

An Efficient High-performance Liquid Chromatography Combined with Electrospray Ionization Tandem Mass Spectrometry Method to Elaborate the Changes of Components Between the Raw and Processed Radix *Aconitum kusnezoffii*

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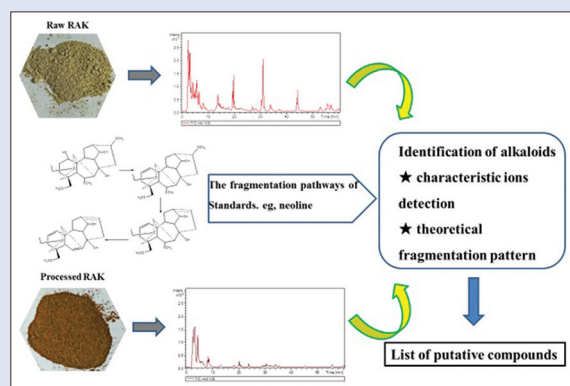
ABSTRACT

Background: Crude radix *Aconitum kusnezoffii* (RAK) has great toxicity. Traditional Chinese medicine practice proved that processing may decrease its toxicity. In our previous study, we had established a new method of RAK processing (Paozhi). However, the mechanism is yet not perfect. **Objective:** To explore the related mechanism of processing through comparing the chemical contents. **Materials and Methods:** A new processing method of RAK named stoving (Hong Zhi) was used. In particular, RAK was stored at 110°C for 8 h, and then high performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC-ESI-MSⁿ) was developed for the detection of the alkaloids of the crude and processed RAK decoction pieces. **Results:** Thirty components of the crude RAK were discovered, among which, 23 alkaloids were identified. Meanwhile, 23 ingredients were detected in the processed RAK decoction pieces, among which, 20 alkaloids were determined yet. By comparison, eight alkaloids were found in both crude and processed RAK decoction pieces, 15 alkaloids were not found in the crude RAK, however, 10 new constituents yield after processing, which are 10-OH-hypaconine, 10-OH-mesaconine, isomer of bullatine A, 14-benzoyl-10-OH-mesaconine, 14-benzoyl-10-OH-aconine, 14-benzoyl-10-OH-hypaconine, dehydrated aconitine, 14-benzoylaconine, chuanfumine, dehydrated mesaconitine. **Conclusion:** The present study showed that significant change of alkaloids was detected in RAK before and after processing. Among them, the highly toxic diester alkaloids decreased and the less toxic monoester alkaloids increased. Moreover, the concentration changes significantly. HPLC-ESI-MSⁿ are Efficient to elaborate the mechanism of reduction of toxicity and enhancement efficacy after processing. **Key words:** Alkaloids, electrospray ionization, mass spectrometry, processing (Paozhi), radix *Aconitum kusnezoffii*

SUMMARY

- Stoving is a simple and effective method for the processing of radix *Aconitum kusnezoffii*.

- In the positive mode, the characteristic fragmentations of *Aconitum* alkaloids were obtained.
- The highly toxic alkaloids have decreased, and the new constituents appeared, which has explained successfully the processing mechanism of radix *Aconitum kusnezoffii* in chemistry.



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INTRODUCTION

Radix *Aconitum kusnezoffii* (RAK), a commonly used Traditional Chinese Medicine (TCM), termed as Caowu, originated from the dried root of *Aconitum kusnezoffii* Reichb. (Family *Ranunculaceae*).^[1] With the efficiency of dispelling wind and dampness, warming the channels and relieving pain, RAK was found to possess many biological activities, such as, the treatment of wind cold damp impediment symptom, joint pain, abdominal pain with cold sensation, and anesthesia pain.^[2-6] Whereas, the crude is with strong toxicity and can generally be used externally.^[7,8] Chemical compositions of the plant contain C-19 (C-18)-diterpenoidalkaloids, lycotonine-type and aconitine-type alkaloids, as well as a small amount of volatile oil, polysaccharides, heavy metals, proteins and other ingredients.^[9-23] Mainly, diester-type alkaloids, including aconitine, hypaconitine, mesaconitine and so on are both the active ingredients and the toxic components, since the therapeutic dose is close to the toxic dose with a small safety range.^[24,25]

