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Rapid identification of herbal toxins using electrospray laser desorption ionization mass spectrometry for emergency care

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ABSTRACT

The unintentional ingestion of toxic compounds in herbs is not uncommon in many parts of the world. To provide timely and life-saving care in the emergency department, it is essential to develop a point-of-care analytical method that can rapidly identify these toxins in herbs. Since electrospray laser desorption ionization mass spectrometry (ELDI/MS) has been successfully used to characterize non-volatile chemical compounds without sample preparation, it was used to identify toxic herbal compounds in this study. The herbal toxins were collected either by sweeping a metallic probe across the surface of a freshly cut herb section or by directly sampling extracts of ground herbal powder. The analytes on the probe were then desorbed, ionized and detected using ELDI/MS, wherein analysis of the herbal toxins was completed within 30 s. This approach allows for the rapid morphological recognition of herbs and early point-of-care identification of herbal toxins for emergency management and is promising in providing important toxicological information to ensure appropriate medical treatment.

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1. Introduction

Traditional Chinese medicine (TCM) and the Indian Ayurveda are the two most ancient systems of traditional medicine, which were developed several millennia ago and are currently still used worldwide [1]. These health practices include herbal- and plant-derived remedies, with such remedies being reported by the World Health Organization (WHO) as the most frequently used therapies around the world. In 2003, the global market for herbal medicines earned over USD $60 billion. In Africa, up to 80% of the population uses traditional medicines including herbal preparations for primary health care, while traditional herbal preparations account for 30–50% of all medicinal consumption in China [2].

Some herbs contain many compounds that are beneficial to human health, while others contain toxins. Misuse of these toxic herbs, especially at high concentrations, can have serious effects on the human body. As a result, the United States is increasingly considering traditional Chinese herbal medications as sources of intentional and unintentional contamination. Due to the lack of transparency in reporting, the risk of herbal medications to the general population has been difficult to assess. In fact, the U.S. Food and Drug Administration (FDA) has little control over the marketing of herbal products in the U.S.A. In many Asian countries, it is not uncommon for the general public to purchase or self-prescribe herbal medications based on unverified sources of medical information. Consequently, acute intoxication with varying degrees of clinical severity frequently occurs after self-treatment with herbs. Thus, it is necessary to establish a rapid and reliable analytical platform for the rapid identification of the toxic constituents in herbs for effective emergency management.

To date, the most commonly applied method for identifying toxins in herbs is through 1) visual morphological matching by experts or 2) referring to photographic descriptions from an atlas or online database for herbs. Since expert advice on herbs is not always available, medical staff can often misidentify a toxic plant as a non-toxic herb. Identification of toxic herbs through ingestion and observation of clinical symptoms is impractical and nearly impossible, since herbal toxins usually affect multiple organ systems (e.g. the nervous, cardiovascular, gastrointestinal, hepatic, renal, and hematological systems).

Conventionally, high-performance liquid chromatography and gas chromatography combined with mass spectrometry (HPLC/MS and GC/MS) have been shown to be useful analytical tools for the identification of toxic and active ingredients in herbs commonly prescribed in TCM regimens [3–5]. However, these traditional methods for elucidating herbal compounds requires laborious and time-consuming sample preparation and are therefore not timely enough for identifying the poisonous herbs consumed by intoxicated patients in an emergency setting.

Alternatively, ambient mass spectrometry (AMS) can rapidly detect analytes in complex organic and biological samples with minimal or no sample pretreatment, and is therefore useful for rapid and convenient sample analysis. Previously, several AMS techniques have been used to directly characterize the biochemical ingredients in plants and herbs. For example, leaf spray was used to identify sugars, amino acids, fatty acids, and lipids in green onions and spinach leaves, as well as alkaloids in potato peels and tubers and in tomato leaves; tissue spray was used to identify ginsenosides, amino acids, and oligosaccharides in ginseng samples; and direct analysis in real time (DART) was used to identify various biological compounds in herbal medicines [6–9]. Although AMS is capable of directly characterizing herbal ingredients, samples usually need to be brought near the ion source for analyte desorption and ionization. This may require additional preparation like cutting the sample to fit in the sampling space, which can render these AMS techniques impractical for emergency management applications during which rapid *in situ* sampling, sample delivery, and analyte detection are required.

Electrospray laser desorption ionization mass spectrometry (ELDI/MS) is an AMS technique that uses a laser to directly desorb analytes from samples in various physical states. The desorbed analytes then flow upward to join an ESI plume, interacting with the charged solvent species for ionization. Whereas many AMS techniques are unable to ionize nonvolatile compounds like herbal toxins, the high-energy laser used in ELDI/MS for analyte desorption also renders it capable of ionizing such compounds. However, as mentioned in the above paragraph, samples may need to be cut to fit in the ELDI/MS sampling space, which necessitates a more convenient sampling method.

The main objective of this study is to develop an analytical method that can rapidly and efficiently provide important toxicological information on herbal toxins for timely decision-making during critical resuscitation in the emergency room. Given the analytical capabilities of ELDI/MS for rapid and direct characterization of non-volatile compounds, the use of a sampling probe enables the analysis of samples that cannot fit in the small sampling space. Moreover, collection of a trace amount of sample avoids excessive matrix effects in the source that can lead to analyte signal suppression.

In this study, we developed a useful point-of-care analytical approach for the rapid identification of toxic ingredients in herbs that were commonly misused by different Asian populations. This approach involves the rapid sampling and direct detection of non-volatile toxins in herbal sections. A sampling probe was used to collect trace amounts of sample from an herbal section. The analytes adsorbed on the probe were characterized using ELDI/MS, where a continuous-wave laser beam was used to irradiate and desorb the non-volatile analytes on the probe; the desorbed analytes were then delivered to and ionized in an ESI plume and detected by a mass analyzer. Herein, we tested the applicability of the above technique for the rapid point-of-care identification of toxic herbal ingredients, demonstrating its promising potential to facilitate the efficiency of emergency care.

2. Materials and methods

2.1. *Samples and chemical reagents*

Standards for toxic herbal compounds including ginkgotoxin, wogonin, aconitine, mesaconitine, and hypaconitine were

purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). Linamarin and senecionine were obtained from Toronto Research Chemicals (Canada), and diosbulbin B was obtained from STB (China). The above standards were used without further purification. According to epidemiological statistics reported by the Taiwanese FDA, the above compounds were the most common herbal toxins ingested by self-treating patients in Taiwan. Eight pure stock solutions of the herbal toxin standards were prepared in methanol at concentrations of 1 mg·mL^{-1} and were serially diluted to working solutions used for analysis.

