

# Quantification and Simplified Detoxification Investigation on Fuzi, Root of *Aconitum carmichaelii*

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## Abstract

Fuzi (lateral root of *Aconitum carmichaelii*) has been used for millennia in Traditional Chinese Medicine (TCM) and Ayurveda to treat cancer and cardiovascular diseases. Fuzi must be processed before use to decrease the concentration of its toxic alkaloids. Detoxification during processing occurs through the transformation of diester-diterpenoid alkaloids (DDAs) to monoester-diterpenoid alkaloids (MDAs). However, traditional detoxification methods are time-consuming and expensive on large-scale production. To develop efficient detoxification protocols to reduce unnecessary processing procedure and keep the maximum functional contents from raw Fuzi, we replicated the traditional procedure and quantified the DDAs and MDAs by UPLC-MS/MS and UPLC-PDA during different steps and conditions of processing. With due consideration of obtained data, we concluded that soaking in Danba solution and the washing steps were inefficient traditional processing methods. The detoxification effect of steaming ( $56.3 \pm 0.27 \mu\text{g/g}$  DDAs, lowest after steaming) was weaker and slower than boiling ( $5.8 \pm 0.33 \mu\text{g/g}$  DDAs, lowest after boiling). Moreover, roasting at  $105^\circ\text{C}$  showed better effect in lowering the DDAs ( $5.8 \pm 0.33 \mu\text{g/g}$  DDAs) and increasing the MDAs ( $729.1 \pm 1.22 \mu\text{g/g}$  MDAs, highest) than roasting at  $60^\circ\text{C}$  ( $17.3 \pm 0.65 \mu\text{g/g}$  DDAs;  $504.0 \pm 0.99 \mu\text{g/g}$  MDAs). With these highly reliable analytic data, we established an efficient and referenceable detoxification protocol for Fuzi TCM products, in which DDAs and MDAs should legitimately follow the safe and specific ranges stipulated in pharmacopeias.

## Keywords

Fuzi, *Aconitum*, aconite, diterpenoid alkaloids, detoxification, Traditional Chinese Medicine processing

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Fuzi, the lateral root of *Aconitum carmichaelii* Debeaux, has been widely used in Chinese medicine for tumors, poor circulation, heart failure, and pain.<sup>1</sup> It was first introduced 2000 years ago in Shennong Bencao Jing, the earliest book of Chinese Materia Medica.<sup>2</sup> Since then, Fuzi was used in many Traditional Chinese Medicine (TCM), Ayurvedic, Kampo, homeopathy, and herbal formulas to treat several clinical conditions such as heart failure, poor circulation, and different tumors.<sup>3</sup> However, the toxicity of raw Fuzi has always been the main concern for healers and patients. There were more than 1000 published cases of *Aconitum* poisoning worldwide.<sup>4,5</sup> The common clinical presentation of *Aconitum* poisoning included cardiac arrhythmias, paresthesia and numbness over the face and limbs, nausea, dizziness, cold sweating, chest tightness, and hypotension.<sup>3</sup> Although most patients could recover without a specific sequela after appropriate

