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Measurement of yunaconitine and crassicauline A in small-volume blood serum samples by LC–MS/MS: Tracing of aconite poisoning in clinical diagnosis

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ABSTRACT

Aconite poisoning is one of the most serious types of herb-related medical emergencies. In Hong Kong, many if not most of these poisoning cases are due to confusion in herbal species; that is, the wrong herbs are used in prescriptions. Such human errors, while inevitable perhaps, can be serious, and sometimes fatal. The chemical components responsible for aconite poisoning are yunaconitine and crassicauline A. In the present study, a rapid and sensitive method for the screening and quantification of yunaconitine and crassicauline A in human serum, using LC–MS/MS, was developed and validated. Methyllycaconitine was chosen as the internal standard. The limit of detection (LOD) of yunaconitine and crassicauline A were found to be 0.022 and 0.021 ng/mL, respectively. The limit of quantification (LOQ) was 0.1 ng/mL for both yunaconitine and crassicauline A. The recovery of yunaconitine and crassicauline A ranged from 78.6% to 84.9% and 78.3% to 87.2%, respectively. The matrix effect of yunaconitine and crassicauline A ranged from 110.0% to 130.4% and 121.2 to 130.0%, respectively. Both yunaconitine and crassicauline A were stable in serum for at least 3 months at −20 °C, and the extracts were stable for at least 7 days. For clinical applications, serum samples of two patients confirmed to have had aconite herbs poisoning in 2008 were quantified using the developed method. The result showed that this method can be utilized in clinical routine applications. This screening method expedites the diagnosis in cases of suspected aconite poisoning, thus enabling doctors to treat the condition more quickly and effectively.

1. Introduction

Aconitum genus is a commonly prescribed traditional Chinese medicine used as an analgesic, anti-inflammatory and cardiotonic agent [1]. It contains aconitine and related alkaloids that are powerful cardiotoxins and neurotoxins. Aconitine is known to suppress the inactivation of sodium channels by binding to the neurotoxin binding site 2 of channel protein [2]. Persistent activation of the sodium channels leads to continuous sodium influx and sustained depolarization, which is lethal to tissues. Poisoning symptoms include paraesthesia, sweating, nausea, vomiting, colicky diarrhea, intense pain and then paralysis of the skeletal muscles with onset at anytime from 20 min to 2 h after ingestion. The most severe cases show cardiac arrhythmia, including ventricular tachycardia and ventricular fibrillation, and lead to death [3,4]. The misuse of aconitum plants has caused cases of severe poisoning and fatalities both locally [5] and in other countries [6–8] in past decades. The overall in-hospital mortality of aconite poisoning in a local hospital was 5.5% [9]. In the past five years, the Toxicology Reference Laboratory of Hong Kong has confirmed more than 40 cases in Hong Kong alone. 30% of such cases were caused by aconite herbs containing yunaconitine (from plants of Yunnan origin) and crassicauline A (Fig. 1). In many of these poisoning incidents, aconite herbs were not specified in the herbal prescriptions; it is suspected that aconite species were either included by mistake by the herbalist, or were misidentified or mislabeled by the herbal collector.

Some aconite species used as folk drugs are known to contain other toxic alkaloids such as yunaconitine and crassicauline A. These two toxins are found in *A. vilmorinianum*, *A. foresstii*, *A. delavayi* and *A. transsectum* [10]. Animal studies have revealed that yunaconitine is as toxic as aconitine, whilst crassicauline A shows moderate toxicity comparable to deoxyaconitine [11]. Therefore, analytical methods for detection and quantification of

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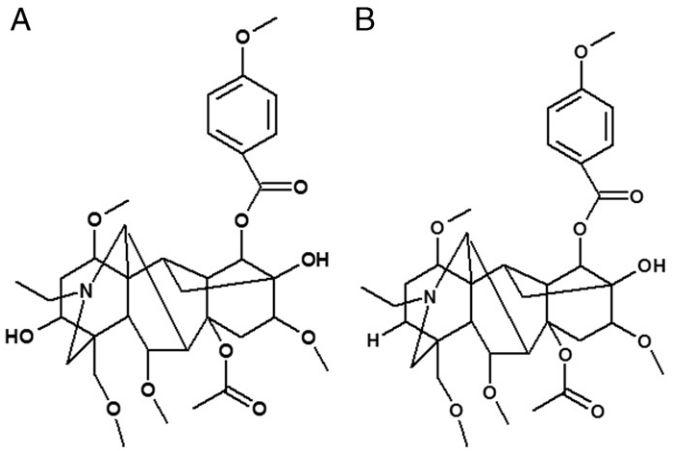

Fig. 1. Chemical structures of (A) yunaconitine and (B) crassicauline A.

aconitum alkaloids are urgently needed for clinical and forensic toxicology. As the serum concentrations correlate best with the pharmacological and toxicological effects, determination of such alkaloids in serum is the most expedient approach to aconite poisoning assessment.

