

High-performance liquid chromatography for the determination of oxymatrine content in radix *Sophorae tonkinensis*

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The purpose of the present study was to establish a high-performance liquid chromatography method for the determination of the oxymatrine content of radix *Sophorae tonkinensis* and to comparatively analyze the content of oxymatrine in radix *Sophorae tonkinensis* from different origins. C18 was used as a chromatographic column (250 mm × 4.6 mm, 5 μm), methanol/3% phosphoric acid in water (80:20) as the mobile phase, 220 nm as the ultraviolet wavelength detection, 1 mL/min as the flow rate and 30°C as the column temperature. All samples were subjected to organic solvent

Radix *Sophorae tonkinensis* is the processed lateral root of *Sophorae tonkinensis* Gapnep (1). It is bitter, cold, toxic, in the lung and stomach meridian. It has been used as a Chinese medicinal herb to treat jaundice, inflammation and aches, and to clear away heat and purging pathogenic fire (2,3). Radix *Sophorae tonkinensis* mainly include alkaloid, flavonoids and triterpenoid, but oxymatrine and matrine are the primary effective components (4,5). However, oxymatrine and matrine are also the primary toxic components. The Pharmacopoeia of the People's Republic of China states that the safe and effective dosage of radix *Sophorae tonkinensis* is 3 g to 6 g. If the dosage exceeds 12 g, it can cause severe adverse reactions such as nausea and vomiting (6,7). Radix *Sophorae tonkinensis* from different origins have different oxymatrine content. The aim of the present study was to establish a high-performance liquid chromatography (HPLC) method for the determination of oxymatrine, and to determine the oxymatrine content of different origins of radix *Sophorae tonkinensis* to provide an evaluation standard for controlling radix *Sophorae tonkinensis* quality.

METHODS

Materials

The reference substance of oxymatrine was purchased from Guangzhou Institute for Drug Control (Guangzhou, China). Radix *Sophorae tonkinensis* was provided by The Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine (Guangzhou, China). Methanol and phosphoric acid were chromatographic grade, and all the other chemicals were analytical grade.

Instrumentation

All HPLC experiments were performed on a self-assembled HPLC (SHIMADZU, Japan), with the C18 chromatographic column (R&C, Guangzhou). The extraction process of radix *Sophorae tonkinensis* used rotary evaporators (EYELA, Shanghai), ultrasonic cleaner (Ningbo, China) and an electronic balance (ACCULAB, Beijing).

extraction with ultrasonic treatment before the oxymatrine content from radix *Sophorae tonkinensis* was measured. Oxymatrine in the range of 30 μg/mL to 150 μg/mL had a good linear relationship ($r=0.9998$), and the average recovery was 99.22%, suggesting that this method is accurate and has high reproducibility. The established high-performance liquid chromatography method for determining oxymatrine content from radix *Sophorae tonkinensis* is efficient and stable. This may be an evaluation criteria used for quality control given that different origins of radix *Sophorae tonkinensis* have wide-ranging differences in oxymatrine content.

Key Words: HPLC; Oxymatrine; radix *Sophorae tonkinensis*

METHODS

Chromatographic conditions

C18 as chromatographic column (250 mm × 4.6 mm, 5 μm), methanol/3% phosphoric acid in water (80:20) as the mobile phase, 220 nm as the ultraviolet wavelength detection, 1 mL/min as the flow rate, 30°C as the column temperature.

Oxymatrine standard preparation

7.5 mg oxymatrine standard was precisely measured and dissolved in methanol in a 50 mL volumetric flask, obtaining a 150 μg/mL oxymatrine standard solution. Subsequently, 2 mL, 4 mL, 6 mL and 8 mL of the solution was diluted with methanol to obtain 30 μg/mL, 60 μg/mL, 90 μg/mL and 120 μg/mL oxymatrine standard solutions.

