19

The MALDI-TOF Analysis of Aconitum Alkaloids in Proprietary Chinese Medicine and in the Concoction of Fuzi

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1. Introduction

In theory, the kinds and relative amounts of aconitum alkaloids in proprietary Chinese medicines should be consistent with those in Fuzi. However, this feature has not been noted enough and no direct experimental evidence to prove it. In this work MALDI-TOF-MS was used to analysis 19 kinds of proprietary Chinese medicines, benzoylmesaconine and hypaconitine have been proved to be the predominant monoester and diester alkaloids respectively. In addition, the MALDI-TOF analysis of the concoction of seven kinds of Chinese medicine has confirmed that the acidity of concoction will improve the aconitines contents and the toxicity of concoction, but this rule was not suited for Lonicera nitida, Rhizoma Chuanxiong or Chaenomeles sinensis.

The Aconitum plants are widely used in China as an analgesic, a cardiotonic, and an anti rheumatism treatment. Among all the species Aconitum, the lateral roots of Aconite (Aconitum Camicheali Debx, Fuzi), main roots of Aconite (Chuanwu) and roots of Aconitum kusnezoffii Reichb (Caowu) are the three kinds of plant medicines that are collected in Chinese Pharmacopoeia, and some of other Aconitum plants have been used as folk medicine. It is well known that diester diterpenoid aconitine (DDA) and its analogues isolated from Aconitum plants contribute to the bioactivity and the high toxicity for the heart and the central nervous system. Fortunately, aconitines are heat-unstable and will be conversed into less toxic monoester diterpenoid alkaloids (MDA) or lipo-alkaloids after processing [1], therefore, the amounts of highly toxic aconitine are lower than those of monoester diterpenoid alkaloids in processed aconite and proprietary Chinese medicines (PCMs).

For monitoring the possible toxicity of Chinese medicines, high-performance liquid chromatography (HPLC) has been frequently used for quantification analysis of aconitines. Generally, only aconitine, mesaconitine and hypaconitine three standards could be purchased and most of research was limited in these 3 alkaloids [2]. Therefore, qualitative analysis is also essential for PCM besides quantitative analysis. In contrast to chromatographic methods, matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) determines the molecular mass of alkaloids, which represents an inherent physical property and is feasible for high-throughput analysis of different samples containing aconitines.

In this work, we have analyzed aconitum alkaloids in 19 kinds PCMs and found that hypaconitine is the dominant DDA in the 12 oral PCMs, which is somewhat different from previous quantitative results of aconitines in PCM reported by using HPLC method [3]. These PCMs include Guifudihuangwan (GFDHW), Fuzilizhongwan (FZLZW), Jinkuishenqiwan(JKSQW), Xiaohuoluowan (XHLW), Sanhanhuoluowan (SHHLW), Zuifengtouguwan (ZFTGW), Narusanwewan (NRSWW), Muguawan (MGW), Xiaojinwan (XJW), Haimabushenwan (HMBSW, Dahuoluowan (DHLW), Panlongqipian (PLQP), Fufangxuelianjiaonang (FFXLJN), Diedazhentonggao (DDZTG), Tiebangcuizhitonggao (TBCZTG), Guzengshengzhentonggao (GZSZTG), Shexiangzuifenggao (SXZFG), Zhentonglingding (ZTLD) and Shangtongding (STD).

On the other hand, the detoxification mechanism of aconite in concoction is far away from clear. For example, acidity is one of the factors to influence the hydrolysis of aconitines [4]. Here we have concocted Fuzi with 7 others herbal Medicines and analyzed the alkaloids in the concoction by MALDI-TOF-MS. We have found that acidity is not the only factor that affects the hydrolysis reaction of DDAs as well as the amounts of aconitines because of the complexity of Chinese medicine.

1.1 Experimental

Prepared root of aconite and all proprietary Chinese medicine (PCM) were purchased from drug store; 5g dried and powered aconite root or PCM were soaked with 60 mL ethanol for 48 h at room temperature, and the resulting solution of alkaloids was diluted with 50% ethanol for further analysis of MALDI-TOF.

1.2 Mass spectrometry

All experiments were performed using a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems) The Voyager DE STR was operated in a positive reflector mode with the following parameters: acquisition mass range, 400-1000 Da; accelerating voltage, 20,000 V; grid voltage, 73%; mirror voltage ratio, 1.14; guide wire, 0.01%; low mass gate set at 300; extraction delay time, 150 ns; and the laser power attenuator set at 2700, total 100 shots/spectrum. Matrix solution was prepared by dissolving 8mg of α-cyano-4-hydroxycinnamic acid (CHCA) in 1 ml of 1:1 mixture of acetonitrile and 0.1% trifluoroacetic acid.

2. Results and discussions

2.1 The MALDI-TOF analysis of nineteen kinds of PCM

For aconitum alkaloids, protonated molecules $(M+H)^+$ were observed by MALDI-MS in positive ion mode. The structures of aconitum alkaloids are very similar and it is reasonable to assume that they have similar ionization efficiencies [5]. The alkaloids of 13 oral administration pills or tablets that using prepared aconite roots have been analyzed firstly. As shown in Fig.1-Fig.13, benzoylhypaconine (BHA, m/z 574), benzoylmesaconine (BMA, m/z 590) and benzoylaconine (BAC, m/z 604) were the major components, hypaconitine (HA, m/z 616) is the most main diester alkaloids, additionally, deoxyaconitine (DA, m/z 630), mesaconitine (MA, mz/ 632) and aconitine (AC, m/z 646) can also be observed. In all 13 PCMs, HMBSW exhibit the most high relative abundance of the highly toxic diester aconitines (Fig.12), however, different with some previous report that obtained by high

performance liquid chromatography [3], it is HA other than MA is the dominant aconitine, in addition, 10-OH-MA (m/z 648) and 10-OH-AC (m/z 662) have been detected also. We believe our result is reasonable because MA is more prone to be hydrolyzed to BMA after boiling [1] and HA is the main aconitine in prepared aconite roots [5]. An exception is Daguoluowan, only BMA was detected with a weak signal. According to the ancient Chinese concept of Yin-Yang, Aconite is one of the most important herbal medicines that related Yang deficiency and relieving pain, so a certain amount of monoester aconitum alkaloids and diester alkaloids is essential for the treatment. Generally, in the mass region of m/z 400-500, talatizidine, talatisamine, neoline, fuziline and 14-acetyl- talatisamine can be detected at m/z 408, m/z422, m/z 438, m/z 545 and m/z 464 respectively owing to they also exist in prepared Fuzi [6]. Secondly, for the vitro dosage form such as plaster or tincture, some obvious changes have been observed. Aconitine at m/z 646 has been detected as the main alkaloids in DDZTG (Fig.14), TBCZTG (Fig.15), GZSZTG (Fig.16), and STD (Fig.17); thirdly, mesaconitine is the most high content alkaloids in SXZFG (Fig.18); fourthly, high abundance of HA, DA, MA and AC have been detected in ZTLD.

In sum, because of the high ionization efficiency of alkaloids, aconitines and their hydrolysis products can be easily analyzed by MALDI-TOF after extracted by chloroform, thus, MALDI-TOF mass spectrometry provides a rapid, sensitive, simple and specific method for the qualitative analysis of alkaloids mixtures in complex systems such as proprietary Chinese. By comparing the relative abundance of DDA and MDA, we can further acquire relatively quantitative information of aconitum alkaloids. Actually, as an analysis method that based on mass isolation, mass spectrometry method is especially suit for the well-known research system such as aconitum alkaloids.

