Asian Journal of Chemistry; Vol. 24, No. 1 (2012), 303-308

ASIAN JOURNAL OF CHEMISTRY

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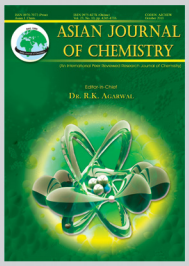

Screening and Identification of Multi Compounds in *Radix aconiti* Using Combination of Liquid Chromatography/Time-of-Flight Tandem Mass Spectrometry

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(*Received*: 21 December 2010; *Accepted*: 19 September 2011) AJC-10422

An approach for screening and identification of multi components in traditional Chinese medicine systems using combination of LC/TOF-MS technique was described in this paper. The chemical profile of *Radix A*., a well-known traditional Chinese medicine, was studied using the established method as for an application and the possibilities of screening complex traditional Chinese medicine systems and identifying these non-target components with modern data acquisition methods of acceleration time of flight mass spectrometers, such as data-dependent MS to MS/MS switching were investigated. As a result, 33 components were identified. This study is expected to provide a rapid, sensitive, economical and systematical method for the identification and further quality evaluation of traditional Chinese medicine.

Key Words: *Radix Aconiti Lateralis*, Screening, Identification, Mass spectrometry, LC/TOF-MS, Traditional Chinese medicine.

INTRODUCTION

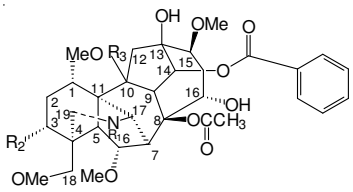
In recent years, the traditional Chinese medicine (TCM) has been given increasing popularity worldwide for their complementary therapeutic effects to the Western drugs but with minimum side effects[1,2]. The effects of traditional Chinese medicine are, of course, brought about by their chemical constituents; thus, the chemical analysis of traditional Chinese medicine is especially important because it helps to understand which chemical components exist inside and which ingredients are the real bioactive ones for certain therapeutic effects and then to establish scientific and rational quality control methods. Each traditional Chinese herb comprises hundreds of different constituents, therefore, systematical and comprehensive analysis of traditional Chinese medicine is a difficult, in some respect even more challenging task.

The classical chemical research method for constituents of herbal prescription is usually time intensive and expensive. No matter the conventional approaches such as LC, NMR or the applications of hyphenated techniques such as GC-MS, CE-MS, *etc.*, the applications of these methodologies are greatly limited by the time-consuming periods and the lack of appropriate standards. Thus, a combinative and powerful metho-dology which could offer higher quality structural information and comprehensive components inside is therefore required for the extensive characterization of traditional Chinese medicine systems.

High performance liquid chromatography/electro spray ionization tandem mass spectrometry (HPLC/ESI MS) had been shown to be a useful analytical tool for the identification of the known compounds in traditional Chinese medicine prescriptions[3-5]. And, to solve the problem of uncertainties of unknown compounds existing in the identification and elucidation, a more powerful methodology, time-of-flight mass spectrometry (TOF-MS), has been developed for the precise and sensitive analysis[6,7]. Benefit from the increased resolving power, accurate mass measurement and high full-scan capability, TOF-MS can provide the elemental compositions of the compounds with low limited accuracy (routinely within 5 ppm). Currently, this strategy has been successfully developed and applied in the analysis of environmental contaminants including pharmaceuticals and pesticide degradates[8-11]. However, to the best of our knowledge, only a few researches on the complex traditional Chinese medicine systems with this technique have been reported yet[12-14].

Radix Aconiti Lateralis is widely distributed over the southwest provinces of China, India and southeast Asia, with the Chinese name Fuzi. Aconitine-type alkaloids from *Radix*

A. possess not only wide bioactivity, such as analgetic, diuretics, antiinflammatory and cardiotonic actions, but also significant toxicity[15-18] (Fig. 1). To our knowledge, there are few published literatures about major aconitine-type alkaloids of *Radix A*. up to now. The aim of this work is to screen and identify the main aconitine-type alkaloids of *Radix A*. using combination of LC/TOF-MS, then to develop a sensitive and accurate method for simultaneous quantitative determination of them for future safety of clinical use. The work of simultaneous quantitative determination is now being carried out in our laboratory.