Various herbs such as *Alocasia macrorrhiza*, *Colocasiaes culenta* (L.) Schott, cv. Mein, *C. culenta* (L.) Schott, cv. Betelnut, *Dioscorea bulbifera*, *Dioscorea hispida*, *Dioscorea japonica*, *Dioscorea alata* L. var. *purpurea*, *Fallopia multiflora*, *Ginkgo biloba*, *Manihotes culenta*, Radix aconiti, Radix aconiti kusnezoffii, Radix aconiti laterali spreparata, and *Scutellaria baicalensis* were purchased from traditional medicine stores and local markets. For qualitative analysis, the herbs were cut into small pieces by a knife prior to ambient mass spectrometric analysis. To test the extraction efficiency, the herbs were ground into powder by a blender. Water and four common organic solvents (methanol, ethanol, isopropyl alcohol and ethyl acetate) were added to extract herbal toxins from Radix aconiti, Radix aconiti kusnezoffii, and Radix aconiti laterali spreparata, respectively. After vortexing for 15 s and centrifuging at 6000 rpm for 5 min, the extracts were then tested to determine the concentration of these toxins by ELDI/MS and LC-MS/MS.

Methanol (MeOH) was purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from Sigma–Aldrich. (St. Louis, MO, U.S.A.). Distilled deionized water (purified with a PURELAB Classic UV from ELGA, Marlow, U.K.) was used to prepare the standard electrospray ionization solution that contained water, methanol, and acetic acid (50/50/0.1% by volume). HPLC-grade solvents such as ethanol (EtOH), ethyl acetate (EA), and isopropyl alcohol (IPA) were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.).

2.2. *Electrospray laser desorption ionization/mass spectrometry (ELDI/MS)*

The ELDI/MS system was built in-house and comprised of a sampling probe (60 mm long, 2.5 mm in diameter; Ming Yuh Scientific Instruments Co. Ltd, Tainan, Taiwan), continuous wave (CW) laser (IR808-1500, Tan-Yu Technology, Taiwan), and homemade electrospray ionization ion source. A linear ion trap mass spectrometer (LTQ XL Thermo Scientific, Waltham, MA, U.S.A.) was attached to the ELDI source to detect the ions generated in the source and perform tandem mass spectrometry (MS/MS) analysis [10–12]. Samples were prepared by (1) cutting an herbal sample into two sections to expose the interior, (2) grinding the herbal sample into powder, and (3) extracting analytes in the herbal powder using an organic solvent. The metallic sampling probe partly comprised of an inoculating loop, which was used to collect analytes from the herbal section, powders, and extracts (Fig. 1a). The metallic probe carrying the sample was inserted into the ELDI source (Fig. 1b), after which the sample was irradiated by a CW laser beam. The CW laser was operated at 808 nm with a flux energy of ca. 2 W, where the CW laser spot was focused to ca. 1 mm. The incident angle between the CW and the metal sampling probe was set at 90°.

An electrospray solution composed of 50% methanol and water with 0.1% acetic acid (v/v/v) was flowed through a fused-silica capillary at a flow rate of 0.2 mL·h^{-1}. A high voltage (4.0 kV) was applied to the capillary to induce electrospray ionization via solution conduction. The exit of the ESI source was 5 mm directly above the sample. The distance between the exit of the ESI capillary and the MS inlet was 5 mm. Analyte ions were detected by the linear ion trap mass analyzer that was operated in positive ion mode.

2.3. *Fourier transform-ion cyclotron resonance/mass spectrometry (FT-ICR/MS)*

An FT-ICR/MS instrument (solariX, Bruker Daltonics, Leipzig, Germany) was used to accurately identify the *m/z* 222 ion observed from the EA extracts of *D. hispida*. The following parameters were used: capillary entrance voltage 4.5 kV, end plate electrode voltage 0.5 kV, dry gas 4 L/min, dry temperature 50 °C, nebulizer gas 0.7 bar. A methanolic electrospray solution was flown through a fused-silica capillary (i.d.

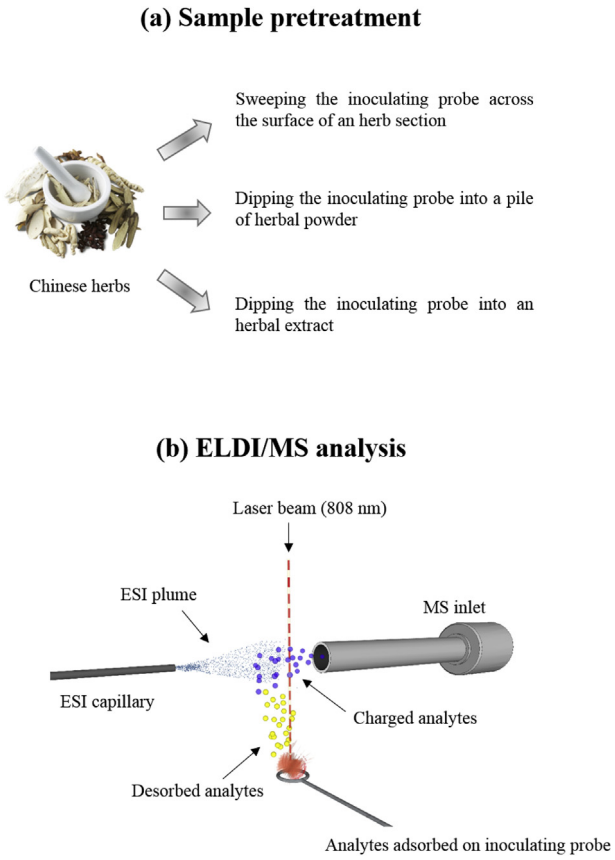

Fig. 1 – Direct metallic probe sampling and ELDI/MS analysis: (a) analyte collection from herbal samples by sweeping the inoculating probe across the surface of an herb section, dipping the inoculating probe into a pile of herbal powder, and dipping the inoculating probe into an herbal extract; (b) laser desorption, electrospray ionization, and detection of analytes collected on the sampling probe.

100 μm) at a flow rate of 120 μL/h. The mass spectrometer was operated in positive ion mode over a scan range of 100–2000 *m/z* (detection mode in narrowband) using polyethylene glycol 200 (PEG 200) calibration standards.

2.4. *Liquid chromatography-tandem mass spectrometry (LC-MS/MS)*

LC-MS/MS analysis was performed with a liquid chromatography system (Shimadzu LCMS-8040) equipped with a triple quadrupole mass spectrometer (Shimadzu LCMS-8040). The aqueous mobile phase (A) consisted of 0.1% acetate acid in deionized water, while the organic mobile phase (B) consisted of 0.1% acetate acid in pure methanol. Five microliter of sample solution was injected into a Shim-pack GIST C18 column (3 μm, 4.6 mm × 150 mm). The flow rate of each mobile phase was 0.8 mL/min, and the isocratic flow gradient of the organic mobile phase (B) was 70%. The entire time of chromatographic analysis is 3 min.

2.5. *Principal component analysis*

For this study, we also used principal component analysis (PCA), a commonly used multivariate statistical method that reduces the dimensionality of a data set while retaining information present in the original mass spectra [13–18]. To distinguish morphologically similar toxic and non-toxic herbs, the experimentally obtained ELDI/MS results were further analyzed using PCA. The resulting ion signals from each sample were processed using PCA in the MATLAB software tool integrated with the Mass Profiler Professional 13.1 suite (Agilent Technologies Inc. and Strand Life Sciences Pvt. Ltd.).