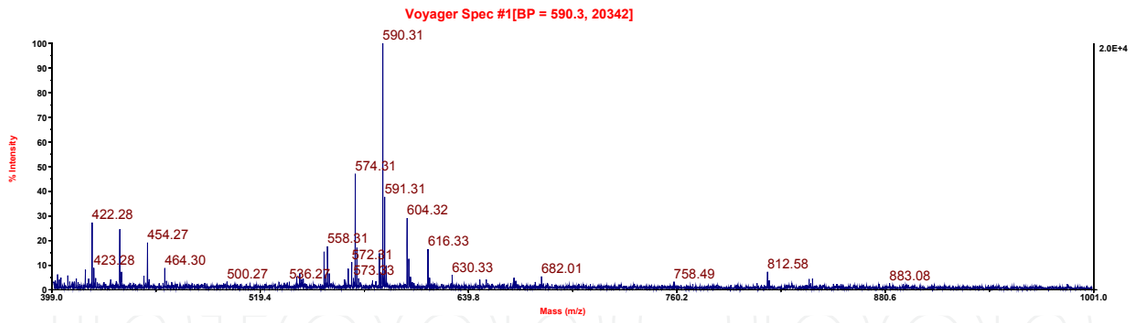

Fig. 1. MALDI-TOF spectrum of GFDHW

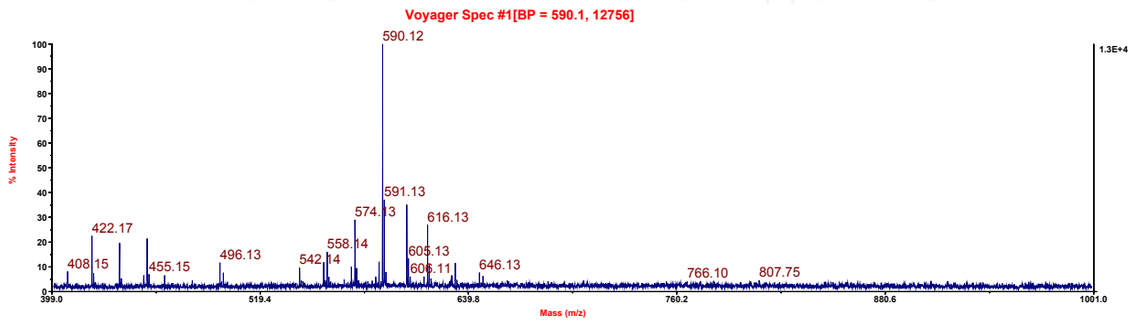

Fig. 2. MALDI-TOF spectrum of FZLZW

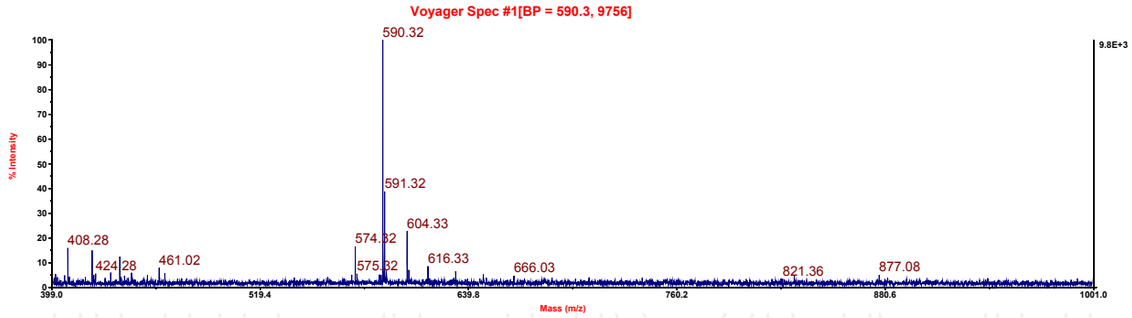

Fig. 3. MALDI-TOF spectrum of JKSQW

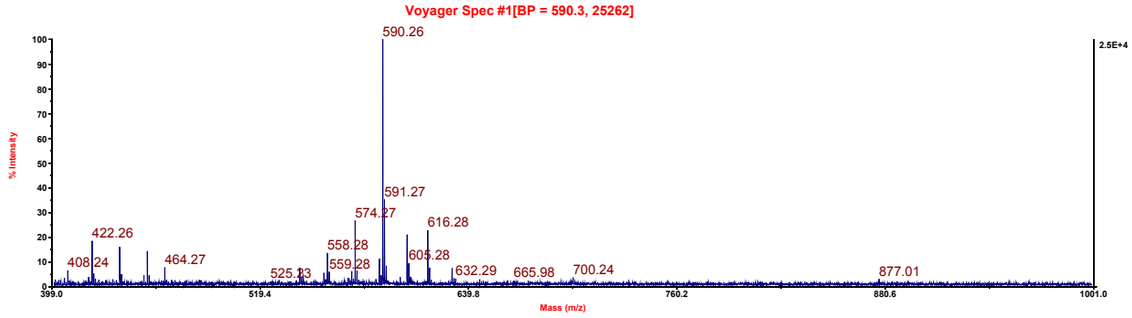

Fig. 4. MALDI-TOF spectrum of XHLW

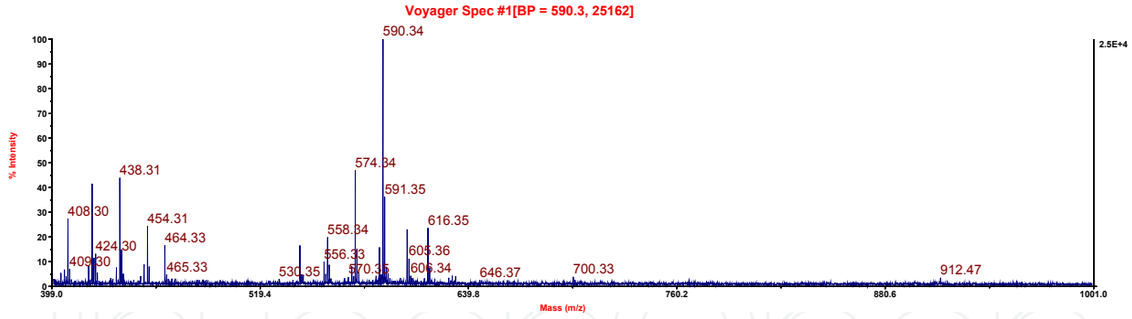

Fig. 5. MALDI-TOF spectrum of SHLLW

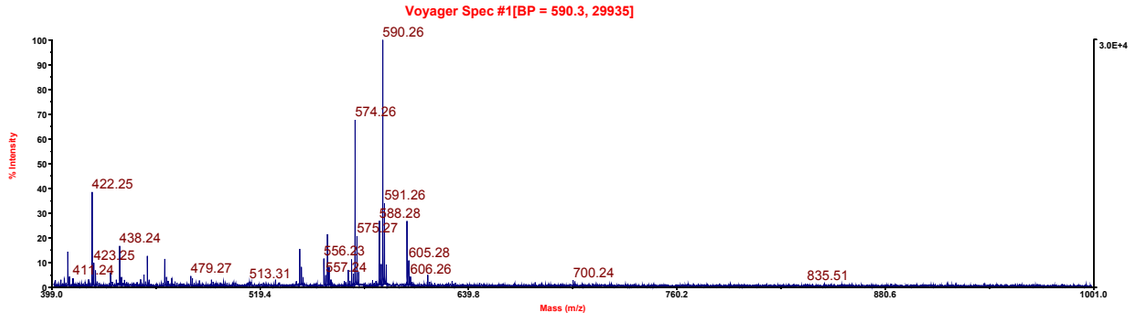

Fig. 6. MALDI-TOF spectrum of ZFTGW

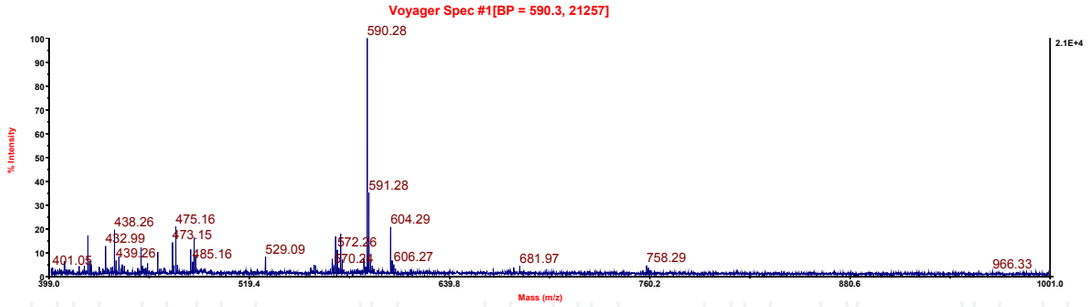

Fig. 7. MALDI-TOF spectrum of NRSWW

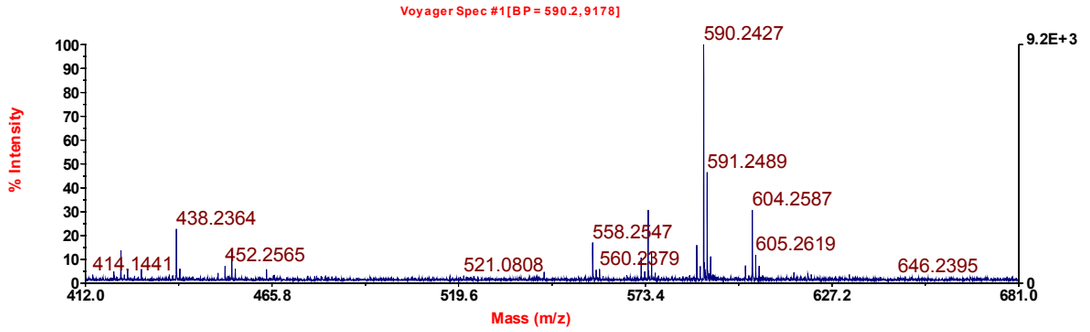

Fig. 8. MALDI-TOF spectrum of MGW

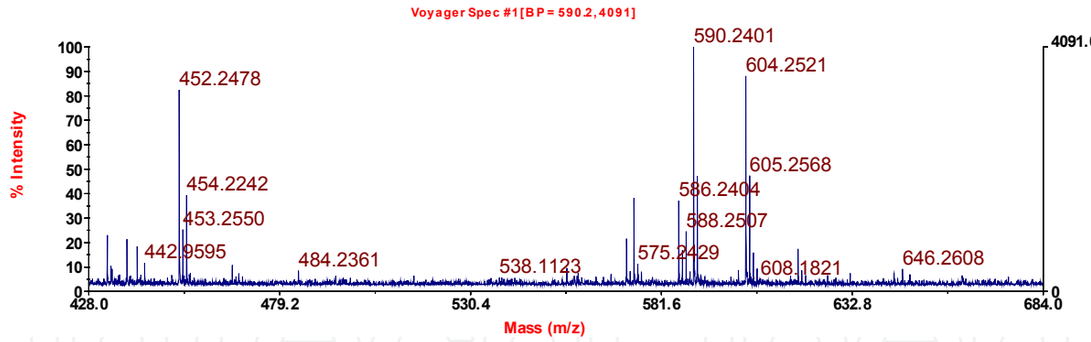

Fig. 9. MALDI-TOF spectrum of PLQP

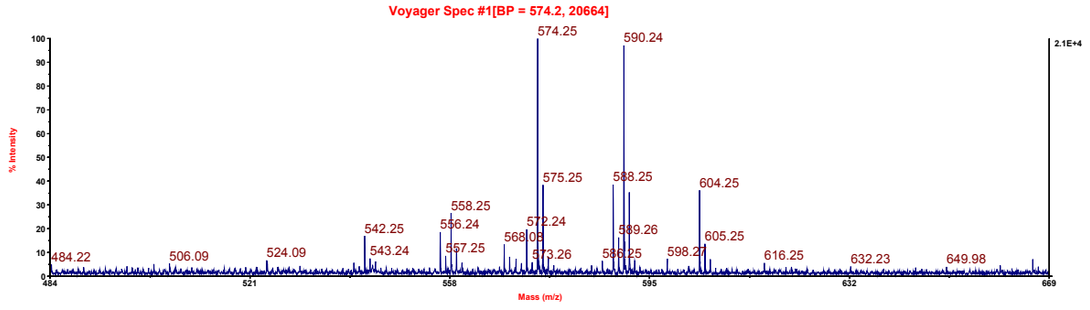

Fig. 10. MALDI-TOF spectrum of XJW

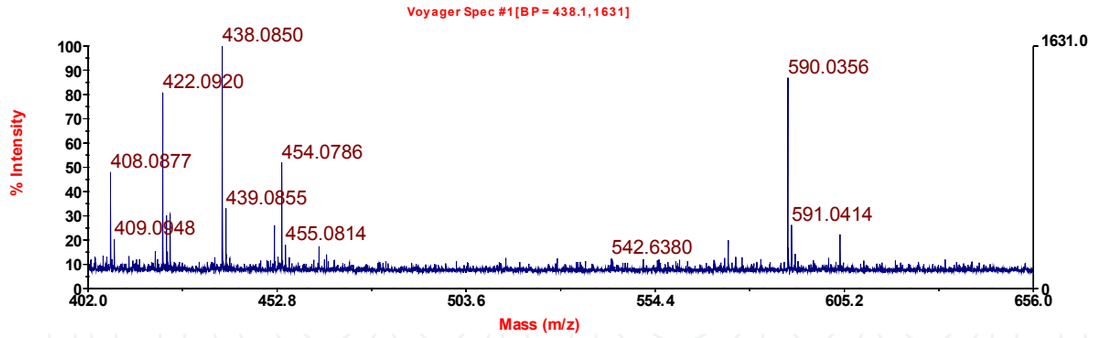

Fig. 11. MALDI-TOF spectrum of FFXLJN

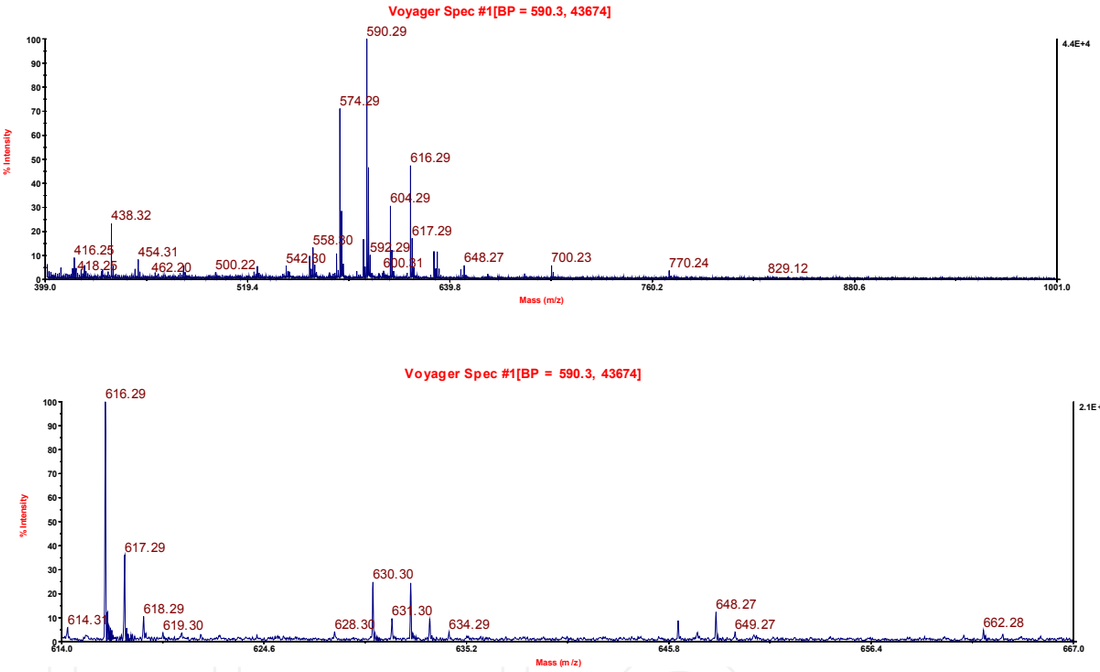

Fig. 12. MALDI-TOF spectrum of HMBSW

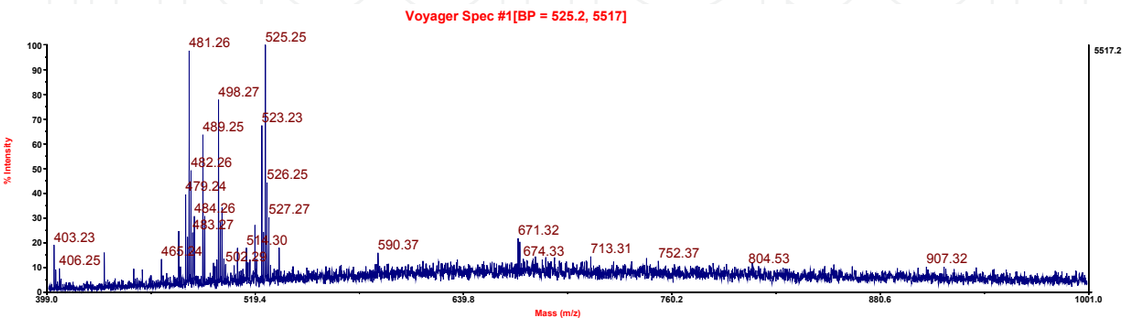

Fig. 13. MALDI-TOF spectrum of DHLW

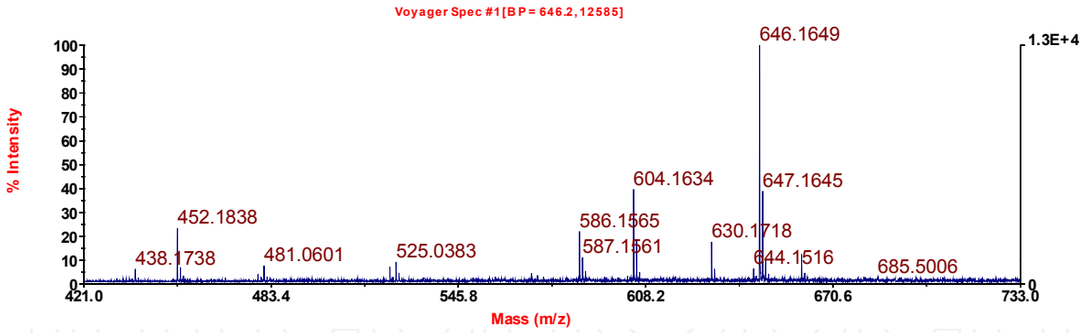

Fig. 14. MALDI-TOF spectrum of DDZTG

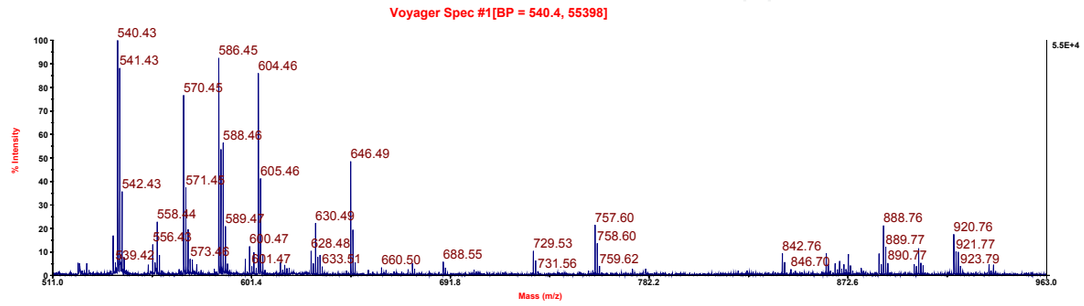

Fig. 15. MALDI-TOF spectrum of TBCZTG