	R_1	R_2	R_3
Hypaconitine	CH_3	H	H
Deoxyaconitine	C_2H_5	H	H
Mesaconitine	CH_3	OH	H
Aconitine	C_2H_5	OH	H
10-OH-mesaconitine	CH_3	OH	OH
10-OH-aconitine	C_2H_5	OH	OH

Fig. 1. Structures of some aconitine-type alkaloids in *Radix A*.

EXPERIMENTAL

HPLC grade acetonitrile was purchased from Caledon Laboratories Ltd. (Georgetown Ont., Canada). Ultrapure water was self-made in our laboratory. Acetic acid was of an analytical grade (YuWang, ShanDong, China).

Radix A. was purchased from Sichuan, China, which had been identified by Pharmacognosist Zengxi Guo and also been kept under certain conditions for future identification.

Sample preparation: The *Radix A*. was pulverized into powder, passed through a 0.45 mm sieve, then 10 mL 10 % ammonia solution and 200 mL diethyl ether was added with ultrasonic batch at room temperature for three times (0.5 h each). The extracts were combined and evaporated to dryness at 40 ºC under a stream of nitrogen. The residue was dissolved to a 10 mL volumetric flask with methanol-0.5 % hydrochloric acid. The solution was ready for chromatographic analysis after passing through a 0.45 μm membrane filter.

HPLC condition: An Agilent 1200 series LC system was employed in this research, which consisted of a G1376A Cap Pump, a G1379B Degasser, a G1365B multi-wave detector, a G1376B Autosampler and a Hystar PP work station. The analysis of the alkaloids was carried out on a Agilent SB C_{18} (250 mm × 4.6 mm I.D., 5 μm, Agilent, USA), which was protected by a RP18 guard column.

The solvents used for HPLC separation were buffer solution (A, containing 40 mm ammonium acetic with pH adjusted to 9.5) and acetonitrile (B) at a flow rate of 1.0 mL min^{-1}. The mobile phase was as the following: the proportion of acetonitrile was increased from 25-70 % in the first 0.5 h, then increased to 100 % in 10 min and remained for 20 min. The column temperature was 35 ºC and the sample injection volume was 10 μL .

LC/TOF-MS: The HPLC system was coupled to an Bruker micrOTOF-Q 125 (Bruker Ltd., USA) equipped with an electrospray interface. The electrospray source includes dual nebulizers-one nebulizer for the LC eluent and the other for the internal reference solution. The reference standards was sodium formiate, introduced into the TOF-MS with a automated calibrant delivery system (CDS), which would be used as the internal standard for acute mass weight calibration. Accurate mass measurements of the components were obtained with this calibrant delivery system and thus achieved with this on-line prompt calibration.

The HPLC conditions for the LC/TOF-MS analysis were the same as the HPLC method, except for that one-second of the eluent was introduced into the TOF-MS system with a split valve. TOF-MS analysis was performed in both positive (ESI^+) and negative (ESI^-) ion mode under the following operation parameters:capillary voltage 4000 V; drying gas 4 L/min; nebulizer 1.0 psig; gas temp 210 ºC; fragmentor voltage 175 V (ESI^+) and 190 V (ESI^-); skimmer voltage 60 V; octopole dc1 33.3 V (ESI^+) and -40.0 V (ESI^-); octopole RF 250V. The full-scan carried out by LC/TOF-MS was recorded across the mass range 50-2000 m/z.

The elemental composition of every peak was calculated by TOF software. Considering the possible elemental composition of potential components existing in *Radix A*., the number and types of the expected atoms were set as follows: carbons ≤ 30, hydrogens ≤ 50, oxygens ≤ 20, nitrogens ≤ 5. The doublebond equivalent (rdb) parameter was set from 0-20 and the option of electron state was selected as "even". The accuracy error threshold was fixed at 5 ppm for a strict criterion.