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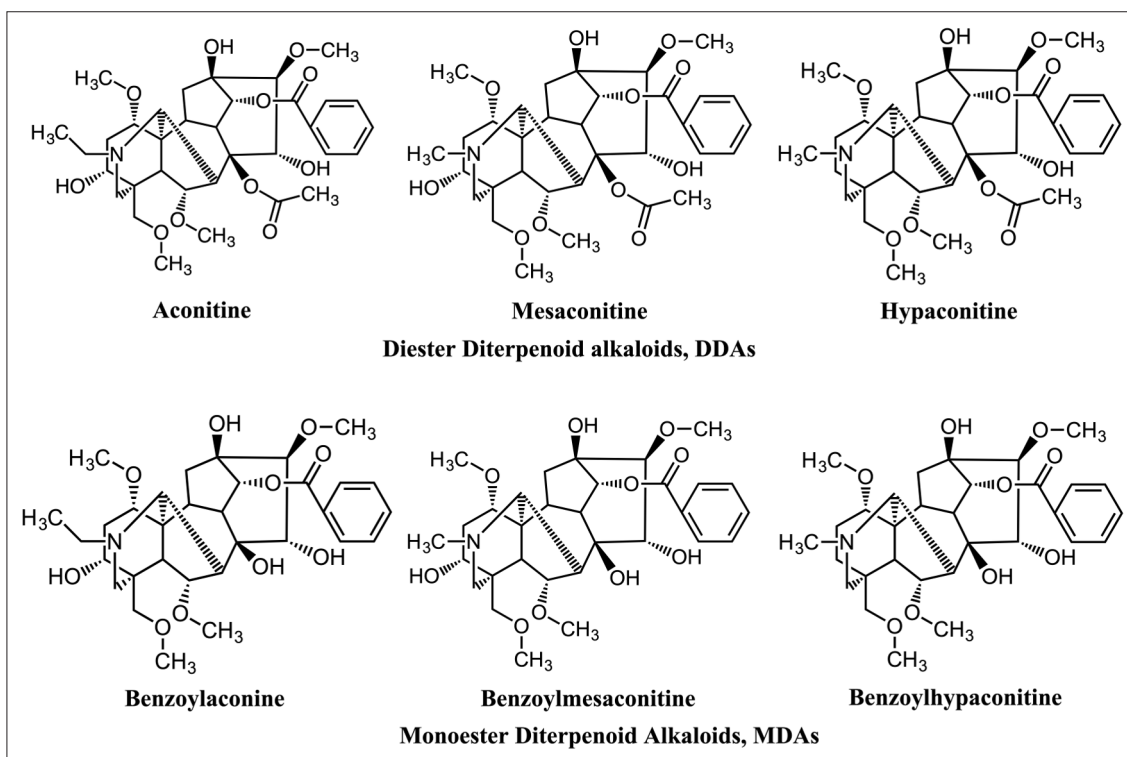
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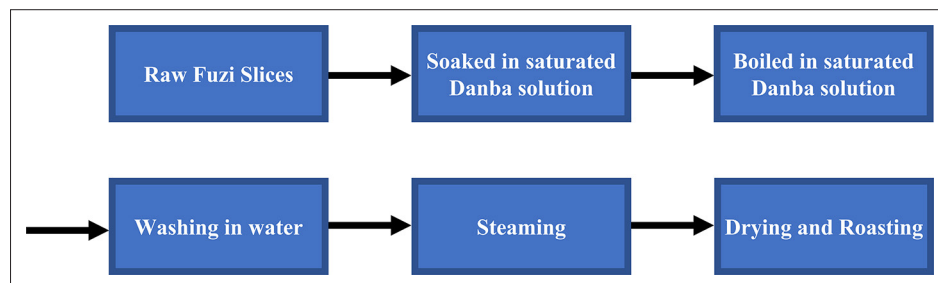
**Figure 1.** The chemical structures of the six representative components in Fuzi: aconitine, mesaconitine, hypaconitine, benzoylaconine (BA), benzoylmesaconine (BM), and benzoylhypaconine (BH).

treatment of *Aconitum* poisoning, the usage of *Aconitum* sp. should be under clinical supervision and strict regulation.

Since ancient times, TCM doctors developed detoxification processes such as soaking in water, stir-frying, boiling, roasting, and steaming to reduce the toxicity of Fuzi.<sup>6</sup> Some practitioners mix Fuzi with the roots of *Glycyrrhiza uralensis* Fisch., *Panax ginseng* C.A. Mey., or *Zingiber officinale* Roscoe to decrease toxicity and enhance efficacy of Fuzi.<sup>2</sup> According to recent studies, detoxification during processing or metabolism occurs through the hydrolysis of the C-8 acylester group of diester-diterpenoid alkaloids (DDAs) to form monoester-diterpenoid alkaloids (MDAs).<sup>7</sup> These monoesters are further debenzoylated at the C-14 to form hydramine-type unesterified diterpenoid alkaloids.<sup>6</sup> According to the Taiwan Herbal Pharmacopeia (Figure 1), the total concentration of DDAs (aconitine, mesaconitine, and hypaconitine) should not be

above 0.02% (200  $\mu\text{g/g}$ ), as well as the total amount of MDAs (benzoylaconine (BA), benzoylmesaconine (BM), and benzoylhypaconine (BH)) should be over 0.01% (100  $\mu\text{g/g}$ ) in the commercial products of Fuzi.<sup>8</sup>

