ g (glycyrrhetic acid).

**Table 1.** Sample recovery test (n=6)

Compound	Sample content (mg)	Addition amount (mg)	Mean measured (mg)	Average recovery (%)	Relative standard deviation (%)
Glycyrrhizic acid	0.612	0.508	0.501	98.6	1.7
Glycyrrhetic acid	0.107	0.110	0.107	97.3	3.2

**Table 2.** Sample content determination results (n=3)

Reference	20140314 (mg/g)	20140315 (mg/g)	20140316 (mg/g)
Glycyrrhizic acid	3.067	3.019	3.041
Glycyrrhetic acid	0.535	0.521	0.530

#### Accuracy test

20  $\mu$ L of reference solutions were precisely drawn, injected continuously six times, and their peak areas were measured, glycyrrhizic acid RSD = 0.23%, glycyrrhetic acid RSD = 1.29%.

#### Reproducibility test

6 aliquots of test solutions were prepared as per the method under item "2.3", 20  $\mu$ L of each solution was

precisely drawn and determined under conditions prescribed in item "2.1", glycyrrhizic acid RSD = 1.98%, glycyrrhetic acid RSD = 3.20%.

#### Stability test

Aliquots of 20  $\mu$ L of the same test solutions were precisely drawn, and determined at 0, 4, 8, 12 and 24 h, respectively, glycyrrhizic acid RSD = 1.37%, glycyrrhetic acid RSD = 2.13%, which indicated that the test solution was stable within 24 h.

### Sample recovery test

6 aliquots of 0.1 g of Weiyanning Granule of known contents were accurately weighed, and added separately with certain amount of reference stock solution, next, test solution was prepared according to the method under item "2.3", and determined under conditions prescribed in item "2.1", the results are shown in Table 1.

### Determination of sample content

Weiyanning Granule with three different batch numbers were taken, and prepared into test solutions as per the method under item "2.3", followed by content determination. The results are shown in Table 2.

### Discussions

The method established in this paper allows simultaneous determination of glycyrrhizic and glycyrrhetic acid contents, which can effectively control the quality of Weiyanning Granule, and provides a new way for the quantitative determination of compound preparations containing Radix Glycyrrhizae.

To ensure the sensitivity of the determination method, the detection wavelength was set to 250 nm, the maximum absorption wavelength of glycyrrhizic and glycyrrhetic acids. As to the selection of mobile phase, the present study has tried acetonitrile-water, acetonitrile-water (1% acetic acid), acetonitrile-ammonium acetate and acetonitrile-methanol systems as well as elution with different gradients, acetonitrile-ammonium acetate system not only allowed better isolation between glycyrrhizic and glycyrrhetic acids, but also rather good isolation of other ingredients in the formulation, so after comprehensive analysis, elution with acetonitrile-ammonium acetate gradient was adopted. For the selection of column temperature and flow rate, flow rates of 0.8, 1.0 and 1.2 mL/min, and column temperatures of 30°C and 20°C were investigated, which found that chromatographic peaks were better separated at 30°C, so column temperature was set as 30°C. Further investigation of flow rate found that comparatively favorable effect was achieved at a flow rate of 1.0 mL/min.

During the course of this experiment, the extraction solvents of 30%, 50% and 80% methanol, as well as mobile phases of acetonitrile-0.03 mol/L ammonium acetate (1:4; 1:2; 1:1) have been investigated, which revealed that the extraction yield was higher for mobile phase (1:1).

Gastritis is a common gastrointestinal disease [9-10], according to the reported literature, glycyrrhizic acid has a gastritis curing effect [8,11], so we have chosen

glycyrrhizic acid and a similar compound, glycyrrhetic acid, in Weiyanning Granule as the index components for determination, which are able to fully reflect the accuracy and reasonableness of the topic in terms of both activity basis and analysis and determination conditions. Weiyanning Granule is favored by the majority of patients, with huge market sales, so this study not only has laid the foundation for the research of quality control of pharmaceutical preparations, but has also provided the necessary guarantee for health and safety of society.

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