

# Analysis of the metabolites of mesaconitine in rat blood using ultrafast liquid chromatography and electrospray ionization mass spectrometry

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## ABSTRACT

**Background:** Mesaconitine is the main active component of genus aconitum plants that are widely used in clinics in China. However, little has been known about the metabolic pathway of mesaconitine. **Objective:** To explore the metabolites and propose the pathway of mesaconitine. **Materials and Methods:** In the present study, mesaconitine (4 mg kg<sup>-1</sup>) was orally administered to male rats. Then, blood samples collected were pretreated using solid-phase extraction technique with C18 cartridges, and analyzed using LC/MS/MS method with electrospray ionization. Both positive ion mode and collision induced dissociation (CID) were used to elucidate the structures of the major metabolites of mesaconitine. **Results:** Ten compounds were identified, among which seven were new metabolites, and the metabolic pathway was proposed. The protonated molecular ions of seven new metabolites were at m/z 648, 618, 616, 602, 572, 468, and 542, multistage fragment ions with neutral loss of 28 u (CO), 60 u (CH<sub>3</sub>COOH), 18 u (H<sub>2</sub>O), and 32 u (CH<sub>3</sub>OH). These new metabolites detected firstly *in vivo*, were named 10-hydroxyl-mesaconitine, hyaconitine, dehydrated mesaconitine 16-O-demethylmesaconitine, 16-O-demethylhyaconitine, and 16-O-demethyl-dehydrated hyaconitine, respectively. Furthermore, the breaking sequence of methoxyl was obtained using quantum chemistry. **Conclusion:** The study proved that the method of solid-phase extraction technique coupled with MS and quantum chemistry can be applied to the analysis of metabolites in plasma quickly and conveniently.

**Key words:** Liquid chromatography-electrospray ion trap-mass spectrometry, mesaconitine, metabolite, quantum chemistry

## INTRODUCTION

Mesaconitine is one of the important alkaloids of genus aconitum plants used in traditional Chinese medicine and has potential toxicity and wide bioactivities. Mesaconitine is classified into diester diterpenoid alkaloids, which share a common C19-norditerpenoid skeleton, and have anti-inflammatory activities and analgesic functions, accompanied by strong toxicity and narrow therapeutic window. At cellular level, aconitine-type compounds interact with activated sodium channels with high affinity and shift the conformational equilibrium toward the activated state.<sup>[1]</sup> Studies on aconitine metabolites have been reported,<sup>[2-9]</sup> indicating the main metabolites include benzoylaconine,

16-O-demethylaconitine, and aconine. However, additional study is needed to confirm whether or not the three metabolites are only ultimate ones. Currently, liquid chromatography mass spectrometry is an extremely sensitive technique that can detect traces of metabolites in a metabolome sample.<sup>[10]</sup> In the present study, we found not only some reported metabolites but also seven compounds that were not detected previously using this method. These seven compounds were identified as 10-hydroxyl-mesaconitine, 16-O-demethylmesaconitine, hyaconitine, 16-O-demethylhyaconitine, dehydrated mesaconitine, 16-O-demethyl-dehydrated hyaconitine, and dehydrated mesaconine, respectively. A novel metabolic pathway was presented based on the present findings.

## MATERIALS AND METHODS

### Standards and samples

Standards of aconitine (>98%) were purchased from the National Institute for the Control of Pharmaceutical

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and Biological Products, Beijing, China. Six male Sprague Dawley rats were supplied from the Animal Service of Health Science Center, Peking University.

### Solvents and reagents

HPLC-grade acetonitrile (MeCN) was purchased from E. Merck (Darmstadt, Germany) and ammonia (AR grade) was obtained from Beihua Fine Chemicals Co., Ltd. (Beijing, China). The water used for HPLC was purified by a Milli-Q system (Millipore, Milford, MA, U.S.A.).

### Rat blood samples preparation

After 12-hour overnight fast, rats were treated with mesaconitine (4 mg kg<sup>-1</sup>, intragastric administration). Fifteen minutes after the dose, the whole blood samples were collected from abdominal aorta, and then the plasma was separated and stored at -20°C prior to analysis by HPLC/MS/MS. Before analysis, 200 µl of each plasma sample was mixed with 200 µl of methanol and centrifuged at 16,000 × g for 10 min, and then the supernatant was transferred to vials for assay.