Several techniques have been reported for separation and quantification of aconitine and its related compounds, e.g. hypaconitine, mesaconitine and their hydrolysis products, using capillary electrophoresis (CE) [12,13], GC–MS [14], HPLC–DAD [15,16], LC–MS/MS [17,18]. For the analysis of aconitum alkaloids in biological specimens, e.g. blood, serum and urine, several GC–MS [3,8,14,19] and LC–MS/MS [20–23] methods have been reported. Although different methods for the detection of aconitine and related alkaloids in herbs or biological specimens have been developed [6,21,23,24], none can simultaneously detect and quantify yunaconitine and its metabolite, crassicauline A, in a blood serum matrix. Due to the low concentrations in serum and fast hydrolytic properties of these two chemicals, the method to detect them must be rapid, sensitive and specific if it is to be useful in clinical diagnosis. LC–MS/MS is the most powerful instrument for identification and structural elucidation of compounds. It enables very sensitive and specific detection of high molecular weight aconitum alkaloids in biological samples. The advantage of LC–MS over GC–MS is the ability to directly measure compounds of interest without derivatization. In comparison with HPLC, LC–MS offers unsurpassed selectivity and superior qualitative and quantitative analysis. In the present study, we developed a rapid and sensitive method for the target screening and quantification of yunaconitine and crassicauline A in blood serum, using LC–MS/MS. Successful development of this test means that rapid clinical diagnosis and appropriate treatment of suspected poisoning victims are now possible.

2. Experimental

2.1. Standards and reagents

Yunaconitine and crassicauline A were purchased from the Hong Kong Jockey Club Institute of Chinese Medicine Limited (Hong Kong, China) and Herbstandard Inc. (Champaign, USA), respectively. The internal standard (IS), methyllycaconitine, was purchased from Tocris Bioscience (Minneapolis, USA). Aconitine and hypaconitine were purchased from LKT Laboratories (St. Paul, USA). Brucine, scopolamine and strychnine were obtained from Sigma (St. Louis, USA). Atropine, colchicines, gelsemine and matrine were obtained from ILUSA (San Francisco, USA), Alexis (Farmingdale, USA), NICPBP (Beijing, China) and Wako (Osaka, Japan), respectively. Mesaconitine was purchased from Wako (Osaka, Japan). Acetonitrile (ACN, HPLC grade) and methanol (MeOH, HPLC grade) were purchased from Merck (Darmstadt,-Germany) and Labscan (Gliwice, Poland), respectively. Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$) was obtained from USB Chemicals (Cleveland, USA). Sodium phosphate dibasic (Na_2HPO_4) and ammonium formate were acquired from Sigma (St. Louis, USA). Formic acid (FA) and acetic acid were obtained from Fluka (Steinheim, Germany).

2.2. Standard preparation

Individual stock standard solutions (1 mg/mL) of yunaconitine, crassicauline A, methyllycaconitine, aconitine, hypaconitine and mesaconitine were prepared in 0.1% formic acid (FA) in acetonitrile (ACN). Other standards were prepared in MeOH. All standard solutions were stored at 4 °C.

2.3. Sample preparation

For method validation, pooled serum was obtained from healthy subjects. The serum samples (100 μL) were spiked with known quantity of analyte, then mixed with 2 mL 0.5 M phosphate buffer and 100 μL IS (2 ng/mL in 0.1% FA in ACN), and finally applied to a bond elute C18 cartridge (Varian), which had been pre-conditioned with methanol, water and equilibrated with 0.5 M phosphate buffer at pH 6.0. The cartridge was then washed with 5% methanol. The analytes were eluted by using 0.1% acetic acid in methanol. The eluate was evaporated to dryness under 37 °C and reconstituted in 100 μL of 50% mobile phase (0.1% FA, 1 mM ammonium formate in 50% ACN). 10 μL of sample was injected into the LC–MS/MS system. In retrospective study, 100 μL patient sample was added.

2.4. Liquid chromatography

An Agilent 1100 series liquid chromatographic system equipped with a Zorbax Eclipse XDB-C8 column ($4.6 \times 150\ mm^2$, 5 μm) was used. Mobile phases A (0.1% FA, 1 mM ammonium formate in 5% ACN) and B (0.1% FA, 1 mM ammonium formate in 95% ACN) were used in the gradient elution programme. The flow rate was 0.5 mL/min, and the gradient was 0–5 min, 60–80% B; 5–6 min, 80–60% B. Total running time was 6 min/sample.

2.5. Mass Spectrometry

The study was carried out by using a quadruple mass spectrometer (Applied Biosystems 4000 QTRAP equipped with a Turbo ion-spray ionization source). MS detection was performed in positive electrospray ionization mode using the following settings: curtain gas, 20; collision gas, high; ionspray voltage, 3000 V; source temperature, 700 °C; ion source gas 1 and 2, 50 and 40 U, respectively; interface heater, on. Multiple reaction monitoring (MRM) mode was used, and two pairs of transitions were selected for each analyte. The setting (collision energy, CE; declustering potential, DP; collision cell exit potential, CXP) for *m/z* transitions of individual analyte were as follows: yunaconitine 660→600 (CE: 49; DP: 106; CXP: 16), 660→135 (CE: 87; DP: 106; CXP: 22); crassicauline A 644→584 (CE: 47; DP: 101; CXP: 16), 644→135 (CE: 83; DP: 101; CXP: 22); methyllycaconitine, IS 683→216 (CE: 50; DP: 66; CXP: 26).

