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Determination of aconitine-type alkaloids as markers in *fuzi* (*Aconitum carmichaeli*) by LC/(+)ESI/MS^3

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ABSTRACT

LC/(+)ESI/MS^3 was used to determine aconitine, mesaconitine, and hypaconitine as target markers in crude methanol extracts of (i) the raw lateral roots of *Aconitum carmichaeli*, (ii) roots treated by three different refining processes, and (iii) eight generally available traditional Chinese medicine (TCM) preparations containing *fuzi* (treated lateral roots of *A. carmichaeli*). The optimal ionization behavior resulted when using electrospray ionization (ESI) in positive-ion mode with 0.005% TFA as an additive in the mobile phase. The consecutive reaction monitoring (CRM) mode provided additional improvements in selectivity, which was exploited to minimize the noise and interference problems.

Employing this approach, aconitine and mesaconitine were found to decompose readily during the refining processes, but hypaconitine remains present at the same content, presumably because of its characteristic chemical structure. Thus, treated and untreated *fuzi* samples can be distinguished by monitoring the ratio of aconitine and mesaconitine to hypaconitine. The limits of detection (LODs) for these three markers were 0.05, 0.08, and 0.03 ng/ml. The linearity range for the three marker compounds was 0.1–1000 ng/ml. The analysis time was 12 min per sample.

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1. Introduction

Aconite ("Carmichael's Monkshood," *Aconitum carmichaeli*) is widely distributed over the southwest provinces of China. Its lateral roots and those of other near-relative species of the same genus share the common name *fuzi*, which is one of the most useful herbal medicines. The raw lateral roots of aconite cannot be used directly because of their high content of aconitine-type alkaloids; hence, a pretreatment process that reduces their toxicity is necessary. Each of the three major kinds of pretreated *fuzi* on the market – *bai-fu-pian*, *hei-shun-pian*, and *yen-fu-zi* – has its own characteristic refining processes [1].

The pharmacological effects of *fuzi* are a regeneration of vigor with dispelled damp; it is used in many Chinese medicinal preparations for the treatment of colds, polyarthralgia, diarrhea, heart failure, beriberi, and edema. The main ingredients in the lateral roots of *A. carmichaeli* are a series of alkaloids sharing a common C_{19}-norditerpenoid skeleton; the major toxic ingredients – aconitine, mesaconitine, and hypaconitine (Fig. 1) – are also active agents of this herbal medicine [2], even though they can result in fatal ventricular fibrillation. The mechanism of its toxicity has been reported to be the activation of the sodium channel function in cells [3]. The reported LD_{50} value of aconitine for mice per oral injection is 1.8 mg/kg body weight [4]. Based on this high toxicity and pharmacological activity, refining processes are necessary to reduce the toxicity in most Chinese medicinal preparations.

Several methods have been developed for the analysis of aconitine-type alkaloids in the lateral roots of aconite, using gas chromatography (GC) [5], high-performance liquid chromatography (HPLC) [6], or capillary electrophoresis (CE) [7]. Nevertheless, these methods have limited applicability because of their low sensitivities and selectivities. In recent years, mass spectrometry (MS) has been employed for the analysis of the alkaloids in the lateral roots of aconite because of its high selectivity. Four toxic aconitine-type alkaloids – aconitine, mesaconitine, hypaconitine, and jesaconitine – were determined in blood and urine by LC/MS operated in the selected ion monitoring (SIM) mode after solid phase extraction (SPE) [8]. Subsequently, a series of these alkaloids was analyzed using matrix-assisted laser desorption ionization (MALDI)/time-of-flight (TOF) mass spectrometry on the basis of their typical fragmentations [9]. The complete fragmentation pathways of such aconitine-type alkaloids were later determined using electrospray ionization tandem mass spectrometry (ESI/MS^n) which was also employed for their qualitative analysis

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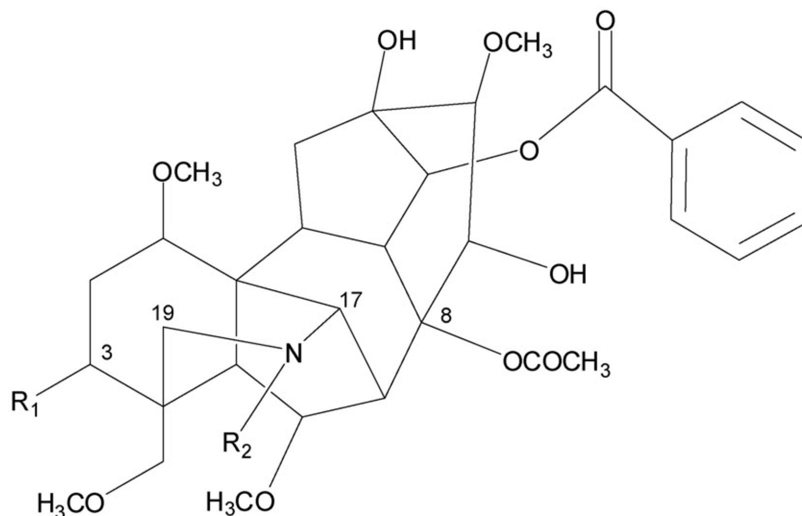
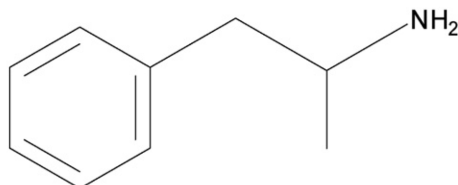