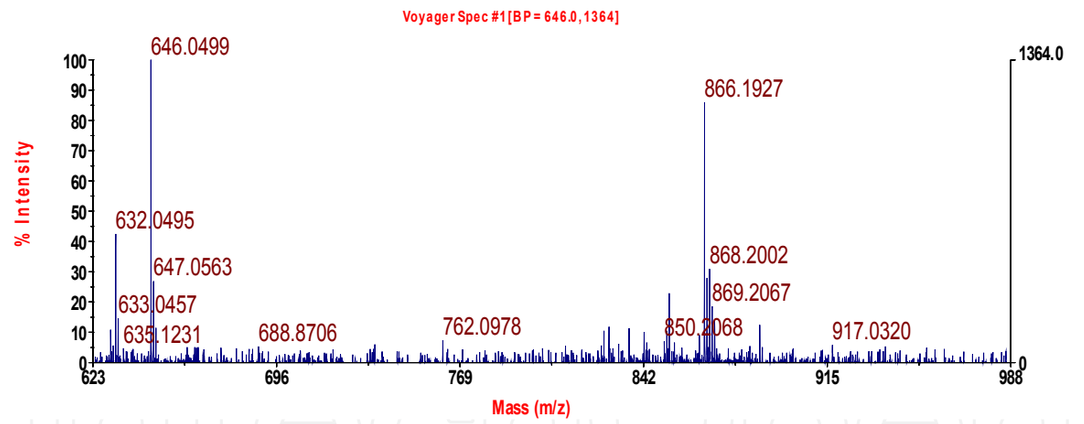

Fig. 16. MALDI-TOF spectrum of GZSZTG

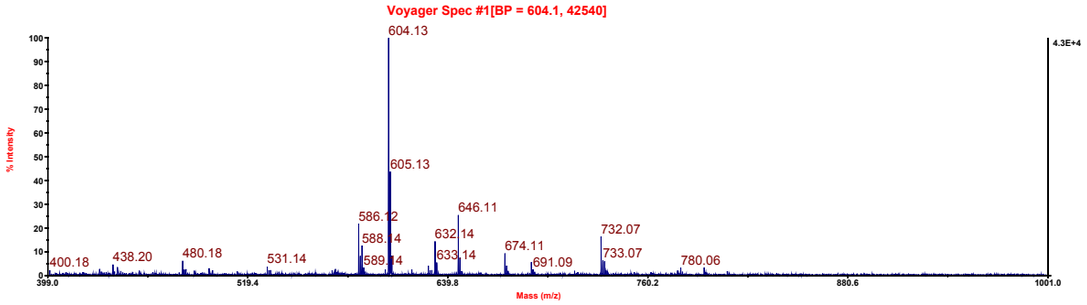

Fig. 17. MALDI-TOF spectrum of STD

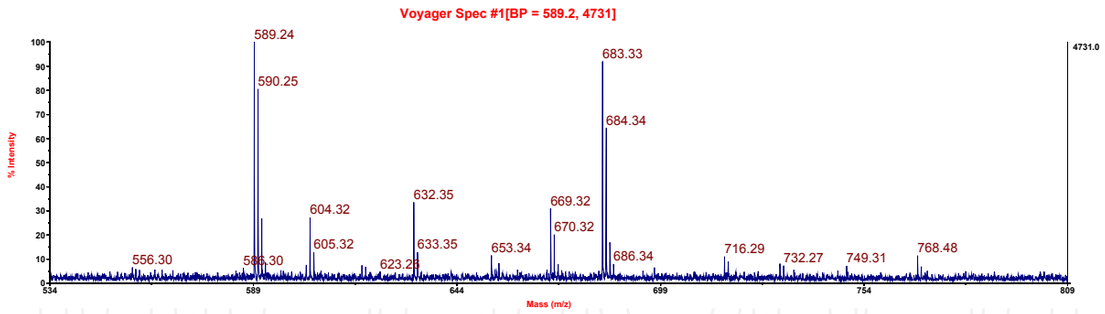

Fig. 18. MALDI-TOF spectrum of SXZFG

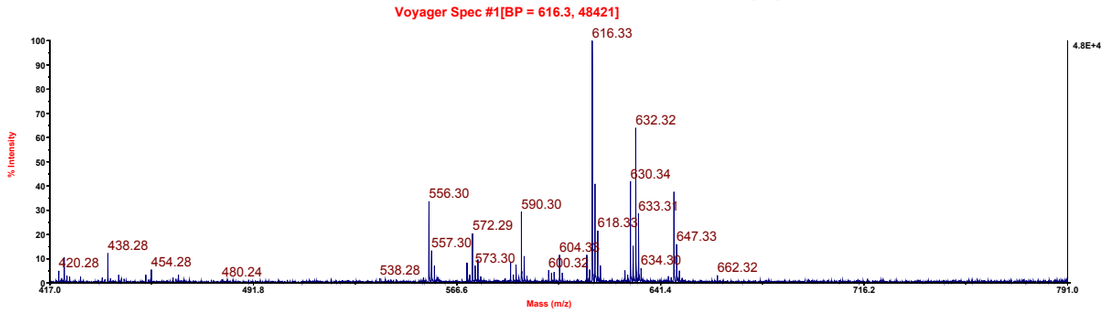

Fig. 19. MALDI-TOF spectrum of ZTLD

2.2 The MALDI-TOF analysis of alkaloids in the concoction of aconite roots with other Chinese medicine

To some extent, the combinational principles of Fuzi can be explained by the chemical reactions in the concoction, namely, DDA will be changed to MDA and lipo-alkaloids in boiling water [1]. In addition, the acidity of solution has played an important role for these chemical reactions and high acidity will inhibit the hydrolysis of aconitines [4]. However, it is still not clear that whether the acidity is the only factor for the amounts of aconitines in concoction. In this study, we have selected 7 kinds of Chinese medicines of which decoction are acidic to answer this question.

As shown in Table 1, the pH value of the Fuzi decoction is 4.73; the pH value of the decoction of Radix Glycyrrhizae is 5.54, the pH value of other 6 kinds of herbal medicine ranged from 3 to 4. The pH value of the concoction of aconite with others is similar with those of decoction.