2.6. Method validation

The method validation criteria follow the recommendation from modified USFDA and IFCC [25,26].

2.6.1. Selectivity and specificity

Selectivity (endogenous interference) was assessed using blood serum samples from ten human volunteers. The samples were analyzed for the presence of peaks within ± 0.5 min corresponding to the retention time of IS and the two analytes. To assess specificity, potential exogenous interference: three aconitum alkaloids, seven toxic plant alkaloids were spiked into pooled serum during analysis.

2.6.2. Linearity

Calibration standards with different concentrations of analytes (yunaconitine and crassicauline A: 0.1, 0.25, 0.5, 1.0, 5.0, 10.0 and 50.0 ng/mL) were assayed ($n=3$). The relationship between response and amount ratio of the analytes and IS was plotted. Linearity was evaluated by the ordinary least squares model for weighted linear regression. All calibration curves should show correlation coefficient $r^2 \geqq 0.99$.

2.6.3. Limit of detection (LOD) and limit of quantification (LOQ)

LOD was determined based on the standard deviation (S.D.) of the serum blank. Blank serum matrices ($n=10$) were used. The background signal of expected RT ± 0.5 min was quantified. LOD is defined as mean + 3 S.D. and was expressed in ng/mL. LOQ includes both lower LOQ (LLOQ) and upper LOQ (ULOQ). For the determination of lower LOQ, three concentrations of analytes (0.1, 0.25 and 0.5 ng/mL) were spiked into blank pooled serum. For upper LOQ, 50 ng/mL of analytes was spiked into blank pooled serum. These samples were analyzed ($n=6$). The accuracy was calculated. The targets of percentage accuracy $\pm 20\%$ and percentage RSD$\leqq$20% were met.

2.6.4. Accuracy and precision

Accuracy and precision of assay were analyzed at three levels: 0.12, 2.5 and 45 ng/mL. For intra-assay precision, three levels of analytes spiked to pooled serum were analyzed within a day ($n=6$). For inter-assay precision, three levels of analytes spiked to pooled serum, were analyzed on six different days. The accuracy was calculated.

2.6.5. Recovery and matrix effect

Recovery and matrix effect were assessed at three levels: 0.12, 2.5 and 45 ng/mL. The analytes and internal standard were spiked in a matrix-free solvent (distilled water) and blank serum from six different sources ($n=6$). For the blank serum, they were spiked with analytes before and after extraction. The concentrations of analytes in the matrix-free solvent (A), spiked blank serum before (B) and after extraction (C) were determined. The recovery and matrix effect of the given matrix can be assessed by comparing their concentrations:

$$Recovery\ (\%) = \frac{\text{Calculated Concentration from } B}{\text{Calculated Concentration from } C} \times 100\%$$

$$Matrix\ Effect\ (\%) = \frac{\text{Calculated Concentration from C}}{\text{Calculated Concentration from A}} \times 100\%$$

2.6.6. Stability

Pre-preparative stability was done by fortifying blank pooled serum with 1 and 10 ng/mL of analytes and stored at -20 °C. These were regularly tested on days 0, 1, 3, 7, 14, 30, 60 and 90. Internal standard was added at the time of analysis. For post-preparative stability, it was assessed at three levels: 0.12, 2.50, and 45 ng/mL. The processed samples were reanalyzed at days 0, 1, 3 and 7.

2.7. Clinical application

Two serum samples from patients confirmed with aconite herbs poisoning in 2008 (using method described by Lai et al. [27]) were quantified using the developed method.

3. Results

In LC–MS/MS analysis, aconitum alkaloids contain characteristic product ions $[M\text{-}60+H]^+$ (loss of CH_3COOH group) and $[M\text{-}60\text{-}32+H]^+$(further loss of CH_3OH group) [28]. In addition, m/z 105 and 135 are common fragments of all aconitum alkaloids in high collision energy. A typical mass spectra of analytes with IS at CE 50 is shown in Fig. 2. Two pairs of the most intense product ions were selected in all the analytes. The first pair transition serves the purposes of identification and establishment of a calibration curve; whereas the second pair transition serves as a confirmatory purpose. The ratio of the 1st pair MRM to the 2nd pair MRM was calculated for each sample, including QCs. The compound of interest was said to be confirmed if such ratio was within $\pm 20\%$ of that of the spiked aqueous standard. A total ion chromatogram (TIC) is shown in Fig. 2.