### Mass spectrometry analysis

All experiments were performed using an AB SCIEX QTRAP® 5500 LC/MS/MS with a Shimadzu UFLC. The chromatogram flow rate was 0.36 ml min<sup>-1</sup> acetonitrile and 0.04 ml min<sup>-1</sup> aqueous phase. The MS parameters were optimized using chemical reference substance and confirmed to be effective in compounds identification. The ion source was electrospray ionization source with the mode of positive ion. The ion-spray voltage was 5500 V, capillary temperature was set at 250°C, ion source gas was 20 V, declustering potential was 80 V, entrance potential was 10 V, and collision cell exit potential was 13 V.

## RESULTS AND DISCUSSION

### Identification of metabolites

It was reported that pseudomolecules [M + H]<sup>+</sup> of aconitine-type alkaloids were often detected easily under the positive ion mode,<sup>[11]</sup> which was further confirmed in this study. The mass spectrum of each metabolite of mesaconitine is shown in Figure 1. The metabolic pathway is shown in Figure 2. The pseudomolecules of reaction products of aconitine were at m/z 648, 632, 618, 616, 602, 590, 572, 542, 468, and 486, respectively [Table 1].

The pseudomolecule of 648 is speculated to be 10-hydroxyl-mesaconitine with the MS<sup>2</sup> at m/z 616 and m/z 588. According to the literature,<sup>[6]</sup> the fragmentation ion was formed by loss of methanol (CH<sub>3</sub>OH) and acetic

acid (CH<sub>3</sub>COOH). The fragmentation pathway is identical to that of aconitine.

The pseudomolecule of 632 is likely to be mesaconitine with MS<sup>2</sup> at m/z 572 and m/z 540. According to the literature,<sup>[6]</sup> the fragmentation ion was loss of methanol and acetic acid.

The pseudomolecule of 618 may be 10-O-demethyl-mesaconitine. Its MS<sup>2</sup> was at m/z 558 and 526; 558 was loss of CH<sub>3</sub>COOH, whereas 526 was loss of CH<sub>3</sub>OH and CH<sub>3</sub>COOH.

The pseudomolecule of 616 is likely to be hyaconitine, which is consistent with the previous study. Its MS<sup>2</sup> was at m/z 556 and 524; 556 was loss of CH<sub>3</sub>COOH, whereas 524 was loss of CH<sub>3</sub>COOH and CH<sub>3</sub>OH.

According to the literature, the pseudomolecule of 602 is 16-O-demethylhyaconitine. Its MS<sup>2</sup> was at m/z 542 and 510; 542 was loss of CH<sub>3</sub>COOH, whereas 510 was loss of CH<sub>3</sub>COOH and CH<sub>3</sub>OH.

According to the literature,<sup>[6]</sup> the pseudo-molecule of 590 is benzoyl-mesaconine. Its MS<sup>2</sup> was at m/z 540 and 508; 508 was loss of CH<sub>3</sub>OH.

The pseudomolecule of 572, according to the literature,<sup>[11]</sup> we think it is dehydrated mesaconitine. Its MS<sup>2</sup> was at m/z 542 and 510; 542 was loss of CH<sub>3</sub>COOH, whereas 510 was loss of CH<sub>3</sub>COOH and CH<sub>3</sub>OH.

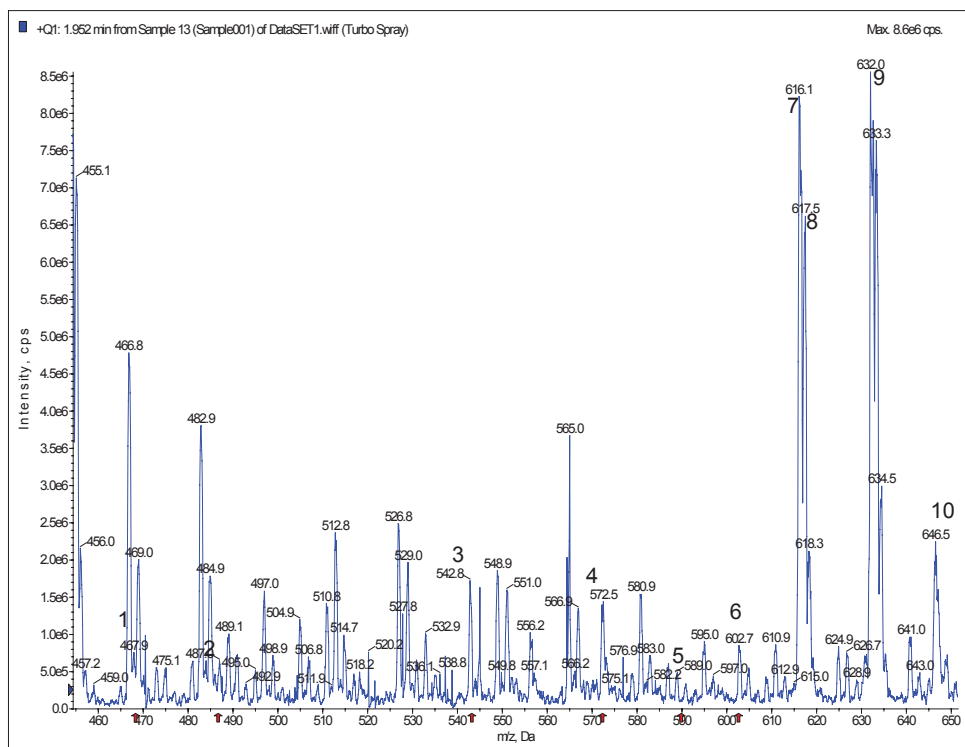
The pseudomolecule of 542, according to the literature,<sup>[11]</sup> we think it is 16-O-demethyl-dehydrated hyaconitine. Its MS<sup>2</sup> was at m/z 482 and 450; 482 was loss of CH<sub>3</sub>OH and CO, whereas 450 was loss of 2 CH<sub>3</sub>OH and CO.