Aconitine: R_1=OH, R_2=C_2H_5 (MW=645) ;

Mesaconitine: R_1=OH, R_2=CH_3 (MW=631) ;

Hypaconitine: R_1=H, R_2=CH_3 (MW=615)



Amphetamine (used as IS)

Fig. 1. Chemical structures of aconitine, mesaconitine, hypaconitine, and amphetamine (IS).

[10]. These toxic alkaloids in aconite pills were determined using LC/MS/MS operated in selected reaction monitoring (SRM) mode [2], although the LOD was fairly high (0.3 μg/ml).

Even though various reports outlined above have appeared in recent years for the analysis of aconitine-type alkaloids, there remains a need to devise a simple quality control method for analyzing *fuzi*-containing Chinese herbal preparations that allows the levels of the three major toxic alkaloids to be estimated simultaneously without sample pretreatment. Thus, the aim of this study was the development of a simple and specific LC/MS^3 method, exhibiting high precision and selectivity, for the identification and quantification of low levels of aconitine-type alkaloids. Using this technique in conjunction with the improved selectivity of the consecutive reaction monitoring (CRM) mode of MS, we analyzed four *fuzi* samples with and without treatment and eight Chinese medicinal preparations containing *fuzi*.

2. Experimental

2.1. Materials

Aconitine was purchased from Sigma (St. Louis, MO, USA). Mesaconitine and hypaconitine were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), China. The purities of the three standards were above 99.8%. Amphetamine was used as the internal standard (IS). Analytical-grade trifluoroacetic acid (TFA), acetic acid (AA), formic acid (FA), methanol, and acetonitrile were purchased from Merck (Gibbstown, NJ, USA). The treated *fuzi* samples *hei-shun-pian* and *bai-fu-pain* were purchased from a local market in Taiwan Taichung city. Raw *fuzi*, the treated *fuzi* sample *yen-fu-zi*, and eight Chinese medicinal preparations containing *fuzi* (*yow-guei-wan*, *shy-nin-tang*, *fu-tzyy-li-chong-tang*, *guey-fuh-dih-huang-wan*, *jen-wu-tang*, *jih-sheng-shenn-chin-wan*, *sheau-shium-ming-tang*, and *guey-jy-shaur-yuh-jy-muu-tang*) were provided by the Chuang-Song-Zong Pharmaceutical Company.

2.2. Preparation of samples

Each ground crude *fuzi* sample powder (0.2 g) was sonicated in methanol (30 ml) at 25 °C for 30 min. The mixture was then centrifuged at 3000 rpm for 5 min. The supernatant was collected, filtered through a 0.2 μm PVDF syringe filter, and concentrated to a final volume of 10 ml. An aliquot (1 ml) of the sample solution was mixed with an equal volume of the IS solution (2 μg/ml) prior to analysis.

Each of the dry powder Chinese medicinal preparations (1.0 g) was treated as described above, except that they were concen-

trated to a volume of 1 ml. The extracts were kept in a refrigerator at −4 °C.

2.3. Liquid chromatographic conditions

The LC analyses were conducted using a Surveyor liquid chromatograph (Thermo Finnigan Corporation, San Jose, CA, USA) equipped with two solvent pumps, a VWD 1100 UV detector (Agilent Technologies, Waldbronn, Germany), a Rheodyne injector (5 μl loop), and a Gemini C18 column (3 μm, 150 mm × 2 mm) (Phenomenex, Torrance, CA, USA), with a Phenomenex Luna Security Guard Cartridge C18 (5 μm, 4 mm × 2.0 mm i.d.). Two mobile phases were employed at a flow rate of 0.1 ml/min. Phase A consisted of water and 0.005% TFA (v/v); phase B comprised ACN and 0.005% TFA (v/v). Both mobile phases were passed through a filter (0.45 μm) and degassed prior to use.

Column separation was performed at room temperature by means of a gradient elution program, which started with 20% of phase B and increased linearly to 25% of phase B over 3 min and then to 28% B linearly over 7 min. Subsequently, the column was washed with phase B for 2 min and then equilibrated with the initial 20% phase B for 10 min. The total analysis time including the washing and equilibrium steps, was 22 min.