Preparing radix *Sophorae tonkinensis* samples

Radix *Sophorae tonkinensis* was crushed and screened, then taking screened powder (approximately 0.5 g) gave a respective weight, denoted M. Trichloromethane-methanol-ammonia (40:10:1) was used to dispose the radix *Sophorae tonkinensis* samples for 30 min, and all samples were subsequently obtained from organic solvent extraction with 30 min ultrasonic treatment. All disposed samples were filtered, then 10 mL of filtrate was measured to recover solvents to dry under decompression at 38°C to obtain the residue, the residue was diluted by methanol, then transferred to a 10 mL volumetric flask. After mixing and filtering with 0.45 μm filter membrane, radix *Sophorae* samples were obtained. The blank groups were treated as the samples but without radix *Sophorae tonkinensis*.

The content of oxymatrine

Twenty microlitres of radix *Sophorae tonkinensis* samples were injected in the HPLC system, the peak area was recorded, mass concentration (C) was obtained and the content of Oxymatrine from radix *Sophorae tonkinensis* was determined. The content of Oxymatrine was calculated by 51C/1000M.

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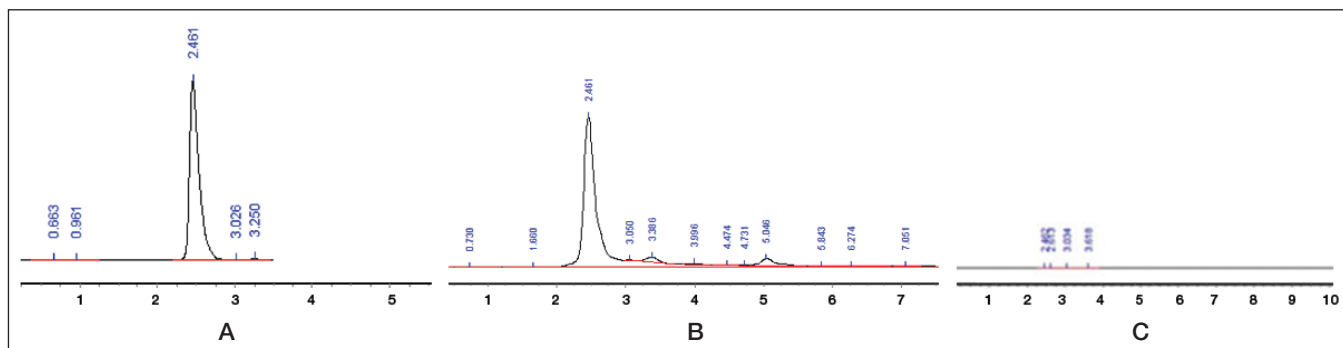


Figure 1) High-performance liquid chromatography chromatograms of oxymatrine. A Standard, B sample, C Blank

TABLE 1
Results of recovery tests (n=3)

NO*	Samples contained, mg	Added, mg	Measured, mg	Recovery, %	Average recovery, %	Relative SD, %
1	5.707	0.150	5.854	98.00	99.22	1.874
2	5.710	0.150	5.859	99.33		
3	5.703	0.150	5.852	99.33		
4	5.703	0.150	5.849	97.33		
5	5.700	0.150	5.854	102.67		
6	5.705	0.150	5.853	98.67		
Total						

*All Radix Sophorae tonkinensis were from Guangxi, China, batch number: 121003; the content of oxymatrine was 22.82 mg/g

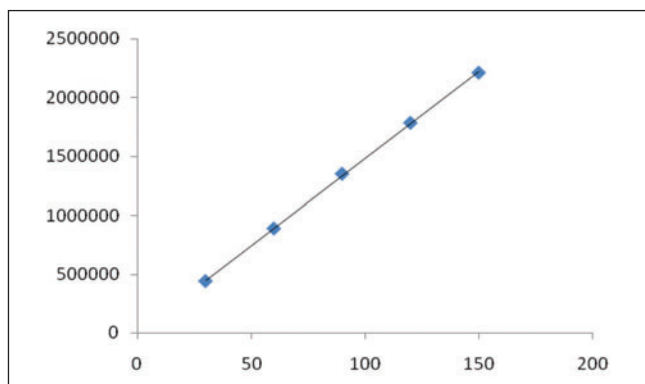


Figure 2) Standard curve of oxymatrine

TABLE 2
Results of different origin of Radix Sophorae tonkinensis sample determination (n=3)

Origin	Batch number	Oxymatrine content, mg/g
Heilongjiang, China	121001	24.72
	121002	24.85
	121003	22.82
Guilin, Guangxi, China	1209021411	7.18
	1209021412	6.98
	1209021413	6.99
Nanning, Guangxi, China	120901	9.28
	120902	8.43
	120903	8.27

RESULTS

Specific inspection study

According to the described chromatographic conditions, oxymatrine standard and radix *Sophorae tonkinensis* samples were effectively separated. The other ingredients did not interfere with the oxymatrine chromatography peak. The method had high specificity; specificity results are shown in Figure 1.