The pseudomolecule of 486, according to the literature,<sup>[11]</sup> we think it is mesaconine. Its MS<sup>2</sup> was at m/z 412; 412 was loss of two CH<sub>3</sub>OH.

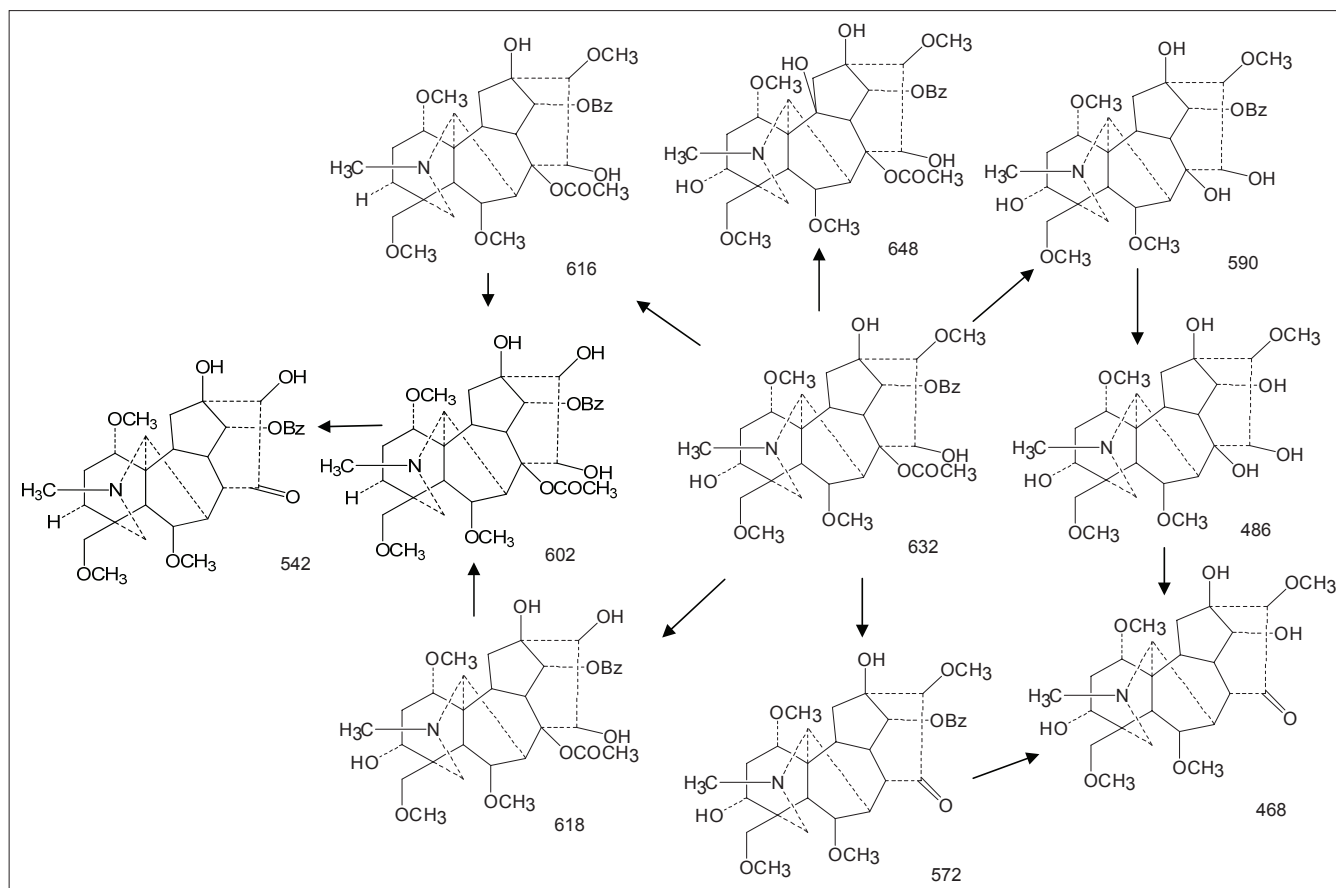
The pseudomolecule of 468, according to the literature,<sup>[12-14]</sup> we think it is dehydrated mesaconine. Its MS<sup>2</sup> was at m/z 352; 352 was loss of two CH<sub>3</sub>OH, two H<sub>2</sub>O, and O.

