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SHORT COMMUNICATION

Development and application of a forensic toxicological library for identification of 56 natural toxic substances by liquid chromatography–quadrupole time-of-flight mass spectrometry

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Abstract

Purpose The present study aims to develop a forensic toxicological library to identify 56 natural toxic substances by liquid chromatography–quadrupole time-of-flight tandem mass spectrometry (LC–QTOF-MS/MS).

Methods For setting up the library of product ion spectra, individual substances (31 plant toxins, 7 mushroom toxins, 5 marine toxins, 5 frog venoms, 4 mycotoxins, and 4 substances derived from plants) were analyzed by LC–QTOF-MS/MS with positive and negative ionization. The product ion spectra were acquired at the collision energies (CEs) of 20, 35, and 50 eV in single enhanced product ion mode and then in collision energy spread mode in which the CE ramp range was set to 35 ± 15 eV.

Results To test the performance of the library, human blood plasma samples were spiked with a mixture of lycorine and domoic acid, extracted by acetonitrile deproteinization and analyzed by LC–QTOF-MS/MS. Identification by our library search could be achieved for these toxins at the purity scores of 79.1 and 67.2, respectively. The method was also applied to postmortem blood from a death case with an aconite intake, and showed that four toxins in an aconite could be identified in the blood sample at the purity scores of 54.6–60.3.

Conclusions This library will be more effective for the screening of natural toxic substances in routine forensic toxicological analysis. To our knowledge, there are no reports dealing with development of library for natural toxic substances by LC–QTOF-MS/MS.

Keywords Natural toxic substances · Forensic toxicological library · Screening and identification · Tetrodotoxin · Aconitine and amanitin · LC–QTOF-MS/MS

Introduction

Natural toxins are chemicals that are naturally produced by living organisms such as some plants, mushrooms, marine animals, and so on [1]. These toxins are not harmful to the

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organisms themselves but they may be toxic to other creatures, including humans, when eaten [1]. For example, tetrodotoxin in pufferfish and some marine animals is a powerful sodium channel blocker in excitable tissues such as nerves and muscles, and is about 10,000 times more lethal than cyanide by weight [2]. Forensic toxicology is a part of the pharmacological science, which is concerned with the identification/quantification and effects of various drugs and poisons in human beings [3]. Natural toxins are very important analytical targets in forensic toxicology [4–6]. It is difficult to attribute a cause of death to natural toxin(s) in routine toxicological analysis because there is currently no effective routine screening method for a variety of natural toxins [7].

In the last decade, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developing its importance for the screening of drugs and/or poisons, most of which is based on triple quadrupole mass spectrometers employing multiple reaction monitoring survey scan followed by a product ion scan by electrospray ionization [8–11]. The application of this method is limited because of disturbances by high matrix burden and co-eluting peaks, indicating that analytes can be detected only if they are abundantly contained in the samples and that the method can easily lead to false positive/negative detection [12]. This drawback prompts the need for additional approaches achieving unambiguous identification of analytes.

Recently, liquid chromatography–quadrupole time-of-flight tandem mass spectrometry (LC–QTOF-MS/MS) has been utilized to develop libraries of compounds relevant to clinical and forensic toxicology [13–21]. However, to the best of our knowledge, there are no reports on development of library for natural toxic substances by LC–QTOF-MS/MS. In this paper, we describe the development and application of forensic toxicological library of 56 natural toxic substances using LC–QTOF-MS/MS.