In Taiwan, Fuzi commercial products should be processed following Taiwan Herbal Pharmacopeia or government-approved traditional protocols. According to traditional processing protocol, the raw Fuzi is soaked in Danba salts ( $\text{CaCl}_2$ ,  $\text{MgCl}_2$ , or  $\text{NaCl}$ ) solution for antiseptic purposes and then washed (Figure 2). The soaking and washing process may reduce the active constituents such as DDAs and MDAs in Fuzi. However, the quantification change of effective compounds during the process was not clearly understood.<sup>9</sup> Moreover, the comparison of detoxification processes such as boiling, steaming, and roasting was not established. Therefore, the current study aimed to establish the quantification data of



**Figure 2.** Standard processing procedures of Fuzi in traditional Chinese method.

DDAs and MDAs during the traditional standard procedures, which may provide scientific evidences to TCM factories to develop efficient modern processing procedures.

Before processing, hypaconitine concentration (264.85  $\mu\text{g/g}$ ) in raw Fuzi was the highest among the 3 DDAs, followed by mesaconitine (107.59  $\mu\text{g/g}$ ) and aconitine (73.37  $\mu\text{g/g}$ ). The mean of sum of DDAs concentration was  $445.8 \pm 3.52 \mu\text{g/g}$ . Among MDAs, BM concentration (78.64  $\mu\text{g/g}$ ) was the highest followed by BA (33.47  $\mu\text{g/g}$ ) and BH (21.53  $\mu\text{g/g}$ ). The mean of sum of MDAs concentration was  $133.6 \pm 0.57 \mu\text{g/g}$  in the raw Fuzi sample.

Soaking in Danba salt was the first step of processing raw Fuzi according to traditional Chinese methods (Figure 2). The duration of traditional soaking process was between 10 and 16 days. We evaluated the concentration change of DDAs and MDAs of 1000 g raw Fuzi slices, which were soaked in saturated Danba salt solution for 10 or 16 days. After that, Fuzi slices were boiled with Danba salt solution for 30 minutes. Sliced Fuzi samples were then dried at 70°C and prepared for analysis.<sup>10</sup>

The sum of DDAs was  $107.8 \pm 5.65 \mu\text{g/g}$  in the 16-day soaking group and  $16.8 \pm 0.08 \mu\text{g/g}$  in the 10-day group. After soaking and boiling, aconitine concentration was reduced to 18.13  $\mu\text{g/g}$  in the 16-day group and to 2.45  $\mu\text{g/g}$  in the 10-day group. Hypaconitine concentration was reduced to 37.42  $\mu\text{g/g}$  in the 16-day and 6.49  $\mu\text{g/g}$  in the 10-day group. Mesaconitine concentration was also reduced to 52.21 and 6.49  $\mu\text{g/g}$ , respectively. As for MDAs, the 16-day soaking group had also higher concentration of MDAs ( $29.9 \pm 0.04 \mu\text{g/g}$ ) than the 10-day soaking group ( $8.9 \pm 0.02 \mu\text{g/g}$ ). BA concentration was reduced to 4.02  $\mu\text{g/g}$  in the 16-day group, and barely undetectable in the 10-day group. BH concentration was similar (3.34  $\mu\text{g/g}$  vs 3.95  $\mu\text{g/g}$ ) in the 16-day and the 10-day group, respectively. BM concentration was significantly higher in the 16-day soaking group (22.52  $\mu\text{g/g}$ ) compared to the 10-day group (4.91  $\mu\text{g/g}$ ). Although soaking in Danba solution decreased the concentrations of DDAs and MDAs, these samples did not meet commercial products qualifications due to low MDAs concentration (8.9-16.8  $\mu\text{g/g}$ ) just after soaking and boiling steps. Surprisingly, DDAs and MDAs increase after soaking for a longer time.