RESULTS AND DISCUSSION

HPLC separation: The representative HPLC chromatogram of *Radix A*. was shown in Fig. 2a. The proposed method was therefore acceptable as well as adequate for further MS analysis.

Procedure for the identification of multi components: The base peak chromatograms (BPC) of *Radix A*. obtained by LC/TOF-MS in positive and negative ion mode were presented in Fig. 2b-c. The accurate mass spectrum of each peak in the HPLC or MS chromatogram and the empirical formulae corresponding to the probable existent compounds were obtained subsequently and then the screening, identification and further confirmation of multi components were then performed by detailed studies of their MS and MS/MS spectral data with the published literatures.

Figs. 3 and 4 showed the MS and MS/MS spectra of peak 16, hypaconitine. The MS spectra in the positive mode exhibited an abundant parent ion $[M + H]^+$ at m/z 616.3112 (calcd. 616.3116). According to this molecular weight, the molecular formula could be deduced as $C_{33}H_{45}NO_{10}$, which was accordant with hypaconitine. Fig. 5 showed the hypothetic fragmentation pathway of hypaconitine in MS^n. The MS/MS spectra of 616 m/z exhibited several fragment ions at m/z 584, 556, 524, 492, 464, 338 and 105. The fragment ion at m/z 556 could be attributed to the cause of i clearage, which cost the loss of acetoxy group from the parent ion. And the i clearage further yielded an ion at m/z 524, signaling the open of heptcycle. Subsequent loss of 122 mass units equated to the clearage of N-cycle and the loss of N,N-diethyl group. This clearage was very common in N-containing compound, because N was a electron-abundant group, which could easy attract proton to rearrange its position for the clearage of this positive-charge group to form a much more stable structure. Also, some other rearrangement, such as α and β rearrangement, could be

