Determination of Tanshinone IIA, Notoginsenoside R1, Ginsenoside Rg1 and Ginsenoside Rb1 in Guanxin Danshen Diwan

Chinese Pharmacopeia 2025 Method

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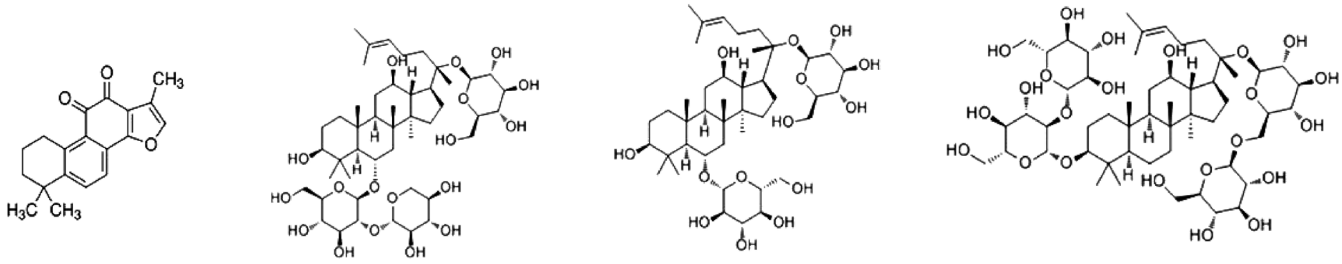
Abstract

Two HPLC methods were developed for the determination of tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 in Guanxin Danshen Diwan, following a public based on a draft monograph released by the Chinese Pharmacopoeia Commission. Following sample preparation, tanshinone IIA was analyzed using a Discovery® C18 column, whereas notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 were determined with a Discovery® HS C18 column. The results demonstrated that both methods satisfied the requirements of the Chinese pharmacopeia draft, confirming their applicability for the quantification of the active ingredients in Guanxin Danshen Diwan.

Introduction

Guanxin Danshen Diwan (or: "Diwan") is formulated from three herbal medicines: Dan Shen, San Qi, and Xiang Fu You.[1,2] It is primarily used for the treatment and relief of chest pain associated with Qi stagnation and blood stasis, with symptoms including chest tightness, chest pain, palpitations, and shortness of breath.[2,3]

In 2024, the official website of the Chinese Pharmacopoeia Commission published a draft method for the detection of four active compounds in Guanxin Danshen Diwan: tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 (**Figure 1**).[4] Upon successful public validation, this monograph will be incorporated into the 2025 edition of the Chinese Pharmacopoeia. In accordance with this draft, two methods were developed and evaluated for the quantitative determination of these four active compounds in a commercially available preparation of Guanxin Danshen Diwan, thereby supporting quality control in accordance with pharmacopeial requirements.



Tanshinone IIA | **Notoginsenoside R1** | **Ginsenoside Rg1** | **Ginsenoside Rb1**

Figure 1. Structures of tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1.

Experimental

Standard and Sample Preparation

Standard Preparation

For the determination of tanshinone IIA:

- **Stock solution:** *Weigh* 10 mg of a tanshinone IIA reference standard into a 10 mL brown volumetric flask. Add ~8 mL methanol and sonicate for 5 minutes. Top up to the mark with the mobile phase and mix well. The resulting concentration for tanshinone IIA stock solution is 1 mg/mL.
- **Standard solutions for external calibration:** Dilute the stock solution with methanol to obtain a series of standard solutions with concentrations of 1,2, 5, 10, 20, and 50 µg/mL for tanshinone IIA.

For the determination of notoginsenoside R1, ginsenoside Rg1 and ginsenoside Rb1:

- **Stock solution:** Separately weigh 2.5 mg of a notoginsenoside, 10 mg of ginsenoside Rg1, and 10 mg of ginsenoside Rb1 reference standard into a 10 mL brown volumetric flask. Add ~8 mL methanol and sonicate for 5 minutes. Top up to the mark with the mobile phase and mix well. The concentrations in the resulting stock solution were 0.25 mg/mL, 1.0 mg/mL, 1.0 mg/mL for notoginsenoside, ginsenoside Rg1, and ginsenoside Rb1, respectively.
- **Standard solutions for external calibration:** Dilute the stock solution with 19% acetonitrile aqueous solution to obtain a series of standard solutions with concentrations of 2.5, 5, 12.5, 25, 50 and 125 µg/mL for notoginsenoside, and 10, 20, 50, 100, 200, and 500 µg/mL for ginsenoside Rg1 and ginsenoside Rb1.

Sample Preparation

Sample: Guanxin Danshen Diwan purchased from a local pharmacy.