The processing of Chinese herbal medicine (TCM)^[26] was strictly required and displayed an efficient effect of efficacy enhancing and toxicity reducing

in TCM clinical application in China.^[27-29] Until now, there are varieties of herbal processing methods. In summary, these methods can be divided into three types: Dealing with water (immersion, bubbling, wetting with water or other liquid excipients), drying with heat (stoving, to a sting, fumigating), (moistening and frying).^[30-34] As for RAK, the traditional processing method is immersion, achieving a phenomenon of tasting with a little numbing, which aims to accelerate hydrolysis of diester-type

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alkaloids, thus reducing its toxicity. However, it is worth noting that many alkaloids are lost by water processing and the RAK efficacy decreases. Steaming and boiling, that is, treatment of RAK with thermal pressure steaming after completely moistening, has varieties of advantages, such as high contents of total alkaloids, lower content of toxic diester-type alkaloids, short production cycle and keep similar analgesic effect with the herb. However, they need high-pressure equipment which is not suitable for large-scale production. In our previous study, we compared seven documented processing methods according to the purpose of RAK processing and proved that the most optimal way is to stove, which facilitates toxic diester-type alkaloids hydrolysis under appropriate temperature. It can not only accelerate hydrolysis of aconitine and decrease its toxicity, but also guarantee the amount of total alkaloids.^[30]

To investigate the mechanism of the new processing method of RAK, termed as stoving, the components change of RAK before and after stoving were detected using high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC-ESI-MSⁿ). This is a sensitive, accurate and rapid technique, which can acquire the mass spectrum information of pure compounds from complex system.^[35-38] In this paper, a HPLC-ESI-MSⁿ method was applied to lay the foundation for the further study of RAK processing.

MATERIALS AND METHODS

The HPLC/MS was recorded on an Agilent 1100 HPLC/MSD Trap XCT/plus mass spectrometer (Wilmington, Germany), which equipped with an ESI source, a diode array detector, a binary pump, an auto-sampler, a column compartment and a Chem Station (Agilent, USA). Samples for detection, benzoyleaconine, neoline and fuziline (HPLC-ELSD >98%), were isolated by our lab (School of Chinese Materia Medica, Beijing University of Chinese Medicine). Samples of RAK were collected from (Xinlinhot, China), and were air-dried. All the samples were authenticated by Prof. Shengsang Na. HPLC-grade (MeCN) was purchased from Fisher (USA). Water for HPLC was double distilled and filtrated by 0.45 µm filter membrane.

High-performance liquid chromatography method

Separation of the samples was performed on an Agilent XDB-C₁₈ (250 mm × 4.6 mm, 5 µm). Solvent A (water/ammonia, 99.6:0.4 v/v), and solvent B (MeCN) were used as mobile phase (gradient eluting), 33% (B) in 0–15 min, 33–50% (B) in 15–45 min, 50–80% (B) in 45–60 min, with the flowing rate of 1.0 ml/min and an column temperature of 30°C. The detection wavelength was 240 nm.

Mass spectrometry method

All experiments were performed on a mass spectrometer equipped with an electro spray source and capable of analyzing ions up to m/z 1000. The ion source temperature was maintained at 350°C. The spray voltage was 40 psi in the positive ion mode. Sheath gas (N₂) was infused at 10 L/min. The capillary voltage was fixed at 3000 ev.

Processing of radix *Aconitum kusnezoffii*

Herbs of RAK were processed, briefly, removing impurity, then placing in a drying oven at an constant temperature (110°C) for 8 h, and took out. The processed sample was reserved in School of Chinese Materia Medica, Beijing University of Chinese Medicine.

Preparation of samples

Preparation of the standards

Benzoyleaconine, neoline and fuziline were weighed and fully dissolved with 15 ml methanol to conical flasks, finally, diluted to

50 ng/ml for reserving. The result of mass spectrometer was shown in Figure 1.

Preparation of the samples

The 0.5 g air-dried powder and 0.5 g processed powder of RAK, accurately weighted, were fully dissolved with 25 ml methanol to conical flasks, separately. Weighted, after circumfluence extraction for 30 min, cooled and weighed again, compensating the losing weight with methanol and amply shaking. Supernatant of the samples was filtrated with a 0.45 µm Nylon filter (Iwaki Glass, Tokyo, Japan) into a HPLC amber sample vial for HPLC-MSⁿ analysis. The result of the mass spectrometer was shown in Figure 2.