2.3 The MALDI-TOF analysis of alkaloids in prepared Fuzi and the decoction of Fuzi

The MALDI-TOF spectrum of the ethanolic extract of prepared Fuzi provides a profile of the alkaloids. BHA, BMA and BAC are the main alkaloids in Prepared Fuzi, HA, DA, MA and 10-OH-AC has been observed as the main aconitines (Fig.20). After decocting, the relative amounts of highly toxic aconitines have been reduced because they are heat-unstable [5,6].

2.4 The MALDI-TOF analysis of alksloids in the concoction of Fuzi with Radix Glycyrrhizae, Lonicera nitida and Rhizoma Chuanxiong

It is well known that Radix Glycyrrhiza has the detoxify effect, so it is reasonable that almost no aconitines has been detected in the concoction of Fuzi with it except for low abundant

HA at m/z 616 (Fig.22). However, to our surprise, in the concoction of Fuzi with Lonicera nitida or Rhizoma Chuanxiong (Fig.23 and Fig.24), the relative intensity of aconitines is also low and other aconitines have been detected at noice lowel. Since the pH value of the concoction of Fuzi with Lonicera nitida and Rhizoma Chuanxiong is respective 3.54 and 3,75, these results have suggest that the acidity of concoction is not the only factor that effect the hydrolysis reactions of aconitines.

Aconite roots	4.73
Radix Glycyrrhizae (甘草)	5.54
Lonicera nitida (金银花)	3.54
Crateagus pinnatifida (山楂)	3.32
Chaenomeles sinensis (木瓜)	3.95
Rhizoma Chuanxiong (川穹)	3.75
Herba Potulacae Oleraceae (马齿苋)	3.84
Galla Chinensis (*五倍子*)	3.63
Aconite roots+ Radix Glycyrrhizae (A+RG)	5.12
Aconite roots+ Lonicera nitida (A+LN)	3.22
Aconite roots+ Crateagus pinnatifida (A+CP)	3.26
Aconite roots+ Chaenomeles sinensis (A+CS)	3.69
Aconite roots+ Rhizoma Chuanxiong (A+RC)	4.10
Aconite roots+ Herba Potulacae Oleraceae (A+HPO)	3.87
Aconite roots+ Galla Chinensis (A+GC)	3.65

Table 1. The pH value of the decoction of eight kinds of Chinese Medicine

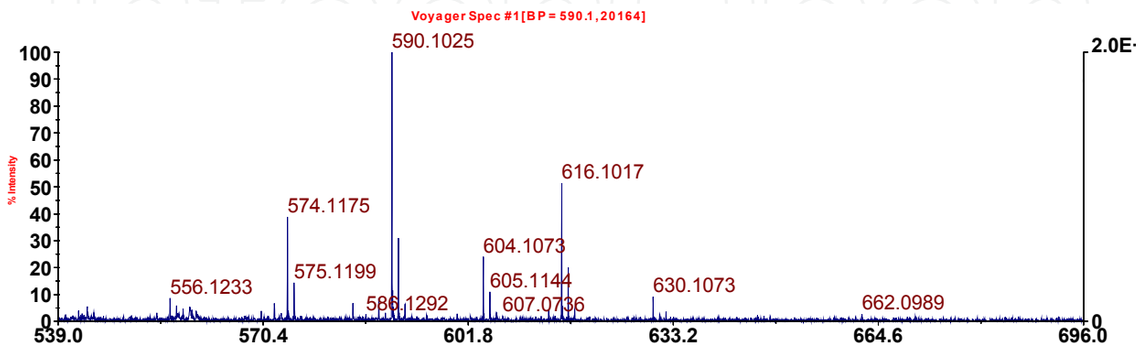

Fig. 20. MALDI-TOF spectrum of the ethanol extract of prepared Fuzi

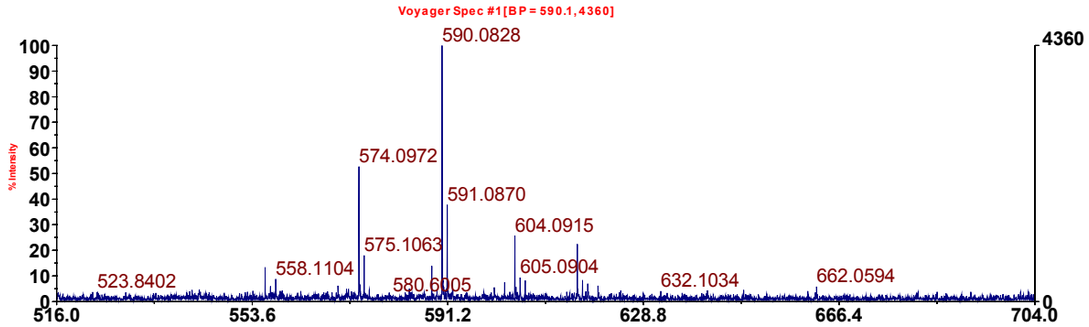

Fig. 21. MALDI-TOF spectrum of the decoction of prepared Fuzi

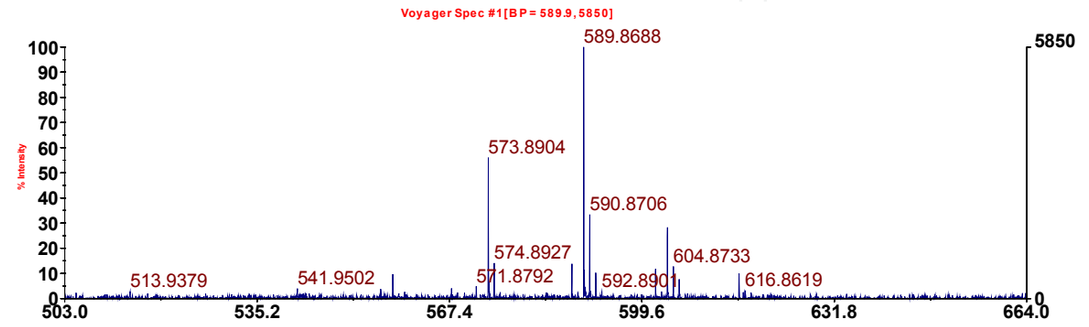

Fig. 22. MALDI-TOF spectrum of the concoction of prepared Fuzi with Radix Glycyrrhizae