3. Results

The ELDI/MS analytical process was developed to perform rapid and efficient analysis for practical use in emergency care, and involves the following steps: (1) direct probe sampling of analytes from solid or liquid herbal samples, (2) desorption of analytes from the sampling probe via CW laser irradiation, (3) ionization of desorbed analytes via their interactions with charged solvent species in an electrospray plume, (4) detection of herbal toxin ions with a linear ion trap mass analyzer, and (5) removal of residual sample on the probe by burning it with a high-temperature flame for a few seconds (Fig. 1).

Fig. 2 shows the ELDI mass spectra for eight herbal toxin standards (1 ppm each). Six toxins were detected as protonated molecular ions ($[MH]^+$; ginkgotoxin, *m/z* 184; wogonin, *m/z* 285; senecionine, *m/z* 336; aconotine, *m/z* 646; mesaconitine, *m/z* 632; hypaconitine, *m/z* 616), while the other two were detected as ammoniated ions (MNH_4^+; linamarin, *m/z* 265; diosbulbin B, *m/z* 362). The MS/MS spectrum for the molecular ion of each herbal toxin is presented in the inset of its respective full scan mass spectrum (Fig. 2). The precursor ion and prominent product ions of each herbal toxin obtained by ELDI/MS and ELDI/MS/MS analyses are summarized in Table 1.

To optimize the efficiency of the rapid detection of trace toxins in herbs, we tested different sampling strategies for samples in different physical states: (1) directly sweeping the sampling probe across the surface of an herbal section for ca. 1 cm, (2) inserting the sampling probe into a pile of herbal powder, and (3) dipping the sampling probe into an herbal extract solution to collect ca. 2 μL of sample within the inoculating loop of the probe. The total turnaround time to conduct sampling and ELDI/MS analysis was about 30 s. The time taken to prepare the herb powder for strategy 2 and the extract solution from the herb powder for strategy 3 ranged between 5 and 10 min.

As shown in Fig. 3, a predominant ion signal at *m/z* 222 was detected in the ELDI mass spectra for *D. hispida* samples collected using the three aforementioned sampling strategies. The experimental results showed that even when a trace amount of sample was collected by sweeping the sampling probe across the herbal section surface for 1 cm, an ion signal for the analyte toxin was still detected (Fig. 3a). Even though the same ion at *m/z* 222 was detected using all three sampling approaches, its ion intensity from the sample directly collected from the herb section surface was lower than that from the samples collected from the herbal powder or extract. This is reasonable since much fewer analyte molecules were probably collected using strategy 1 than when using strategies 2 and 3. Since interferences from sample matrix effects can still affect ELDI/MS analysis, the direct collection of analytes from solid samples seems to be the most feasible sampling strategy for expeditiously identifying toxic ingredients in herbs while still providing comparable analytical capabilities. This sampling strategy combined with ELDI/MS analysis seems to be a promising approach to rapidly and efficiently identify herbal toxins for timely emergency management.

To identify the ion at *m/z* 222 via its elemental composition, we analyzed *D. hispida* extracts in EA using direct infusion ESI followed by FT-ICR/MS analysis. The accurate mass and chemical composition of the ion was determined as *m/z* 222.14870 and $[C_{13}H_{19}NO_2+H]^+$, respectively (Inset in Fig. 3). The MS/MS analysis of the ion indicated that several fragment ions at *m/z* 140, *m/z* 178, and *m/z* 204 were observed. The experimentally obtained molecular weight and fragment ion pattern were respectively in agreement with those of dioscorine, a major toxin found in *D. hispida*. Other toxins were also detected in different herbal sections using ELDI/MS. Figure S2 shows the ELDI mass spectra for samples collected directly from the surface of different herbal sections. Ion signals for herbal toxins from four herbal sections were successfully detected: (1) diosbulbin B ($[MNH_4]^+$, *m/z* 362), a toxin found in *D. bulbifera* (Figure S2d); (2) dioscorine ($[MH]^+$, *m/z* 222), a toxin found in *D. hispida* (Figure S2e); (3) ginkgotoxin ($[MH]^+$, *m/z* 184), a toxin found in *G. biloba* (Figure S2i); and (4) wogonin ($[MH]^+$, *m/z* 285), a toxin found in *S. baicalensis* (Figure S2k). No obvious toxin signals were detected on the surfaces of the herbal sections of *Colocasia esculenta* (L.) Schott, cv. Mein; *C. esculenta* (L.) Schott, cv. Betelnut; *D. japonica*; *D. alata* L. var. *purpurea*; and *F. multiflora* (Figures S2b, c, f, g, and h) indicating these herbs are non-toxic. The predominant ions detected in the solvent extracts of these non-toxic herbs are mainly for carbohydrates, lipids, and other unknown compounds.

Fig. 4a shows the results of within-run tests (n = 17) performed via ELDI/MS analysis of an herbal standard solution for aconitine (10 ppm), with a relative standard deviation (RSD)