2.4. Optimal conditions for mass spectrometry

Mass spectra were recorded using a quadrupole ion trap instrument equipped with an ESI source (LCQ™, Thermo Finnigan Corporation, San Jose, CA, USA). The data acquisition software was X-calibur (v. 1.2). Helium was the damping and collision gas. The LC/ESI/MS^n analysis was run in the positive-ion mode and operated under the following optimal conditions: sheath gas flow, 1.35 l/min; ion spray voltage, 4.5 kV; capillary temperature, 175 °C; capillary voltage, 44 V; tube lens offset, 0 V; first multiplier offset, −5.00 V; second multiplier offset, −6.50 V; inter-multipole lens voltage, −18.00 V.

The analysis process was separated into two segments: segment I (from 0 to 7.5 min) was set for the detection of the IS; segment II (from 7.5 to 12 min) was set for the measurement of the three alkaloids. The CRM mode was used to select the protonated molecular ions ($[M+H]^+$) of aconitine, mesaconitine, hypaconitine, and the IS as parent ions and to monitor the fragmentations to their corresponding daughter ions (m/z 646 → 586 → 526, m/z 632 → 572 → 512, m/z 616 → 556 → 496, and m/z 136 → 119 → 91, respectively).

2.5. Calibration curves and recovery tests

Stock solutions of the three standards (aconitine, mesaconitine, and hypaconitine) were dissolved individually in appropriate volumes of methanol to provide 1000 μg/ml solutions, which were kept in a refrigerator at −4 °C. The working solution of the three standards was prepared by diluting with methanol to obtain a concentration of each component of 10 μg/ml. Mixed standard solutions of nine different concentrations (0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 ng/ml), each containing the IS (1000 ng/ml) and the extract of the medicinal preparation *guey-fuh-dih-huang-wan* as a matrix [prepared from TCM sample powder (0.05 g) extracted with methanol (1 ml)], were used to construct calibration curves in which the peak ratio was plotted against the analyte concentration over the range from 0.1 to 1000 ng/g.

The recoveries of the full experimental procedure were determined by adding three different concentrations of the analytes (5, 20, and 100 ng/ml; 1 ml) to sample powders of medicinal preparation *guey-fuh-dih-huang-wan* (1.0 g), re-drying them, and then analyzing each sample using the procedure described in Section 2.2. The extract obtained from each concentration was analyzed through three consecutive injections under the optimal conditions and then another three consecutive injections performed after 48 h. The recoveries were calculated by comparing the analyte-to-IS peak area ratios; they were regressed to concentrations by using the calibration curve, taking into consideration the original concentration of the spiked analytes. A blank test was used to calibrate the content of the original analytes contained in the medicinal preparation sample.

3. Results and discussion

3.1. Performance of HPLC-UV method

Although HPLC is a common method used to detect the presence of aconitine, mesaconitine, and hypaconitine in single-herb samples [6], our preliminary investigations indicated that it could not be used for accurate qualitative and quantitative analyses of Chinese medicinal preparations because of the presence of the complicated matrix. Chinese medicinal preparations usually comprise several herbs; therefore, the sensitivity and selectivity of the HPLC method are often insufficient to detect those markers. Fig. 2 presents the HPLC chromatogram of *guey-jy-shaur-yuh-jy-muu-tang*, that contains nine herbs, including processed *fuzi*, *gui-zhi*, dried *jiang*, *bai-shao*, *fang-feng*, and *zhi-mu*. It is clear that HPLC methods for quantitative analysis suffer from too much interference.

3.2. ESI/MS^n spectra of the markers

We observed the MS fragmentation processes in (+)ESI and optimized the MS parameters of aconitine, mesaconitine, and hypaconitine using flow injection analysis. The protonated molecular ions $[M+H]^+$ of aconitine, mesaconitine, and hypaconitine appeared as base ions at m/z 646, 632, and 616 Da, respectively.

From MS^2 experiments in which the collision energy was set at 33%, we observe in Fig. 3a–c that the corresponding product ions of aconitine, mesaconitine, and hypaconitine appeared at m/z 586, 572, and 556, respectively. For each of the alkaloids, the most intense product ion was generated characteristically through the loss of one molecule of acetic acid ($[M+H-60]^+$).

