Determination of Benzoylaconitine Compounds in the Decoctions of Aconiti Lateralis Radix Praeparata (Heishun Pieces), Trichosanthis Fructus and Their Combination

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Abstract [**Objectives**] This study was conducted to determine the contents of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine in the decoctions of Heishun pieces, Trichosanthis Fructus and their combination. [**Methods**] Heishun pieces, Trichosanthis Fructus and their combination were extracted for different time periods, and then grouped. HPLC was performed using an Agilent ZORBAX SB-C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm) and acetonitrile-0.02 mol/L sodium dihydrogen phosphate as the mobile phase at a flow rate of 1 mL/min and a column temperature of 30 ℃, and the sample volume was 20 μL. The detection wavelength was 230 nm. [**Results**] The total amounts of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine in the single decoction group of Heishun pieces were all significantly different from those in the combined decoction group at corresponding time. [**Conclusions**] The total content of the benzoylaconitine type increased significantly after the combined decoction of Heishun pieces and Fructus Trichosanthis, which proves the scientificity of "eighteen incompatible medicaments, 19 counteraction" in traditional Chinese medicine to some extent.

Key words Aconiti Lateralis Radix Praeparata, Trichosanthis Fructus, Benzoylaconitine compound, Content determination

1 Introduction

Traditional Chinese medicine is an important means of treating diseases, and proper combination of Chinese medicinal materials can achieve synergistic treatment and increase drug efficacy. However, if they are combined improperly or the compatibility is violated, the efficacy will be reduced and even toxic and side effects will occur. For example, the "18 incompatible medicaments" points out the adverse reactions and even toxicity caused by 18 kinds of concomitant application of Chinese medicinal materials. Practice has also proved that if some medicinal materials are used incorrectly, it will lead to serious adverse consequences, so that many doctors dread the contradictory use of Chinese medicinal materials. The "18 incompatible medicaments" thinks that Aconiti Lateralis Radix Praeparata (mostly processed products such as Heishun pieces and Baifu pieces used in clinic) and Trichosanthis Fructus are incompatible medicinal materials, which are highly toxic when used together and should not be used in combination. With the deepening of the research on the compatibility of Chinese medicinal materials, it is found that some medicinal materials in the "18 incompatible medicaments" will have better effects on the treatment of some intractable diseases as long as they are properly matched. In this paper, focusing on the changes in the contents of toxic active ingredients, changes in the contents of toxic active ingredients in the combined decoction of Aconiti Lateralis Radix Praeparata (Heishun pieces) and Trichosanthis Fructus were objectively analyzed[1-5].

2 Materials

2.1 Instruments High performance liquid chromatograph (model: e2695, Waters, USA); electronic balance (model: GH-252, Japan AND Electronic Balance Co., Ltd.); chromatographic column (model: Agilent ZORBAX SB-C_{18} column (4.6 mm × 250 mm, 5 μm), Agilent Technologies Inc.).

2.2 Reagents Benzoylaconitine reference substance (batch No.: MUST-23102120), benzoylmesaconine reference substance (batch No.: MUST-23110314) and benzoylhypacoitine reference substance (batch No.: MUST-22121912) were all purchased from Chengdu Must Biotechnology Co., Ltd. Acetonitrile of chromatographic grade was purchased from Fisher Company, USA. Water used in the experiment was ultrapure water. Trichosanthis Fructus (batch No.: 23102120) and Heishun pieces (batch No.: 23101209) were purchased from Guangxi Xianzhu Traditional Chinese Medicine Technology Co., Ltd.

3 Determination of contents of benzoylaconitine compounds[6-7]

3.1 Chromatographic conditions Chromatographic column: Agilent ZORBAX SB-C_{18} column (4.6 mm × 250 mm, 5 μm); flow rate: 1 mL/min; column temperature: 30 ℃; detection wavelength: 230 nm; sample volume: 20 μL; mobile phase: acetonitrile-0.02 mol/L sodium dihydrogen phosphate (25 ∶ 75).

3.2 Preparation of test solution First, 100 g of Trichosanthis Fructus and Heishun pieces were accurately weighed, respectively. Next, 10 times of water was added to extract the materials for 2 h. Next, filtration was performed with 300-mesh filter cloth.

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The extract was added into a 1 000 mL volumetric flask, diluted to constant volume and mixed well. Subsequently, 5.0 mL was measured and centrifuged at 4 000 r/min for 10 min. Finally, the liquid was filtered through a 0.22 μm microfiltration membrane to get a test solution (Fig. 1).

