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Application Note

Determination of Glycyrrhizic Acid, Honokiol and Magnolol in Huoxiang Zhengqi Shui

Chinese Pharmacopeia 2025 Method Draft

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Abstract

This study describes the development of an HPLC method for the determination of the three active components, honokiol, magnolol, and glycyrrhizic acid, in Huoxiang Zhengqi Shui, based on a draft monograph method released by the Chinese Pharmacopoeia Commission. Following sample preparation, honokiol and magnolol were analyzed using an Ascentis® Express C18 superficially porous particle HPLC column, whereas glycyrrhizic acid was determined using a Purospher® STAR RP-18 endcapped column designed for extended pH stability. Both methods fulfilled the requirements specified in the draft, confirming their suitability for the determination of these three active ingredients in Huoxiang Zhengqi Shui.

Introduction

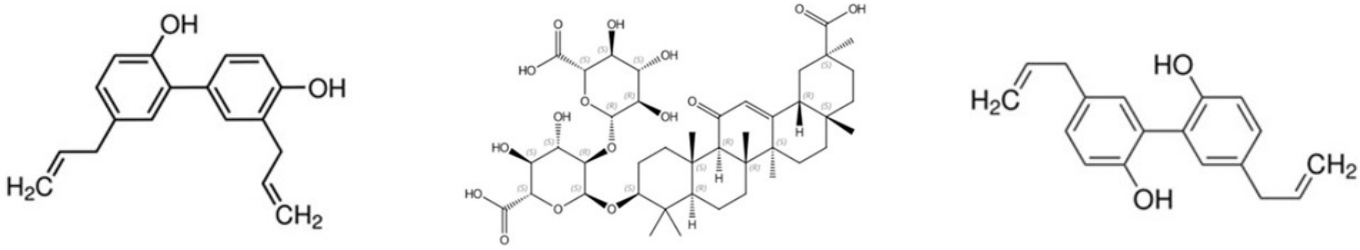
Huoxiang Zhengqi Shui is a well-known liquid herbal formulation in traditional Chinese medicine. It has historically been used to treat various ailments, including colds caused by external wind and cold, internal disorders arising from damp stagnation, and symptoms associated with summer heat and dampness, such as headache, heaviness, chest fullness and discomfort, abdominal distension and pain, vomiting, diarrhea, and gastrointestinal-type colds.[1,2]

The formulation is composed primarily of traditional Chinese herbs, including Atractylodes, dried tangerine peel, magnolia bark (processed with ginger), angelica dahurica, poria, dafupi, raw pinellia, licorice extract, patchouli oil, and perilla leaf oil, with dried ginger and ethanol serving as excipients.[2,3]

In 2024, the Chinese Pharmacopoeia Commission released a draft method for the detection of three marker components in Huoxiang Zhengqi Shui.[4] Following public validation, this method is intended for inclusion in the 2025 edition of the Chinese Pharmacopoeia.

The draft method specifies honokiol, magnolol, and glycyrrhizic acid as target analytes for detection. The content of glycyrrhizic acid is calculated by dividing the measured value of ammonium glycyrrhizinate by 1.0207. The chemical structures of these three compounds are shown in **Figure 1**.

In the draft method, a C18 stationary phase is specified for the analysis of three compounds using two compound specific methods, one for honokiol



Honokiol | Glycyrrhizic acid | Magnolol

Figure 1. Chemical structures of three active components honokiol, glycyrrhizic acid, and magnolol in Huoxiang Zhengqi Shui herbal formula.

and magnolol, and another one for ammonium glycyrrhizinate. In the present study, honokiol and magnolol were analyzed using a superficially porous particle (SPP) Ascentis® Express C18 column to enhance separation efficiency, which allowed the use of a shorter column. Glycyrrhizic acid was determined with a fully porous particle (FPP) column offering extended pH stability (1.5–10.5), the Purospher® STAR RP-18e. The composition of a commercially available preparation of Huoxiang Zhengqi Shui was assessed using these two methods.

Experimental

Standard and Sample Preparation

Standards and samples were prepared according to below procedures. The Huoxiang Zhengqi Shui was purchased from a local pharmacy.

Standard Preparation

For quantification of honokiol and magnolol:

- **Mixed stock solution:** Weigh 2.5 mg of honokiol and 5 mg of magnolol into a 10 mL brown glass volumetric flask. Add approximately 8 mL of methanol and sonicate for 5 minutes. Top up to the mark with methanol and mix well. The resulting concentrations of honokiol and magnolol in the stock solution are 250 µg/mL and 500 µg/mL, respectively.
- **Standard solutions for external calibration:** Pipette 2, 4, 10, 20, 40, 100, 200, 400 and 1000 µL, respectively, of mixed stock solution into 1.5 mL centrifuge tubes. Dilute each to a final volume of 1 mL with methanol to obtain a series of standard solutions with concentrations of 0.5, 1, 2, 5, 10, 25, 50, 100 and 250 µg/mL for honokiol, and concentrations of 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/mL for magnolol.