To further identify the fragmentation pathways, we obtained the (+)ESI/MS^3 spectra of the three aconitine-type alkaloids by selecting the base peaks from the MS^2 spectra (Fig. 3a′–c′). After optimizing the collision energy, we obtained two intense prod-

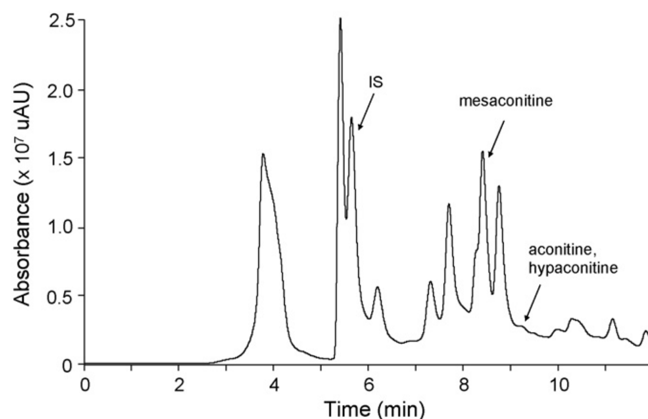


Fig. 2. HPLC chromatogram of the extract of *guey-jy-shaur-yuh-jy-muu-tang* (sample 12 in Table 4), detected at 254 nm.

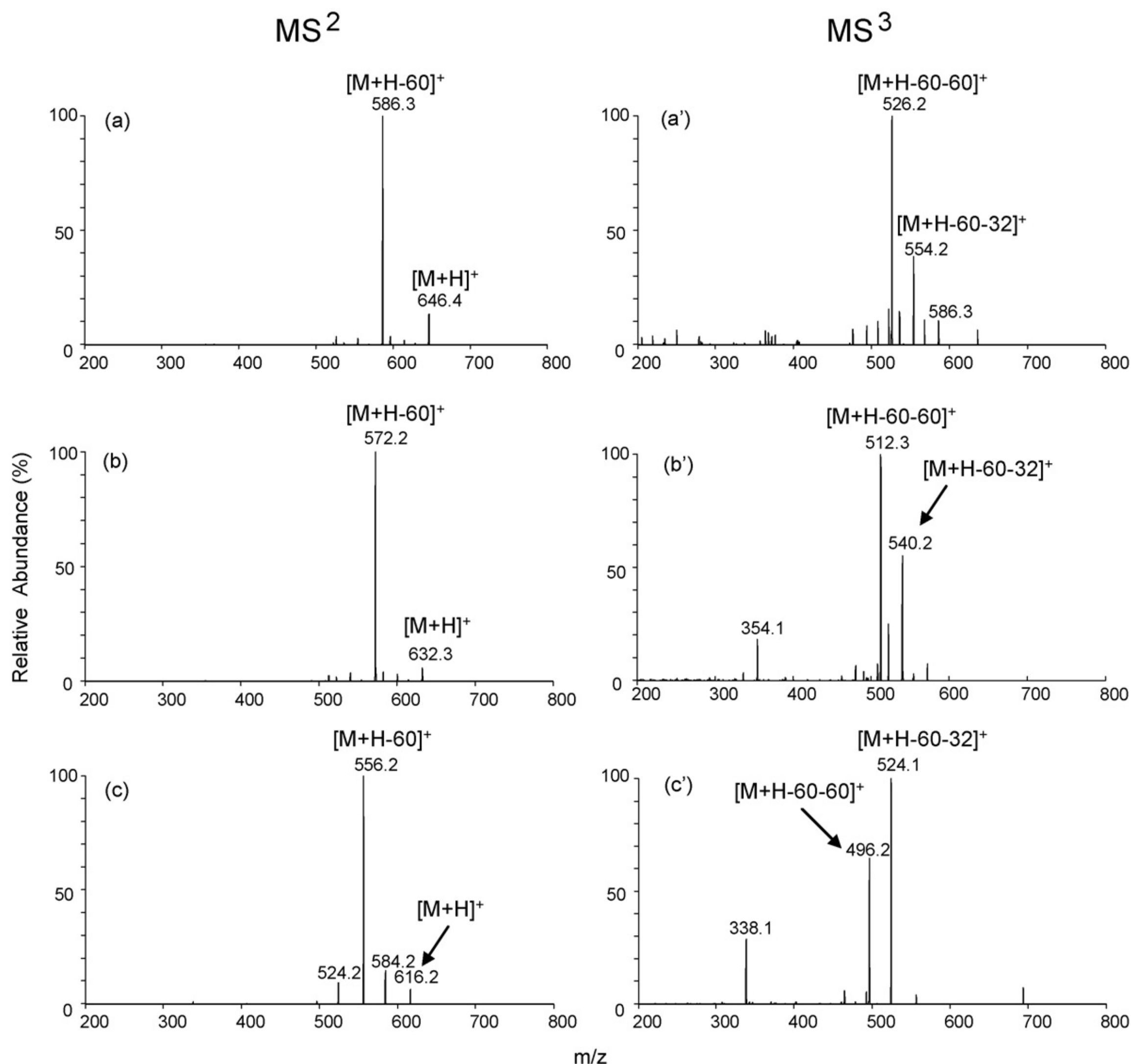


Fig. 3. MS^2 and MS^3 spectra: (a and a′) aconitine; (b and b′) mesaconitine; (c and c′) hypaconitine.

uct ions, at [M+H−60−60]$^+$ and [M+H−60−32]$^+$, respectively, for each alkaloid. We assume that these ions resulted from losses of CO plus CH_3OH and of a CH_3OH group, respectively from the precursor [M+H−60]$^+$ ion [10].