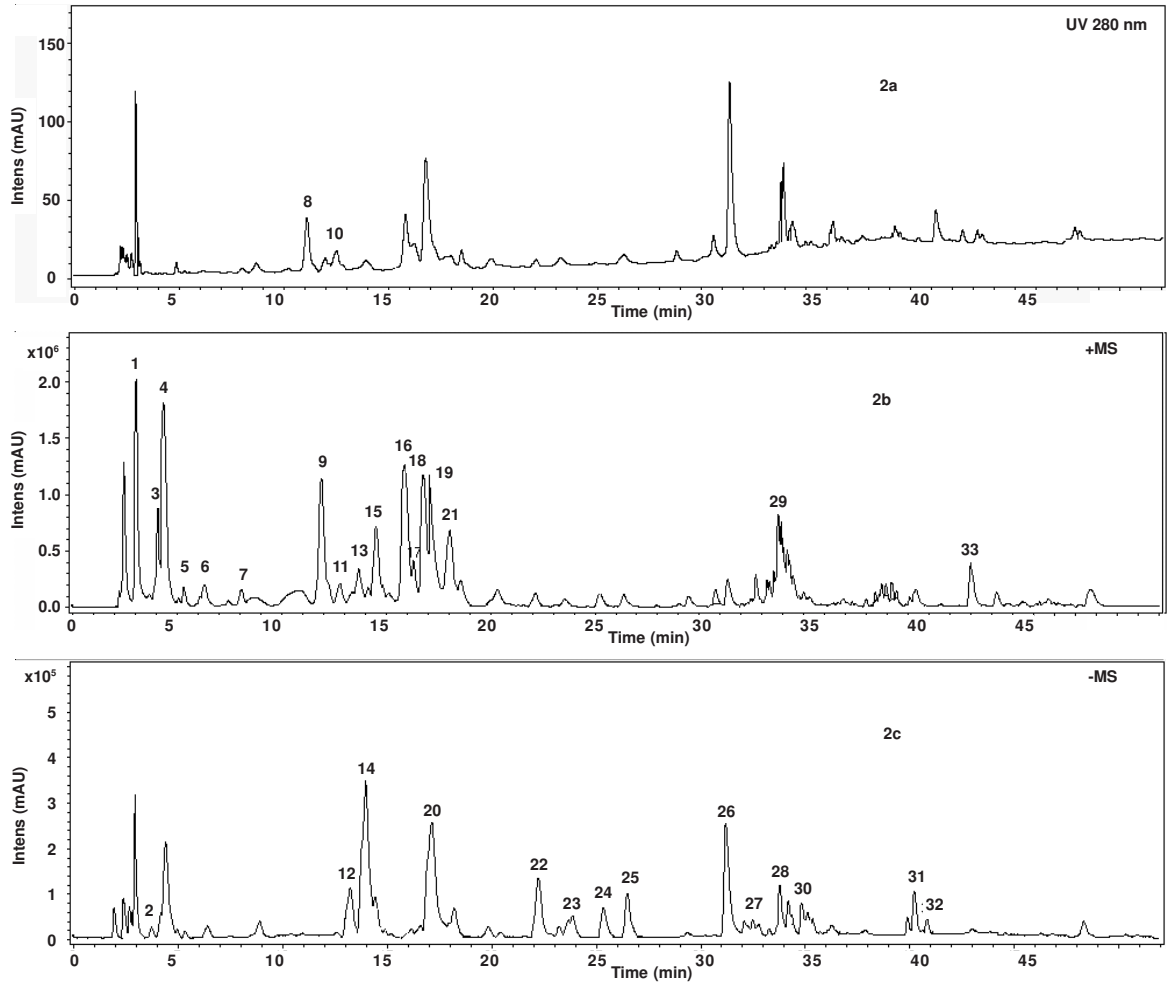


Fig. 2. Chromatograms of multi compounds in *Radix A*. (a) UV chromatogram at 280 nm; (b) BPC chromatogram in positive mode; (c) BPC chromatogram in negative mode

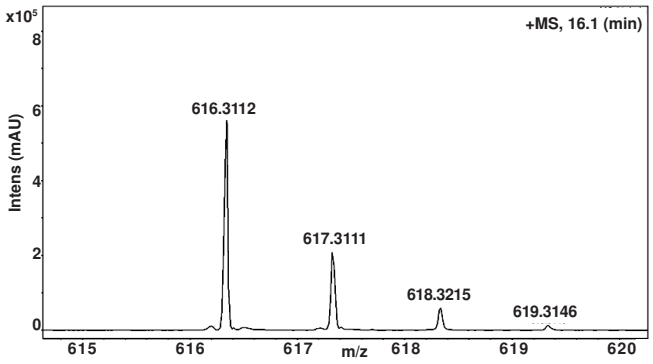


Fig. 3. TOF-MS data of hypaconitine

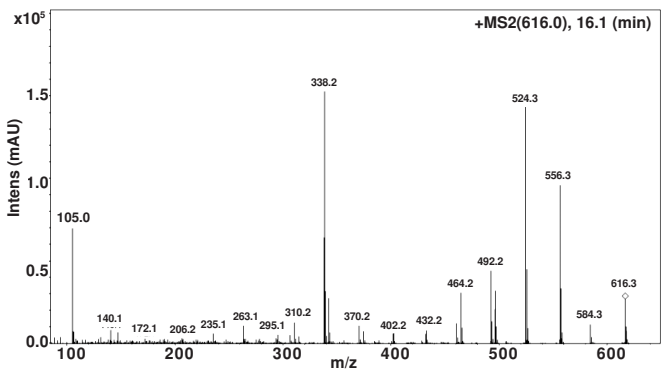


Fig. 4. TOF-MSn data of hypaconitine

seen among the total fragment pathway. These rules could be concluded as the rules of aconitine-type alkaloids from *Radix A*. And according to these rules, some other aconitine-type alkaloids were also deduced.

As a result, 33 components in *Radix A*. were identified with the relative data in Tables 1-2. The results listed in these tables exhibited excellent coherence with the mass spectrometries.