However, the mechanism was unclear. In our speculation, Danba salt was not washed out clearly in the first 2 steps, which might interfere with the release of DDAs and MDAs in Fuzi slices for analysis.<sup>11</sup> Therefore, we recommended to add some time points, such as 5-day, 8-day, and 13-day groups, to obtain the change trend of alkaloids concentration in the future study.

After soaking, Danba salt was washed out clearly because of its possible toxicity (Figure 2).<sup>3</sup> Traditionally, Fuzi slices were picked up from saturated Danba solution and then put into water to wash out Danba salt. The water was changed regularly within the washing procedures, which persisted for about 3 to 4 days.<sup>12</sup> After washing out Danba salt, Fuzi was steamed and prepared to undergo further processing procedure. Therefore, the second experiment aimed to illustrate the effect of washing and steaming on the change of concentration of diterpenoid alkaloids. Raw Fuzi slices were soaked in saturated Danba salt solution for 10 days and boiled for 30 minutes. Samples underwent washing procedures for 3 days and were steamed for 1, 2, or 3 hours. Samples were then dried at 70°C and prepared for analysis.

The current study proved that washing and steaming were effective steps for detoxification. The concentration of total DDAs was not significantly different after 10-day soaking and boiling ( $16.8 \pm 0.08 \mu\text{g/g}$ ) compared to 12.5 to 27.5  $\mu\text{g/g}$  after washing and steaming (Table 1). We noticed that the concentration of hypaconitine increased by the steaming duration. We supposed that other alkaloids could be turned into hypaconitine by steaming procedure. In contrast, the concentration of total MDAs significantly increased from 8.9 to 50.0 to 125.8  $\mu\text{g/g}$  in different steaming time. The samples after steaming for 3 hours were qualified as commercial products, because the total amount of MDAs was above 0.01% (100  $\mu\text{g/g}$ ). Moreover, the results indicated that MDAs might not have only originated from the hydrolysis of DDAs. Therefore, the transformation of diterpenoid alkaloids is worth further studies.

In China and Japan, there are some commercial Fuzi products sold on the market by Chinese companies, in which Danba salt is not used during processing and no washing is applied. Some of the products were processed by boiling and roasting, and the others were processed by steaming and roasting.

**Table 1.** The Change of Diterpenoid Alkaloids Concentration After Washing and Steaming Procedure.

Steaming time	Diester-diterpenoid alkaloids			
	Aconitine ( $\mu\text{g/g}$ )	Hypaconitine ( $\mu\text{g/g}$ )	Mesaconitine ( $\mu\text{g/g}$ )	Mean of sum ( $\mu\text{g/g}$ )
1 h	1.26	8.71	2.57	$12.5 \pm 0.02$
2 h	1.64	9.65	3.02	$14.3 \pm 0.22$
3 h	1.31	22.88	3.29	$27.5 \pm 1.47$
Steaming time	Monoester-diterpenoid alkaloids			
	Benzoylaconine ( $\mu\text{g/g}$ )	Benzoylhypaconine ( $\mu\text{g/g}$ )	Benzoylmesaconine ( $\mu\text{g/g}$ )	Mean of sum ( $\mu\text{g/g}$ )
1 h	7.51	6.83	35.69	$50.0 \pm 0.07$
2 h	14.21	10.33	62.17	$86.7 \pm 0.07$
3 h	20.42	23.81	81.58	$125.8 \pm 0.07$

However, we did not know whether DDAs of these products were above the upper limitation of the regulation in Taiwan. Raw Fuzi samples were divided into 2 groups. One group was processed by boiling and the other by steaming for 1, 2, and 6 hours. All samples were then roasted at 60°C or at 105°C for 8 hours.<sup>10</sup>

The boiling process was more effective to decrease the amount of DDAs than the steaming process. Roasting at 105°C could further decrease the concentrations of DDAs compared to roasting at 60°C (Tables 2 and 3).