3.3. Effect of modifier on LC/MS

In this experiment, we chose an acid for use as the modifier because the standards were all alkaloids. Addition of an acid at optimal concentration improved the efficiency of the (+)ESI/MS analysis, due to protonation of various N atoms. In the LC system, the presence of an acid as the modifier prevented tailing, thereby providing sharp peaks. In addition, the lower pH reduced the strength of the interactions between the analytes and stationary phase of the C18 columns, thereby shortening the analysis time.

The peak areas obtained from the sample incorporating 0.1% AA were higher than those featuring either 0.1% FA or 0.001% TFA. The highest ionization efficiency occurred when the concentration of TFA was 0.005%. Therefore this was selected as the modifier for the mobile phase in subsequent experiments.

3.4. Selection of the IS

Amphetamine was selected as the IS (Fig. 1). Its molecular ion [M+H]$^+$appears at *m*/*z* 136 after direct infusion; it formed a stable base peak at *m*/*z* 119 ([M+H−17]$^+$) in the (+)ESI/MS/MS spectrum upon extrusion of NH_3 at the collision energy of 24.5%. We observed an ion at *m*/*z* 91 ([$C_6H_5CH_2$]$^+$) in the (+)ESI/MS^3 spectrum due to the fragmentation of the precursor ion at *m*/*z* 119 when the collision energy was set at 24%.

3.5. Performance of LC/MS^2 and LC/MS^3

After optimizing the MS and LC conditions, we analyzed solutions of the mixed standards and the extracts of real samples. The ions in the SRM mode of the mass chromatogram for aconitine, mesaconitine, hypaconitine, and the IS were set at *m*/*z* 646 → 586, *m*/*z* 632 → 572, *m*/*z* 616 → 556, and *m*/*z* 136 → 119, respectively. The LC/(+)ESI/MS/MS mass chromatograms of the mixed standards and the single-herb *fuzi* sample both exhibited well-shaped peaks for each extract ion; the corresponding retention times were 9.1, 8.7, 9.2, and 5.8 min, respectively.

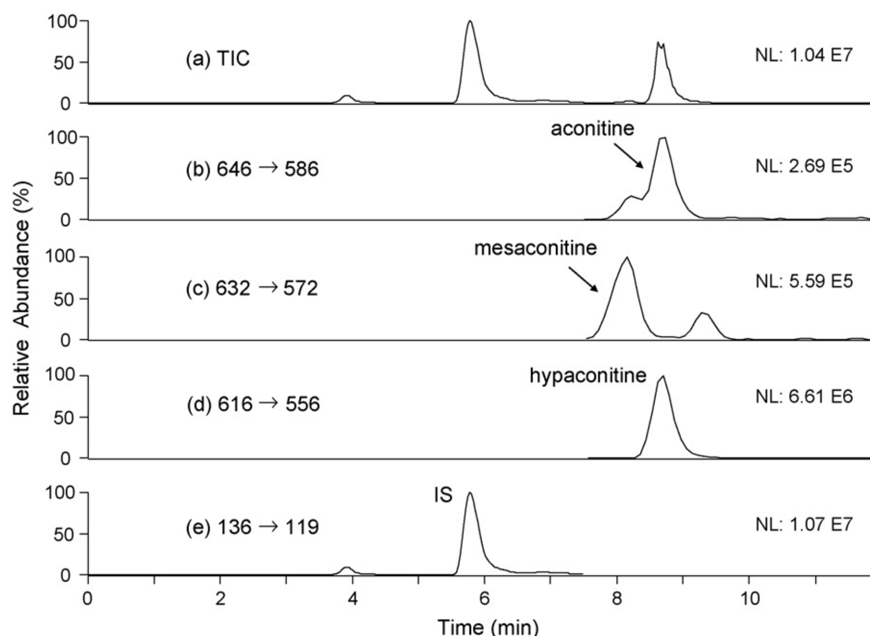


Fig. 4. Mass chromatograms of (a) the total ion current (TIC), (b) aconitine, (c) mesaconitine, (d) hypaconitine, and (e) the IS; obtained using LC/(+)ESI/MS/MS (SRM) from an extract of *guey-jy-shaur-yuh-jy-muu-tang*.