Conclusion

In this work, a reliable and powerful analytical method by using LC/TOF-MS for rapid screening and identification of multi components in *Radix A*.was established. As a result, 33 components were identified. According to the literature, most of the identified compounds possess pharmacological activities related to the clinical application. So the identification of the 33 components equals to identify the main pharmacological substances in this traditional Chinese medicine. Meanwhile, the application of the method to the commercial products of *Radix A*. also provided the chemical support for the chromatographic fingerprint technology and facilitates to improve the quality control standard. On the whole, the LC/TOF-MS has a powerful capability for screening and identification of multi components. This method could identify multi components in traditional Chinese medicine without long-time-consuming isolation and purification period, just relying on

TABLE-1
IDENTIFICATION RESULTS OF *Radix A*. BY LC/TOF-MS

No	t_R (min)	Formula	Calcd. $[M + H]^+$	Observed $[M + H]^+$	Compound
1	3.0	$C_{24}H_{39}N\,O_5$	422.2901	422.2905	Talatizamine
2	3.8	$C_{24}H_{39}NO_7$	454.2799	454.2792	Fuziline
3	4.2	$C_{24}H_{39}NO_6$	438.2850	438.2857	Neoline
4	4.8	$C_{25}H_{41}NO_6$	452.3007	452.3011	Chasmanine
5	5.5	$C_{51}H_{75}NO_{13}$	910.5311	910.5315	3-Acetyl-8-lino-10-O*H*-benzoylmesaconine
6	6.3	$C_{54}H_{85}NO_{12}$	940.6145	940.6149	3-Acetyl-8-esc-benzoylaconine
7	8.2	$C_{53}H_{81}NO_{12}$	924.5832	924.5839	3-Acetyl-8-ndn-benzoylaconine
8	11.2	$C_{25}H_{41}NO_9$	500.2854	500.2851	Aconine
9	11.9	$C_{31}H_{43}NO_9$	574.3011	574.3018	Benzyolhypaconine
10	12.5	$C_{32}H_{45}NO_9$	588.3167	588.3160	Beyzoylaconitine
11	12.8	$C_{33}H_{45}NO_{12}$	648.3015	648.3060	10-OH-Mesaconitine
12	13.1	$C_{33}H_{45}NO_{11}$	632.3065	632.3113	Mesaconitine
13	13.6	$C_{50}H_{75}NO_{11}$	866.5413	866.5467	8-Lino-benzoylaconine
14	14.0	$C_{50}H_{73}NO_{11}$	864.5256	864.5249	8-Linolen-benzoylaconine
15	14.5	$C_{49}H_{71}NO_{10}$	834.5151	834.5148	8-Lino-hypaconine
16	15.9	$C_{49}H_{71}NO_{11}$	882.5362	882.5381	hypaconitine
17	16.3	$C_{50}H_{77}NO_{11}$	852.5620	852.5680	8-Ole-benzoyldeoxyaconine
18	16.9	$C_{46}H_{71}NO_{11}$	814.5099	814.5094	8-Pdc-benzoylmesaconine
19	17.2	$C_{46}H_{71}NO_{10}$	798.5151	798.5157	8-Pdc-benzoylhypaconine
20	17.3	$C_{49}H_{75}NO_{11}$	836.5207	836.5352	8-Lino-benzoylhypaconine
21	18.0	$C_{49}H_{71}NO_{11}$	850.5099	850.5155	8-Lino-Benzoylmesaconine
22	22.2	$C_{50}H_{75}NO_{12}$	854.5413	854.5363	8-Ole-benzoylmesaconine
23	23.9	$C_{49}H_{73}NO_{10}$	828.5256	828.5317	8-Pal-benzoylmesaconine
24	25.3	$C_{49}H_{75}NO_{11}$	842.5413	842.5476	8-Pal-benzoylaconine
25	26.7	$C_{47}H_{73}NO_{11}$	812.5307	812.5363	8-Pal-benzoylhypaconine
26	31.3	$C_{48}H_{75}NO_{11}$	838.5464	838.5516	8-Lino-benzoylHypaconine
27	32.5	$C_{48}H_{75}NO_{10}$	826.5464	826.5469	8-Pal-benzoyldeoxyaconine
28	33.8	$C_{49}H_{77}NO_{11}$	856.5569	856.5572	8-Str-benzoylmesaconine
29	34.0	$C_{49}H_{77}NO_{10}$	840.5620	840.5624	8-Str-benzoylhypaconine
30	35.0	$C_{51}H_{81}NO_{11}$	884.5882	884.5887	8-esc-benzoylmesaconine
31	40.3	$C_{51}H_{77}NO_{12}$	896.5519	896.5523	3-Acetyl-8-ole-benzoylmesaconine
32	41.9	$C_{51}H_{77}NO_{13}$	912.5468	912.5461	3-Acetyl-8-ole-10-OH-benzoylmesaconine
33	43.0	$C_{53}H_{83}NO_{12}$	926.5988	926.5992	3-Acetyl-8-esc-benzoylmesaconine