In contrast, the boiling and steaming processes were both effective to increase the number of MDAs. Steaming for 6 hours and roasting at 105°C for 2 hours had the highest concentration of MDAs (729.1 µg/g) among all groups (Tables 2 and 3). The amount of increased MDAs was more than the decreased DDAs, so it proved that BA, BH, and BM could not have originated only from aconitine, hypaconitine, and mesaconitine. To the best of our knowledge, the current study was the first to demonstrate that raw Fuzi could be qualified even without the Danba soaking and washing process.

In order to decrease the cost and improve production, herbal Good Manufacturing Practice (GMP) factories need to find an efficient way to detoxify Fuzi and maintain the concentration of its active components. Under formal regulations, the detoxification process should follow the procedures described in ancient texts, which might not be the most effective way. Although ultrasonic bath and microwave-assisted extraction were applied on small scale to shorten the extraction time,<sup>13,14</sup> these procedures are not economically feasible for mass production.

The current study showed that soaking in Danba solution and washing process were unnecessary to produce qualified commercial products. Moreover, boiling or steaming more than 2 hours could decrease the total concentration of DDAs to less than 0.02% (200 µg/g). Roasting at 105°C could save time compared to roasting at 60°C. The factories could develop and improve their processing procedures based on the current study.

“Processing” is an important technique in Chinese Herbology. The purpose of it is to alter the properties of crude medicinal materials, so as to enhance the active ingredients responsible for pharmacodynamic effects. Oppositely, it also plays a very crucial role in reducing or decomposing poison components to prevent any possible abnormal, adverse, or harmful risks in clinical administration. Different ways of heating, even combined with accompanying materials, are the most common and traditional approaches conducted for preparation. Both DDAs and MDAs are active compounds of Fuzi. However, the therapeutic windows of DDAs, eg aconitine, are extremely narrow with an estimated lethal dose around 2 to 6 mg for humans.<sup>15</sup> Its poisoning symptoms were well known as hypotension, palpitation, ventricular tachyarrhythmia, asystole, and numbness of the face and limbs.<sup>16</sup> In contrast, the toxicity of the MDAs was about 1000-fold weaker, with the LD<sub>50</sub> values (oral, mice) of benzoylaconine,

benzoylmesaconine, and benzoylhypaconine as 1.50, 0.81, and 0.83 g/kg, respectively.<sup>17</sup>

In this work, we were aiming to quantify and monitor the trajectory of diterpenoid alkaloid changes during processing of Fuzi. Washing, boiling, steaming, and roasting now can be commonly controlled/standardized by professional equipment in TCM pharmaceutical companies issued with GMP certificate. With the highly reliable analytic data collected by validated UPLC-MS/MS and UPLC-PDA, we established an efficient and referenceable protocol for the manufacture of commercial Fuzi TCM products, in which DDAs and MDAs should legitimately follow the safe and specific ranges.

In conclusion, we suggested that the competent authority of Chinese Medicine Manufacturing should not simply follow the traditional processing method, and there might be effective scientific methods to improve the quality of processing of all kinds of Chinese herbs.

## Experimental

### Plant Material

Raw Fuzi is the daughter root of *Aconitum carmichaelii* Debeaux, which was cultivated and brought from Jiangyou, Sichuan, China. The raw plant material was identified by Dr Shu-Tuan Chiang. A voucher specimen (code no. Aconitum 001) was stored in the Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University and Chuang Song Zong Pharmaceutical Co. Ltd., Taiwan. Raw Fuzi was cut into slices and dried. Danba salt was purchased from Sichuan Xinhehua Company (Sichuan, China).