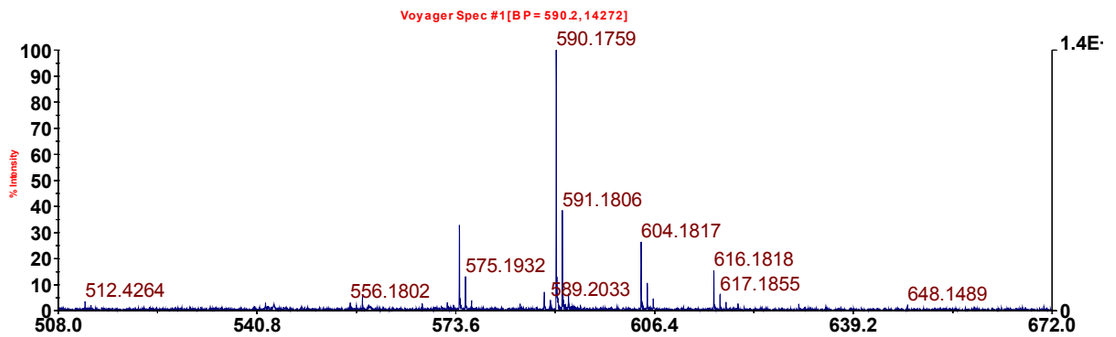

Fig. 23. MALDI-TOF spectrum of the concoction of prepared Fuzi with Lonicera nitida

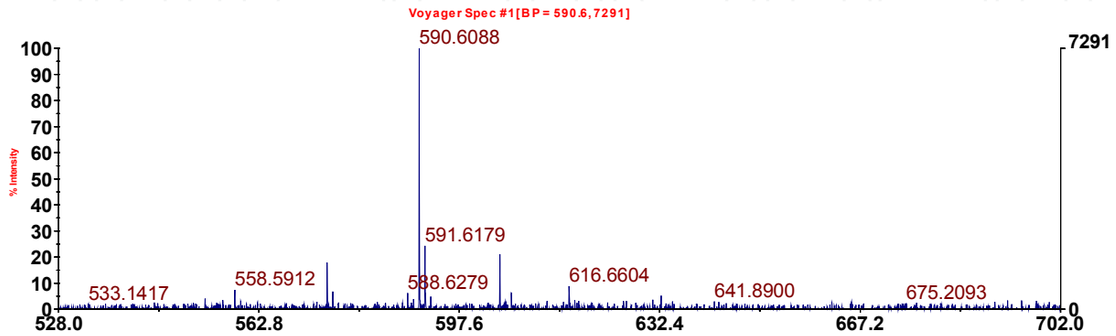

Fig. 24. MALDI-TOF spectrum of the concoction of prepared Fuzi with Rhizoma Chuanxiong

2.5 The MALDI-TOF analysis of alkaloids in the concoction of Fuzi with other 4 kinds of acidic herbal medicines

As shown in Fig.25-Fig.28, when Fuzi was concocted with Crateagus pinnatifida, Galla Chinensis, Chaenomeles sinensis or Herba Potulacae Oleraceae respectively, the relative amounts of DDA were higher that those in decoction, which suggested that these four herbal medicines should not be concocted with Fuzi. However, owing to the point-to-point difference of MALDI source, a statistical analysis is necessary. All the results have been shown in Table 3-Table 11 and summarized in Table 12.

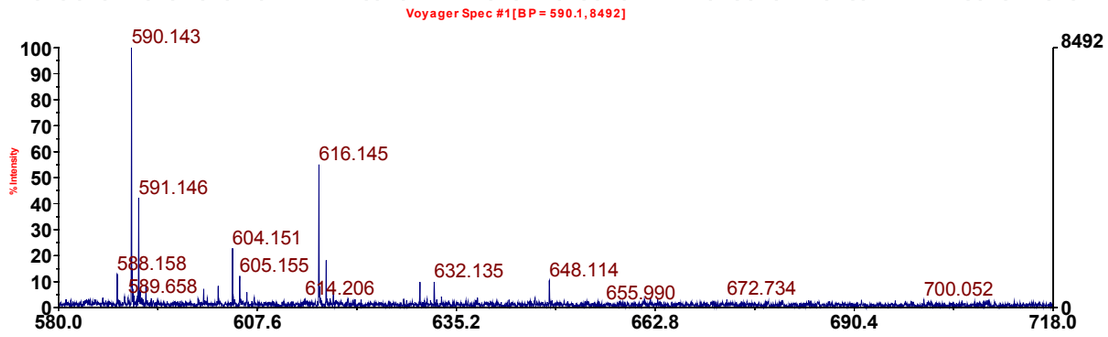

Fig. 25. MALDI-TOF spectrum of the concoction of prepared Fuzi with Crateagus pinnatifida

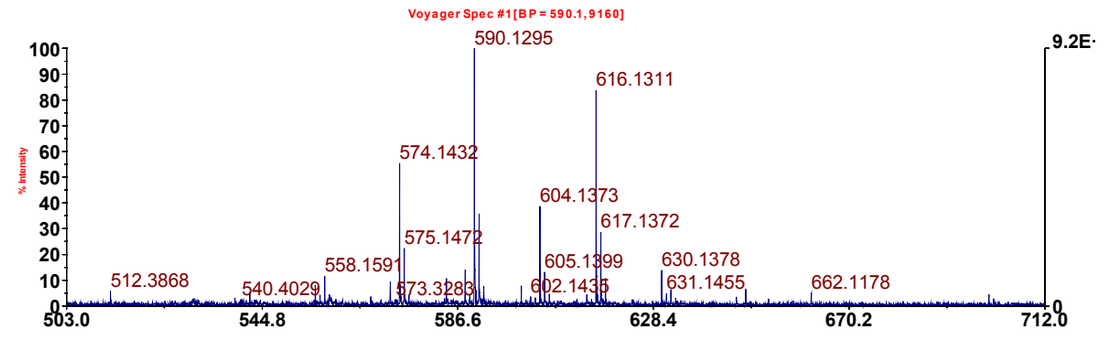

Fig. 26. MALDI-TOF spectrum of the concoction of prepared Fuzi with Galla Chinensis

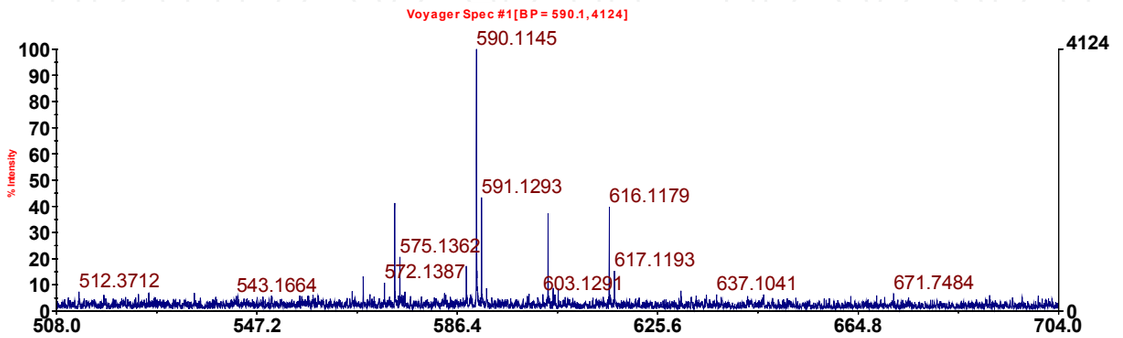

Fig. 27. MALDI-TOF spectrum of the concoction of prepared Fuzi with Chaenomeles sinensis

