Determination of Sinomenine Hydrochloride in Zhengqing Fengtongning Tablets

Chinese Pharmacopeia 2025 Method

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Abstract

A method for the determination of sinomenine in Zhengqing Fengtongning tablets, following one public draft of the monograph method for Zhengqing Fengtongning tablets proposed by the Chinese Pharmacopeia, was developed. After sample preparation, using solvatization in ethanol and filtration, sinomenine was determined by HPLC-UV using a Discovery® C18 column. Results showed that the developed method complied with and exceeded the requirements of the draft monograph method.

Introduction

Sinomenine (**Figure 1**), a bioactive alkaloid, is an active ingredient extracted from the traditional Chinese medicinal plant Qinfengteng.[1] *In vivo* and *in vitro* experiments have confirmed that sinomenine has biological effects such as anti-inflammatory, antioxidant, inhibition of cell apoptosis, and immunosuppression.[2] Zhengqing Fengtongning tablets are derivatives related to sinomenine, which have been approved by the National Medical Products Administration of China and the National Health Insurance Directory for the treatment of Rheumatoid Arthritis (RA). Huang et al.[3] and Liu et al.[4] confirmed that Zhengqing Fengtongning tablets can inhibit the progression of RA inflammation by regulating the secretion levels of inflammatory factors and macrophage subpopulations by utilizing collagen-induced arthritis mouse models and peripheral blood from RA patients.

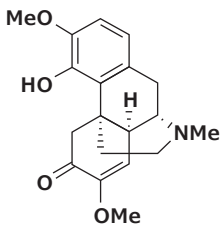

Figure 1. Chemical structure of sinomenine.

In 2024, the official website of the Chinese Pharmacopoeia Commission released a draft of a detection method for sinomenine hydrochloride in Zhengqing Fengtongning tablets.[5] Once it passes method validation, this method will be included in the 2025 edition of the Chinese Pharmacopoeia. Accordingly, this work describes the development and evaluation of a reversed-phase HPLC method using a Supelco® Discovery® C18 column for the quantification of sinomenine in a commercially available brand of Zhengqing Fengtongning tablet.

Experimental

Standards and samples were prepared and analyzed according to the following procedures:

Standard Preparation

- **Stock solution**: Weigh 10 mg of a sinomenine reference standard into a 10 mL amber glass volumetric flask. Add approximately 3 mL of ethanol and sonicate for 5 minutes. Top up to the mark with the mobile phase and mix well. The concentration of sinomenine in the resulting stock solution is 1 mg/mL.
- **Standard solutions for external calibration**: Separately pipette 2, 4, 8, 20, 40, 80, 160, 400, and 800 µL of the stock solution into 1 mL vials, and fill up to 1 mL with mobile phase to obtain a series of standard solutions with sinomenine concentrations of 2, 4, 8, 20, 40, 80, 160, 400, and 800 µg/mL.

Sample Preparation

Weigh ten tablets of Zhengqing Fengtongning (~1.6 g) precisely to 0.1 mg and grind to powder. Transfer ~ 0.16 g of powder into a 100 mL volumetric flask to get the equivalent quantity of 20 mg sinomenine hydrochloride (the label indicated a concentration of 20 mg/tablet). Add 30 mL of ethanol and 0.1 mL of ammonia solution (400 mL of concentrated ammonia, diluted with water to a total volume of 1000 mL). Sonicate the mixed solution for 20 minutes at 300 W and 25 kHz. Cool to room temperature, then add mobile phase to the mark and shake well. Finally, filter through a 0.45 µm membrane before HPLC analysis.

HPLC UV Method

For the HPLC-UV analysis, a Discovery® C18 column was used (**Table 1**).

Table 1. HPLC conditions utilized for the analysis of sinomenine

LC Conditions	
Column:	Discovery® C18, 5 µm, 250 x 4.6 mm I.D. (**504971**)
Mobile phase:	[A] Acetonitrile; [B] 0.78% sodium dihydrogen phosphate solution (12:88 A:B); isocratic
Flow rate:	1.0 mL/min
Pressure:	1,590 psi (110 bar)
Column temp.:	25 °C
Detector:	UV, 265 nm
Injection:	5 µL

Acceptance Criteria for Standard Solution

Theoretical plate number calculated from the peak of sinomenine: NLT 2,000.

Results and Discussion

Zhengqing fengtongning tablets were ground into a powder and then extracted with ethanol. The resulting solution was then filtered and analyzed for sinomenine on a Discovery® reversed-phase C18 HPLC column using an external calibration. Then the content of sinomenine hydrochloride in the tablet samples was calculated by multiplying the content of sinomenine by 1.219.

Chromatographic results for the HPLC analysis of the sinomenine standard and blank solution are displayed in **Figures 2–3**. The developed method showed a plate count of 12,601 and therefore meets the criteria of NLT 2,000 (**Table 2**). The eluting peak was very symmetrical with a tailing factor of 1.1.