### Chemicals and Reagents

Chemical standards of aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylhypaconine, and benzoylmesaconine were purchased from Kishida Chemical Co., Ltd (Osaka, Japan). LC/MS and HPLC grade methanol (J.T. Baker, Phillipsburg, USA), LC/MS grade acetonitrile (J.T. Baker), LC/MS grade 0.1% formic acid/acetonitrile (J.T. Baker), ACS grade diethyl ether (Echo, Leiden, Netherlands), HPLC grade acetic acid (Echo), ACS grade ammonium hydroxide solution (28%-30%; Tedia, Fairfield, USA), ACS grade 0.1% formic acid (Sigma-Aldrich, St Louis, USA), GR grade isopropanol (Tedia), ACS grade ethyl acetate (Echo), GR grade dichloromethane, *ortho*-phosphoric acid (85%, Merck, Darmstadt, Germany), and deionized water (resistivity  $\geq 16$  million ohm-cm) were used for the preparation of standard solutions, sample solutions, and mobile phases for UPLC.

### Sample Preparations

The preparation of extract for investigation of DDA analysis was as follows<sup>18</sup>: Accurately weigh 1.0 g sample and put it into a 50 mL centrifuge tube. Add 1 mL 10% ammonium hydroxide solution. Stir well to ensure that the entire sample is wet. Then

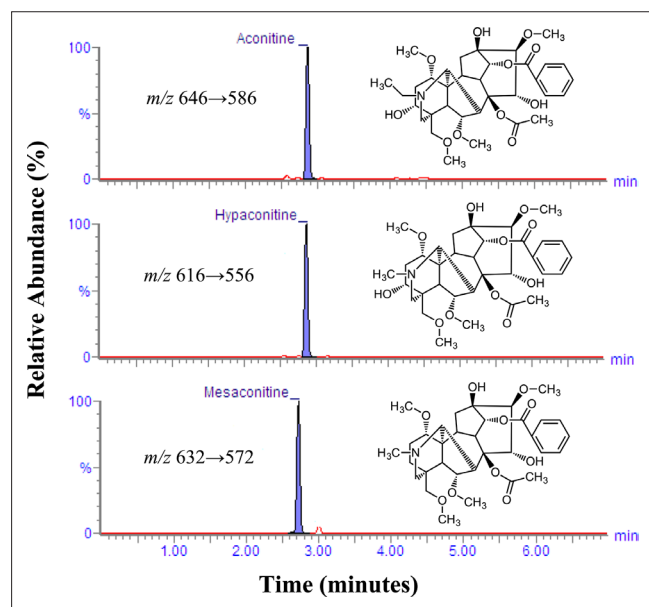
**Table 2.** The Change of Diterpenoid Alkaloids Concentration After Boiling or Steaming and Then Roasting at 60°C.

Roasting at 60°C									
Boiling time	Diester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )	Steaming time	Diester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )
	Aconitine ( $\mu\text{g/g}$ )	Hypaconitine ( $\mu\text{g/g}$ )	Mesaconitine ( $\mu\text{g/g}$ )			Aconitine ( $\mu\text{g/g}$ )	Hypaconitine ( $\mu\text{g/g}$ )	Mesaconitine ( $\mu\text{g/g}$ )	
<b>1 h</b>	1.27	80.0	3.45	84.7 $\pm$ 0.58	<b>1 h</b>	20.46	205.35	89.99	315.8 $\pm$ 0.66
<b>2 h</b>	0.35	45.23	0.94	46.5 $\pm$ 1.23	<b>2 h</b>	6.42	165.99	32.13	204.5 $\pm$ 0.62
<b>6 h</b>	0	17.10	0.23	17.3 $\pm$ 0.65	<b>6 h</b>	1.13	114.12	3.96	119.2 $\pm$ 1.08
Boiling time	Monoester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )	Steaming time	Monoester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )
	Benzoylaconine ( $\mu\text{g/g}$ )	Benzoylhypaconine ( $\mu\text{g/g}$ )	Benzoylmesaconine ( $\mu\text{g/g}$ )			Benzoylaconine ( $\mu\text{g/g}$ )	Benzoylhypaconine ( $\mu\text{g/g}$ )	Benzoylmesaconine ( $\mu\text{g/g}$ )	
<b>1 h</b>	23.52	54.77	154.27	232.6 $\pm$ 1.10	<b>1 h</b>	17.89	30.11	157.02	205.0 $\pm$ 0.57
<b>2 h</b>	23.45	47.91	125.16	196.5 $\pm$ 1.85	<b>2 h</b>	22.02	48.11	260.00	330.1 $\pm$ 1.38
<b>6 h</b>	75.18	167.31	261.50	504.0 $\pm$ 0.99	<b>6 h</b>	58.50	123.81	246.35	428.7 $\pm$ 0.57