Materials and methods

Chemicals and reagents

Target natural toxic substances were selected on the basis of previously reported poisoning cases as follows: 31 plant toxins (coniine, lycorine, galantamine, atropine, picrotoxinin, scopolamine, picrotin, strychnine, colchicine, veratramine, cyclopamine, jervine, amygdalin, aconine, cymarin, convallatoxin, cucurbitacin E, oleandrin, benzoylmesaconine, benzoylaconine, tubocurarine, hypaconitine, mesaconitine, 14-anisoylaconine, aconitine, jesaconitine, digitoxin, digoxin, α-chaconine, α-solanine, and dioscin), 7 mushroom toxins (muscimol, ibotenic acid, muscarine, phalloidin, γ-amanitin, α-amanitin, and β-amanitin), 4 mycotoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2), 5 marine toxins (domoic acid, tetrodotoxin, okadaic acid, dinophysistoxin-1, and brevetoxin b), and 5 frog venoms (bufotenine, resibufogenin, bufalin, cinobufagin, and batrachotoxin). In addition to the natural toxins, berberine, cinchonidine, diosgenin, and quinine were selected as target substances, which are considered to be important materials of herbal medicines. Scopolamine, aflatoxin B1, aflatoxin B2, tetrodotoxin, quinine, aflatoxin G1, aflatoxin G2, resibufogenin, bufalin, colchicine, cyclopamine, diosgenin, cinobufagin, amygdalin, benzoylmesaconine, tubocurarine, mesaconitine, 14-anisoylaconine, jesaconitine, okadaic acid, and dinophysistoxin-1 were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan); muscimol, coniine, ibotenic acid, muscarine, bufotenine, cinchonidine, strychnine, cymarin, convallatoxin, cucurbitacin E, oleandrin, digitoxin, digoxin, and α-solanine from Sigma-Aldrich (St. Louis, MO, USA); picrotoxinin, picrotin, domoic acid, γ-amanitin, and β-amanitin from Abcam Biochemicals (Cambridge, UK); berberine, aconine, and benzoylaconine from Cayman Chemical (Ann Arbor, MI, USA); galantamine, atropine, and jervine from Tokyo Chemical Industry (Tokyo, Japan); α-chaconine and dioscin from Extrasynthese (Lyon, France); batrachotoxin and aconitine from Latoxan (Valence, France); phalloidin and α-amanitin from Merck Millipore (Billerica, MA, USA); lycorine, hypaconitine, brevetoxin b, and veratramine from Enzo Life Sciences (New York, NY, USA); Kishida Chemical (Osaka, Japan), LKT Laboratories (St. Paul, MN, USA), and Toronto Research Chemicals (Toronto, Canada), respectively. The stock solutions of all substances were prepared at a concentration of 10–1000 μg/mL. Muscarine, lycorine, cinchonidine, scopolamine, tetrodotoxin, quinine, berberine, resibufogenin, cinobufagin, amygdalin, hypaconitine, and α-amanitin were dissolved in distilled water (DW). Aconitine and benzoylaconine were dissolved in acetonitrile. Other toxins were dissolved in methanol solution. Stock solutions were stored at − 80 °C until analysis. Methanol, acetonitrile and DW of the HPLC grade were purchased from Kanto Chemical (Tokyo, Japan). Other common chemicals used were of the highest purity commercially available. Human whole blood was obtained from Tennessee Blood Services (Memphis, TN, USA).

LC–QTOF-MS (/MS) conditions

Sciex Triple TOF 5600 mass spectrometer (Sciex, Framingham, MA, USA) and Shimadzu NexeraX2 LC system (Shimadzu Co., Kyoto, Japan) were used for analysis. The column used for chromatographic separation was L-column ODS (150 × 1.5 mm i.d., particle size 5.0 μm; Chemicals Evaluation and Research Institute, Sugito, Saitama, Japan). The column temperature was maintained at 40 °C, and the gradient system was used with a mobile phase (A) 10 mM ammonium formate in 5% methanol aqueous solution and

(B) 10 mM ammonium formate in 95% methanol solution. Linear gradient elution was started from 100% A to 100% B over 15 min. The 100% B was held for 5 min. It was then returned to 100% A over 10 min for the next run. The autosampler was maintained at 4 °C and the injection volume was 10 µL. Electrospray ionization was used in both positive and negative modes. The optimal MS parameters were declustering potential at 80 V and information dependent acquisition (IDA) criteria set at over 50 cps. The LC–QTOF-MS system allowed the acquisition of highly sensitive full scan MS spectra with high resolution and mass accuracy. In addition, IDA can be used to collect MS/MS spectra for compound identification based on MS/MS library searching.