In contrast, when we analyzed the extract from *guey-jy-shaur-yuh-jy-muu-tang*, we observed interfering peaks, most notably in the channels of the extract ions of aconitine and mesaconitine (Fig. 4b and c), making quantitative analysis very difficult. Therefore, we employed the CRM mode to solve this problem, setting the ions in the mass chromatogram for aconitine, mesaconitine, hypaconitine, and the IS at m/z 646 → 586 → 526, m/z 632 → 572 → 512, m/z 616 → 556 → 496, and m/z 136 → 119 → 91, respectively. Fig. 5 displays the mass chromatograms of the extract ions obtained using LC/(+)ESI/MS^3. The interference found in the SRM mode vanished when using the CRM mode for LC/MS^3 analysis (Figs. 4b and 5b), but the cost of increasing the selectivity was a decrease in sensitivity. Although the intensity of the signals was reduced, the detection limits remained quite low, allowing to determine very small amounts of analytes.

3.6. Detection ranges and limits of detection (LODs)

We constructed calibration curves by spiking each alkaloid into the extract of the above mentioned medicinal preparation. Triplicate injections were performed for each analyte at various concentrations and then the CRM mode was employed for quantitative analysis. We varied the level of each analyte between 0.1 and 1000 ng/ml to fit the large distribution of their quantities in

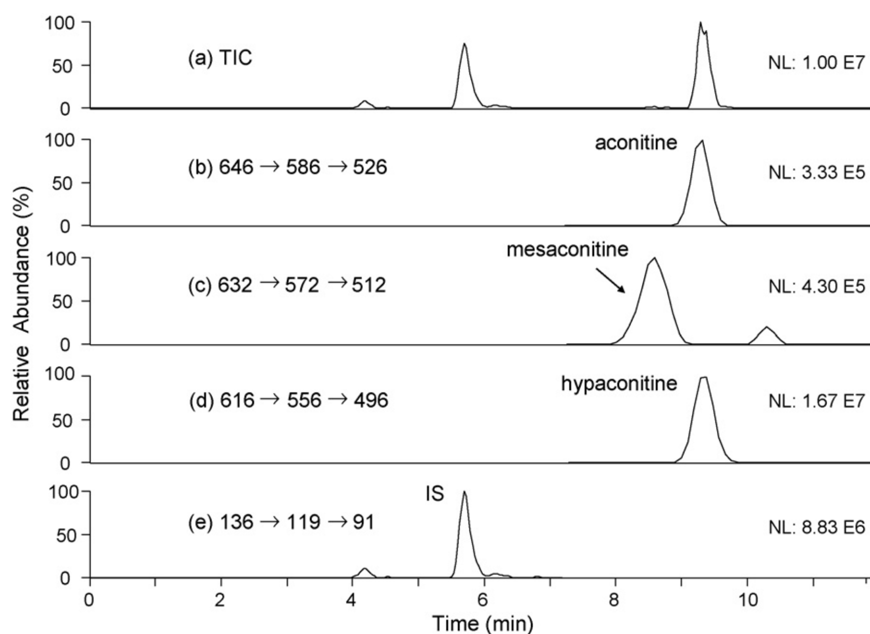


Fig. 5. Mass chromatograms of (a) the TIC, (b) aconitine, (c) mesaconitine, (d) hypaconitine, and (e) the IS; obtained using LC/(+)ESI/MS^3 (CRM) from an extract of *guey-jy-shaur-yuh-jy-muu-tang*.

Table 1
Linear ranges, regression equations, correlation coefficients, and detection limits for aconitine, mesaconitine, and hypaconitine, obtained using LC/(+)ESI/MS^3

Compound	Linear range (ng/ml)[a]	Regression equation	r^2	LOD (ng/ml)
Aconitine	0.1–10	$y = 0.2250x + 0.1445$	0.9986	0.05
	10–1000	$y = 0.0690x + 3.7539$	0.9969	
Mesaconitine	0.1–10	$y = 0.1735x + 0.1021$	0.9987	0.08
	10–1000	$y = 0.0785x + 3.2253$	0.9979	
Hypaconitine	0.1–10	$y = 0.1785x + 0.1109$	0.9982	0.03
	10–1000	$y = 0.0580x + 2.9685$	0.9975	

[a] Prepared in the extract of *quey-fun-dih-huang-wan*.

real samples. Table 1 reveals that the slopes of the three calibration curves, with the linear range between 10 and 1000 ng/ml, were very similar (between 0.06 and 0.08) with squared correlation coefficients (r^2) all higher than 0.996, suggesting sufficient accuracy for quantitative analysis. Nevertheless, these curves were not absolutely straight lines, with ion depression appearing at concentrations greater than 10 ng/ml. For more accurate quantitative analysis, we separated the calibration curves into two parts, ranging from 0.1 to 10 ng/ml and from 10 to 1000 ng/ml, respectively, for measurements of low and high quantities, respectively. When the concentrations of a sample were close to the meeting point, i.e.10 ng/ml, the best way was to dilute the sample to fit the calibration curve of 0.1–10 ng/ml.