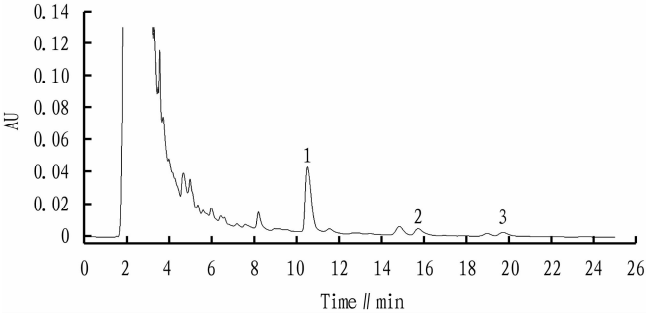
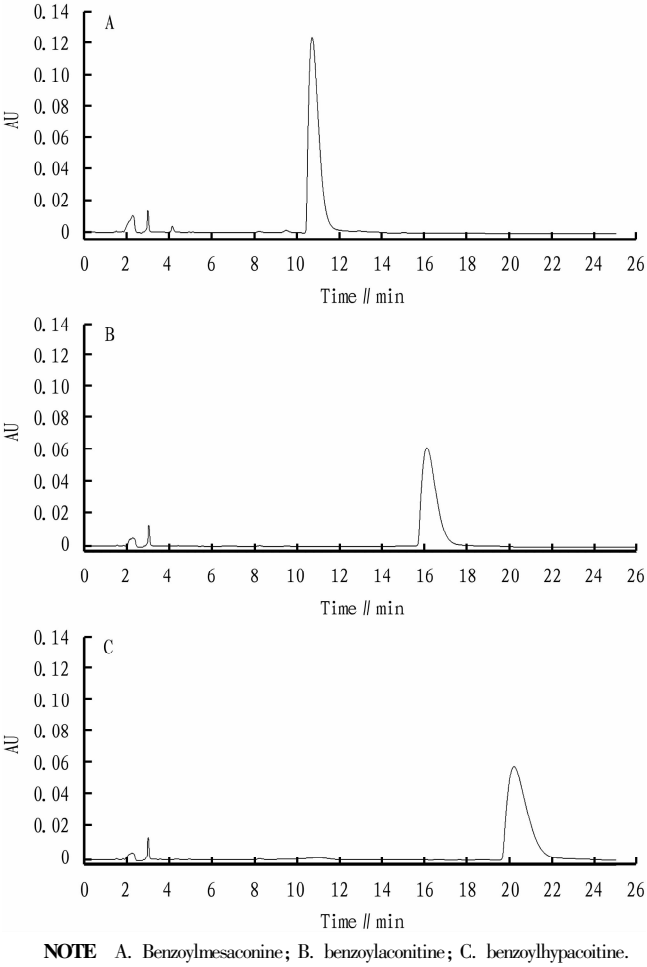


Fig. 1 Chromatogram of test solution

3.3 Preparation of reference solution Proper amounts of benzoylmesaconine reference substance, benzoylaconitine reference substance and benzoylhypacoitine reference substance were accurately weighed. Next, methanol was added to prepare mixed solutions with concentrations of 0.20, 0.044 0 and 0.04 mg/mL respectively, and they were shaken evenly to get the final products (Fig. 2).



NOTE A. Benzoylmesaconine; B. benzoylaconitine; C. benzoylhypacoitine.

Fig. 2 Chromatogram of reference solution

3.4 Preparation of negative sample solution A negative sample lacking Heishun pieces were prepared into a negative reference solution lacking Heishun pieces according to the method in Section **3.2** (Fig. 3).

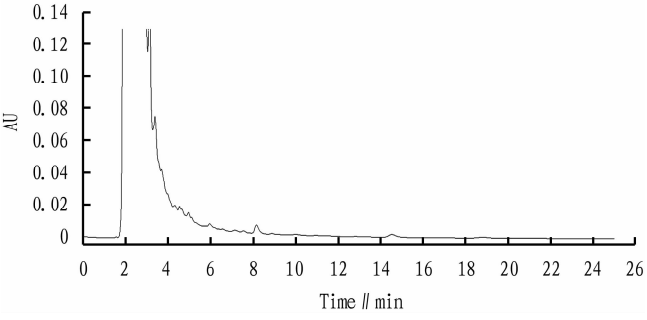


Fig. 3 Chromatogram of negative sample solution

3.5 Methodological investigation

3.5.1 Investigation of linear relationship. First, 5.0 mg of benzoylmesaconine reference substance, 1.1 mg of benzoylaconitine reference substance and 1.0 mg of benzoylhypacoitine reference substance were accurately weighed, and added in a 25 mL volumetric flask, which was then added with methanol to obtain a solution, which was diluted to constant volume to obtain a mixed stock solution of reference substances. Next, 0.5, 1, 2, 3, 4 and 5 mL of the mixed stock solution of reference substances were accurately pipetted into 5 mL volumetric flasks, respectively, and diluted to constant volume with methanol to get six mixed reference substance solutions of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine with different concentrations, which were then shaken evenly. Subsequently, 20 μL of each solution were injected into HPLC for separation and detection. Finally, linear regression equations and linear ranges were obtained with the content (μg) as the abscissa and the peak area as the ordinate. The results of regression equations are shown in Table 1.