This LC–QTOF-MS (/MS) method had several advantages for accurate detection of natural toxic substances. For instance, the mass spectrometer used in this study, triple TOF 5600, had high throughput which enabled very fast MS/MS acquisition rates at as low as 20 ms accumulation time in IDA mode. To fully leverage the instrument speed and obtain the best depth of coverage, the IDA workflow was optimized such that software overhead is minimized. The IDA method consisted of a high-resolution TOF-MS survey scan could follow up to 50 MS/MS ions. The combined use of high-resolution MS and IDA were extremely effective for the simultaneous detection of natural toxic substances in forensic samples. The instrument gave the resolution of 35,000.

Data acquisition and processing were performed by Analyst software and Peak View incorporated with the XIC Manager application (Sciex). The XIC Manager can be used for targeted processing of high-resolution MS and MS/MS data allowing for screening and identification with the highest confidence based on retention time (RT), mass error of molecular ion, isotopic pattern, and automatic MS/MS library searching.

Construction of library of natural toxic substances by LC–QTOF-MS (/MS)

All target substances were analyzed to investigate their retention properties, isotopic ratios and high-resolution MS/MS spectra obtained by collision induced dissociation (CID) with the injected amount of each compound of 0.1 µg. The four spectra were acquired at the collision energy (CE) at 20, 35, and 50 eV in single enhanced product ion (EPI) mode together with collision energy spread (CES) mode, in which the CE ramp range was set to 35 ± 15 eV. The CES parameter, in conjunction with the CE, determined the collision energy applied to the precursor ion in a product ion scan. The CE is ramped from low to high energies. The selection ranges of the precursor ion and RT of each compound for acquiring the library search were 20 mDa and 4.0 min, respectively. Compound identification was based on chromatographic and mass spectrometric information, including RT error, mass error, isotope matching, and library search results. The product ion for library search could be chosen from four spectra by CID energies of (±) 20, 35, 50, and 35 ± 15 eV, automatically.

Limits of detection and recovery rates

To determine the limits of detection (LODs), 5 plots with different concentrations of each substance spiked into blank blood plasma were used. The LODs were defined as the concentrations giving a signal-to-ratio of 3:1. The recovery rates were calculated by the ratio of peak area obtained from a target substance spiked into ante-extraction matrix to that obtained from the substance spiked into post-extraction matrix.

Analysis of spiked samples

The blood plasma samples spiked with lycorine (1 µg/mL) and domoic acid (10 µg/mL) were prepared. A 100-µL volume of blood plasma containing the target substances was mixed with 100 µL methanol and 300 µL acetonitrile. The mixture was then mixed by vortexing for 30 s and centrifuged at 15,000 *g* for 10 min. The supernatant was transferred to another tube and evaporated with a centrifugal evaporator (CVE-2000; Tokyo Rikakikai, Tokyo, Japan). The residue was reconstituted in 100 µL of 10 mM ammonium formate in 5% methanol solution and mixed by vortexing for 1 min. A 10-µL of the extract solution was analyzed by LC–QTOF-MS/MS using our newly developed library.

Application to forensic autopsy samples

A 45-year-old male with groan was found at his home. He was taken to hospital by an ambulance, but died shortly afterward. Beside the body in the room, there were dried roots of an aconite plant. Femoral vein and right and left heart blood samples were collected at autopsy performed in our laboratory and stored at − 80 °C until analysis. The blood samples were treated and analyzed in the same way as spiked samples described above.