Standard curve drawing

With peak area (y) as the ordinate and mass concentration (x, µg/mL) as the abscissa, the standard curves for oxymatrine were linear in the concentration range of 30 µg/mL to 150 µg/mL ($y=14739x+9487.6$, $r=0.9998$); a standard curve is shown in Figure 2.

Precision, stability, repeatability and recovery tests

Taking the same concentration of oxymatrine standard solution, continuously investigated six times, the RSD result of precision test was 0.941%; taking screened radix *Sophorae tonkinensis* powder (approximately 0.5 g), disposing samples as described, then investigating its peak area every 2 h, the RSD result of stability test was 1.423% in 12 h; taking screened radix *Sophorae tonkinensis* powder

(approximately 0.5 g), disposing samples as described, then continuously investigated its peak area six times, the RSD result of repeatability test was 1.879%; taking screened radix *Sophorae tonkinensis* powder (approximately 0.25 g) for the six groups, disposing them as described, then adding 0.150 mg oxymatrine standard solution to the six radix *Sophorae tonkinensis* samples and investigating its peak area, the average recovery was 99.22%, and RSD was 1.874%. The results of recovery tests are shown in Table 1. Precision, stability, repeatability and recovery tests were well within the 5% acceptable range.

Oxymatrine content of different origin radix *Sophorae tonkinensis* samples

Approximately 0.5 g of radix *Sophorae tonkinensis* powder from different origins disposed them as described, recording the peak area, the content of oxymatrine from radix *Sophorae tonkinensis* was obtained and is presented in Table 2.

As the results in Table 2 demonstrate, radix *Sophorae tonkinensis* coming from Heilongjiang origin had the highest oxymatrine content

24.85 mg/g. Radix *Sophorae tonkinensis* of Guilin origin had the lowest content, and the highest content was approximately four times the lowest (a considerable difference).

DISCUSSION

Oxymatrine has a unique oxygen structure; it can lower blood pressure, resist viruses, strengthen the heart, resist arrhythmia, and treat jaundice, inflammation and aches. Existing research shows that oxymatrine has antitumour activity, mainly by inhibiting DNA synthesis in tumour cells and restraining enzyme activity to inhibit the growth of tumour cells (8). Oxymatrine is the one of the main effective ingredients of radix *Sophorae tonkinensis*; studying the different oxymatrine content from different origins of radix *Sophorae tonkinensis*, the peak concentration was 24.85 mg/g, but the lowest concentration was 7.18 mg/g. The reasons are as follows: different geographical environment causes the different oxymatrine content, and different growth periods lead to the difference (9,10).

Choosing amino as chromatographic column, acetonitrile/isopropyl alcohol/3% phosphoric acid in water (80:5:15) as the mobile phase,

chromatographic peak of oxymatrine appeared overlapping; choosing C18 as chromatographic column, methanol/2.5% phosphoric acid in water (5:95) as the mobile phase, the chromatography of oxymatrine appeared baseline drift; choosing C18 as chromatographic column, methanol/water (75:25) as the mobile phase, the chromatography of oxymatrine could not be separated. Finally, choosing C18 as chromatographic column (250 × 4.6 mm, 5 μm), methanol/3% phosphoric acid in water (80:20) as the mobile phase, 220 nm as the ultraviolet wavelength detection, 1 mL/min as the flow rate and 30°C as the column temperature, good specificity was obtained.

The present study established a HPLC methodology for determination of oxymatrine content from radix *Sophorae tonkinensis*. Because oxymatrine is one of the main effective ingredients and is stable, the content of oxymatrine can be a standard for controlling and evaluating the quality of radix *Sophorae tonkinensis* to offer theoretical basis.

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