For quantification of ammonium glycyrrhizinate:

- **Stock solution (1 mg/mL):** Weigh 10 mg of ammonium glycyrrhizinate into a 10 mL brown glass volumetric flask. Add approximately 8 mL of a 50% aqueous solution of ethanol and sonicate for 5 minutes. Top up to the mark with 50% aqueous solution of ethanol and mix well. The concentration of ammonium glycyrrhizinate in the resulting stock solution is 1 mg/mL.
- **Standard solutions for external calibration:** Pipette 1, 2, 5, 10, 20, 50, 100, 200 and 500 µL, respectively, of stock solution into 1.5 mL centrifuge tubes. Dilute each to a final volume of 1 mL with 50% aqueous ethanol solution to obtain a series of standard solutions with concentrations of 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/mL of ammonium glycyrrhizinate.

Sample Preparation

Accurately transfer 5 mL of Huoxiang Zhengqi Shui into a 20 mL volumetric flask. Add 10 mL of ethanol, shake well, and dilute to volume with ethanol. Mix thoroughly and filter through a 0.45 µm membrane filter prior to HPLC analysis.

HPLC Methods

The quantification of honokiol, magnolol was performed using an Ascentis® Express 90 Å C18 column (**Table 1**), while ammonium glycyrrhizinate was analysed on a Purospher® STAR RP-18 endcapped column (**Table 2**). Chromatographic conditions for each method are summarized in the respective tables.

Table 1. HPLC conditions used for the analysis of honokiol and magnolol

LC Conditions	
Column:	Ascentis® Express 90 Å C18, 5 µm, 150 x 4.6 mm I.D. (**50537-U**)
Mobile phase:	[A] Methanol; [B] acetonitrile; [C] water; (40:20:40 A:B:C); isocratic
Flow rate:	1.0 mL/min
Pressure:	3990 psi (275 bar)
Column temp.:	25 °C
Detector:	UV 294 nm
Injection:	10 µL

Table 2. HPLC conditions applied for the analysis of ammonium glycyrrhizinate

LC Conditions	
Column:	Purospher® STAR RP-18 endcapped, 5 µm, 250 x 4.6 mm I.D. (**1.51456**)
Mobile phase:	[A] Acetonitrile; [B] 0.1% phosphoric acid aqueous solution; (34:66 A:B); isocratic
Flow rate:	1.0 mL/min
Pressure:	1930 psi (133 bar)
Column temp.:	25 °C
Detector:	UV 253 nm
Injection:	10 µL

Acceptance Criteria

The standard method requires that the theoretical plate numbers for magnolol and glycyrrhizic acid be NLT 5,000, and the resolution between the three analytes and their respective impurities must not be less than 1.5.

Results & Discussion

The chromatographic results of the HPLC analysis of honokiol, magnolol (Ascentis® Express C18 column) and ammonium glycyrrhizinate (Purospher® STAR RP-18 endcapped column) are shown in **Figures 2** & **3** (standards) and **Figures 5** & **6** (samples). Theoretical plate counts for all analytes exceeded 5,000, thereby fulfilling the requirements of the standard method (**Tables 3** & **4**).

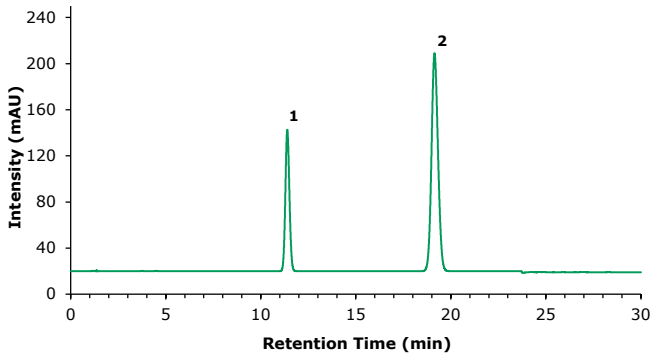


Figure 2. Chromatogram (294 nm) for the standard solution containing 100 µg/mL of honokiol (1) and 200 µg/mL of magnolol (2) obtained using the Ascentis® Express C18 column.