### The loss order of four methoxyl groups

At first, we speculated that the loss neutral molecule (m/z 32) was CH<sub>3</sub>OH. However, there are four methoxyl groups in mesaconitine, and the loss order of the four methoxyl groups was unknown. In the present study, quantum chemistry software Gaussian 03W was used to reveal the law of loss. Using density functional theory and the 6-31G\* basis sets, geometry promoting and shaking rate were analyzed under HF/STO-3G level, we



**Figure 1:** The MS of blood sample in rat: 1: dehydrated mesaconitine; 2: mesaconitine; 3: 16-O-demethyl-dehydrated hyaconitine; 4: dehydrated mesaconitine; 5: Benzoyl-mesaconitine; 6:16-O-demethylhyaconitine; 7: hyaconitine; 8: 16-O-demethylmesaconitine; 9:mesaconitine; 10: hydroxyl-mesaconitine



**Figure 2:** The metabolic pathway of mesaconitine in rat

**Table 1: Identification of metabolites by MS<sup>n</sup>**

Compounds	MS <sup>1</sup>	MS <sup>2</sup>	MS <sup>3</sup>
10-hydroxyl-mesaconitine	648	616	588
Mesaconitine	632	572	540
16-O-demethylmesaconitine	618	558	526
Hypaconitine	616	556	524
16-O-demethylhypaconitine	602	542	510
Benzoyl-mesaconine	590	540	508
Dehydrated mesaconitine	572	540	-
16-O-demethyl-dehydrated Hypaconitine	542	482	450
Mesaconine	486	412	-
Dehydrated mesaconine	468	352	-

used the B3LYP/6-31G\* technology, and the geometrical model standard is the aconitine model, and the bond length U of aconitine and mesaconitine is international standard. The results showed that the length order of the bonds that connects the four methoxy groups is as follows: C16 (1.440 Å) > C1 (1.422 Å) > C6 (1.411 Å) > C18 (1.408 Å); the results of mesaconitine are C16 (1.421 Å) > C1 (1.422 Å) > C6 (1.411 Å) > C18 (1.408 Å). According to these data, the conclusion is obtained that the breaking sequence of chemical bond is probably C16 or C1.

## CONCLUSIONS

Numerous aconitum plants, such as aconite root, *Aconitum carmichaeli* Debx., *Aconitum szechenyianum*, are widely used in clinics, especially in China.<sup>[15-18]</sup> However, these herbs may be fatal if improperly used or used as poisons. To clarify the mechanism of toxicity, the metabolites of alkaloids, which are the main bioactive compounds of those herbs mentioned above, are perfect proof for taking these herbs. It is widely believed that aconitine-type alkaloids are unstable, especially *in vivo*, and thus it is extremely important to detect more metabolites and elucidate the metabolic pathway. In this study, seven new metabolites and two metabolic pathways are found *in vivo* for the first time. Since this method in the study is exploratory, more effort is needed to confirm the findings using this method. For example, the identification of these compounds should be further studied.

In the present study, blood samples at a series of time points were detected, and the results indicated that varied sample data appeared at different time points.<sup>[19,20]</sup> For example, there was no mesaconine in the sample of 0.5 hour, whereas it was found in 2-hour sample. This result further validated the instability of the alkaloid. So it is very important that a database of metabolites was built, which is the goal of our next work. During

the experiment, we found an interesting thing that different fragments were produced under different CID voltage; for example, mesaconine was broken under 40 eV, whereas mesaconine under 50 eV. These data tell us that CID voltage is an important parameter in MS experiment.

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