RESULTS

Establishment of mass spectrometry cracking regulation

A sensitive detector of MS was developed for the detection of benzoyleaconine, neoline and fuziline, with the MS¹⁻⁴ spectral data shown in Figure 1. After further analysis, regulations were represented as follows: Peaks of [M + H]⁺ is prone to appear, easy to lose CH₃OH, H₂O, CH₃COOH, as well as C₆H₅COOH, which laid the foundation for rapid identification of alkaloids.

Identification of alkaloids in radix *Aconitum kusnezoffii* by liquid chromatography-mass spectrometry

In the experiments, 30 alkaloids in RAK were discovered, and 23 compounds were tentatively identified by detailed study of their fragmentation regulations [Table 1]; whereas 23 alkaloids were detected in the processed product, and 20 compounds identified [Table 2], simultaneously. The comparison between the compounds identified from RAK before and after processing on MS¹⁻⁴ shows, 15 alkaloids were not observed in the processed part, including sachaconitine, dehydrated deoxyhyaconine, chasmanine, karakolidine, aconitine, neoline, dehydrated hyaconitine, 10-OH-mesaconitine, 10-OH-hyaconitine, dehydrated-3,13-deoxyaconine, mesaconitine, 10-OH-aconitine, 14-acetylneoline, hyaconitine and deoxymesaconitine. However, 10-OH-mesaconitin, 10-OH-hyaconitine, mesaconitine, 10-OH-aconitine, aconitine, hyaconitine, deoxymesaconitine, these seven compounds were diester-type alkaloids with great toxicity and easily hydrolyzed after heating. Ten new compounds were identified in processed RAK, including 10-OH-mesaconine, 14-benzoyl-10-OH-mesaconine, 14-benzoyl-10-OH-hyaconine, 14-benzoyl-10-OH-aconine, 14-benzoyleaconine, chuanfumine, isomer of bullatine A, 10-OH-hyaconine, dehydrated mesaconitine and dehydrated aconitine, among which the first five compounds are hydrolyzates of diester-type alkaloids and two compounds dehydrated mesaconitine, dehydrated aconitine, are decomposition products of diester-type alkaloids. The results provide direct evidence that hydrolysis and decomposition reactions of the components with great toxicity occurred in the processing program. By this way, components with little toxicity produce and the toxicity of the herbs decreases.

CONCLUSIONS AND DISCUSSION

According to the references, there are 26 alkaloids from RAK have been reported, which are aconitine, hyaconitine, mesaconitine, deoxyaconitine, beiwutin,^[41] beiwusines A, beiwusine B,^[9] beiwudine,^[15] acsonine,^[10] 10-aconifine, benzoyleaconine, benzoyl mesaconitine, neoline, 15- α -hydroxyneoline, chasmanine, talatizamine, foresticine, lycotonine, anthranoyllycotonine,^[42] lepenine, denudatine,^[13]