Results

Development of library of natural toxic substances

Registered data consisted of 56 natural toxic substances with compound name, source, formula, exact mass, polarity, exact mass of precursor ion, ion form, RT, LOD, and recovery rate (Table 1). Extracted ion chromatograms of simultaneous determination of 56 substances are shown in Fig. 1. Product

Table 1 Registered data in a forensic toxicological library for natural toxic substances for liquid chromatography–quadrupole time-of-flight mass spectrometry listed according to the retention times

No	Compound name	Source	Molecular formula	Exact mass (Da)	Polarity	Extracted mass (ionized form) calculated	Ion form	Retention time (min)	LOD (ng/mL)	Recovery rate (%)
1	Tetrodotoxin	*Tetraodontidae*	$C_{11}H_{17}N_3O_8$	319.1016	Pos	320.1088	$[M+H]^+$	1.9	100	75.5
2	Muscimol	*Amanita pantherina*	$C_4H_6N_2O_2$	114.0429	Pos	115.0502	$[M+H]^+$	2.1	500	88.4
3	Ibotenic acid	*Amanita pantherina*	$C_5H_6N_2O_4$	158.0328	Pos	159.0400	$[M+H]^+$	2.1	100	98.6
4	Muscarine	*Amanita pantherina*	$C_9H_{20}NO_2$	174.1494	Pos	174.1489	$[M]^+$	3.2	5	68.2
5	Domoic acid	*Chondria armata*	$C_{15}H_{21}NO_6$	311.1369	Pos	312.1442	$[M+H]^+$	4.2	10	92.8
6	Bufotenine	*Bufo gargarizans*	$C_{12}H_{16}N_2O$	204.1263	Pos	205.1335	$[M+H]^+$	6.2	0.5	69.1
7	Amygdalin	*Prunus armeniaca*	$C_{20}H_{27}NO_{11}$	457.1584	Pos	475.1922	$[M+NH_4]^+$	8.4	0.5	70.2
8	Coniine	*Conium maculatum*	$C_8H_{17}N$	127.1361	Pos	128.1434	$[M+H]^+$	9.0	5	81.4
9	Aconine	*Aconitum*	$C_{25}H_{41}NO_9$	499.2781	Pos	500.2854	$[M+H]^+$	9.5	0.5	71.7
10	Lycorine	*Lycoris radiata*	$C_{16}H_{17}NO_4$	287.1158	Pos	288.1230	$[M+H]^+$	9.6	0.05	90.7
11	Galantamine	*Lycoris radiata*	$C_{17}H_{21}NO_3$	287.1521	Pos	288.1594	$[M+H]^+$	9.7	0.05	88.5
12	α-Amanitin	*Amanita phalloides*	$C_{39}H_{54}N_{10}O_{14}S$	918.3542	Pos	919.3615	$[M+H]^+$	10.2	10	70.5
13	β-Amanitin	*Amanita phalloides*	$C_{39}H_{53}N_9O_{15}S$	919.3382	Pos	920.3455	$[M+H]^+$	10.2	10	71.0
14	Atropine	*Datura metel*	$C_{17}H_{23}NO_3$	289.1678	Pos	290.1751	$[M+H]^+$	10.7	0.05	75.0
15	Tubocurarine	*Chondrodendron tomentosum*	$C_{37}H_{41}N_2O_6$	609.2965	Pos	609.2959	$[M]^+$	10.7	5	73.8