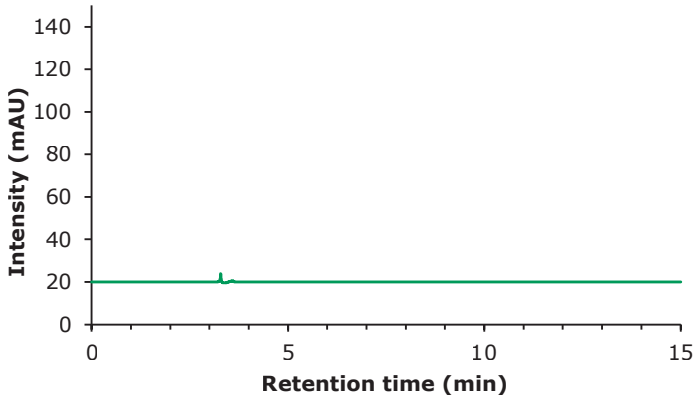

Figure 2. Analysis of a blank solution (mobile phase: [A] acetonitrile; [B] 0.78% sodium dihydrogen phosphate solution, A:B 12:88).

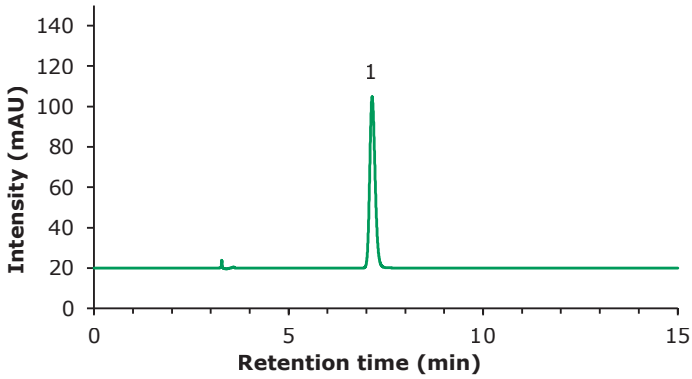

Figure 3. Standard solution containing 160 µg/mL of sinomenine (1).

Table 2. Chromatographic data of standard solution containing 160 µg/mL of sinomenine

Peak	Compound	Retention time (min)	Plates (USP)	Tailing factor (USP)	Peak purity
1	Sinomenine	7.15	12,601	1.1	1

Calibration and Repeatability

The calibration curve for sinomenine is shown in **Figure 4**. The calibration in the range of 2 to 800 µg/mL showed a linearity value R^2 of 1.000. The method also showed excellent repeatability with an RSD value of 0.07% for the 160 µg/mL standard solution (**Table 3**).

Table 3. Repeatability data obtained by the HPLC analysis of five standard solution(160 µg/mL) injections