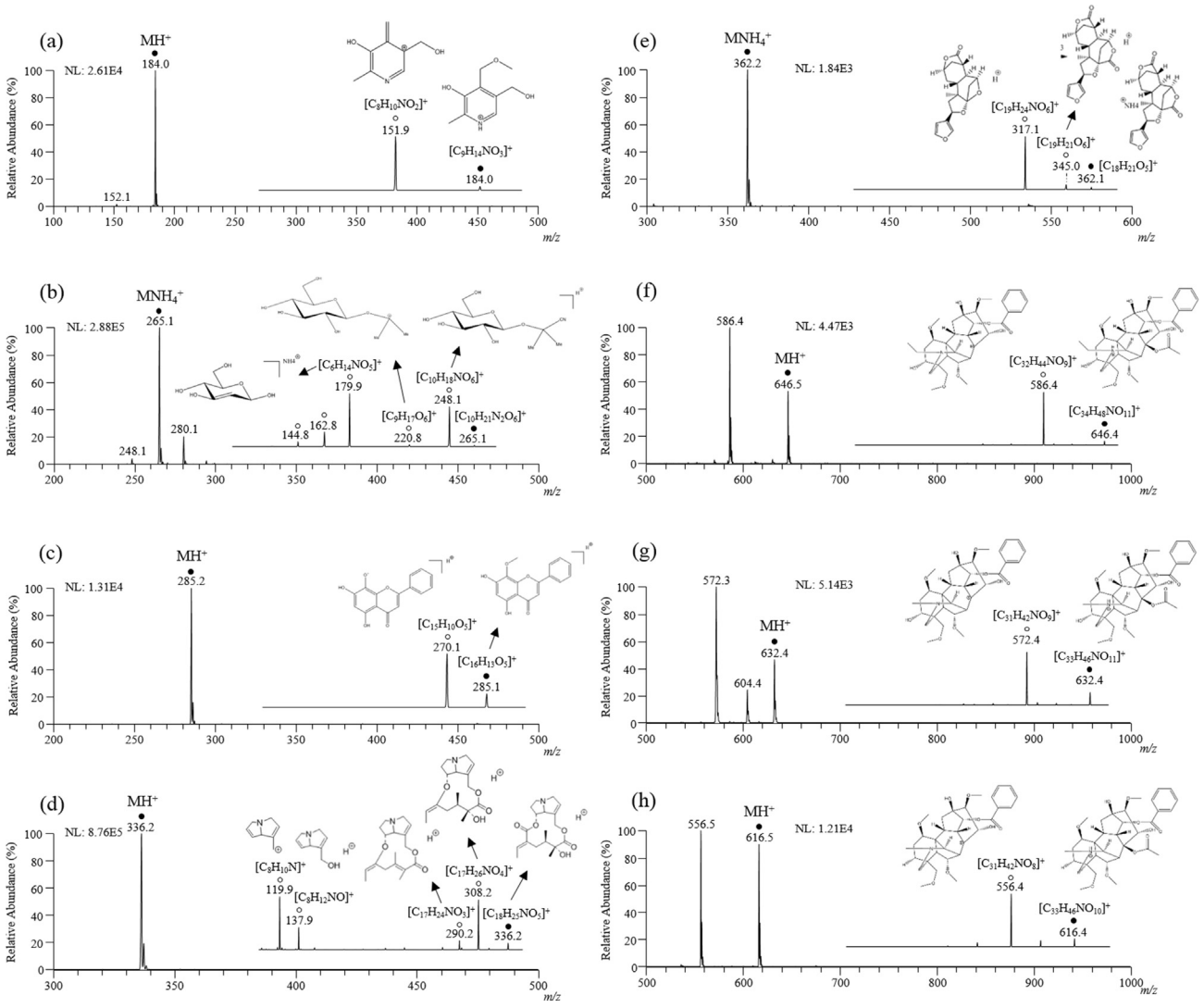

Fig. 2 – Positive mode ELDI mass spectra for the herbal toxin standards (prepared in MeOH, 10 ppm each): (a) ginkgotoxin, (b) linamarin, (c) wogonin, (d) senecionine, (e) diosbulbin B, (f) aconitine, (g) mesaconitine, and (h) hepaconitine. Insets show the corresponding MS/MS mass spectra for each analyte. Precursor ions of the herbal toxins are labeled ●, while product ions are labeled ○. NL: normalized level expressed in absolute intensity counts.

Table 1 – Results of ELDI/MS and ELDI/MS/MS analyses of herbal toxin standards.

Herb	Detected toxin	Precursor ion (*m/z*)	Product ion (*m/z*)
Ginkgo biloba	Ginkgotoxin	184.0 $[M+H]^+$	151.9[a]
Dioscorea hispida	Dioscorine	222.1 $[M+H]^+$	178.1, 140.1[a]
Manihot esculenta	Linamarin	265.1 $[M+NH_4]^+$	248.1, 220.8, 179.9[a], 162.8, 144.8
Scutellaria baicalensis	Wogonin	285.2 $[M+H]^+$	270.1[a]
Senecio vulgaris L.	Senecionine	336.2 $[M+H]^+$	308.2[a], 290.2, 137.9, 119.9
Dioscorea bulbifera	Diosbulbin B	362.2 $[M+NH_4]^+$	345.0, 317.1[a]
Radix aconiti, Radix aconiti	Aconotine,	646.5 $[M+H]^+$	586.4[a]
kusnezoffii, Radix aconiti	Mesaconitine,	632.4 $[M+H]^+$	572.4[a]
lateralis preparata	Hypaconitine	616.5 $[M+H]^+$	556.4[a]

[a] Major product ion observed during quantitative mass spectrometric analysis.

for 17 analyses of the aconitine ion was 9.44%. To mimic the use of ELDI/MS in a clinical setting, between-run tests were also performed. Fig. 4b–d shows that reproducible results were obtained when toxic herbal extracts of radix aconiti were analyzed by three different operators. In these cases, the RSD of the aconitine ion signal was found to be less than 15%. The relatively good within-run and between-run stability of ELDI/MS makes it a reliable technique that is applicable for use in

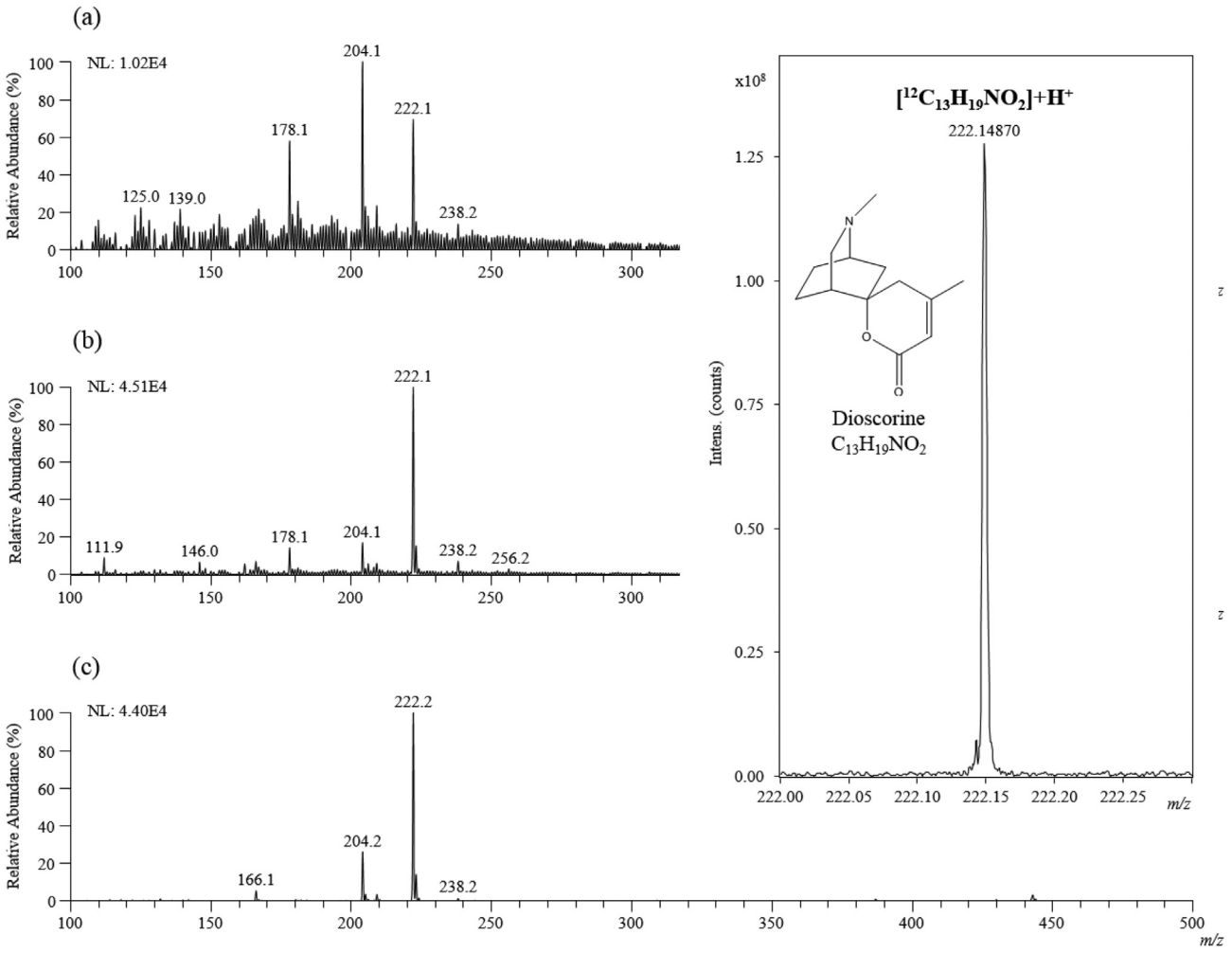

Fig. 3 – Evaluation of different ELDI/MS sampling methods for *Dioscorea hispida*: (a) directly sweeping the surface of the herb section, (b) sampling the ground powder, and (c) sampling the solvent extract of the ground powder. Inset: the mass spectrum for a *Dioscorea hispida* extract analyzed using FT-ICR/MS. (NL: normalized level expressed in absolute intensity counts).

emergency management, providing data that are reliable between different operators.