16	γ-Amanitin	*Amanita phalloides*	$C_{39}H_{54}N_{10}O_{13}S$	902.3593	Pos	903.3665	$[M+H]^+$	11.0	10	69.0
17	Scopolamine	*Datura metel*	$C_{17}H_{21}NO_4$	303.1471	Pos	304.1543	$[M+H]^+$	11.1	0.1	73.3
18	Picrotin	*Anamirta cocculus*	$C_{15}H_{18}O_7$	310.1053	Neg	309.0980	$[M-H]^-$	11.2	100	96.0
19	Picrotoxinin	*Anamirta cocculus*	$C_{15}H_{16}O_6$	292.0947	Pos	293.1020	$[M+H]^+$	11.5	50	71.1
20	Strychnine	*Strychnos nux-vomica*	$C_{21}H_{22}N_2O_2$	334.1681	Pos	335.1754	$[M+H]^+$	11.7	0.05	78.6
21	Berberine	*Coptis japonica*	$C_{20}H_{18}NO_4$	336.1236	Pos	336.1230	$[M]^+$	12.8	5	76.1
22	Cinchonidine	*Cinchona pubescens*	$C_{19}H_{22}N_2O$	294.1732	Pos	295.1805	$[M+H]^+$	12.9	5	83.1
23	Benzoylaconine	*Aconitum*	$C_{32}H_{45}NO_{10}$	603.3044	Pos	604.3116	$[M+H]^+$	12.9	5	75.5
24	Phalloidin	*Amanita phalloides*	$C_{35}H_{48}N_8O_{11}S$	788.3163	Pos	789.3236	$[M+H]^+$	12.9	100	83.4
25	Aflatoxin G2	*Aspergillus flavus*	$C_{17}H_{14}O_7$	330.0740	Pos	331.0812	$[M+H]^+$	13.0	1	87.5
26	Benzoylmesaconine	*Aconitum*	$C_{31}H_{43}NO_{10}$	589.2887	Pos	590.2960	$[M+H]^+$	13.1	0.05	79.8
27	Aflatoxin G1	*Aspergillus flavus*	$C_{17}H_{12}O_7$	328.0583	Pos	329.0656	$[M+H]^+$	13.4	1	82.0
28	Convallatoxin	*Convallaria majalis*	$C_{29}H_{42}O_{10}$	550.2778	Pos	551.2851	$[M+H]^+$	13.6	10	69.1
29	14-Anisoylaconine	*Aconitum*	$C_{33}H_{47}NO_{11}$	633.3149	Pos	634.3222	$[M+H]^+$	13.6	1	79.9
30	Aflatoxin B2	*Aspergillus flavus*	$C_{17}H_{14}O_6$	314.0790	Pos	315.0863	$[M+H]^+$	13.8	1	90.8
31	Quinine	*Cinchona pubescens*	$C_{20}H_{24}N_2O_2$	324.1838	Pos	325.1911	$[M+H]^+$	13.8	5	78.6
32	Colchicine	*Colchicum autumnale*	$C_{22}H_{25}NO_6$	399.1682	Pos	400.1755	$[M+H]^+$	13.9	0.1	97.5
33	Veratramine	*Veratrum album*	$C_{27}H_{39}NO_2$	409.2981	Pos	410.3054	$[M+H]^+$	14.1	0.05	79.2
34	Jervine	*Veratrum album*	$C_{27}H_{39}NO_3$	425.2930	Pos	426.3003	$[M+H]^+$	14.1	50	87.2
35	Batrachotoxin	*Phyllobates terribilis*	$C_{31}H_{42}N_2O_6$	538.3043	Pos	539.3116	$[M+H]^+$	14.1	1	86.3
36	Digoxin	*Digitalis lanata*	$C_{41}H_{64}O_{14}$	780.4296	Neg	825.4278	$[M+HCOO]^-$	14.1	500	86.0