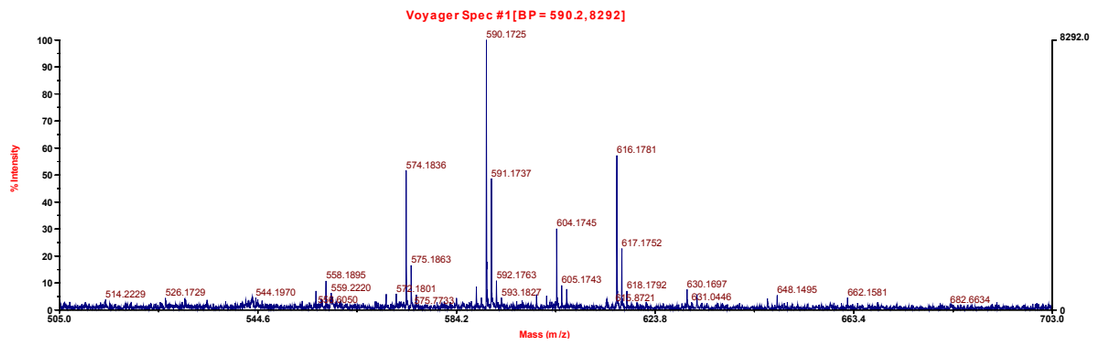

Fig. 28. MALDI-TOF spectrum of the concoction of prepared Fuzi with Herba Potulacae Oleraceae

Aconitum alkaloids	Relative intensities							Average value	RSD
	1	2	3	4	5	6	7		
Benzoylhypaconine (m/z574)	25.19	36.56	38.83	37.19	30.25	28.09	36.77	33.27	5.33
Benzoylmesaconine (m/z590)	100	100	100	100	100	100	100	100	0.00
Benzoylaconine (m/z604)	23.65	39.82	24.04	32.98	39.86	15.10	17.85	27.61	10.07
Hypaconitine (m/z616)	22.16	21.88	51.37	30.43	41.87	50.18	45.13	37.57	12.65
Deoxyaconitine (m/z630)	3.16	5.69	9.14	8.97	10.82	9.43	5.91	7.59	2.71
Mesaconitine (m/z632)	2.63	3.78	3.58	3.24	4.94	8.27	6.22	4.67	1.98
Aconitine (m/z646)	0.00	4.78	2.27	2.98	11.76	6.99	2.74	4.50	3.86
10-OH-mesaconitine (m/z648)	1.43	4.77	1.08	2.48	11.40	7.15	2.93	4.46	3.71
10-OH-aconitine (m/z662)	0.00	3.98	2.67	4.57	4.37	10.39	4.18	4.31	3.12

Table 3. The average value of relative intestines of the detected peaks from prepared Fuzi for seven times

Aconitum alkaloids	Relative intestines							Average value	RSD
	1	2	3	4	5	6	7		
Benzoylhypaconine (m/z574)	65.08	54.99	52.73	56.36	61.57	60.03	74.30	60.72	7.31
Benzoylmesaconine (m/z590)	100	100	100	100	100	100	100	100	0.00
Benzoylaconine (m/z604)	28.22	29.91	25.67	21.63	25.71	28.18	23.78	26.16	2.86
Hypaconitine (m/z616)	38.76	31.93	22.43	22.25	30.74	38.88	22.85	29.69	7.39
Deoxyaconitine (m/z630)	4.12	5.00	3.00	11.71	4.14	3.98	6.82	5.54	2.97
Mesaconitine (m/z632)	2.44	3.20	4.06	6.51	2.26	2.80	6.68	3.99	1.87
Aconitine (m/z646)	0.00	3.45	2.87	4.57	2.66	0.00	6.21	2.82	2.27
10-OH-mesaconitine (m/z648)	0.00	3.65	4.52	7.91	2.11	1.71	7.20	3.87	2.90
10-OH-aconitine (m/z662)	0.00	0.00	5.87	3.41	0.00	2.85	7.43	2.79	3.02

Table 4. The average value of relative intetie of the detected peaks from the detection of Fuzi for seven times

Aconitum alkaloids	Relative intestines							Average value	RSD
	1	2	3	4	5	6	7		
Benzoylhypaconine (m/z574)	57.73	40.16	40.63	51.59	55.97	53.19	52.16	50.20	7.04
Benzoylmesaconine (m/z590)	100	100	100	100	100	100	100	100	0
Benzoylaconine (m/z604)	23.91	24.03	21.44	38.33	28.22	28.27	36.77	28.71	6.53
Hypaconitine (m/z616)	31.67	5.56	11.75	28.82	9.85	17.67	23.82	18.45	9.97
Deoxyaconitine (m/z630)	9.91	0	0	8.33	0	0	2.62	2.98	4.33
Mesaconitine (m/z632)	8.08	0	0	3.7	0	0	6.08	2.55	3.42
Aconitine (m/z646)	5.51	1.91	2.13	2.21	0	0	4.13	2.27	2.02
10-OH-mesaconitine (m/z648)	6.71	0	1.63	2.21	0	0	5.65	2.31	2.80
10-OH-aconitine (m/z662)	4.4	0	2.2	2.5	0	0	10.13	2.75	3.66

Table 5. The average value of relative intestines of the detected peaks from the concoction of Fuzi with Radix Glycyrrhiza for seven times

Aconitum alkaloids	Relative intestines						Average value	RSD
	1	2	3	4	5	6		
Benzoylhypaconine (m/z574)	32.81	44.01	24.66	32.52	30.84	38.10	33.82	6.60
Benzoylmesaconine (m/z590)	100	100	100	100	100	100	100	0.00
Benzoylaconine (m/z604)	26.40	23.12	25.97	21.72	26.86	26.06	25.02	2.09
Hypaconitine (m/z616)	15.43	11.11	12.24	23.16	12.01	13.42	14.56	4.47
Deoxyaconitine (m/z630)	2.59	1.74	0.00	3.67	0.00	2.40	1.73	1.48
Mesaconitine (m/z632)	0.00	4.72	2.68	3.75	0.00	2.90	2.34	1.95
Aconitine (m/z646)	0.00	3.97	0.00	2.95	0.00	0.00	1.15	1.82
10-OH-mesaconitine (m/z648)	2.33	9.11	0.00	3.49	0.00	2.71	2.94	3.35
10-OH-aconitine (m/z662)	0.00	5.40	0.00	2.66	0.00	0.00	1.34	2.25

Table 6. The average value of relative intestines of the detected peaks from the concocction of Fuzi with Lonicera nitida for six times

Aconitum alkaloids	Relative intestines			Average value	RSD
	1	2	3		
Benzoylhypaconine (m/z574)	37.02	17.91	21.42	25.45	10.17
Benzoylmesaconine (m/z590)	100.00	100.00	100.00	100.00	0.00
Benzoylaconine (m/z604)	22.79	21.14	15.60	19.84	3.77
Hypaconitine (m/z616)	19.69	8.84	15.17	14.57	5.45
Deoxyaconitine (m/z630)	0.00	0.00	0.00	0.00	0.00
Mesaconitine (m/z632)	2.98	5.20	3.90	4.03	1.12
Aconitine (m/z646)	0.00	0.00	0.00	0.00	0.00
10-OH-mesaconitine (m/z648)	3.61	1.76	0.00	1.79	1.81
10-OH-aconitine (m/z662)	0.00	0.00	1.76	0.59	1.02

Table 7. The average value of relative intestines of the detected peaks from the concocction of Fuzi with Rhizoma Chuanxiong for three times

Aconitum alkaloids	Relative intestines						Average value	RSD
	1	2	3	4	5	6		
Benzoylhypaconine (m/z574)	35.39	38.94	21.33	40.68	40.24	28.24	34.14	7.79
Benzoylmesaconine (m/z590)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00
Benzoylaconine (m/z604)	22.79	18.42	21.20	22.46	21.01	24.09	21.66	1.95
Hypaconitine (m/z616)	54.98	56.09	38.57	80.39	51.51	53.42	55.83	13.61
Deoxyaconitine (m/z630)	9.86	5.56	8.57	7.49	13.16	12.54	9.53	2.94
Mesaconitine (m/z632)	9.92	8.74	11.58	10.90	6.60	9.80	9.59	1.76
Aconitine (m/z646)	3.86	3.56	2.42	4.54	3.85	4.56	3.80	0.79
10-OH-mesaconitine (m/z648)	10.46	6.37	5.08	6.64	6.01	7.20	6.96	1.85
10-OH-aconitine (m/z662)	3.71	4.33	0.00	3.62	4.71	3.06	3.24	1.69