3.1. Method validation

3.1.1. Selectivity and specificity

No endogenous and exogenous interference peaks were identified within ± 0.5 min corresponding to the retention time of the internal standard and the two analytes. Therefore, the selected transitions m/z for each analyte were considered to be sufficiently specific. For specificity, potential exogenous interference: three aconitum alkaloids (aconitine, hypaconitine, and mesaconitine) and seven toxic plant alkaloids (atropine, brucine, colchicine, gelsemine, matrine, scopolamine, and strychnine) were spiked into pooled serum for analysis. No peak was observed within ± 0.5 min corresponding to the retention time of the internal standard and the two analytes.

3.1.2. Linearity

The relationship between response and amount ratio of the analytes and IS was plotted. The calibration curves for yunaconitine and crassicauline A in serum were linear over the range of 0.1–50 ng/mL ($r^2 \geqq 0.99$), calculated by weighted ($1/x$) linear regression.

3.1.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LODs of yunaconitine and crassicauline A were 0.022 and 0.021 ng/mL, respectively. The LLOQs of both yunaconitine and crassicauline A were 0.1 ng/mL, with accuracy of 87.32% and 95.60%, respectively. For ULOQ at 50 ng/mL, of yunaconitine and crassicauline A, the accuracy percentages were 99.30% and 99.60%, respectively.

3.1.4. Accuracy and precision

The intra- and inter-assay accuracy and precision data for the quantification of yunaconitine and crassicauline A in pooled serum samples are summarized in Table 1. For intra-assay, the percentage accuracies of yunaconitine and crassicauline A ranged from 103.5% to 114.4% and 101.6% to 112.5%, respectively. For inter-assay, the percentage accuracies of yunaconitine and crassicauline A ranged from 95.0% to 107.8% and 96.6% to 101.0%, respectively.