Table 1 (continued)

No	Compound name	Source	Molecular formula	Exact mass (Da)	Polarity	Extracted mass (ionized form) calculated	Ion form	Retention time (min)	LOD (ng/mL)	Recovery rate (%)
37	Aflatoxin B1	*Aspergillus flavus*	$C_{17}H_{12}O_6$	312.0634	Pos	313.0707	$[M+H]^+$	14.2	1	86.3
38	Hypaconitine	*Aconitum*	$C_{33}H_{45}NO_{10}$	615.3044	Pos	616.3116	$[M+H]^+$	14.6	0.1	90.4
39	Cyclopamine	*Veratrum album*	$C_{27}H_{41}NO_2$	411.3137	Pos	412.3210	$[M+H]^+$	14.8	50	76.8
40	Mesaconitine	*Aconitum*	$C_{33}H_{45}NO_{11}$	631.2993	Pos	632.3065	$[M+H]^+$	14.9	5	85.9
41	Aconitine	*Aconitum*	$C_{34}H_{47}NO_{11}$	645.3149	Pos	646.3222	$[M+H]^+$	14.9	0.5	80.1
42	Jesaconitine	*Aconitum*	$C_{35}H_{49}NO_{12}$	675.3255	Pos	676.3328	$[M+H]^+$	14.9	1	75.6
43	Cymarin	*Adonis ramosa*	$C_{30}H_{44}O_9$	548.2985	Pos	549.3058	$[M+H]^+$	15.0	10	82.6
44	Bufalin	*Bufo gargarizans*	$C_{24}H_{34}O_4$	386.2457	Pos	387.2530	$[M+H]^+$	15.1	0.5	80.1
45	Cucurbitacin E	*Lagenaria siceraria*	$C_{32}H_{44}O_8$	556.3036	Pos	574.3374	$[M+NH_4]^+$	15.3	50	72.3
46	α-Solanine	*Solanum tuberosum*	$C_{45}H_{73}NO_{15}$	867.4980	Neg	912.4962	$[M+HCOO]^-$	15.9	5000	93.9
47	α-Chaconine	*Solanum tuberosum*	$C_{45}H_{73}NO_{14}$	851.5031	Neg	896.5013	$[M+HCOO]^-$	16.0	5000	68.6
48	Oleandrin	*Nerium oleander*	$C_{32}H_{48}O_9$	576.3298	Neg	575.3226	$[M-H]^-$	16.7	100	97.4
49	Cinobufagin	*Bufo gargarizans*	$C_{26}H_{34}O_6$	442.2355	Pos	443.2428	$[M+H]^+$	16.8	5	83.1
50	Resibufogenin	*Bufo gargarizans*	$C_{24}H_{32}O_4$	384.2301	Pos	385.2373	$[M+H]^+$	16.9	5	72.1
51	Digitoxin	*Digitalis purpurea*	$C_{41}H_{64}O_{13}$	764.4347	Neg	809.4329	$[M+HCOO]^-$	17.5	100	73.0
52	Okadaic acid	*Halichondria okadai*	$C_{44}H_{68}O_{13}$	804.4660	Pos	805.4733	$[M+H]^+$	17.9	100	94.7
53	Dinophysistoxin-1	*Dinophysis fortii*	$C_{45}H_{70}O_{13}$	818.4816	Pos	819.4889	$[M+H]^+$	18.6	50	87.2
54	Brevetoxin b	*Karenia brevis*	$C_{50}H_{70}O_{14}$	894.4766	Pos	895.4838	$[M+H]^+$	18.9	50	82.4
55	Dioscin	*Dioscorea quinqueloba*	$C_{45}H_{72}O_{16}$	868.4820	Neg	913.4802	$[M+HCOO]^-$	19.8	5000	84.0
56	Diosgenin	*Dioscorea quinqueloba*	$C_{27}H_{42}O_3$	414.3134	Pos	415.3207	$[M+H]^+$	24.3	100	89.8

Reference standards (100 ng each) were used for accurate mass measurements of 56 toxic substances except for the limit of detection (LOD) and recovery rate measurements. Each LOD values was obtained using 5 plots with different concentrations of each substance spiked into blank blood plasma. For recovery rate measurement, 0.1–10 μg/mL in plasma for each substance spiked into ante-extraction and post-extraction matrices was used