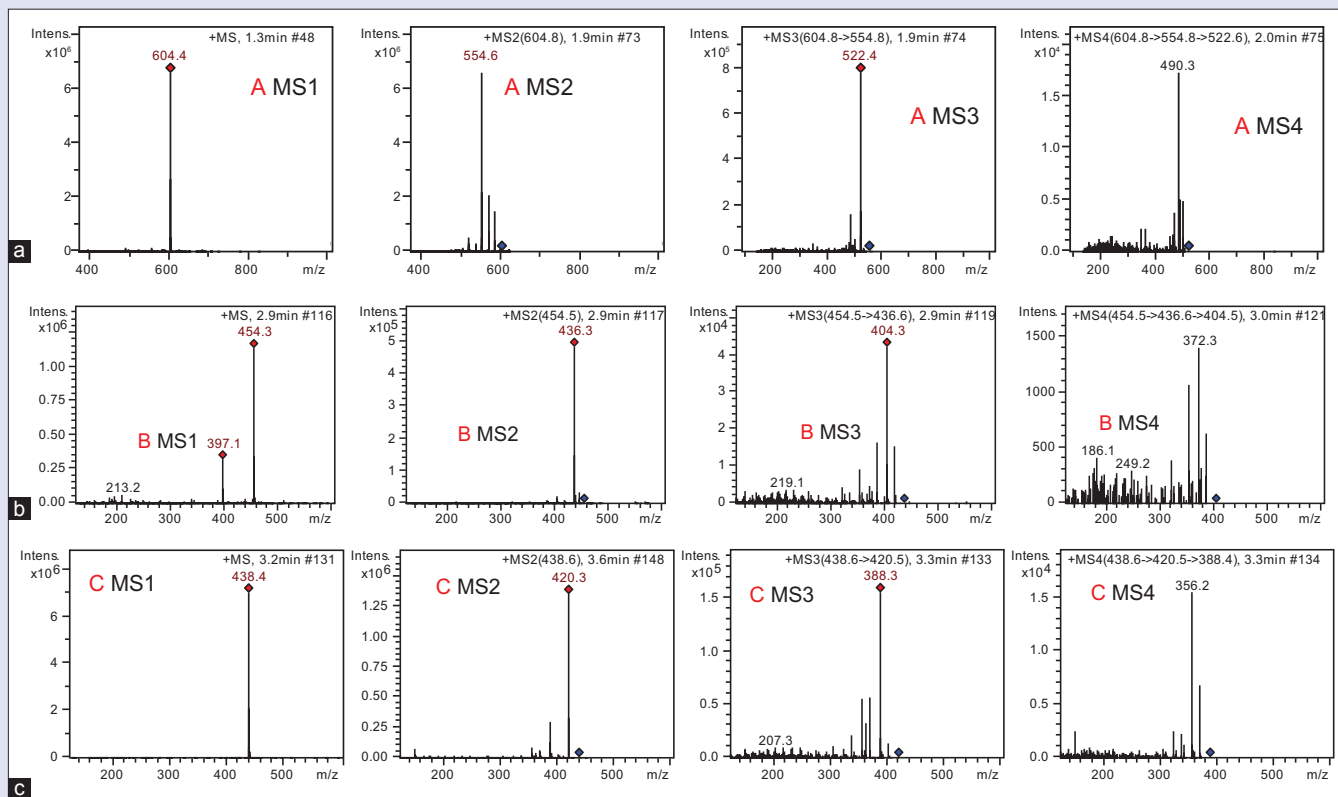


Figure 1: Mass spectrometer of the benzoylaconine (a), fuziline (b), and neoline (c)

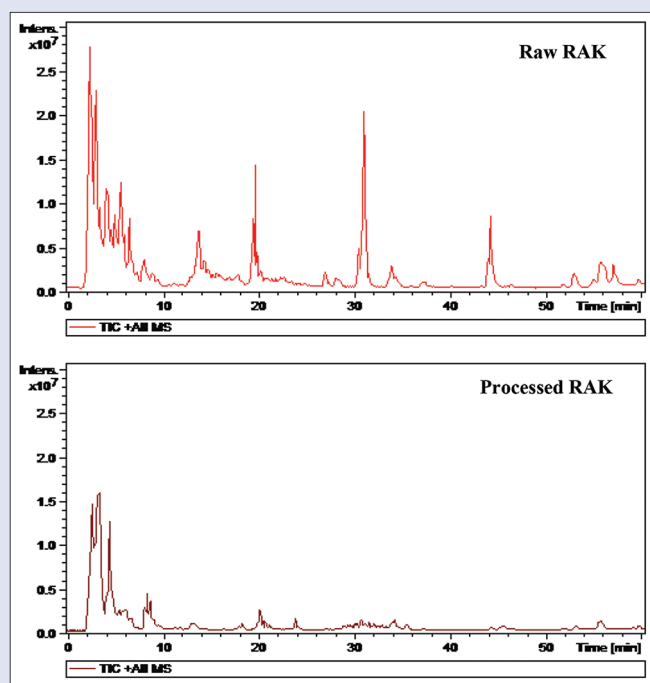


Figure 2: Total ion-chromatogram of the raw and processing radix *Aconitum kusnezoffii*