The linear-range experiments provided the necessary information to estimate the LODs, which we calculated from the slopes of the calibration curves in the low concentration ranges and the three-fold standard deviation of the noise. Thus, the LODs of aconitine, mesaconitine, and hypaconitine were 0.05, 0.08, and 0.03 ng/ml, respectively, with values of r^2 exceeding 0.996 for both ranges of each compound (Table 1).

3.7. Precisions and recoveries

We conducted precision tests after spiking the analytes at three different concentrations (5, 20, and 100 ng/ml) into the extract of the Chinese medicinal preparation *guey-fuh-dih-huang-wan* and then performing triplicate injections for each solution. A blank control was used for calibration of the quantity of the analytes to access the ideal spiked concentration. The precisions of the analytes, expressed in terms of the relative standard deviations (R.S.D.s), ranged from 1.53 to 7.73%; Table 2 lists the detailed values.

We performed the recovery experiments as described in Section 2. The recoveries (at the 5 ng/ml level) of aconitine, mesaconitine, and hypaconitine were 69.2, 70.5, and 64.1% (intraday), respectively, and 74.0, 74.1, and 69.7% (interday), respectively. The recoveries became greater at higher spiked concentrations, suggesting that the sample powder adsorbed some of the analytes, i.e. because the quantity of the sample powder remained fixed. The interday and intraday recoveries were similar, each with R.S.D.s below 9.25%; see Table 3 for details.

Table 2
Precision tests (R.S.D., %) for the analyses of aconitine, mesaconitine, and hypaconitine at three different concentrations

Analyte	R.S.D. (%)[a]		
	5 ng/ml	20 ng/ml	100 ng/ml
Aconitine	2.7	1.5	3.1
Mesaconitine	3.3	1.8	4.6
Hypaconitine	6.4	7.7	2.1

[a] Three replicate analyses in a single day.

3.8. Application to real samples

We tested the effectiveness of this LC/(+)ESI/MS^3 method operated in the CRM mode for determining the amounts of aconitine, mesaconitine, and hypaconitine in an untreated raw *fuzi* sample and three treated *fuzi* samples. The CRM chromatograms of the extracts of these single herbs displayed well-shaped peaks for each extract ion, from which we could clearly identify and quantify the three analytes in each sample (Tables 1–4). In the treated *fuzi* samples, we detected hypaconitine in very high amounts, whereas the contents of aconitine and mesaconitine were reduced dramatically in comparison with those in the raw *fuzi* sample. The small differences in the levels of hypaconitine (from 92.6 to 98.9 μg/g) in these samples suggested only slight differences in their geographical origins and small variations among plant batches. Moreover, a comparison of the various alkaloids' contents in the treated and untreated *fuzi* samples revealed that only hypaconitine did not decompose during the repetitive heating process used to prepare the treated samples. The amounts of aconitine and mesaconitine in the samples decreased by up to 200-fold relative to those in the raw *fuzi*, especially in the processed *yen-fuzi*, suggesting that the presence of the C-3 OH group in the aconitine-type alkaloids may cause these compounds to degenerate readily during the refining process. The amounts of aconitine and mesaconitine decreased from 59.5 to 0.3 μg/g and from 87.4 to 0.4 μg/g, respectively, in the raw *fuzi* and the treated *yen-fuzi*.

Entries 5–12 in Table 4 provide the amounts of the three marker alkaloids in eight Chinese medicinal preparations containing *fuzi* as one of its ingredients. These eight preparations each contain

Table 3
Average recovery tests and R.S.D.s (%) for the analyses of aconitine, mesaconitine, and hypaconitine, spiked at three different concentrations

Analyte	Recovery (R.S.D., %)					
	Day 1[a]			Day 3[b]		
	5 ng/ml	20 ng/ml	100 ng/ml	5 ng/ml	20 ng/ml	100 ng/ml
Aconitine	69.2 (4.3)	83.2 (6.0)	86.4 (3.8)	74.0 (6.3)	84.3 (4.5)	89.3 (5.7)
Mesaconitine	70.5 (5.1)	78.3 (8.4)	80.7 (2.5)	74.1 (3.2)	83.8 (7.3)	89.1 (2.9)
Hypaconitine	64.1 (4.3)	80.4 (9.3)	83.1 (2.8)	69.7 (7.1)	84.3 (7.1)	86.3 (8.1)

[a] Three replicate analyses in a single day.
[b] Three replicate analyses after 48 h.