**Table 3.** The Change of Diterpenoid Alkaloids Concentration After Boiling or Steaming and Then Roasting at 105°C.

Roasting at 105°C									
Boiling time	Diester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )	Steaming time	Diester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )
	Aconitine ( $\mu\text{g/g}$ )	Hypaconitine ( $\mu\text{g/g}$ )	Mesaconitine ( $\mu\text{g/g}$ )			Aconitine ( $\mu\text{g/g}$ )	Hypaconitine ( $\mu\text{g/g}$ )	Mesaconitine ( $\mu\text{g/g}$ )	
<b>1 h</b>	0.45	28.46	1.14	$30.1 \pm 0.35$	<b>1 h</b>	3.36	83.78	20.55	$107.7 \pm 1.69$
<b>2 h</b>	0.30	37.76	0.93	$39.0 \pm 0.61$	<b>2 h</b>	3.00	59.54	23.43	$86.0 \pm 0.65$
<b>6 h</b>	0	5.64	0.16	$5.8 \pm 0.33$	<b>6 h</b>	0.84	51.88	3.59	$56.3 \pm 0.27$
Boiling time	Monoester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )	Steaming time	Monoester-diterpenoid alkaloids			Mean of sum ( $\mu\text{g/g}$ )
	Benzoylaconine ( $\mu\text{g/g}$ )	Benzoylhypaconine ( $\mu\text{g/g}$ )	Benzoylmesaconine ( $\mu\text{g/g}$ )			Benzoylaconine ( $\mu\text{g/g}$ )	Benzoylhypaconine ( $\mu\text{g/g}$ )	Benzoylmesaconine ( $\mu\text{g/g}$ )	
<b>1 h</b>	31.81	93.92	172.03	$297.8 \pm 1.53$	<b>1 h</b>	54.17	103.17	291.20	$448.5 \pm 1.57$
<b>2 h</b>	26.54	101.53	144.12	$272.2 \pm 0.03$	<b>2 h</b>	52.19	179.56	497.31	$729.1 \pm 1.22$
<b>6 h</b>	48.06	211.49	252.22	$511.8 \pm 0.19$	<b>6 h</b>	87.09	134.68	403.61	$625.4 \pm 1.59$



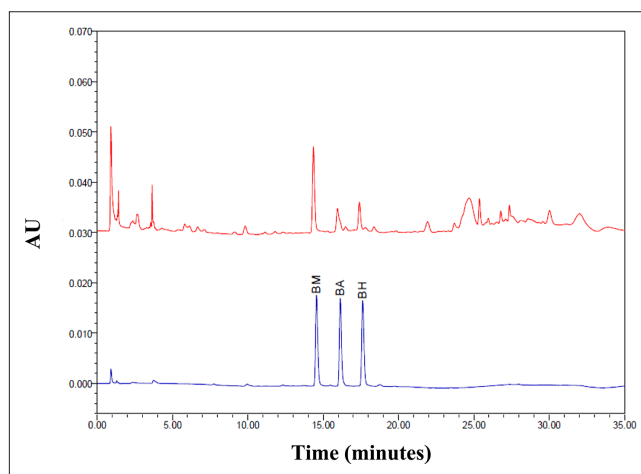
**Figure 3.** UPLC-ESI-MS/MS chromatogram of aconitine, hyaconitine, and mesaconitine from Fuzi.

add 25 mL diethyl ether to the tube. Shake the tube on a platform shaker for 1 hour at a speed of 300 rpm. Centrifuge the sample at 4000 rpm for 10 minutes to settle the solids. Decant the diethyl ether into another centrifuge tube. Repeat the sample extraction twice each time with 10 mL diethyl ether, and shake the tube for 30 and 10 minutes, respectively. Combine the extracts and evaporate to complete dryness at  $\leq 40^{\circ}\text{C}$  under a stream of nitrogen. Add 5 mL acetonitrile-0.1% acetic acid (1 + 1) and ultrasonicate for 20 minutes to re-dissolve the residues. The solution is ready for SPE cleanup after it is passed through a 0.45  $\mu\text{m}$  membrane filter.