Table 3. Chromatographic data of the standard solution containing 100 µg/mL of honokiol and 200 µg/mL of magnolol

Peaks	Compound	Retention time (min)	Resolution	Plates (USP)	Tailing factor
1	Honokiol	11.39	--	13,226	1.08
2	Magnolol	19.14	14.78	14,673	1.08

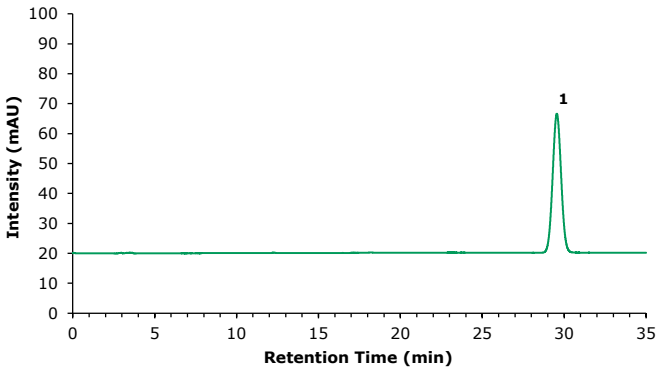


Figure 3. Chromatogram (253 nm) for the standard solution containing 200 µg/mL of ammonium glycyrrhizinate obtained using the Purospher® STAR RP-18 endcapped column.

Table 4. Chromatographic data of the standard solution containing 200 µg/mL of ammonium glycyrrhizinate

Peaks	Compound	Retention time (min)	Resolution	Plates (USP)	Tailing factor
1	Ammonium glycyrrhizinate	29.57	--	15288	1.03

Calibration, Sensitivity, and Repeatability

The calibration curve for honokiol is shown in **Figure 4** as a representative example; similar results were obtained for magnolol and glycyrrhizic acid (calibration curves not shown). An overview of the calibration data for all compounds is provided in **Table 5**.

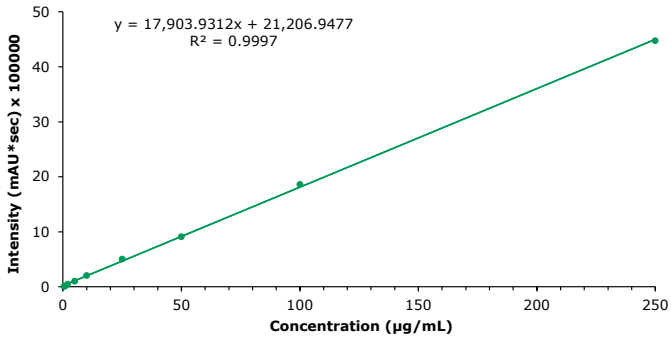


Figure 4. Calibration curve obtained for the analysis of nine honokiol standard solutions (c=0.5–250 µg/mL).

Table 5. Calibration data for honokiol, magnolol and glycyrrhizic acid obtained from the analysis of the respective standard solutions

Compound	Calibration range (µg/mL)	No. of calibrators	R^2
Honokiol	0.5–250	9	0.9997
Magnolol	1–500	9	0.9997
Glycyrrhizic acid*	0.98–490*	9	0.9999

* The linear range of glycyrrhizic acid was obtained by dividing the concentrations of a series of standard solutions of ammonium glycyrrhizinate (1–500 µg/mL) by 1.0207.

The LOD and LOQ for honokiol within the calibration range of 0.5 to 250 µg/mL were 2.13 and 6.46 µg/mL, respectively. For magnolol, the LOD and LOQ were 1.18 and 3.57 µg/mL within the range of 1 to 500 ng/mL. For glycyrrhizic acid, the LOD and LOQ were 0.40 µg/mL and 1.22 µg/mL within the range of 0.98–490 µg/mL (**Table 6**).

Table 6. LOD and LOQ obtained from the analysis of standard solutions of honokiol, magnolol and glycyrrhizic acid

Compound	LOD (µg/mL)	LOQ (µg/mL)
Honokiol	2.13	6.46
Magnolol	1.18	3.57
Glycyrrhizic acid*	0.40	1.22

* The linear range of glycyrrhizic acid was obtained by dividing the concentrations of a series of standard solutions of ammonium glycyrrhizinate by 1.0207.

Repeatability was evaluated using five replicate injections of the standard solutions at concentrations of 100 µg/mL for honokiol and magnolol, and 200 µg/mL for ammonium glycyrrhizinate. The resulting Relative Standard Deviation (RSD) values ranged from 0.13% to 0.64% (**Table 7**). These results comply with the general rule 0512 of the Chinese Pharmacopoeia, which

specifies that RSD values should be less than 2% unless otherwise stated, and provided RSD values between 0.13 and 0.64% (**Table 7**).