Table 1: The MS¹⁻⁴ data and the identification of alkaloids before processing

Name	Time	MS ¹	MS ²	MS ³	MS ⁴
Mesaconine	3.1	486	436	404	372
Sachaconitine ^[39]	3.5	392	356	338	-
Unknown	3.7	440	422	390	372
Mesaconine	3.8	486	436	404	372
10-OH-aconine	4.1	516	466	434	402
Aconine	4.3	500	450	418	386
Fuziline	4.6	454	436	404	372
Unknown	4.7	376	358	340	322
Dehydrated deoxyhypoconine	5.1	436	418	386	360
Karakolidine	6.1	394	376	358	-
Chasmanine ^[40]	6.2	452	434	402	370
Neoline	7.2	438	420	388	356
Unknown	7.9	452	434	402	370
14-benzoylmesaconine	8.2	590	540	508	476
Neoline	11.2	438	420	388	-
Unknown	15.2	432	372	354	-
Unknown	17.3	390	358	340	-
Dehydrated aconine	17.7	482	450	418	386
10-OH-mesaconitine	19.3	648	588	528	478
Unknown	20.5	416	356	338	310
10-OH-hypoconitine	22.2	632	572	512	480
Dehydrated-3,13-deoxyaconine	22.6	450	418	386	342
Dehydrated deoxyaconine	28.0	466	434	402	370
Mesaconitine	30.6	632	572	512	480
10-OH-aconitine	33.9	662	602	542	492
dehydrated hypoconitine	37.0	452	388	356	324
14-acetylneoline	43.2	480	448	416	384
Aconitine	43.8	646	586	526	494
Unknown	43.9	466	344	316	-
Hypoconitine	54.9	616	556	496	478
Deoxyimesaconitine	56.8	616	556	496	464

MS: Mass spectrometry

songorine, 1, 15-dimethoxy-3-hydroxy-14-benzoyl-16-ketoneoline, 8-ethoxyl-14-benzoyl-15-methoxyaconine,^[11] songoramine and karakoline.^[24] In our paper, another 16 alkaloids were identified

Table 2: The MS¹⁻⁴ data and the identification of alkaloids after processing

Name	Time	MS ¹	MS ²	MS ³	MS ⁴
Isomer of bullatine A	2.4	344	298	266	238
10-OH-hypaconine	3.3	486	436	404	372
Mesaconine	3.7	486	436	404	372
10-OH-mesaconine	3.9	502	452	420	392
10-OH-aconine	4.2	516	466	434	402
Fuziline	4.6	454	436	404	372
Aconine	4.8	500	450	418	386
Unknown	4.9	376	358	340	-
14-benzoyl-10-OH-mesaconine	5.8	606	556	524	-
Chuanfumine ^[41]	6.8	394	364	346	-
Dehydrated hypaconitine	8.0	452	434	402	370
14-benzoylmesaconine	8.3	590	540	508	476
Unknown	11.8	420	402	370	292
14-benzoyl-10-OH-aconine	12.9	620	570	538	506
14-benzoyl-10-OH-hypaconine	13.2	588	538	506	474
Dehydrated aconine	18.1	482	450	418	386
14-benzoylaconine	19.8	604	554	522	490
14-benzoyl-10-OH-hypaconine	23.8	572	522	490	458
Dehydrated mesaconitine	24.2	572	522	490	458
Dehydrated deoxyaconine	33.8	466	434	402	370
Dehydrated aconitine	35.3	586	536	504	472
Unknown	44.1	466	344	272	-

MS: Mass spectrometry

in both crude RAK and the processed product for the first time, including mesaconine, sachaconitine, 10-OH-aconine, aconine, fuziline, dehydrated deoxyhypaconine, karakolidine, dehydrated aconine, 10-OH-mesaconitine, 10-OH-hypaconitine, dehydrated-3, 13-deoxyaconine, dehydrated deoxyaconine, 10-OH-aconitine, dehydrated hypaconitine, 14-acetylneoline, and deoxymesaconitine.

Chinese medicine processing is the important means to ensure clinical medication safety. In this paper, we demonstrated once again that HPLC-ESI-MSⁿ was rapid and effective to clarify the mechanism of processing from the perspective of the chemical compositions. As far as RAK is concerned, both chemical constituents and content change significantly before and after processing. However, it is necessary to obtain all kinds of standard materials, so that the changes of chemical constituents can be analyzed accurately and quantitatively.

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Conflicts of interest

There are no conflicts of interest.

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