To efficiently distinguish morphologically similar toxic and non-toxic herbs, we further analyzed our results using principal component analysis (PCA). The results of PCA showed that *D. bulbifera* and *F. multiflora* could be clearly distinguished based on their different chemical fingerprints (Fig. 5a). Using the same approach, we were also able to distinguish *D. hispida*, *D. japonica*, *D. alata* L. var. *purpurea*, and *Manihot esculenta* (Fig. 5b), herbs that have similar colors and appearances and are thus undistinguishable to the general public. We were also able to distinguish *Alocasia macrorrihize*, *C. esculenta* (L.) Schott cv. Betelnut, and *C. esculenta* (L.) Schott cv. Mein samples (Fig. 5c).

Although this study focuses on the rapid qualitative analysis of herbal toxins in an emergency setting, such an analytical approach can also be used to semi-quantify these herbal toxins. However, since the distribution of the toxin on the surface of herb sections may not be homogenous, different toxin ion signal intensities would be observed if samples were collected at different locations on the herb surface. To overcome this shortcoming for quantitative analysis, each herb section was ground into a powder and then extracted using an organic solvent so that the toxins were homogenously distributed in solution. The extraction efficiency of toxins when using different organic solvents was studied (Figure S3), where EA yielded the highest signal intensities for extracted aconitine, mesaconitine, and hypaconitine from three herbal powders (R. aconiti, R. aconiti kusnezoffii, and R. aconiti lateralis preparata, respectively). Even though the extraction efficiency of water was not the best among the examined organic solvents, water is usually used when cooking herbs, so it was used in subsequent studies for extracting herbal ingredients to mimic real-life situations.

Fig. 6 shows the ELDI mass spectra for the extracts from the powders of three Chinese herbs (R. aconiti, R. aconiti kusnezoffii, and R. aconiti lateralis preparata). Toxins such as aconitine (*m/z* 646), mesaconitine (*m/z* 632), and hypaconitine (*m/z* 616) were detected in all herbal extracts. The highest ion signals for these toxins were found in the R. aconiti extracts (Fig. 6a), while the lowest ion signals for these toxins were found in the R. aconiti lateralis preparata extracts (Fig. 6c). The

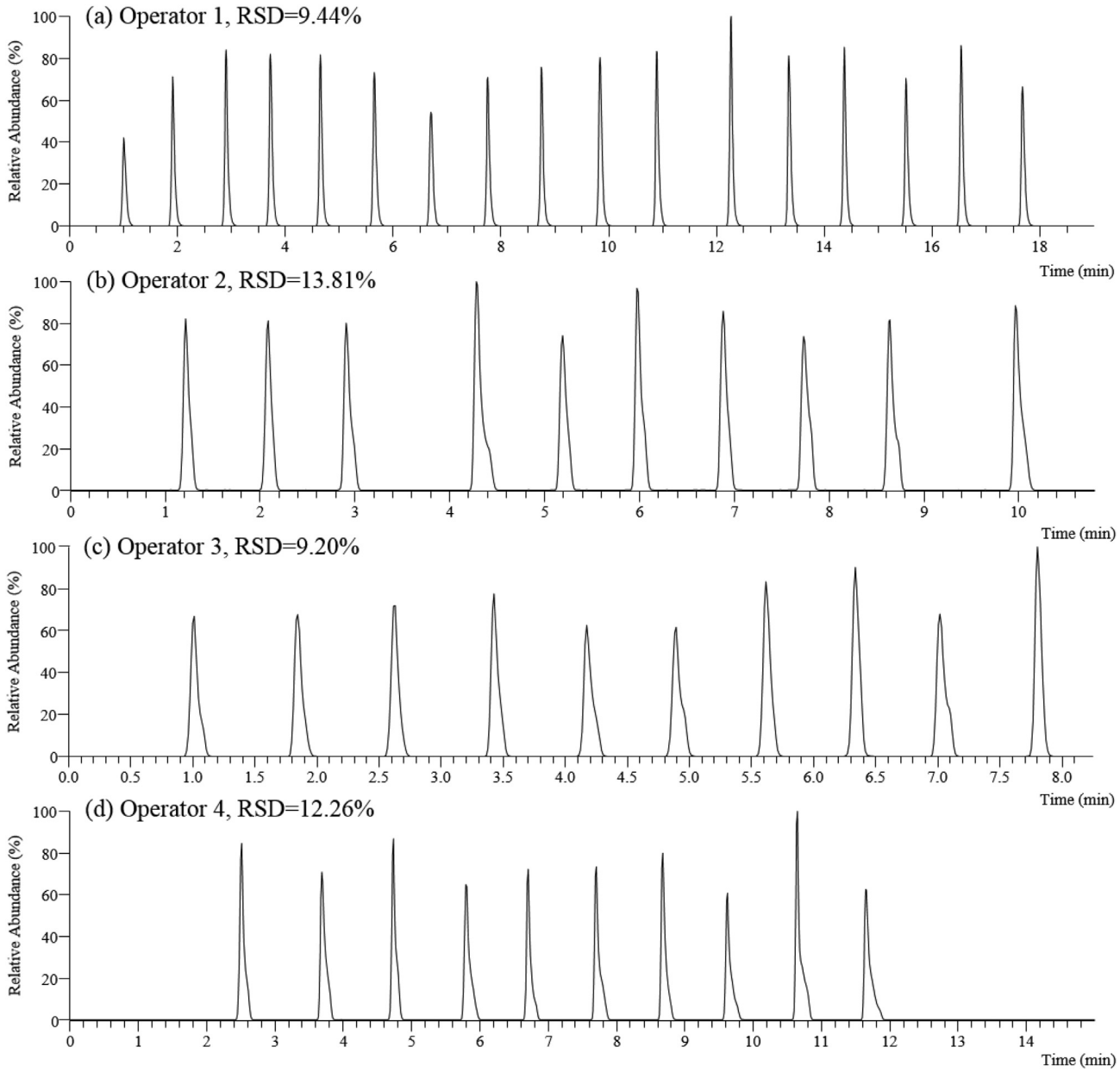

Fig. 4 – (a) Within-run ELDI/MS tests (n = 17) of aconitine standard solution (10 ppm). (b–d) Between-run ELDI/MS tests (n = 10) of Radix aconiti extracts based on the detection of aconitine by three different operators.

results indicated that the R. aconiti sample may be an undomesticated variant due to its high abundance of toxins.