For tanshinone IIA:

- Precisely weigh 50 tablets of Guanxin Danshen Diwan, and grind to a fine powder.
- Precisely weigh about 0.8 g of the powdered sample, and transfer to an amber glass bottle.
- Accurately add 25 mL of 80% methanol (80:20 methanol:water, v/v).
- Weigh the sample, sonicate at 250 W/40 kHz for 30 minutes, allow to cool, and weigh again.
- Use 80% methanol to make up for the weight loss and mix well.
- Filter through a 0.45 µm membrane filter before HPLC analysis.

For notoginsenoside R1, ginsenoside Rg1 and ginsenoside Rb1:

- Precisely weigh 50 tablets of Guanxin Danshen Diwan, and grind to a fine powder.
- Precisely weigh about 0.4 g of the powdered sample, and transfer to an amber glass bottle.
- Accurately add 50 mL of 80% methanol (80:20 methanol:water, v/v).
- Weigh the sample, sonicate at 250 W/40 kHz for 30 minutes, allow to cool, and weigh again.
- Use 80% methanol to make up for the weight loss and mix well.
- Filter through a 0.45 µm membrane filter before HPLC analysis.

HPLC-UV Analysis

The Diwan sample extracts, and corresponding standards were analyzed by HPLC-UV. Tanshinone IIA was determined using a Discovery® C18 (**Table 1**), whereas notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 were analyzed on a Discovery® HS C18 HPLC column (**Table 2**).

Table 1. HPLC conditions for the analysis of tanshinone IIA

LC Conditions	
Column:	Discovery® C18, 5 µm, 150 x 4.6 mm I.D. (**504955**)
Mobile phase:	Methanol/water (75:25, v:v), isocratic
Flow rate:	1.0 mL/min
Pressure:	1290 psi (88.9 bar)
Column temp.:	25 °C
Detector:	UV, 270 nm
Injection:	10 µL

Table 2. HPLC conditions for the analysis of notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1

LC Conditions			
Column:	Discovery® HS C18, 5 µm, 250 x 4.6 mm I.D. (**568523**)		
Mobile phase:	[A] Acetonitrile; [B] water		
Gradient:	**Time (min)**	**A%**	**B%**
	0.0	19	81
	12.0	19	81
	35.0	25	75
	60.0	40	60
	60.1	19	81
	70.0	19	81
Flow rate:	1.0 mL/min		
Pressure:	1830 psi (126 bar)		
Column temp.:	25 °C		
Detector:	UV, 203 nm		
Injection:	10 µL		

Acceptance Criteria

Acceptance criteria for standard solutions

- Theoretical plate number calculated for the peak of tanshinone IIA: NLT (not less than) 3000.
- Theoretical plate number calculated for the peak of ginsenoside Rg1: NLT 4000.
- The resolution between tanshinone IIA, notoginsenoside R1, ginsenoside Rg1 and, ginsenoside Rb1 and their closest sample impurity peaks must not be less than 1.5 (Ch. P. HPLC method general rule 0512).

Results & Discussion

Chromatograms obtained for the developed HPLC methods for the standard solutions are displayed in **Figures 2** & **3**, and the corresponding chromatographic data for tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 is summarized in **Tables 3** and **4**. The theoretical plate count was 11,114 for tanshinone IIA and 73,624 for ginsenoside Rg1. Both values exceeded the requirements of the Chinese pharmacopeia draft (NLT 3000 and 4000 respectively), confirming that the two developed methods can be utilized for the quantification of the four active ingredients in Guanxin Danshen Diwan in accordance with the draft monograph.

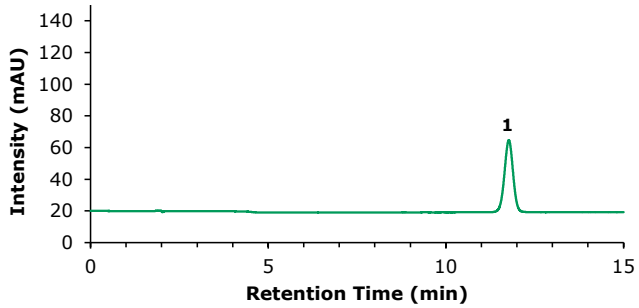


Figure 2. HPLC-UV chromatogram of the standard solution containing 20 µg/mL of tanshinone IIA (**1**).