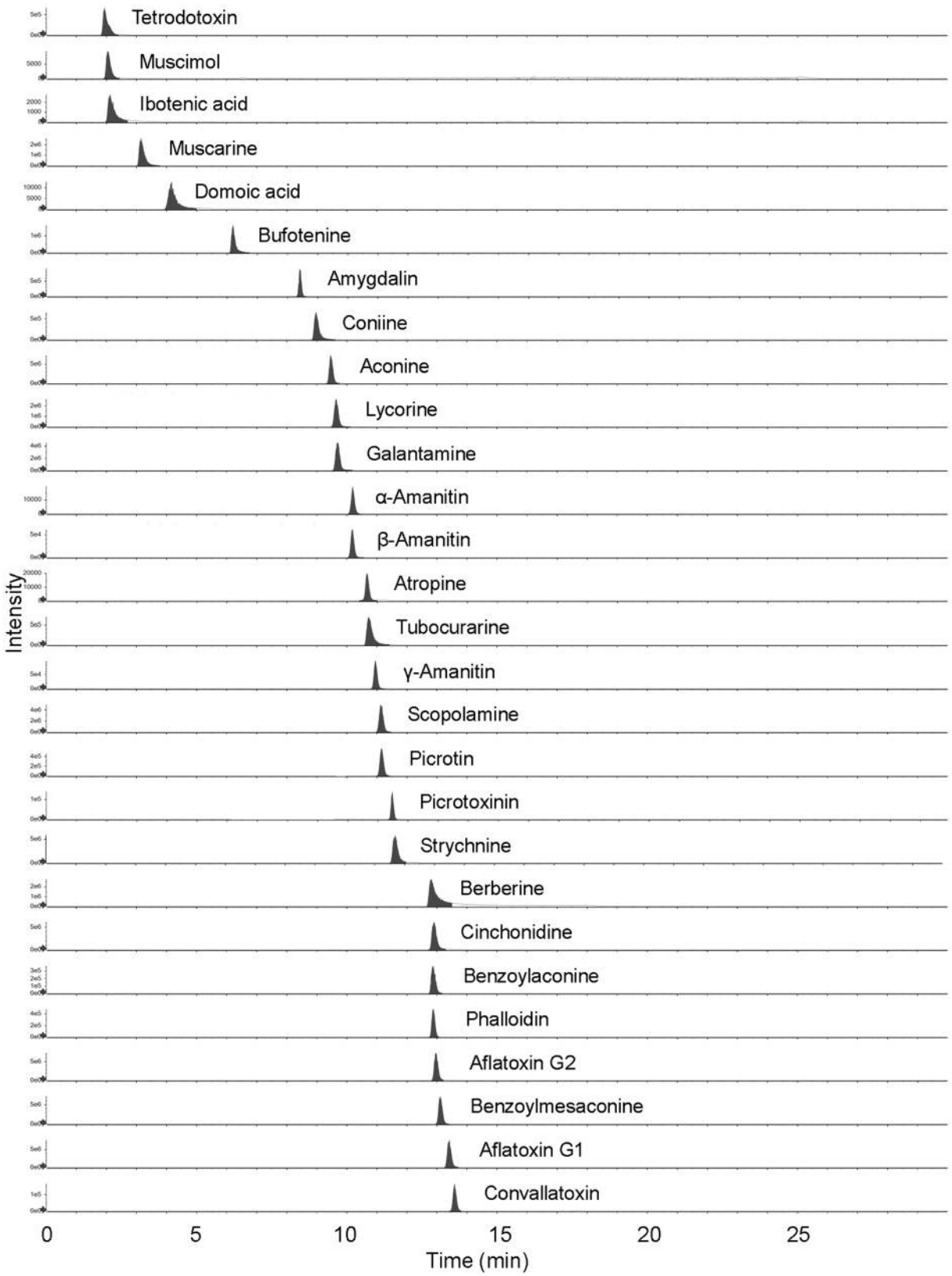

Fig. 1 Extracted ion chromatograms (XICs) of simultaneous determination of 56 natural toxic substances by liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOF-MS). The reference standards (100 ng each) were injected to the instrument