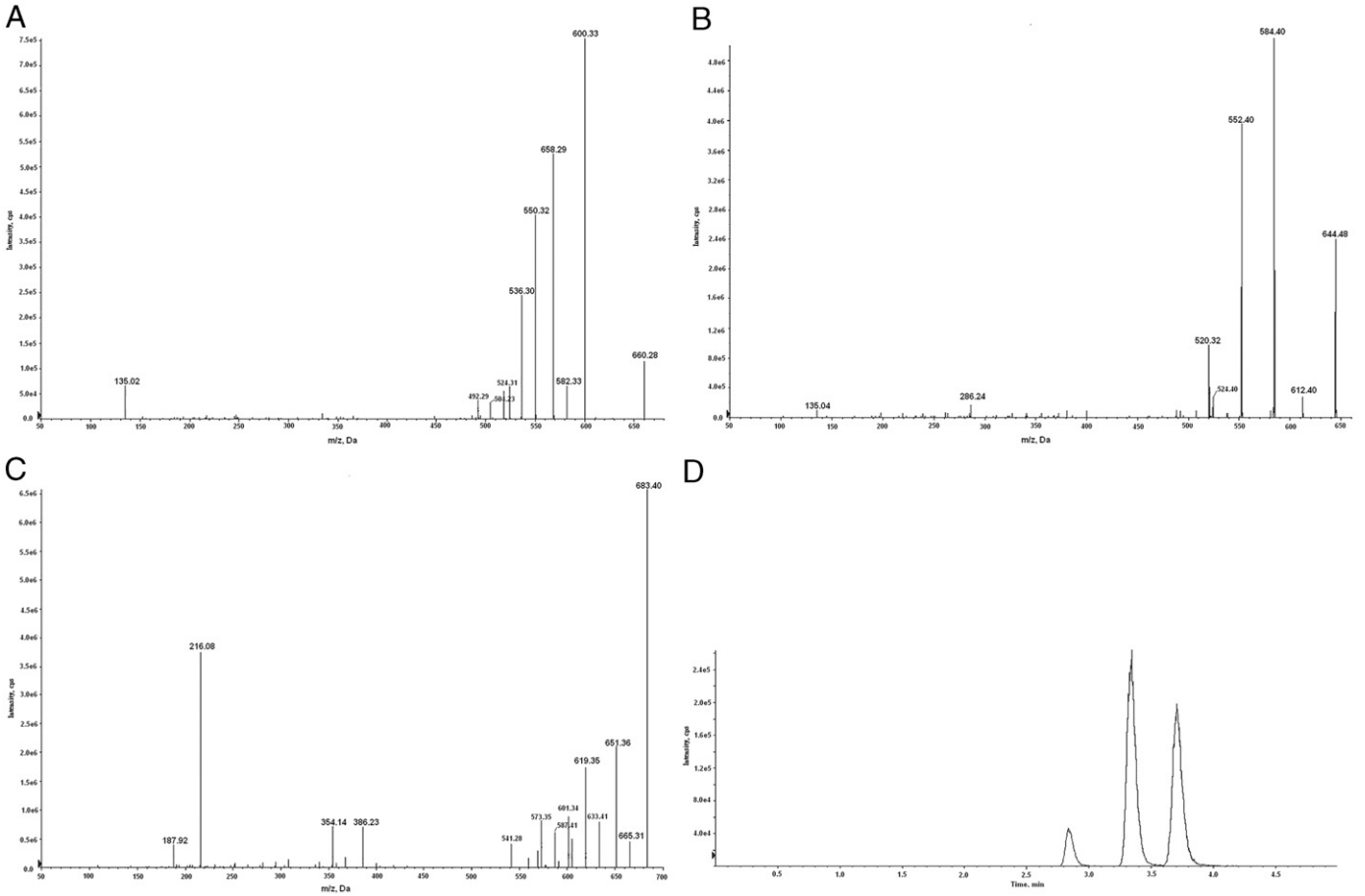

Fig. 2. Mass spectra of (A) yunaconitine, (B) crassicauline A and (C) methyllycaconitine, at CE 50. (D) Total ion chromatogram showing yunaconitine (Rt: 3.4 min), crassicauline A (Rt: 3.8 min) and IS.

Table 1
Intra- and inter-day accuracy and precision of yunaconitine and crassicauline A in human serum.

Nominal conc. (ng/mL)	Yunaconitine			Crassicauline A		
	Mean calculated conc. (ng/mL)	% Accuracy	% RSD	Mean calculated conc. (ng/mL)	% Accuracy	% RSD
Intra-day ($n=6$)						
0.12	0.14	114.44	7.86	0.14	112.50	2.66
2.5	2.59	103.53	6.90	2.54	101.60	4.10
45	47.57	105.70	2.80	46.43	103.19	3.35
Inter-day ($n=6$)						
0.12	0.13	107.78	6.79	0.12	100.00	6.08
2.5	2.55	102.00	2.06	2.53	101.04	2.61
45	42.76	95.02	4.53	43.46	96.58	5.59

Table 2
Recovery and matrix effect for yunaconitine, crassicauline A and IS in human serum.

Nominal conc. (ng/mL)	Analyte		
	Yunaconitine (%)	Crassicauline A (%)	IS (%)
Recovery ($n=6$)			
0.12	78.6 ± 5.9	78.3 ± 4.4	77.5 ± 2.4
2.5	84.9 ± 4.1	87.2 ± 8.2	
45	82.9 ± 2.7	81.5 ± 3.3	
Matrix effect ($n=6$)			
0.12	130.4 ± 16.1	130.0 ± 15.5	
2.5	110.0 ± 10.1	124.6 ± 10.8	
45	113.1 ± 2.8	121.2 ± 4.3	

3.1.5. Recovery and matrix effect

The recovery and matrix effect at three different levels are summarized in Table 2. The recovery of yunaconitine and crassicauline A ranged from 78.6% to 84.9% and 78.3% to 87.2%, respectively; whereas the mean recovery of IS was 77.5%. The matrix effects of yunaconitine and crassicauline A ranged from 110.0% to 130.4% and 121.2% to 130.0%, respectively.

3.1.6. Stability

The pre-preparative stability of analytes in human serum is shown in Fig. 3. At two different concentration levels, the target analytes show similar stability profiles over the 3-month storage period. To achieve the best stability, the sample should be analyzed within the first seven days upon preparation. For post-preparative stability (Fig. 4), the stability profiles were found similar at medium and high analytes concentration. The stability of low concentration (i.e. 0.12 ng/mL) was found fluctuated within the 7-day of storage period.

3.2. Clinical applications

Serum samples from two patients with confirmed aconite herbs poisoning was quantified by the developed method. Yunaconitine and crassicauline A were detected in both patients'