Table 4
Contents (ng/g) of aconitine, mesaconitine, and hypaconitine in *fuzi* samples and eight Chinese medicinal preparations containing *fuzi*

Entry	Sample	Aconitine (ng/g)[a]	R.S.D. (%)	Mesaconitine (ng/g)[a]	R.S.D. (%)	Hypaconitine (ng/g)[a]	R.S.D. (%)
1	Raw *fuzi*	59,467	9.3	87,423	7.3	95,721	8.8
2	*Bai-fu-pian*[b]	12,233	1.5	17,869	4.5	97,694	6.0
3	*Hei-shun-pian*[b]	18,484	7.8	50,492	8.5	98,949	7.2
4	*Yen-fuzi*[b]	298	12.2	382	13.8	92,563	9.7
5	*Yow-guei-wan*	ND[c]	–	ND[c]	–	6,620	8.0
6	*Shy-nin-tang*	ND[b]	–	26	9.5	3,295	8.4
7	*Fu-tzyy-li-chong-tang*	85	7.7	2940	7.6	9,647	4.9
8	*Guey-fuh-dih-huang-wan*	ND[c]	–	ND[c]	–	1,968	9.9
9	*Jen-wu-tang*	267	3.2	1153	8.2	6,605	9.4
10	*Jih-sheng-shenn-chin-wan*	476	5.0	934	4.3	7,158	2.3
11	*Sheau-shium-ming-tang*	174	8.4	1004	3.1	2,724	6.0
12	*Guey-jy-shaur-yuh-jy-muu-tang*	2152	4.9	2984	13.8	7,432	9.6

[a] Regressed amount from 1 g of sample powder.
[b] *Bai-fu-pian*, *hei-shun-pian*, and *yen-fuzi* (entries 2–4) are processed *fuzi* samples.
[c] Not detected or lower than the calibration curve range.

Table 5
Regressed percentages of *fuzi* in eight Chinese medicinal preparations, and the ideal percentage predicted from its recipe

Entry	Sample	Hypaconitine (ng/g)	*Fuzi* contained (%)[a]	Ideal percentage (%)[b]
1	*Yow-guei-wan*	6620	7.2	6.1
2	*Shy-nin-tang*	3295	3.6	27.6
3	*Fu-tzyy-li-chong-tang*	9647	10.4	20.0
4	*Guey-fuh-dih-huang-wan*	1968	2.1	3.7
5	*Jen-wu-tang*	6605	7.1	5.9
6	*Jih-sheng-shenn-chin-wan*	7158	7.7	2.9
7	*Sheau-shium-ming-tang*	2724	2.9	3.5
8	*Guey-jy-shaur-yuh-jy-muu-tang*	7432	8.0	6.7

[a] Based on the amount of hypaconitine detected in the *yen-fuzi* sample.
[b] Calculated from each recipe for the preparations provided by the Chuang-Song-Zong Pharmaceutical Company.

3–13 herbs, with 8 herbs on average. The levels of these alkaloids in the eight preparations ranged from undetectable to 2.2 μg/g for aconitine, from undetectable to 3.0 μg/g for mesaconitine, and from 2.0 to 9.6 μg/g for hypaconitine. The R.S.D.s of these eight Chinese medicinal preparations were all less than 13.8%.

Because we obtained all of these eight Chinese medicinal preparations from the same source, and because the processed *fuzi* used in these preparations was solely *yen-fuzi*, we can calculate the percentage of treated *fuzi* in each of the medicinal preparations by comparing the content of hypaconitine in each analysis. In Table 5 we compare our analytical results with the recipes provided by the pharmaceutical company. In most cases, our regressed weight percentages (w/w, %) were close to the values recorded in the recipes, except for preparations 2 and 3, in which the amounts of hypaconitine were lower than predicted. The logical reason to explain this is that, as the ancient medicinal book *Shang-han-lun* reports, one of the ingredients (*Glycyrrhiza uralensis*) in these two preparations can prevent or destroy the toxicity of treated *fuzi*. Furthermore, several researchers have revealed recently that the ingredients of *G. uralensis* can quench aconitine-type alkaloids and act as the antidote for *fuzi* poisoning [11–13]. This quenching effect may explain the decreased level of hypaconitine that occurred after increasing the ratio of *G. uralensis* in preparation 2; presumably it had little effect in preparations 7 and 8 because the ratio was quite low.

4. Conclusion

Our analyses of eight Chinese medicinal preparations by the new rapid, sensitive, and selective LC/(+)ESI/MS^3 method in the SRM mode demonstrated the superior selectivity to the CRM mode. The crude methanol extracts of the medicinal preparations that contained complicated mixtures of ingredients were analyzed with high accuracy and precision without the need for any preliminary clean-up procedure. Our quantitative analyses of these three marker alkaloids also revealed that the presence of certain ingredients in the preparation could reduce the toxicity of *fuzi*. This new analytical method appears to be a useful and widely applicable tool for detecting and determining these marker compounds in single-herb *fuzi* and *fuzi*-containing medicinal preparations and could serve as the basis for batch-to-batch quality control of these preparations.

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