Table 7. Repeatability data for five replicate injections (n=5) of standard solutions at concentrations of 100 µg/mL (honokiol, magnolol) and 200 µg/mL (ammonium glycyrrhinizate)

	Honokiol (100 µg/mL)	Magnolol (200 µg/mL)	Ammonium glycyrrhizinate
	Peak area (mAu*s)		
Mean	1,840,372	4,509,351	1,678,390
Standard deviation	11,793	25,970	2,240
RSD (%)	0.64	0.58	0.13

Sample Analysis

Real Huoxiang Zhengqi Shui samples were analysed, and the results are displayed in **Figures 5 & 6** and **Tables 8 & 9**. In the method for ammonium glycyrrhizinate (**Figure 6**), an impurity peak was observed at 27.7 min. The resolution between ammonium glycyrrhizinate and the impurity was 2.3, thereby fulfilling the draft method requirement of >1.5. The determined content of honokiol, magnolol, and glycyrrhizic acid in the investigated Huoxiang Zhengqi Shui sample, obtained using the two developed methods, are summarized in **Table 10**.

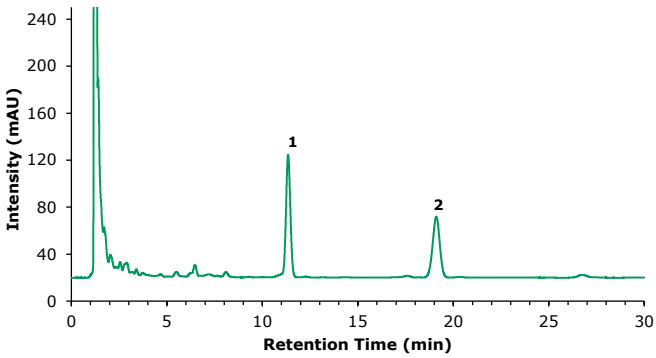


Figure 5. Chromatogram (294 nm) of the sample solution showing (1) honokiol, (2) magnolol.

Table 8. Chromatographic data of honokiol and magnolol in real Huoxiang Zhengqi Shui samples

Peaks	Compound	Retention time (min)	Resolution	Plates (USP)	Tailing factor
1	Honokiol	11.38	--	13,175	1.08
2	Magnolol	19.12	13.70	14,599	1.10

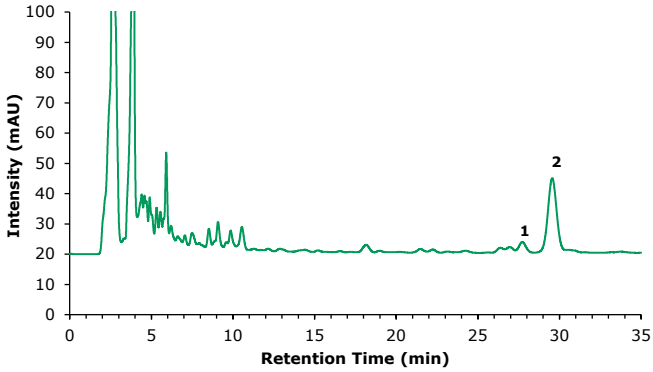


Figure 6. Chromatogram (253 nm) of the sample solution showing (1) unknown impurity, (2) ammonium glycyrrhizinate.

Table 9. Chromatographic data of ammonium glycyrrhizinate in sample solution

Peaks	Compound	Retention time (min)	Resolution	Plates (USP)	Tailing factor
1	Unknown impurity	27.72	NA	18478	NA
2	Ammonium glycyrrhizinate	29.53	2.3	14572	0.98

Table 10. Content of honokiol, magnolol, and glycyrrhizic acid determined in the used Huoxiang Zhengqi Shui sample

Comound	Amount (µg/mL)
Glycyrrhizic Acid	410.67
Honokiol	376.93
Magnolol	236.96

Conclusion

The analysis of the three active compounds honokiol, magnolol, and glycyrrhizic acid in a Huoxiang Zhengqi Shui formulation was demonstrated in accordance with the draft detection methods released by the Chinese Pharmacopoeia (Ch P) Commission. The sample was diluted with ethanol, filtered, and subsequently analysed by HPLC-UV using an Ascentis® Express 90 Å C18 for honokiol and magnolol and a Purospher® STAR C18 endcapped column for glycyrrhizic acid. The developed methods fulfilled the acceptance criteria specified in the Ch P commission's published draft regarding minimum theoretical plate count and resolution. These findings confirm the suitability and compliance of the two employed columns for the analysis of Huoxiang Zhengqi Shui in line with the pharmacopeial requirements.

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Methanol, gradient grade, suitable for HPLC, LiChrosolv®, reag. Ph. Eur.	**1.06007**
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