Even though the objective of this study focuses on the qualitative determination of herbal toxins in Chinese herbs, ELDI/MS can still be used for quantitative toxin analysis. The results of repetitive analysis (n = 17) indicate that stable ion signals were obtained and therefore the peak areas of the analytes were adopted for calibration. Fig. 7 shows the linearity of ELDI/MS over a range of 10 ppb – 10 ppm for the detection of three toxin standards. Three replicates were performed for each sample solution. The R^2 values for aconitine, mesaconitine, and hypaconitine were 0.9959, 0.9988, and 0.9988, respectively. To assess the applicability of ELDI/MS for quantitative analysis, we compared the quantitative results obtained by ELDI/MS and LC/MS using R. aconiti as a reference. A sample of R. aconiti was ground and extracted with water (20 mg sample/1 mL solution). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was also performed to characterize the toxins contained in R. aconiti. The toxin ion signals from the water extract were compared with those from the aconitine, mesaconitine, and hypaconitine standard solutions. The concentrations of aconitine, mesaconitine, and hypaconitine in the water extract determined by ELDI/MS were 0.42 ± 0.039 ppm, 0.61 ± 0.015 ppm and 4.16 ± 0.095 ppm, respectively. On the other hand, the concentrations of aconitine, mesaconitine, and hypaconitine in the water extract determined by LC-MS/MS was 0.33 ± 0.002 ppm, 0.44 ± 0.002 ppm and 3.26 ± 0.042 ppm, respectively (Table 2).

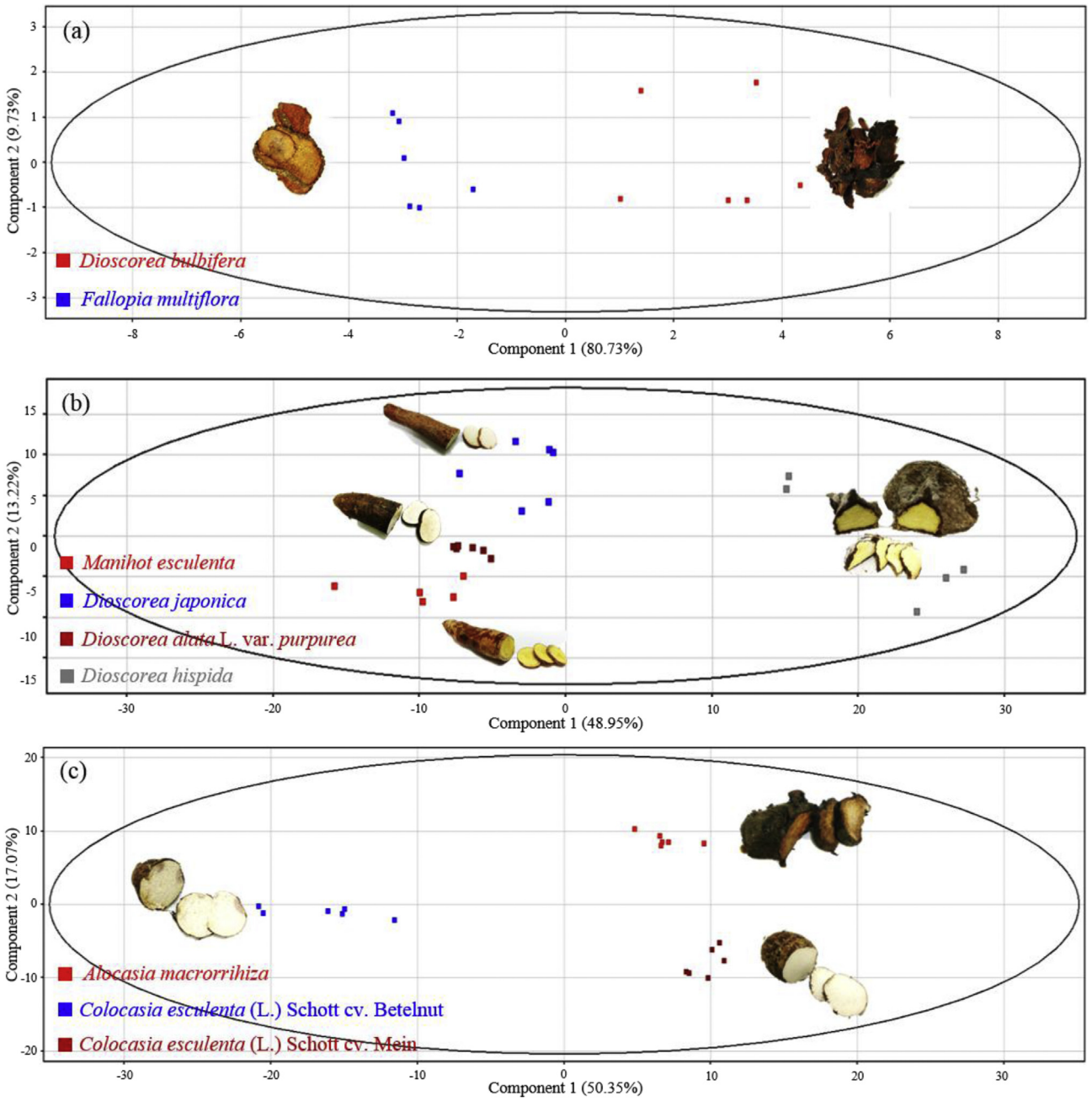

Fig. 5 – Principal component analysis of the ELDI mass spectra (*m/z* 100 to 1000) for (a) *Dioscorea bulbifera* and *Fallopia multiflora*, (b) *Dioscorea hispida*, *Dioscorea japonica*, *Dioscorea alata* L. var. *purpurea*, and *Manihot esculenta*, and (c) *Alocasia macrorrihiza*, *Colocasia esculenta* (L.) Schott cv. Betelnut, and *Colocasia esculenta* (L.) Schott cv. Mein samples.

The concentrations of aconitine, mesaconitine, and hypaconitine as obtained by both approaches are consistent, indicating that ELDI/MS is capable of providing quantitative results for the extracts of the herbal powders.