Table 8. The average value of relative intestines of the detected peaks from the concocction of Fuzi with Crateagus pinnatifida for six times

Aconitum alkaloids	Relative intestines							Average value	RSD
	1	2	3	4	5	6	7		
Benzoylhypaconine (m/z574)	54.45	77.33	50.76	64.26	55.34	51.57	75.68	61.34	11.26
Benzoylmesaconine (m/z590)	89.18	98.06	84.35	84.77	100	100	81.59	91.14	8.03
Benzoylaconine (m/z604)	41.47	36.27	25.70	59.23	38.73	44.37	39.13	40.70	10.07
Hypaconitine (m/z616)	100	100	100	100	83.70	71.44	100	93.59	11.50
Deoxyaconitine (m/z630)	11.65	13.77	12.18	27.91	13.89	7.47	11.48	14.05	6.47
Mesaconitine (m/z632)	13.28	8.18	8.29	5.87	6.38	3.81	8.90	7.82	2.98
Aconitine (m/z646)	3.11	4.25	9.76	10.47	3.46	0.00	8.90	5.71	4.00
10-OH-mesaconitine (m/z648)	3.96	4.78	10.82	13.53	6.57	6.87	10.06	8.08	3.48
10-OH-aconitine (m/z662)	6.98	7.57	11.76	11.32	4.93	4.54	8.15	7.89	2.82

Table 9. The average value of relative intestines of the detected peaks from the concocction of Fuzi with Galla Chinensis for seven times

Aconitum alkaloids	Relative intestines							Average value	RSD
	1	2	3	4	5	6	7		
Benzoylhypaconine (m/z574)	41.22	33.60	25.68	29.67	48.78	44.39	33.30	36.66	8.34
Benzoylmesaconine (m/z590)	1000	100	100	100	1000	100	100	100	0.00
Benzoylaconine (m/z604)	37.32	23.85	39.65	27.92	27.20	18.33	33.73	29.71	7.60
Hypaconitine (m/z616)	39.79	15.07	24.40	26.92	24.88	15.75	19.98	23.83	8.39
Deoxyaconitine (m/z630)	7.76	2.94	9.67	6.85	4.92	6.86	8.78	6.83	2.29
Mesaconitine (m/z632)	5.29	2.26	5.82	6.61	4.02	3.64	2.73	4.34	1.62
Aconitine (m/z646)	6.28	2.90	7.55	9.68	4.84	3.85	2.00	5.30	2.71
10-OH-mesaconitine (m/z648)	4.97	2.61	6.57	7.25	6.26	5.04	3.95	5.24	1.61
10-OH-aconitine (m/z662)	3.59	2.84	5.97	8.17	5.83	2.78	4.09	4.75	1.99

Table 10. The average value of relative intestines of the detected peaks from the concocction of Fuzi with Chaenomeles sinensis for seven times

Aconitum alkaloids	Relative intestines					Average value	RSD
	1	2	3	4	5		
Benzoylhypaconine (m/z574)	30.49	51.63	20.61	43.12	30.06	35.18	12.19
Benzoylmesaconine (m/z590)	100	100	100	100	100	100	0.00
Benzoylaconine (m/z604)	15.28	30.10	18.81	19.65	18.81	20.53	5.61
Hypaconitine (m/z616)	33.70	57.20	34.36	92.86	46.45	52.91	24.34
Deoxyaconitine (m/z630)	5.85	7.59	9.45	8.27	14.36	9.10	3.21
Mesaconitine (m/z632)	5.56	5.71	4.61	7.46	10.20	6.71	2.21
Aconitine (m/z646)	0.00	4.29	5.57	4.02	4.30	3.64	2.12
10-OH-mesaconitine (m/z648)	3.21	5.45	5.76	4.79	3.32	4.51	1.19
10-OH-aconitine (m/z662)	2.95	4.55	6.10	5.93	4.16	4.74	1.31

Table 11. The average value of relative intestines of the detected peaks from the concoction of Fuzi with Herbal Potulacae Oleraceae for five times

Concoction	Relative Intensities								
	BHA	BMA	BAC	HA	DA	MA	AC	10-OH-MA	10-OH-AC
Fuzi	33.27±5.33	100	27.6±10.07	37.57±12.65	7.59±12.65	4.76±1.98	4.50±3.86	4.46±3.71	4.31±3.12
Fuzi decoction	60.72±7.31	100	26.16±2.86	29.69±2.86	5.54±2.97	3.99±1.87	2.82±2.90	3.87±2.90	2.79±3.02
Concoction of A+RG	50.20±7.04	100	28.71±6.53	18.45±9.97	2.98±4.33	2.55±3.42	2.27±2.02	2.31±2.80	2.75±3.66
Concoction of A+LN	33.82±6.60	100	25.02±2.09	14.56±4.47	1.73±1.48	2.34±1.95	1.15±1.82	2.94±3.35	1.34±2.25
Concoction of A+RC	25.4±10.17	100	19.84±3.77	14.57±5.45	0	4.03±1.12	0	1.79±1.81	0.59±1.02
Concoction of A+CS	36.66±8.34	100	29.71±7.60	23.83±8.39	6.83±2.29	4.34±1.62	5.30±2.71	5.24±1.61	4.75±1.99
Concoction of A+GC	61.3±11.26	91.1±8.03	40.7±10.07	93.56±11.50	14.05±6.47	7.82±2.98	5.71±4.00	8.08±3.48	7.89±2.82
Concoction of A+CP	34.14±7.79	100	21.66±1.95	55.83±13.61	9.53±2.94	9.59±1.76	3.80±0.79	3.69±1.85	3.24±1.69
Concoction of A+HPO	35.1±12.19	100	20.53±5.61	52.91±24.34	9.10±3.21	6.71±2.21	3.64±2.12	4.51±1.19	4.74±1.31

Table 12. The average value of relative intestines of the detected peaks from the concocction and decoction of Fuzi

Taken the ethanol extract of Fuzi as a standard, we will find that the DDAs have been reduced after decocting or concocting with Radix Glycyrrhizae, Lonicera nitida, Rhizoma Chuanxiong or Chaenomeles sinensis, respectively. Coincidentally, both Radix Glycyrrhizae and Lonicera nitida is the most popular medicine for detoxification. However, when Fuzi has been concocted with Crateagus pinnatifida, Galla Chinensis or Herba Potulacae Oleraceae, the relative amounts of all DDAs, especially of hypaconitine, have been increased significantly despite that the pH value of Lonicera nitida is smaller than that of Galla Chinensis and Herba Potulacae Oleraceae. The results have indicated that the detoxification mechanism of traditional Chinese medicine such as Fuzi is very complex and can not be explained by a simple reason such as pH value. In addition, our studies have suggested that although Crateagus pinnatifida, Galla Chinensis and Herba Potulacae Oleraceae is beyond the list that should not be combined with aconite according to traditional Chinese theory or experience, but they do increase the toxicity of Fuzi.

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During the recent years, traditional Chinese medicine (TCM) has attracted the attention of researchers all over the world. It is looked upon not only as a bright pearl, but also a treasure house of ancient Chinese culture. Nowadays, TCM has become a subject area with high potential and the possibility for original innovation. This book titled Recent Advances in Theories and Practice of Chinese Medicine provides an authoritative and cutting-edge insight into TCM research, including its basic theories, diagnostic approach, current clinical applications, latest advances, and more. It discusses many often neglected important issues, such as the theory of TCM property, and how to carry out TCM research in the direction of TCM property theory using modern scientific technology. The authors of this book comprise an international group of recognized researchers who possess abundant clinical knowledge and research background due to their years of practicing TCM. Hopefully, this book will help our readers gain a deeper understanding of the unique characteristics of Chinese medicine.

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