Table 3. Chromatographic data obtained for the standard solution containing 20 µg/mL of tanshinone IIA

Peaks	Compound	Retention time (min)	Plates (USP)	Resolution	Tailing factor
1	Tanshinone IIA	11.78	11,114*	na-	0.99

*Acceptance criteria: NLT 3,000

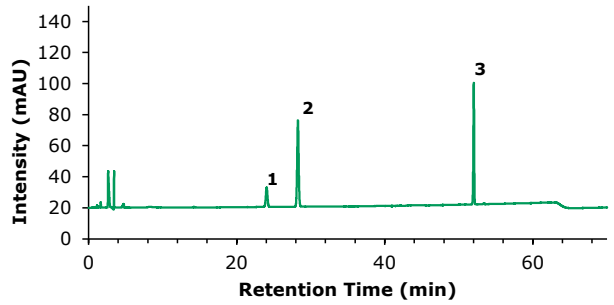


Figure 3. HPLC-UV chromatogram of the standard solution containing 50 µg/mL of notoginsenoside R1 (**1**), and 200 µg/mL of ginsenoside Rg1 (**2**) and ginsenoside Rb1 (**3**), respectively.

Table 4. Chromatographic data obtained for the standard solution containing 50 µg/mL of notoginsenoside R1, and 200 µg/mL each of ginsenoside Rg1 and of ginsenoside Rb1

Peak no.	Compound	Retention time (min)	Plates (USP)	Resolution	Tailing factor
1	Notoginsenoside R1	22.99	44,271	-	1.01
2	Ginsenoside Rg1	28.30	73,624*	10.5	1.00
3	Ginsenoside Rb1	51.90	867,512	78.6	0.92

*Acceptance criteria Ginsenoside Rg1: NLT 4,000

Calibration and Repeatability

Calibration was performed in accordance with the draft monograph method. The curve for tanshinone IIA is shown as a representative example in **Figure 4**. Similar results were obtained for the other compounds, with the exception of notoginsenoside R1, where the 2.5 µg/mL standard produced a signal too low for quantification and was therefore excluded from the calibration range. A summary of the calibration data is provided in **Table 5**. All compounds demonstrated good linearity, with R^2 values greater than 0.99. Repeatability testing for the two methods yielded relative standard deviation (RSD) values ranging from 0.34% to 1.63% (**Tables 6** and **7**).

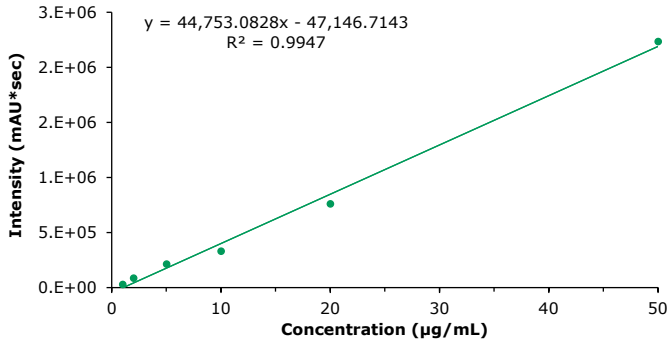


Figure 4. Calibration curve obtained for tanshinone IIA.

Table 5. Linearity of tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 standard solution injections

Compound	Concentration range (µg/mL)	No. of calibrators	R^2
Tanshinone IIA	1–50	6	0.9947
Notoginsenoside R1	5–125	5	0.9999
Ginsenoside Rg1	10–500	6	0.9999
Ginsenoside Rb1	10–500	6	0.9947

Table 6. Repeatability data for the injection of a standard solution of tanshinone IIA (c = 20 µg/mL)

Repeatability	Tanshinone IIA
Mean Peak Area, n = 5 (mAu*s)	772,067
Standard deviation	12,569
RSD (%)	**1.63**

Table 7. Repeatability data for the injection of standard solutions of notoginsenoside R1 (c = 50 µg/mL), ginsenoside Rg1 (c = 200 µg/mL), and ginsenoside Rb1 (c = 200 µg/mL)

Repeatability	Notoginsenoside R1	Ginsenoside Rg1	Ginsenoside Rb1
Mean Peak Area, n = 5 (mAu*s)	199,422	817,950	642,292
Standard deviation	1774	2753	2355
RSD (%)	**0.89**	**0.34**	**0.37**

Sensitivity

The sensitivity of the developed methods is summarized in **Table 8**. The LOD and LOQ were determined based on the lowest concentrations of the calibration standards.

Table 8. LOD and LOQ for tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 in tablet samples (µg/g) obtained by the two developed methods

Compound	LOD (µg/g)	LOQ (µg/g)
Tanshinone IIA	16.38	49.63
Notoginsenoside R1	234	709
Ginsenoside Rg1	1065	3227
Ginsenoside Rb1	996	3017

Tablet Sample Analysis

Chromatographic separations of the tablet sample preparations are shown in **Figures 5** & **6**, and the corresponding chromatographic data is summarized in **Tables 9** & **10**. All neighboring impurity peaks originating from the tablet matrix were adequately resolved from the analytes of interest, with a resolution >1.5, thereby meeting the requirements of the Ch.P. HPLC method general rule 0512.