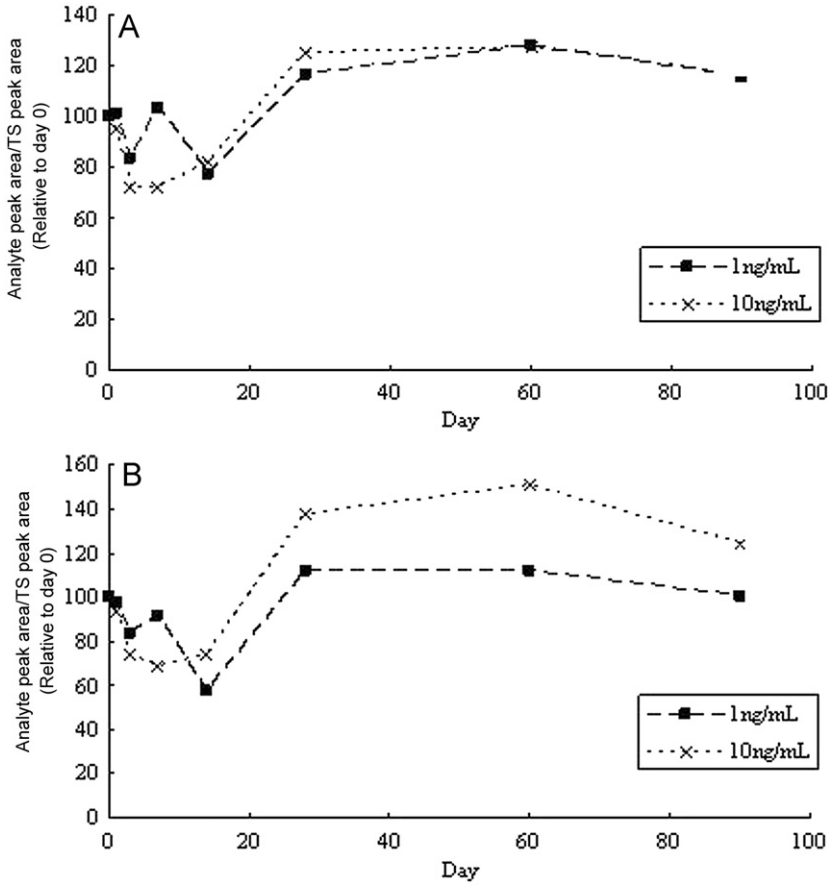

Fig. 3. Pre-preparation stability of (A) yunaconitine and (B) crassicauline A in human serum spiked with 1 and 10 ng/mL of standard and stored at −20 °C until analysis. Analysis was done on days 0, 1, 3, 7, 14, 30, 60, and 90.

serum, and the results are shown in Table 3. The target compound of interest in serum samples was said to be confirmed if the calculated ion ratio of first to second pair MRM matched within 20% of the spiked aqueous standard (Table 4). Fig. 5 shows the extracted ion chromatogram (XIC) with integration of peak area of the two patient samples.

4. Discussion

In this study, a LC–MS/MS method for the identification and quantification of yunaconitine and crassicauline A in human serum was developed and fully validated in accordance with international guidelines [25,26].

During the method development, the LC–MS/MS conditions were optimized to obtain the best signal during analysis, e.g. compound optimization by syringe infusion for selection of ionization mode and MRM transition, source/gas optimization to select suitable compound-specific parameters and the LC profile. The LC profile was set to be 6 min to allow complete separation of the two analytes and the internal standard. Therefore, high sample throughput can be achieved for routine screening and quantification work in a clinical laboratory. Sample extraction is a crucial step for serum analysis. In this method, SPE using Varian Bond elute C18 cartridge was adopted. It shows better extraction efficiency than other cartridges with minimal noise background in the TIC (data not shown).

Methyllycaconitine was chosen as an internal standard because its structure is similar to that of yunaconitine and crassicauline A. It is presumed that their chemical and physical behaviors were similar. An internal standard can compensate for the variation in sample preparation, processing and measurement. Isotope-labeled analogues of target analytes would be the best choice for MS analysis; but they could not be used in this case because they were not available on the market. Ito et al. [14] prepared deuterium-labeled aconitine and mesaconitine in a series of steps. They applied these deuterium-labeled analogues for clinical use. However, home-made isotopically-labeled analogues may contain impurities, such as non-labeled compound, and contain same fragmented product ions as the target analytes. In such cases, peak areas may be over-estimated, and the accuracy of quantification could be compromised.

For method validation, selectivity, specificity, linearity, LOD and LOQ, accuracy and precision, recovery and matrix effect were assessed. All of the results fulfilled the acceptance criteria in the reference guideline. The present method, using MRM detection mode, is certainly specific and sensitive for the target analytes. The specificity of the method can reduce endogenous and exogenous interference from the complicated serum matrix. Therefore, it can be applied to complicated biological samples. The working range of the present method is 0.1–50 ng/mL, which should cover most clinical needs. In some fatal case reports of aconite poisoning, the post-mortem blood concentration ranged from 10.0–12.1 ng/mL [6,7]. In a toxicokinetics study of aconite poisoning, the plasma concentrations were 1.75, 0.75, 0.35 and 0.02 ng/mL after 7, 9, 14 and 26 h post ingestion [29]. As the toxicity of yunaconitine and crassicauline A was similar to that of aconitine and deoxyaconitine [11], we hypothesized that the lethal dose would also be similar. Therefore, this method appears to be sensitive enough for clinical analysis. For the linearity,