4. Discussion

The herbs used in TCM often contain a complex mixture of both beneficial and toxic phytochemicals and are prepared for use from raw materials with little or no separational procedures. The inappropriate use of TCM herbs has resulted in many fatalities, especially for young children [19–26]. It is therefore necessary to promptly identify the toxins in these herbs for emergency management. In practice, the diagnosis of herbal toxicity is often solely based on clinical grounds since sophisticated assays for targeted toxicological screening may not be readily accessible. Patients with a history of ingesting plants or herbal medicines that affect multiple systems (e.g. neurological, cardiovascular, and gastrointestinal effects) should be diagnosed to determine whether the patient is suffering from herbal poisoning [27]. Both herbal residues and written prescriptions should be provided by the patient and analyzed to verify the diagnosis and nature of the health

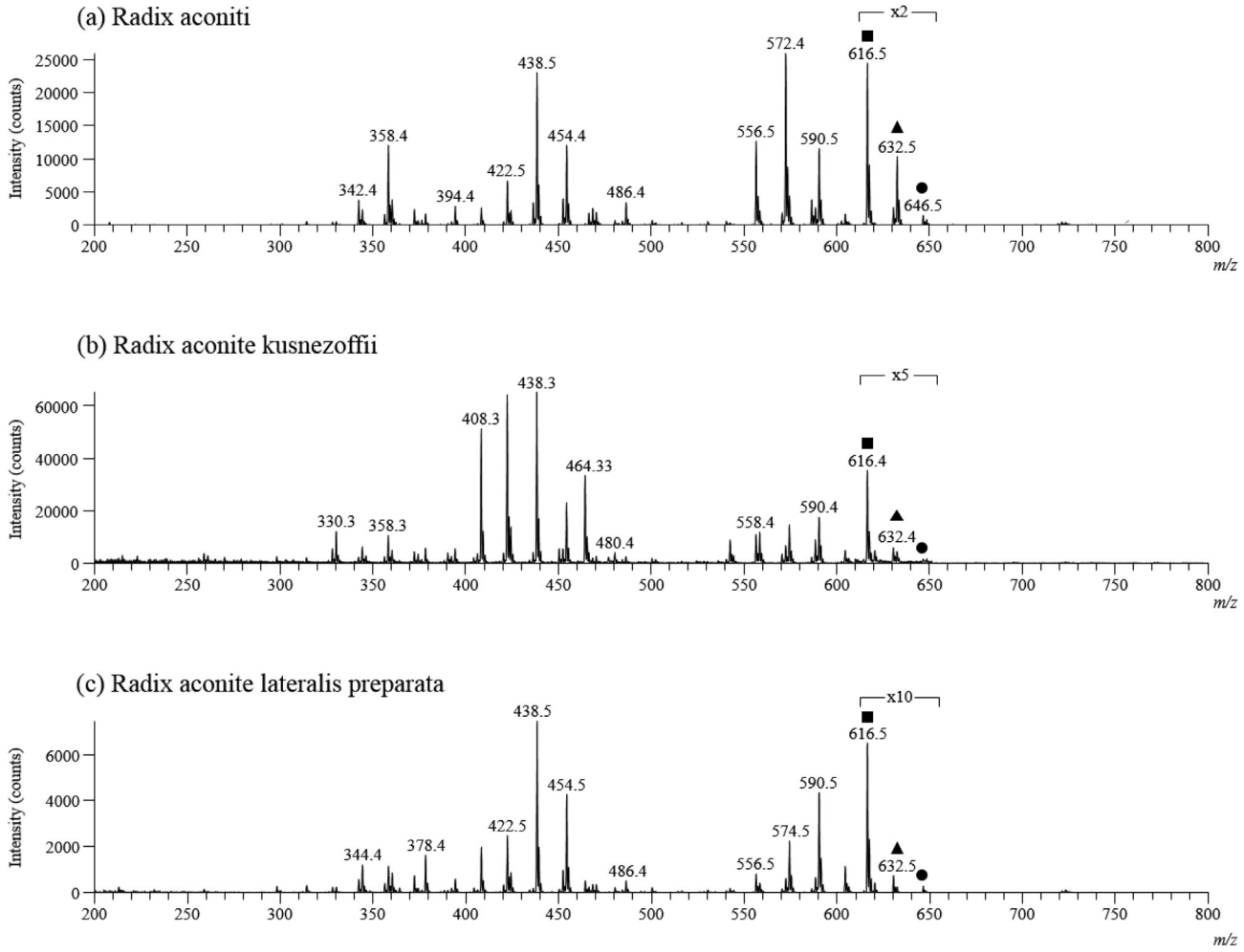

Fig. 6 – ELDI mass spectra for three similar Chinese herbal extracts (a) Radix aconiti, (b) Radix aconite kusnezoffii, and (c) Radix aconite lateralis preparata (●: aconitine, ▲: mesaconitine, ■: hypaconitine).

problem (e.g. use of a larger-than-recommended dose, dispensing errors, and substitution with other herbs) [28]. If the written prescriptions from the herbalist are not available for review, the diagnosis of herbal poisoning should be confirmed by toxicological analysis of the herbal residues and biological samples. Nevertheless, these conventional approaches are sophisticated and time-consuming and are unable to provide toxicological information for timely emergency care.

It is a rule of thumb in many emergency medicine textbooks that "If poisoning is probable, treat the patient immediately. Do not wait for laboratory confirmation". This reflects the fact that the current process of laboratory confirmation is not timely enough to provide an early, rapid, and precise identification of the ingested poisons to save lives. However, it is evident that treating an intoxicated patient with full knowledge of the exact identity of the toxic substances ingested is certainly a preferred option to mere guess-work bases on indirect clues. Although the remains of the herbs or their decoctions are usually brought to the emergency department with the intoxicated patients, herbal experts are not always available on site for immediate identification of the herbs, let alone the toxic compounds in them. The outcome of our study indicated that probe sampling combined with ELDI/MS for the rapid point-of-care identification of toxic herbal compounds to treat poisoning is a promising alternative to conventional diagnostic methods in an emergency setting.

In this study, nine commonly misused herbs (*A. macrorrhiza*, *D. bulbifera*, *D. hispida*, *G. biloba*, *M. culenta*, R. aconiti, R. aconiti kusnezoffii, R. aconiti laterali spreparata, and *S. baicalensis*) and their toxic standards were analyzed using the developed analytical approach. The most commonly found toxins in *R. aconiti*, *R. aconiti kusnezoffii*, and *R. aconiti lateralis preparata* are aconitine (*m/z* 646), mesaconitine (*m/z* 632) and hypaconitine (*m/z* 616). Previous studies indicate that the toxic compounds commonly found in aconiti roots are C_{19}-diterpenoid alkaloids such as aconitine, mesaconitine, and hypaconitine [29,31], which is in accordance with our experimental results. Mesaconitine is similar to aconitine in terms of toxicity, while hypaconitine is more potent than both aconitine and mesaconitine in blocking neuromuscular transmission [35]. In Asia, aconite poisoning is much more common than other parts of the world for continuing use of aconite roots in traditional medicine as analgesic, anti-inflammatory, and cardiotonic agents [30,31]. However, with its accessibility and the increasing popularity of herbal