Table 1 Linear regression equations and linear ranges

Component	Linear equation	*R* value	linear range // μg
Benzoylmesaconine	$y = 10^6x + 272\ 5$	0.999 9	0.396 6 – 3.966 4
Benzoylaconitine	$y = 822\ 737x - 541\ 4.4$	0.999 7	0.087 7 – 0.877 2
Benzoylhypacoitine	$y = 913\ 456x - 803.8$	0.999 8	0.080 0 – 0.799 6

3.5.2 Precision test. Appropriate amounts of Trichosanthis Fructus and Heishun pieces were taken and prepared into a test solution according to the method in Section **3.2**. The solution was injected continuously for 6 times according to chromatographic conditions in Section **3.1**, and the *RSD* values of various components were investigated and calculated, respectively. The results showed that the *RSD* values of peak areas of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were 2.45%, 1.82% and 2.02%, respectively, indicating that the precision of the instrument was good.

3.5.3 Reproducibility test. Appropriate amounts of Trichosanthis Fructus and Heishun pieces were taken and prepared into six test solutions according to the method in Section **3.2**. The solutions were determined according to chromatographic conditions in

Section **3.1**. The results showed that the *RSD* values of peak areas of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were 2.32%, 1.99% and 2.42%, respectively.

3.5.4 Stability test. Appropriate amounts of Trichosanthis Fructus and Heishun pieces were taken and prepared into a test solution according to the method in Section **3.2**. The solution was injected and determined according to chromatographic conditions in Section **3.1** at 0, 4, 8, 12, 16, 20 and 24 h, respectively. The results showed that the *RSD* values of peak areas of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were 2.71%, 1.72% and 1.84%, respectively, indicating that the test solution was stable within 24 h.

3.5.5 Recovery test. First, 100 g of Trichosanthis Fructus and 100 g of Heishun pieces were weighed in three parallel, respectively, and test solutions were prepared according to the method in Section **3.2**. Next, 0.5, 1.0 and 1.5 times of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were precisely added, respectively. Subsequently, the solutions were determined according to chromatographic conditions in Section **3.1**. Finally, the recovery was calculated: Recovery (%) = (Measured amount − Original amount)/Added amount ×100%. The average recovery values of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were 100.06%, 100.77% and 100.84%, respectively, and the *RSD* values of the recovery were 1.96%, 2.43% and 2.36%, respectively, indicating that the recovery of this method was good. The results are shown in Table 2.

3.5.6 Sample determination and results. Three test solutions were prepared according to Section **3.2**. They were injected into a liquid chromatograph to determine the contents of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine according to chromatographic conditions in Section **3.1**. The results are shown in Table 3.

Table 2 Test results of sample recovery

Component	Time	Original amount mg	Added amount mg	Measured amount mg	Yield %	*RSD* %
Benzoylmesaconine	0.5	2.310 0	1.22	3.501 2	97.64	1.96
	1.0	2.310 0	2.34	4.643 3	99.71	
	1.5	2.310 0	3.52	5.894 9	101.84	
	0.5	2.294 2	1.26	3.594 6	103.21	
	1.0	2.294 2	2.35	4.667 5	100.99	
	1.5	2.294 2	3.51	5.880 5	102.17	
	0.5	2.323 2	1.24	3.548 7	98.83	
	1.0	2.323 2	2.33	4.622 9	98.70	
	1.5	2.323 2	3.55	5.783 0	97.46	
Benzoylaconitine	0.5	0.420 3	0.21	0.629 4	99.57	2.43
	1.0	0.420 3	0.42	0.838 1	99.48	
	1.5	0.420 3	0.65	1.062 0	98.72	
	0.5	0.416 6	0.20	0.618 7	101.05	
	1.0	0.416 6	0.43	0.863 5	103.93	
	1.5	0.416 6	0.66	1.080 9	100.65	
	0.5	0.429 6	0.22	0.659 9	104.68	
	1.0	0.429 6	0.43	0.844 4	96.47	
	1.5	0.429 6	0.65	1.095 3	102.42	
Benzoylhypacoitine	0.5	0.362 2	0.19	0.549 3	98.47	2.36
	1.0	0.362 2	0.38	0.750 8	102.26	
	1.5	0.362 2	0.55	0.922 0	101.78	
	0.5	0.358 4	0.18	0.542 8	102.44	
	1.0	0.358 4	0.37	0.714 9	96.35	
	1.5	0.358 4	0.55	0.914 4	101.09	
	0.5	0.370 8	0.18	0.548 7	98.83	
	1.0	0.370 8	0.37	0.758 2	104.70	
	1.5	0.370 8	0.56	0.939 9	101.63	

