Chinese Chemical Letters Vol. 15, No. 9, pp 1071-1074, 2004 1071
http://www.imm.ac.cn/journal/ccl.html

Analysis of the Aconitine Alkaloids in Chuanwu by Electrospray Ionization / Tandem Mass Spectrometry

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Abstract: An electrospray ionization / tandem mass spectrometric (ESI/MS/MS) method was developed for the simultaneous identification and analysis of three aconitine alkaloids [mesacontine (MA), hypaconitine (HA), and aconitine (A)] as intact molecules at low nanogram level in Chinese traditional medicine Chuanwu decoction as well as in human whole blood extract without chromatographic separation.

Keywords: Chuanwu, aconitine, masaconitine, hypaconitine, ESI/MS/MS.

Chuanwu and Caowu are aconites commonly used in Chinese traditional medicine for the treatment of rheumatalgia, and for relieving pain in joints[1]. MA, HA and A contain severely poisonous alkaloids with a very narrow safety margin. In Hong Kong from 1989 to 1995, over 35 cases of aconite poisoning have been documented[2~3]. Such adverse reactions to Chuanwu and Caowu are also reported in China and other countries including Japan and Australia[4]. In support of clinical laboratory diagnosis as well as forensic investigation of aconites poisoning, the sensitive and reliable methods for analysis and identification of these alkaloids in Chinese traditional medicine are necessary. Various methods for identificating separately and assay of aconitum alkaloids have been published, which include high performance liquid chromatography (HPLC)[5], capillary electrophoresis (HPCE)[6], HPLC/MS and GC/MS[7]. However, in these methods the aconites have to be converted to TMS derivatives. Because of their large molecular size (>600) the GC oven has to be programmed to 300°C. High-resolution mass spectrometry in selected ion monitoring mode was employed to increase sensitivity by eliminating high background due to heavy column bleed. Electrospray ionization/tandem mass spectrometry is widely used for characterization of compounds. Because of soft ionization, the molecular ion of the compound of interest usually predominates and forms the base peak in the mass spectrum. In this report, we have developed an electrospray ionization / tandem mass spectrometry method for the simultaneous identification and analysis of three aconitine alkaloids in Chinese

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traditional medicine Chuanwu decoctions and in human whole blood without chromatographic separation.

Experimental

Mesaconitine, hypaconitine and aconitine were purchased from Sigma Co. The entire investigation was preformed using electrospray ionization(ESI) system, interfaced to a model 700 tandem mass spectrometer. The ESI system was tuned and calibrated at mass range of 262~942 amu with a freshly prepared solution of 5 pmol/ul horse skeletal muscle apomyoglobin and 20 pmol/μL of L–methionyl–arginyl–phenyl–alanay–alanine acetate in 50/50 methanol /water containing 0.5% acetic acid.

In the all experiments, Chuanwu sample dissolved in 50/50 methanol /water containing 0.5% acetic acid was directly infused at a rate of 5 μL/min through a 0.1 mm inner diameter fused silica capillary into the ESI ion source. The sample was sprayed by the needle assembly into the ion source where molecules were ionized at atmospheric pressure. The mass spectrometer was scanned from 100-700 amu per second.

Results and Discussion

Acontine, mesacontine and hypacotine are C_{19}-diterpenoid alkaloids containing a nitrogen atom. In the present study, aconitum alkaloids were analyzed using the ESI/MS/MS system. The mass spectra showed fragments of protonated molecules ($[M+H]^+$) and characteristic losses of side chain CH_3COOH(60) and C_6H_5COOH(122) and *m/z* 152 and 279 in **Table 1**. The mass spectra showed $[M+H]^+$ ion at *m/z* 646 for acontine, at *m/z* 632 for mesacontine, and at *m/z* 616 for hypacontine.

Table 1 Protonated molecular ions and characteristic fragment ions of aconitum alkaloids obtained by using ESI/MS/MS

Compound	$[M+H]^+$(*m/z*)	Major fragment ion (*m/z*)	(Neutral loss 60，122，152，279)		
Aconitine	646	586$[M+H-CH^3COOH]^+$	524	494	367
Mesaconitine	632	572$[M+H-CH^3COOH]^+$	510	480	353
Hypaconitine	616	556$[M+H-CH^3COOH]^+$	494	464	337

Figure 1 (a) showed the ESI/MS spectrum of a mixed aconitines standard at 1 ug/mL level and each $[M+1]^+$ peak becomes the characteristic peak for identification of each aconitine alkaloids simultaneously without chromatographic separation. **Figure 1** (b) showed the ESI/MS spectrum of all three aconitine alkaloids existing in a cured Chuanwu decoction after boiling in water for half an hour, and showed the relative amount of semi–hydrolyzed products (*m/z* 603 benzoyl-aconitine, *m/z* 589 benzoyl-mesaconitine and *m/z* 573 benzoyl-hypaconitine)in the MS spectrum. The peaks of all the aconitine alkaloids significantly intensified than those of raw Chuanwu, especially the content of hypaconitine , which is the most poisonous of the three. Prolonged boiling could reduce the amount of aconitines. These compounds were quite stable under the used conditions. The protonated molecular ion has minimal

fragmentation because of soft ionization. This method for analysis of aconitine alkaloids in Chuanwu decoctions indicated HA as the major component. The identity of each alkaloid can further be confirmed by ESI/MS/MS. The protonated molecular ion of parent ion selected by the first mass analyzer undergoes CDI(collision-induced decomposition) by argon gas to fragments which constitute to the finger printing identification of the molecule of parent ion.