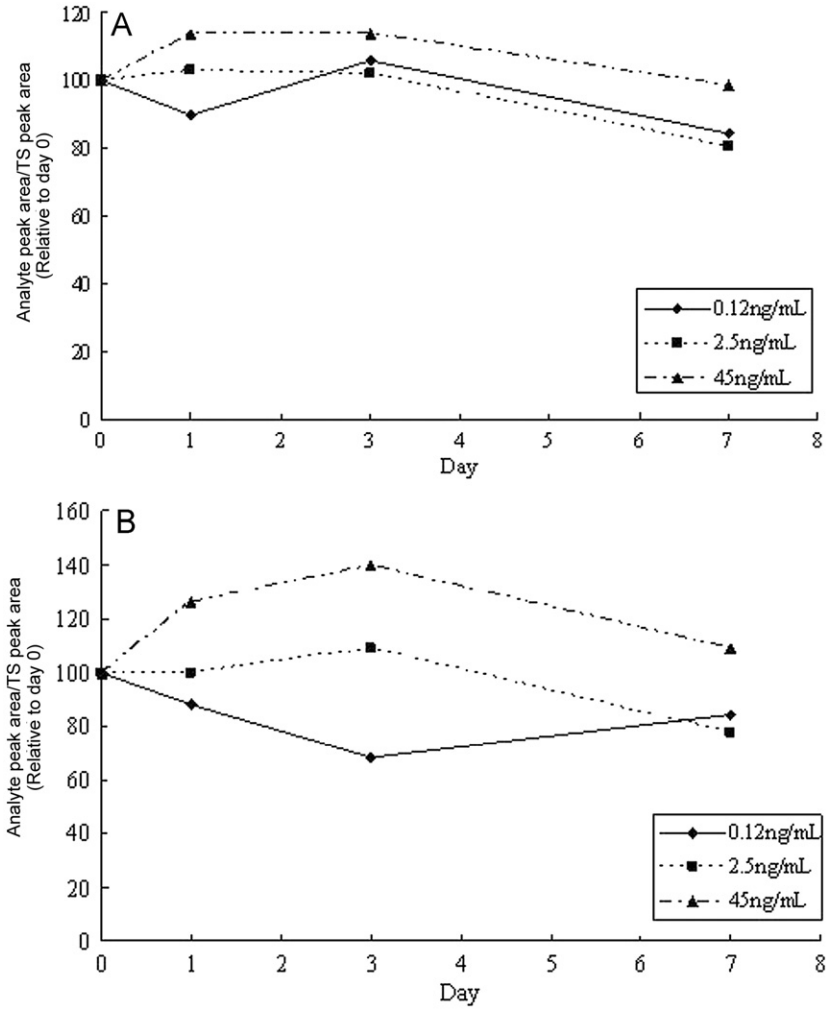

Fig. 4. Post-preparation stability of (A) yunaconitine; (B) crassicauline A in human serum spiked with 0.12, 2.5, and 45 ng/mL of standard and stored at 4 °C and injected on days 0, 1, 3 and 7.

Table 3
Calculated concentrations of serum samples from two patients confirmed with aconite herbs poisoning.

Sample	Analyte concentration (ng/mL)	
	Yunaconitine	Crassicauline A
Patient A	2.56	0.48
Patient B	1.22	6.42

weighted least square model ($1/x$) was applied, as the working range covered three orders of magnitude which will result in significant heteroscedasticity. A weighted model can compensate for this phenomenon [30]. The study of matrix effect is an integral part of method validation using LC–MS/MS because it may affect other validation parameters, e.g. LOD, LLOQ, linearity, accuracy and precision, etc [31]. In the evaluation of matrix effects of the serum in this study, the calculated percentages fulfilled the acceptance criteria (within $\pm 30\%$). Hence, no ion suppression/enhancement was observed. For the analytes stability, no decay was observed in both pre- and post-analysis. The results indicated that both yunaconitine and crassicauline A were stable in serum for at least three months at -20 °C, and the extracts were stable for at least seven days. The importance of the stability study is that it determines whether the samples can be stored and reanalyzed, with accurate results, should the need arise.

Table 4
Calculated ion ratio of 1st pair MRM to 2nd pair MRM, of aqueous standard mix and the patients' samples. The compound of interest was said to be confirmed (✓) if such ratio was within $\pm 20\%$ of that of the spiked aqueous standard mix (shown in bracket).

Analyte peak name	Peak area	Calculated ion ratio (expected value) (%)	Ratio confirms ID
Patient A			
Yunaconitine 1	340,000		
Yunaconitine 2	45,800	13.5 (12.0)	✓
Crassicauline A 1	66,100		
Crassicauline A 2	62,200	94.1 (89.7)	✓
Patient B			
Yunaconitine 1	150,000		
Yunaconitine 2	18,100	12.1 (12.0)	✓
Crassicauline A 1	674,000		
Crassicauline A 2	612,000	90.8 (89.7)	✓

Although some relevant methods have been developed for the analysis of aconitum alkaloids in biological specimens [6,21,23,24], none of these can simultaneously detect and quantify yunaconitine and crassicauline A in serum matrix. A related report by Wang et al. [21] developed a method for simultaneous determination of yunaconitine, crassicauline A and foresaconitine in urine. Herein, we developed a method for analyzing serum matrix with a rapid running time of 6 min/sample, comparable to