Table 3 Contents of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine mg/g

Sample No.	Benzoylmesaconine	Benzoylaconitine	Benzoylhypacoitine	Total content
1	0.371 2	0.073 6	0.033 0	0.477 8
2	0.373 4	0.070 5	0.030 7	0.474 6
3	0.376 6	0.071 9	0.031 1	0.479 6

4 Determination of content in different extracts

First, 100 g of Trichosanthis Fructus was accurately weighed in three parallel, and marked as single Trichosanthis Fructus experiments 1, 2 and 3, respectively. Next, 100 g of Heishun pieces was accurately weighed in three parallel, and marked as single Heishun piece experiments 1, 2 and 3, respectively. Subsequently, 100 g of Trichosanthis Fructus and 100 g of Heishun pieces were weighed in three parallel, respectively, and the three mixtures were marked as combined prescription experiments 1, 2 and 3, respectively. Each sample was added with 10 times of water, respectively, and refluxed in a water bath. A 5 mL of sample was taken at 1, 2 and 3 h, respectively, and the same volume of purified water was added after sampling. Each sample was centrifuged at 4 000 r/min for 5 min, and filtered through a 0.22 μm microfiltration membrane. Finally, the contents of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine were determined, and the total amounts and $\bar{x} \pm s$ of each group were calculated (Table 4).

Table 4 Contents of benzoylmesaconine, benzoylaconitine and benzoylhypacoitine ($\bar{x} \pm s$) in different extracts

Group	Sampling time//h	Benzoylmesaconine//mg/g	Benzoylaconitine//mg/g	Benzoylhypacoitine//mg/g	Total amount//mg/g
Single decoction of Trichosanthis Fructus	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Single decoction of Heishun pieces	1	0.138 8 ±0.002 3*	0.022 8 ±0.001 2*	0.020 4 ±0.001 7*	0.182 0 ±0.002 0*
	2	0.232 9 ±0.003 7*	0.040 9 ±0.001 0*	0.035 8 ±0.002 7*	0.309 6 ±0.005 4*
	3	0.333 1 ±0.004 3*	0.066 0 ±0.002 1*	0.058 5 ±0.001 4*	0.457 6 ±0.001 9*
Combined decoction of Trichosanthis Fructus and Heishun pieces	1	0.189 6 ±0.002 1	0.033 9 ±0.000 8	0.015 2 ±0.000 7	0.238 7 ±0.002 5
	2	0.373 7 ±0.002 2	0.072 0 ±0.001 3	0.031 6 ±0.001 0	0.477 3 ±0.002 1
	3	0.435 1 ±0.003 2	0.095 2 ±0.002 2	0.045 3 ±0.001 6	0.575 5 ±0.001 8

NOTE * indicates that there is a significant difference between the single decoction group of Heishun pieces and the combined decoction group at corresponding time ($P<0.05$).

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5 Conclusions

At present, Aconiti Lateralis Radix Praeparata products are mostly used in clinic, mainly Heishun pieces and Baifu pieces. The aconine type of toxic components in decoction pieces of Aconiti Lateralis Radix Praeparata are greatly reduced, and it is difficult to detect them in actual measurement. Moreover, the effective toxic components after processing are also transformed into the benzoylaconitine type, which greatly reduces the toxicity. Therefore, this study mainly determined changes in the content of the benzoylaconitine type. According to the results, compared with the single decoction of Aconiti Lateralis Radix Praeparata (Heishun pieces), the contents of benzoylmesaconine and benzoylaconitine significantly increased in the combined decoction with Trichosanthis Fructus at corresponding decoction time, while the content of benzoylhypacoitine decreased. However, when comparing the total amount, the content of the benzoylaconitine type in the combined decoction was still improved significantly. This result proves to a certain extent the characteristic that "Pinelliae Rhizoma, Trichosanthis Fructus, Fritillariae Cirrhosae Bulbus, Ampelopsis Radix, and Rhizoma Bletillae have severe side effects when used together with *Aconitum* materials" in the "18 incompatible medicaments"[8-13].

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