Figure 2 showed ESI/MS/MS spectra of HA standard (a) and HA in Chuanwu decoctions sample(b). It shows protonated molecules ($[M+H]^+$) ion for HA at *m/z* 616 and major fragment ion (Neutral loss 60, 122, 152, 279) at *m/z* 556, 494, 464, 337. In addition to the characteristic symptoms of intoxication, the presence of aconitum alkaloids in a patient's blood is a direct diagnostic indication of accidential herbal induced poisoning. **Figure 3** the mass spectra showed the presence of the three aconitine alkaloids spiked to human whole blood (a) and human whole blood control (b). The method is linear over the range from 10 ng to 10 μg of alkaloids per injection. The detection limits of three aconitine alkaloids were 4~10 ng per injection.

Figure 1 ESI/MS spectra of mixed aconitines standard (a) and Chuanwu decoction sample (0.5 h) (b)

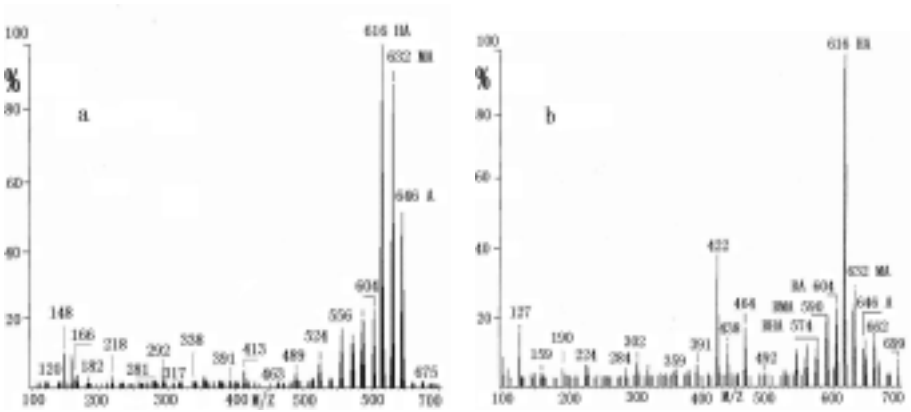


Figure 2 ESI/MS/MS spectra of hypaconitine standard (a) and hypaconitine in Chuanwu decoction sample (b)

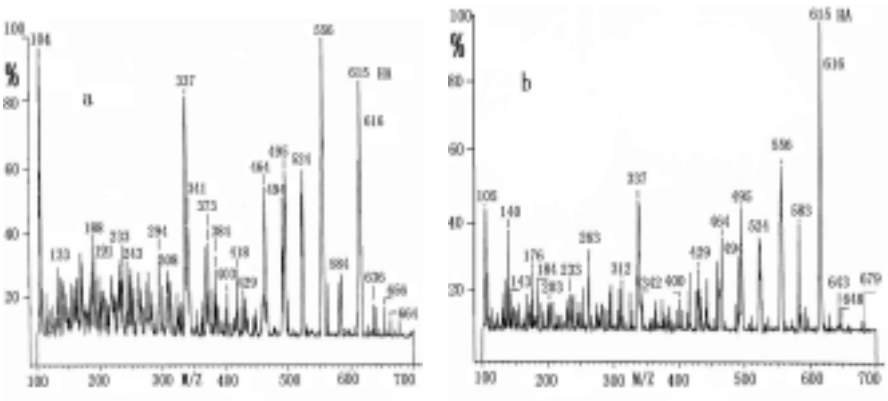
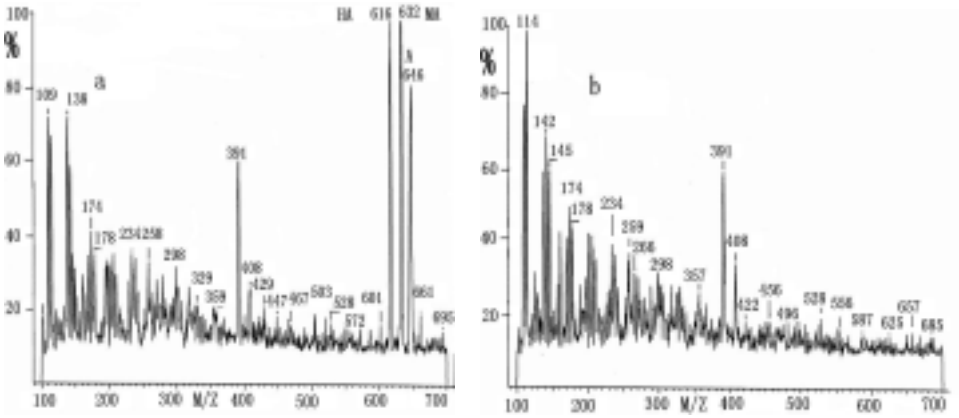


Figure 3 The mass spectra of human whole blood sample poisoned by aconitine alkaloids (a) and control of human whole blood (b)



We also have taken preliminary study to identify the benzoylaconitines, hydrolyzed from the three alkaloids both in raw and in processed Chuanwu and Caowu by HPLC. After processing, most of mesaconitine and aconitine would be transformed to benzoylaconitines analogues. However, hypaconitine has little change.

This is the first report on the direct mass spectrometric simultaneous identification of mesaconitine, hypaconitine and aconitine as intact molecules at low nanogram level in aconites herbal decoction as well as in human whole blood extract without chromatographic separation. The result of the two methods indicated that among the three cured aconitine alkaloids, hypaconitine played a major role in the toxicity. The identity of each aconitine alkaloid can further be confirmed through their MS/MS by CID (collision-induced decomposition).

Acknowledgments

Partial support was received from the Croucher Foundation and the China Medical Board of New York. Assistance from the Chinese Medicinal Material Research Centre, Chinese University of Hong Kong, and from the Government Laboratory is most appreciated.

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Received 18 August, 2003