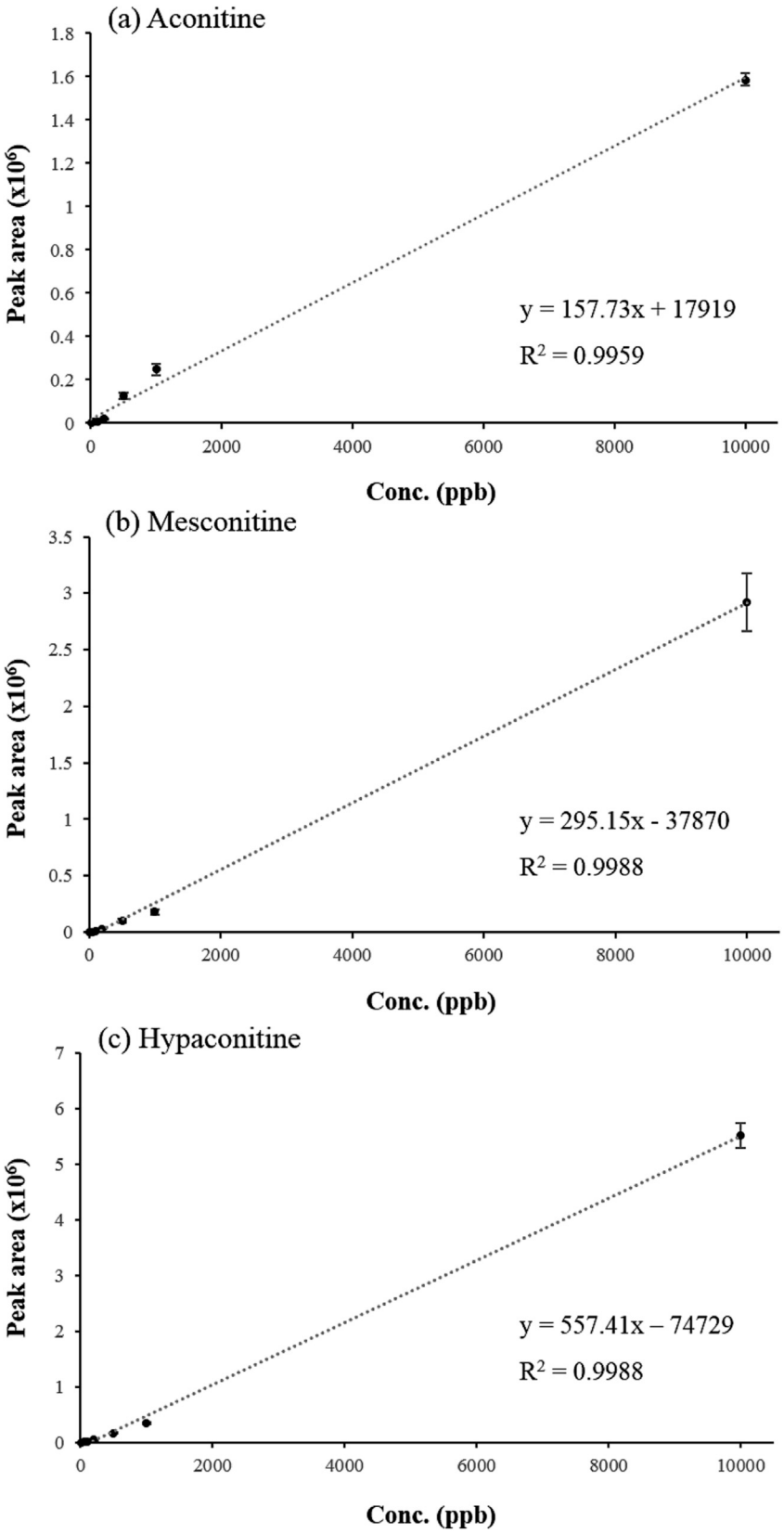

Fig. 7 – Linearity test using standard solutions of (a) aconitine, (b) mesconitine, and (c) hypaconitine (10 ppb - 10 ppm). ELDI/MS was operated in positive mode for analysis.

Table 2 – The results of aconitine, mesaconitine, and hypaconitine extracted from R. aconiti by two mass spectrometric approaches - TD-ESI/MS and LC-MS/MS.

Analyte	ELDI/MS (LC-MS/MS)		
	Aconitine	Mesaconitine	Hypaconitine
Dilution factor	100 (100)	100 (100)	100 (100)
Sample volume (μL)	2 (5)	2 (5)	2 (5)
Conc. (ppm)	0.42 ± 0.039 (0.33 ± 0.002)	0.61 ± 0.015 (0.44 ± 0.002)	4.16 ± 0.095 (3.26 ± 0.042)

medicines in western societies, aconite poisoning can occur anywhere in the world [32–34].

The most common toxic compounds found in *D. bulbifera* are diosbulbin A, diosbulbin B, diosbulbin C, diosbulbin D, diosgenin, and dioscin. Hepato-toxicity induced by diosbuins in *D. bulbifera* has greatly reduced its use in clinical medicine [36,37]. *A. macrorrhiza* contains calcium oxalate which can cause serious symptoms when ingested in excessive amounts — more than 2 g of oxalate at a time is thought to be fatal for humans [38]. This may be why acridity, inflammation and occasional toxicity symptoms can be observed in patients who have ingested wild yam tubers. The main toxin found in *D. hispida* is dioscorine, a toxic alkaloid also found in other yam species [39–41]. Dioscorine can trigger the fatal paralysis of the nervous system when a piece of the tuber weighing more than 100 g is ingested [42].

M. Esculenta, a class of cassava, produces two cyanogenic glucosides, linamarin and a small amount of lotaustralin (methyl linamarin). These cyanogenic glucosides are hydrolyzed by linamarase to produce cyanohydrin and glucose, after which the cyanohydrin breaks down spontaneously above pH 5 to yield hydrogen cyanide (HCN) and a ketone. The ingestion of linamarin in *M. Esculenta* can lead to cyanide intoxication, even though the cassava is arguably one of the most important food sources for humans. Cyanide intake from consumption of cassava can (1) cause acute intoxication with symptoms of dizziness, headache, nausea, vomiting, stomach pain, diarrhea and sometimes death [43,44]; (2) exacerbate goiter and cretinism due to iodine deficiency [45]; (3) contribute to the onset of tropical ataxic neuropathy (TAN), which causes unsteady gait, loss of sensation in the hands, loss of vision, deafness, and weakness [46,47]; and (4) lead to konzo, an irreversible paralysis of the legs which occurs mainly in children and women of child-bearing age [48,49].

5. Conclusion

In this study, we combined probe sampling with ELDI/MS to accomplish the rapid identification of non-volatile toxins in herbal samples. Since no sample pretreatment was required, the time to complete one single analysis is about 30 s. The identity of each toxin detected using ELDI/MS was further confirmed using FT-ICR/MS and MS/MS analyses. The detection sensitivity of herbal toxins through this approach was in the sub-ppm level. Toxic and non-toxic herbs were efficiently distinguished by PCA using experimentally obtained ELDI/MS data. The developed technique was also demonstrated to be useful in semi-quantifying the toxins extracted from herbal powder samples. The aforementioned merits of ELDI/MS contributed to the feasibility of its point-of-care applications in the emergency department for early and accurate identification of herbal toxins, proving it a highly efficient, sensitive, and precise tool for guiding appropriate clinical management of intoxication by herbal toxins.

As a conclusion, in this study, we achieved our goal to develop an analytical approach that enables the rapid identification of toxins in herbs. Since the typical time required to complete an ELDI/MS analysis is about 30 s, our technique is useful for point-of-care applications in emergency management. Based on the results described in this study, the exact identities of ingested herbal toxins can be accurately identified in the emergency department, bypassing the need to rely on potentially unreliable descriptions from patients, undependable morphological recognition of the herbs or plants involved, and time-consuming diagnostic identification of the toxins.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfda.2018.11.001.

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