The preparation of extract for investigation of MDA analysis was as follows<sup>8</sup>: Samples were shredded by shredding machine for 20 minutes to make sample powders. Weigh accurately 2.0 g of powdered sample, transfer to a conical flask with stopper, add 3 mL of ammonia solution, accurately add 50 mL of a solution of isopropanol and ethyl acetate (1:1), weigh, ultrasonicate for 30 minutes, cool, weigh again, replenish the loss of the weight with a solution of isopropanol and ethyl acetate (1:1), mix well, filter, transfer 25 mL of successive filtrate, evaporate the filtrate to dryness on  $40^{\circ}\text{C}$ , dissolve the residue in a solution of isopropanol and dichloromethane (1:1) to 3 mL, filter, and use the filtrate.

#### Instrumentation, UPLC-MS/MS, and UPLC-PDA Analysis

An ACQUITY UPLC system (Waters, Milford, MA, USA) consisting of binary or quaternary solvent manager, sample manager, column manager, ACQUITY TQ detector, and PDA detector was used for acquiring chromatograms, UV spectra, and plots of retention time-absorbance-wavelength. All the



**Figure 4.** UPLC-PDA chromatogram of benzoyleaconine (BA), benzylhyaconitine (BH), and benzoylemesaconine (BM) from Fuzi.

instruments used for analyses were regularly and well validated by the quality control department of Chuang Song Zong Pharmaceutical Co. Ltd. A DC600H ultrasonic processor (Delta, Taipei, Taiwan) was used for sample extraction and a Universal 32 (Hettich, Tuttlingen, Germany) centrifuge for sample preparation.

The standard solutions of aconitine, hyaconitine, and mesaconitine were prepared through procedures for analysis.<sup>18</sup> UPLC operating conditions were as follows: injection volume: 2  $\mu\text{L}$ ; TQ detector was set to selected reaction monitoring mode with positive electrospray ionization. The analytical column for DDA analysis was an ACQUITY UPLC BEH C18 column (1.7  $\mu\text{m}$ , 50 mm  $\times$  2.1 mm) (Waters). The mobile phase consisted of water and 0.1% formic acid (A) and acetonitrile and 0.1% formic acid (B). The flow rate was 0.5 mL/min. Separation was performed at  $35^{\circ}\text{C}$  by means of a gradient elution program. Elution started with 90% A. The concentration of phase A decreased linearly to 20% within 30 minutes. Aconitine, mesaconitine, and hyaconitine precursor ions ( $[M + H]^+$ ) and their corresponding fragments were monitored ( $m/z$  646 $\rightarrow$ 586,  $m/z$  632 $\rightarrow$ 572, and  $m/z$  616 $\rightarrow$ 556)<sup>18</sup> (Figure 3).

The standard solutions of benzoyleaconine, benzoylehyaconitine, and benzoylemesaconine were prepared through procedures for analysis.<sup>8</sup> The analytical column for MDAs was an ACQUITY UPLC HSS C18 column (1.8  $\mu\text{m}$ , 100 mm  $\times$  2.1 mm) (Waters). UV detection was carried out at 235 nm. The mobile phase consisted of water and 0.2% phosphoric acid (A) and 0.2% phosphoric acid in methanol (B). Column separation was performed at  $30^{\circ}\text{C}$  by means of a gradient elution with a flow rate of 0.3 mL/min. Elution started with 75% A and the ratio of A decreased linearly to 50% within 30 minutes (Figure 4).

### Data Analysis

Data are reported as mean  $\pm$  standard deviation values of at least 3 independent experiments.

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### Declaration of Conflicting Interests


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