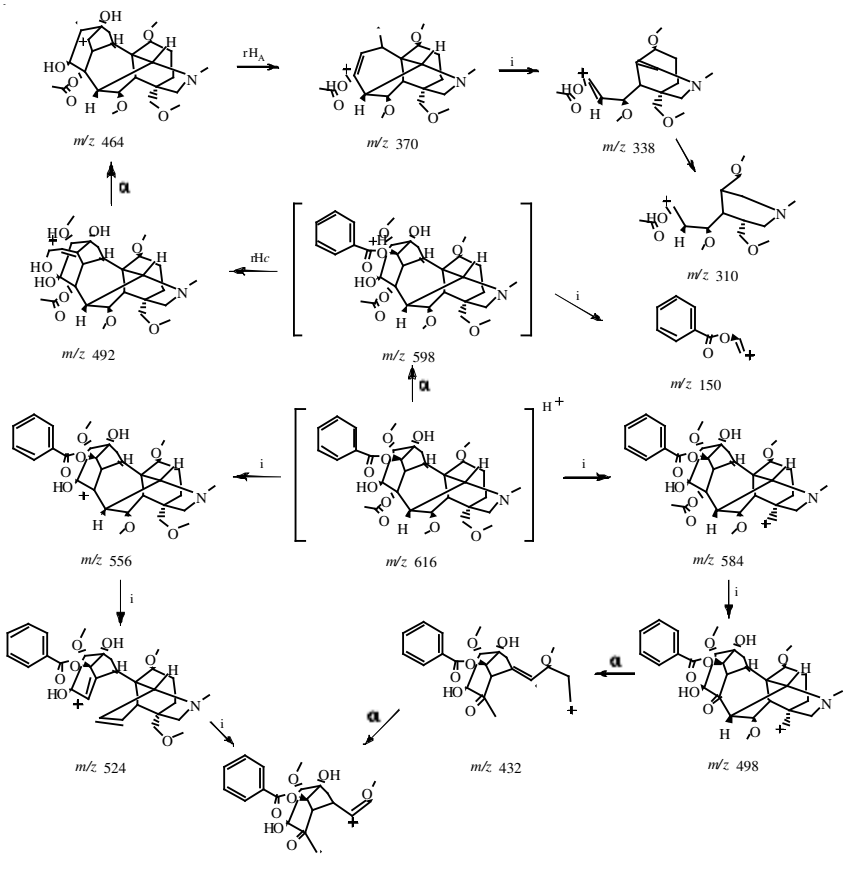


Fig. 5. Possible fragmentation pathway of hypaconitine

TABLE-2
MS^n RESULTS OF *Radix A*. BY TOF-MS^n

No	t_R (min)	Formula	Compound	Daughter ion
1	3.0	$C_{24}H_{39}NO_5$	Talatizamine	404.3 [M+H-H_2O], 376.3[M+H-H_2O-CO]
2	3.8	$C_{24}H_{39}NO_7$	Fuziline	436.3[M+H-H_2O], 404.2 [M+H-H_2O-CO]
3	4.2	$C_{24}H_{39}NO_6$	Neoline	420.3 [M+H-H_2O], 388.3 [M+H-H_2O-CH_3OH], 356.3 [M+H-H_2O- 2CH_3OH]
4	4.8	$C_{25}H_{41}NO_6$	Chasmanine	438.3 [M+H-H_2O], 406.2[M+H-H_2O-CO]
5	5.5	$C_{51}H_{75}NO_{13}$	3-Acetyl-8-lino-10-OH-benzoylmesaconine	570.3[M+H-Linoleic acid-acetyl], 538.2[M+H-oleic acid-acetyl-CH_3OH], 352.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
6	6.3	$C_{54}H_{85}NO_{12}$	3-Acetyl-8-esc-benzoylaconine	572.3[M+H-Eicosenoic acid-acetyl], 540.2[M+H-oleic acid-acetyl- CH_3OH], 354.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
7	8.2	$C_{53}H_{81}NO_{12}$	3-Acetyl-8-ndn-benzoylaconine	556.3[M+H-Nonadecenoic acid-acetyl], 524.2[M+H-monadecenoic acid-acetyl-CH_3OH], 354.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
8	11.2	$C_{25}H_{41}NO_9$	Aconine	483.3[M+H-H_2O], 455.2[M+H-H_2O-CO], 352.2[M+H-linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
9	11.9	$C_{31}H_{43}NO_9$	Benzyolhypaconine	538.3[M+H-Palmic acid-CH_3OH], 352.2[M+H-linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
10	12.5	$C_{32}H_{45}NO_9$	Beyzoylaconitine	556.3[M+H-Nonadecenoic acid-acetyl], 524.2[M+H-nonadecenoic acid-acetyl-CH_3OH], 354.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
11	12.8	$C_{33}H_{45}NO_{12}$	10-OH-Mesaconitine	588.3[M+H-CH_3COOH], 556.2[M+H-CH_3COOH-CH_3OH], 370.2[M+H-CH_3COOH-benzoic]
12	13.1	$C_{33}H_{45}NO_{11}$	Mesaconitine	572.3[M+H-CH_3COOH], 354.2[M+H-CH_3COOH-benzoic acid-3CH_3OH], 105.0 $[C_6H_5CO]^+$
13	13.6	$C_{50}H_{75}NO_{11}$	8-Lino-benzoylaconine	586.3[M+H-Linoleic acid], 554.3[M+H-linoleic acid-CH_3OH], 368.2[M+H-linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