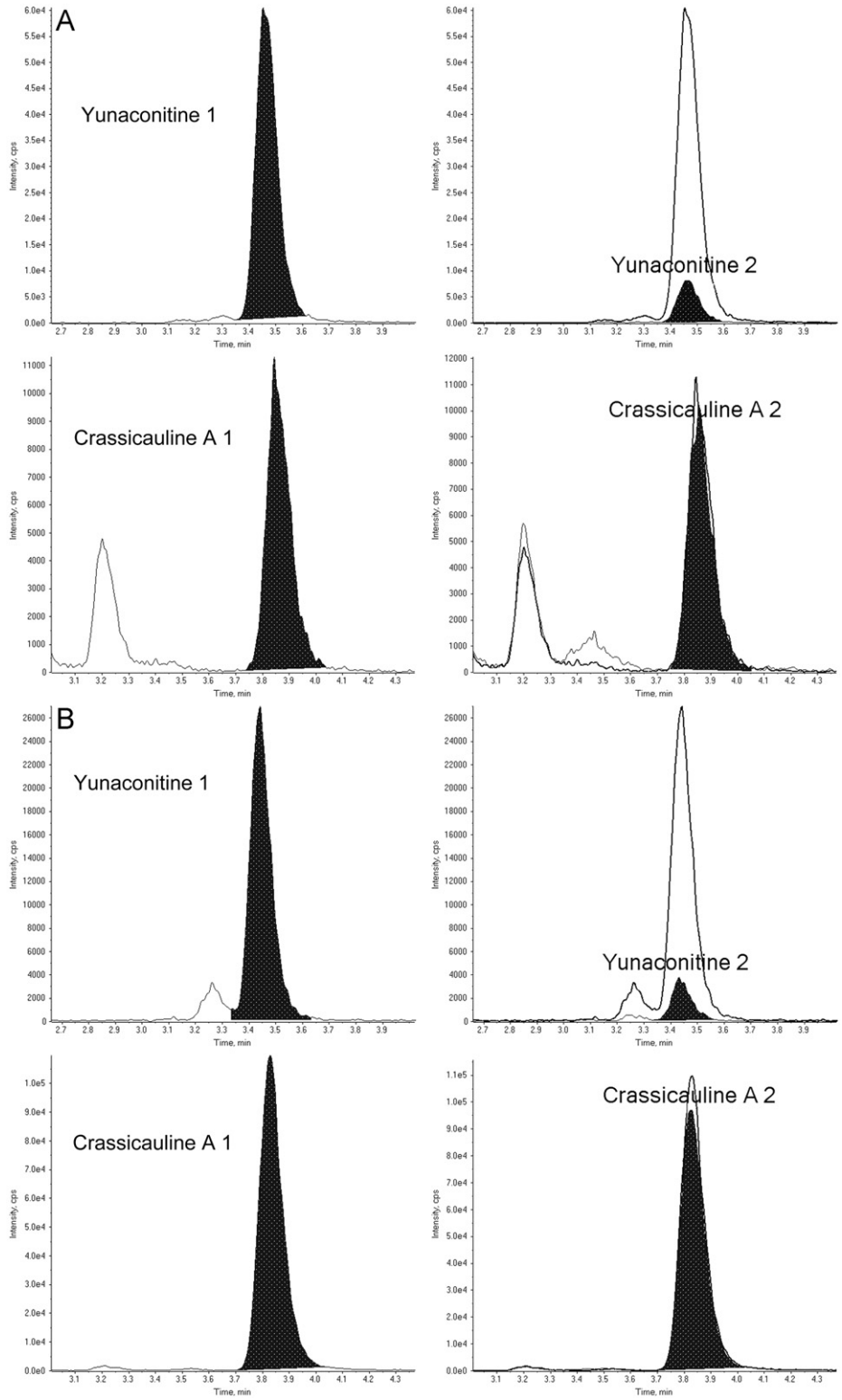

Fig. 5. Extracted ion chromatogram (XIC) of (A) patient A's sample; (B) patient B's sample. Yunaconitine 1: 660→600; yunaconitine 2: 660→135; crassicauline A 1: 644→584. The calculated ion ratio of 1st pair MRM to 2nd pair MRM of each analyte is shown in Table 4.

UPLC system. The LOD and LOQ for both analytes were 3–5 times and 1.5–2 times lower than that of previous work. Xiong et al. [23] developed another method for the quantification of crassicauline A in serum matrix. Our present method developed for simultaneous quantification of yunaconitine and crassicauline A in a single run with marked reduction (to 100 μL) in sample volume required. All of these factors mean that this method not only saves time but also represents less discomfort to patients in blood sampling.

Successful development of the present analytical method is important for rapid diagnosis thereby expediting appropriate treatment of aconite herbs poisoning cases. Since in some cases, the leftover herbal broth or residuals are not available for review. In many cases, even these materials were available, the source of

poisoning incident still cannot be found. Therefore, biological samples, e.g. serum and urine, are ideal for toxicological analysis.

The present study not only establishes a quick, precise method for assessing aconite poisoning, it also serves as a pioneering study in the toxicokinetics of aconitum alkaloids. With this method, the relationship between severity and type of clinical symptoms can be correlated with levels of various chemicals. This knowledge could be valuable in designing treatments for such poisoning.

5. Conclusion

A rapid and sensitive LC–MS/MS method for the screening and quantification of yunaconitine and crassicauline A in human blood serum has been developed. This target screening method facilitates rapid and precise laboratory diagnosis of suspected aconite herb poisoning.

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