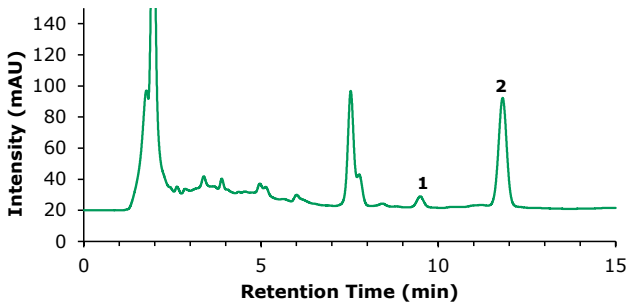


Figure 5. HPLC-UV chromatogram of the sample solution used for the quantification of tanshinone IIA (**2**).

Table 9. Chromatographic data of sample solution used for the quantification of tanshinone IIA

Peak	Compound	Retention time (min)	Plates (USP)	Resolution	Tailing factor
1	Unknown impurity	9.50			
2	Tanshinone IIA	11.82	12,054*	5.67	1.00

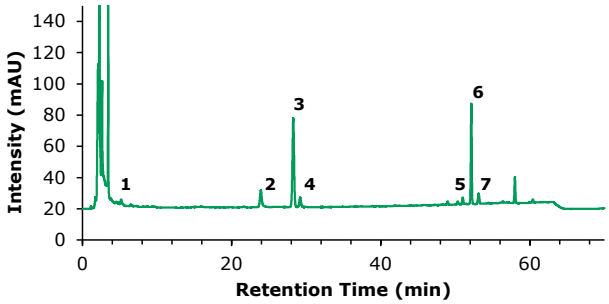


Figure 6. HPLC-UV chromatogram of the sample solution used for the quantification of notoginsenoside R1 (**2**), ginsenoside Rg1 (**3**), and ginsenoside Rb1 (**6**).

Table 10. Chromatographic data obtained for the sample solution used for the quantification of notoginsenoside R1, ginsenoside Rg1 and ginsenoside Rb1

Peak no.	Compound	Retention time (min)	Plates (USP)	Resolution	Tailing factor
1	Unknown impurity 1	6.48			
2	Notoginsenoside R1	23.92	45,379	48.0	1.06
3	Ginsenoside Rg1	28.27	78,325	10.0	0.99
4	Unknown impurity 2	29.20		2.3	
5	Unknown impurity 3	51.00			0.92
6	Ginsenoside Rb1	52.16	833,821	4.5	0.92
7	Unknown impurity 4	53.14		3.4	

Table 11. Content of tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 determined in the Guanxin Danshen Diwan tablet sample

Compound	Amount (mg/g)	Amount (µg/tablet)
Tanshinone IIA	0.77	30.70
Notoginsenoside R1	5.75	229.86
Ginsenoside Rg1	26.83	1073.34
Ginsenoside Rb1	21.96	878.26

Conclusion

Two HPLC-UV methods were developed in accordance with the draft Chinese pharmacopeia monograph for the analysis of the four active compounds tanshinone IIA, notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 in Guanxin Danshen Diwan. The powdered samples were extracted with 80% aqueous methanolic solution, and the resulting extracts were filtered and analyzed using Discovery® C18 and Discovery® HS C18 columns.

The developed method exceeded the theoretical plate counts required by the Chinese Pharmacopoeia acceptance criteria and fulfilled the resolution criteria outlined in the Ch. P. HPLC method general rule 0512. These results confirm the applicability of the two columns for the efficient, sensitive, and reproducible analysis of the active ingredients in Guanxin Danshen Diwan, supporting their use for pharmacopeial quality control.

References

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Description	Cat. No.
HPLC Column	
Discovery® C18, 5 µm, 150 x 4.6 mm I.D.	**504955**
Discovery® HS C18, 5 µm, 250 x 4.6 mm I.D.	**568523**
Solvents, Reagents, and Accessories	
Methanol, suitable for HPLC, LiChrosolv®, Reag. Ph. Eur.	**1.06007**
Acetonitrile, gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**1.00030**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7000T0C**
Millex™ PVDF Syringe Filter, pore size 0.45 µm, diam. 33 mm, non-sterile, hydrophilic	**SLHV033N**
Reference Materials	
Tanshinone IIA, phyproof® Reference Substance	**PHL89320**
Tanshinone IIA, United States Pharmacopeia (USP) Reference Standard	**1643339**
Notoginsenoside R1, phyproof® Reference Substance	**PHL89743**
Ginsenoside Rb1, primary reference standard	**00170580**
Ginsenoside Rg1, certified reference material, pharmaceutical secondary standard	**PHR2200**

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