14	14.0	$C_{50}H_{73}NO_{11}$	8-Linolen-benzoylaconine	586.3[M+H-Palmitic acid], 554.3[M+H-palmic acid-CH_3OH], 336.2[M+H-Palmic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
15	14.5	$C_{49}H_{71}NO_{10}$	8-Lino-hypaconine	554.3[M+H-Linolenic acid], 522.3[M+H-linolenic acid-CH_3OH], 304.3[M+H-linolenic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
16	15.9	$C_{49}H_{71}NO_{11}$	hypaconitine	602.3[M+H-Linoleic acid], 570.3[M+H-linoleic acid -CH_3OH], 352.2[M+H-linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
17	16.3	$C_{50}H_{77}NO_{11}$	8-Ole-benzoyldeoxyaconine	570.3[M+H-Oleic acid], 354.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
18	16.9	$C_{46}H_{71}NO_{11}$	8-Pdc-benzoylmesaconine	572.3[M+H-Pentadecanoic acid], 540.3[M+H-palmic acid -CH_3OH], 322.2[M+H-pentadecanoic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
19	17.2	$C_{46}H_{71}NO_{10}$	8-Pdc-benzoylhypaconine	556.3[M+H-Pentadecanoic acid], 524.3[M+H-linolenic acid-CH_3OH], 306.3[M+H-pentadecanoic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
20	17.3	$C_{49}H_{75}NO_{11}$	8-Lino – benzoylhypaconine	556.3[M+H-Linoleic acid], 524.3[M+H-linoleic acid -CH_3OH], 338.2[M+H -linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
21	18.0	$C_{49}H_{71}NO_{11}$	8-Lino-Benzoylmesaconine	570.3[M+H-Palmic acid], 538.3[M+H-palmic acid-CH_3OH], 510.3[M+H-palmic acid-CH_3OH-H_2O], 292.2[M+H-palmic acid-4CH_3OH-H_2O-benzoic acid], 105.0 $[C_6H_5CO]^+$
22	22.2	$C_{50}H_{75}NO_{12}$	8-Ole-benzoylmesaconine	572.3[M+H-Oleic acid], 540.3[M+H-palmic acid -CH_3OH], 354.2[M+H-oleic acid-2CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
23	23.9	$C_{49}H_{73}NO_{10}$	8-Pal-benzoylmesaconine	572.3[M+H-Linoleic acid], 540.3[M+H-linoleic acid -CH_3OH], 322.2[M+H-linoleic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
24	25.3	$C_{49}H_{75}NO_{11}$	8-Pal–benzoylaconine	586.3[M+H-Palmitic acid], 554.3[M+H-palmic acid-CH_3OH], 336.2[M+H-palmic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