Injection no.	Area
Mean (n=5)	817,138
Standard deviation	560
RSD (%)	0.07

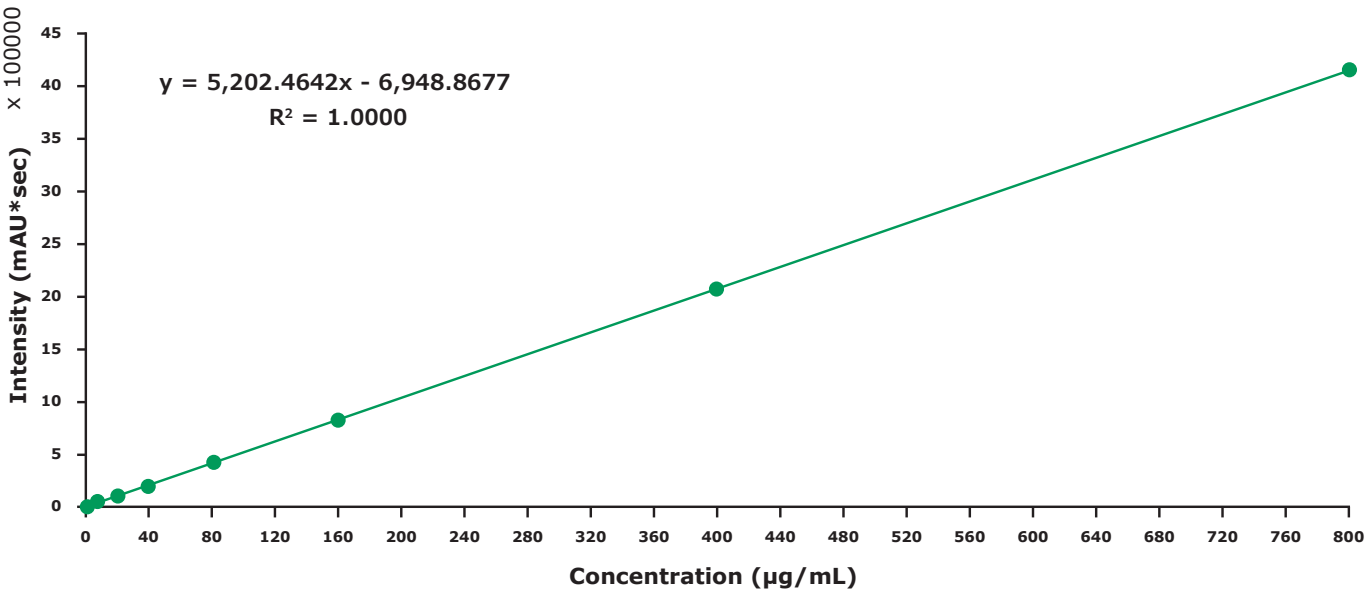

Figure 4. Calibration curve for sinomenine obtained by the analysis of nine calibration solutions.

Method Sensitivity

From the calibration range of sinomenine (2 to 800 µg/mL), the low concentration standards were used for sensitivity determination. The obtained results for the derived limit of detection (LOD) and limit of quantification (LOQ) for sinomenine hydrochloride dihydrate in the tablet sample were 125.7 and 380.8 µg/g.

Table 4. Derived LOD and LOQ for tablet sample obtained by the analysis of sinomenine standard solutions

Compound	LOD (µg/g)	LOQ (µg/g)
Sinomenine hydrochloride	125.7	380.8

Sample Analysis

The analysis of a sample solution representing a tablet with 20 mg sinomenine hydrochloride in 100 mL (**Figure 5**) showed a content of 17.85 mg/tablet (**Table 5**).

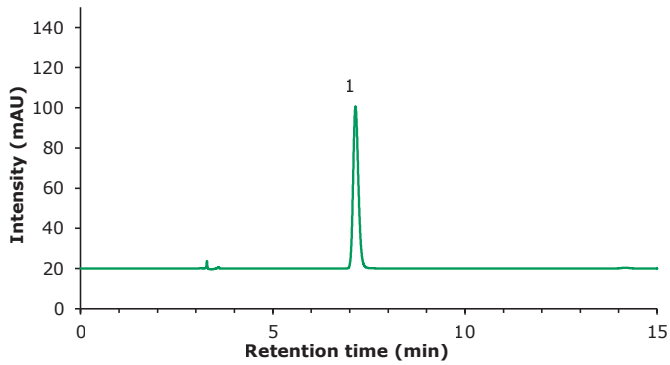

Figure 5. Sample solution for determination of sinomenine (1).

Table 5. Determined sinomenine hydrochloride content in analyzed Zhengqing Fengtongning tablets

Compound	Content in sample solution (µg/mL)	Calculatedcontent per tablet (mg)
Sinomenine	146.45	14.65
Sinomenine hydrochloride	178.52	17.85

Conclusion

An HPLC-UV method was developed for the quantification of sinomenine in Zhengqing Fengtongning tablets, in accordance with the draft monograph published by the Chinese Pharmacopoeia. Sample preparation involved ethanolic extraction of ground tablet material, followed by chromatographic analysis on a Discovery® C18 column.

The developed method yielded a theoretical plate count of 12,601 (USP) for the sinomenine peak, thereby meeting the Chinese Pharmacopoeia draft monograph requirement of not less than 2,000 plates. The determined RSD for the method complied with general requirements of Chromatographic Method 0512 of the Chinese Pharmacopoeia, which stipulates an RSD of less than 2%, unless otherwise specified. This further underlines its applicability in the analysis of the active ingredient sinomenine in Zhengqing Fengtongning. The calculated Limits Of Detection (LOD) and Quantification (LOQ) for sinomenine hydrochloride were 125.7 µg/g and 380.8 µg/g of tablet sample, respectively. These results confirm the suitability of the method for routine quality control and quantification of sinomenine in Zhengqing Fengtongning tablets.

References

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Related Products

Description	Cat. No.
HPLC Column	
Discovery® C18 (5 µm), 25 cm × 4.6 mm I.D.	**504971**
Solvents, Accessories	
Acetonitrile, gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**1.00030**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7000T0C**
Ethyl alcohol, gradient grade, suitable for HPLC, gradient grade, LiChrosolv®	**1.11727**
Ammonia solution, 28-30%, for analysis EMSURE® ACS, Reag. Ph Eur	**1.05423**
Sodium dihydrogen phosphate anhydrous, for HPLC LiChropur™	**5.43840**
Millex™ PVDF syringe filter, pore size 0.45 µm, diam. 33 mm, non-sterile, hydrophilic	**SLHV033N**
Reference Standards	
Sinomenine, European Pharmacopoeia (EP) Reference Standard	**Y0001208**
Sinomenine hydrochloride, phyproof® Reference Substance	**PHL89794**

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