25	26.7	$C_{47}H_{73}NO_{11}$	8-Pal-benzoylhypaconine	556.3[M+H-Palmitic acid], 524.3[M+H-palmic acid-CH_3OH], 306.2[M+H-palmic acid-4CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
26	31.3	$C_{48}H_{75}NO_{11}$	8-Lino-benzoylHypaconine	558.3[M+H-Linolenic acid], 526.3[M+H -Linolenic acid-CH_3OH], 308.2[M+H –Linolenic acid - 4CH_3OH-Benzoic acid], 105.0 $[C_6H_5CO]^+$
27	32.5	$C_{48}H_{75}NO_{10}$	8-Pal-benzoyldeoxyaconine	570.3[M+H -Palmitic acid], 538.3[M+H -Palmic acid - CH_3OH], 320.2[M+H -Palmic acid- 4 CH_3OH -Benzoic acid], 105.0 $[C_6H_5CO]^+$
28	33.8	$C_{49}H_{77}NO_{11}$	8-Str-benzoylmesaconine	572.3[M+H -Palmitic acid], 540.3[M+H -Palmic acid - CH_3OH], 322.2[M+H -Palmic acid- 4 CH_3OH -Benzoic acid], 105.0 $[C_6H_5CO]^+$
29	34.0	$C_{49}H_{77}NO_{10}$	8-Str-benzoylhypaconine	556.3[M+H -Palmitic acid], 524.3[M+H -Palmic acid - CH_3OH], 306.2[M+H -Palmic acid- 4 CH_3OH -Benzoic acid], 105.0 $[C_6H_5CO]^+$
30	35.0	$C_{51}H_{81}NO_{11}$	8-esc-benzoylmesaconine	574.3[M+H -Eicosenoic acid], 542.3[M+H-palmic acid-CH_3OH], 324.2 [M+H -Palmic acid- 4 CH_3OH-benzoic acid], 105.0 $[C_6H_5CO]^+$
31	40.3	$C_{51}H_{77}NO_{12}$	3-Acetyl-8-ole-benzoylmesaconine	556.3[M+H-Oleic acid-acetyl], 524.2[M+H-oleic acid-acetyl-CH_3OH], 338.2[M+H-oleic acid- 3 CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
32	41.9	$C_{51}H_{77}NO_{13}$	3-Acetyl-8-ole-10-OH-benzoylmesaconine	572.3[M+H-Oleic acid-acetyl], 540.2[M+H-oleic acid-acetyl-CH_3OH], 354.2[M+H-oleic acid-3CH_3OH-benzoicacid], 105.0 $[C_6H_5CO]^+$
33	43.0	$C_{53}H_{83}NO_{12}$	3-Acetyl-8-esc-benzoylmesaconine	572.3[M+H-Eicosenoic acid-Acetyl], 540.2[M+H-Oleic acid-acetyl- CH_3OH], 354.2[M+H-oleic acid- 3 CH_3OH -benzoicacid], 105.0 $[C_6H_5CO]^+$

the abundant MS and MS/MS data acquired by LC/TOF-MS, the comprehensive investigation of the previous literatures and a much smaller amount of relative standards. It would provide a rapid, sensitive, economical and systematical method for the improvement of quality control of traditional Chinese medicine in future.

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