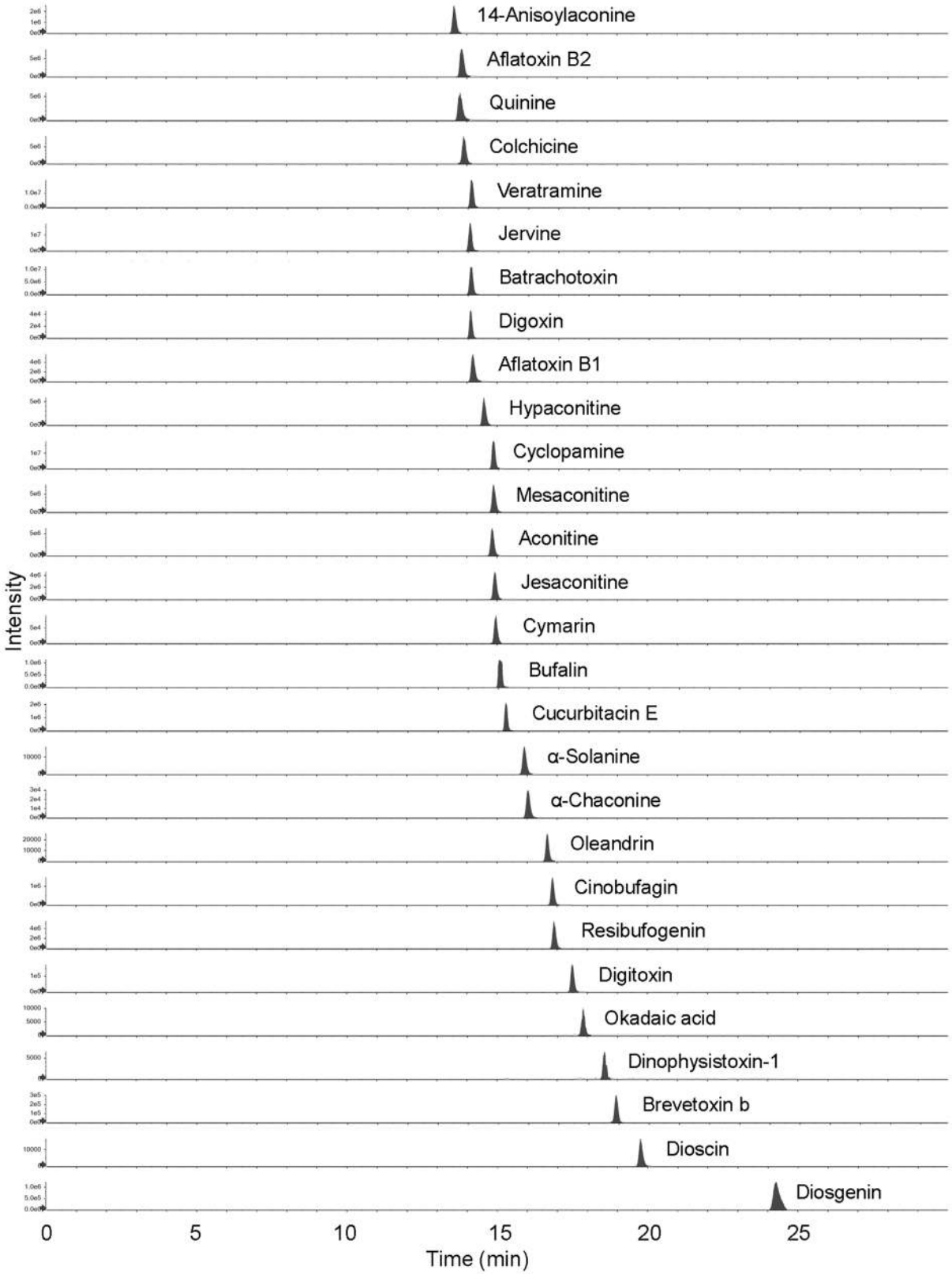

Fig. 1 (continued)

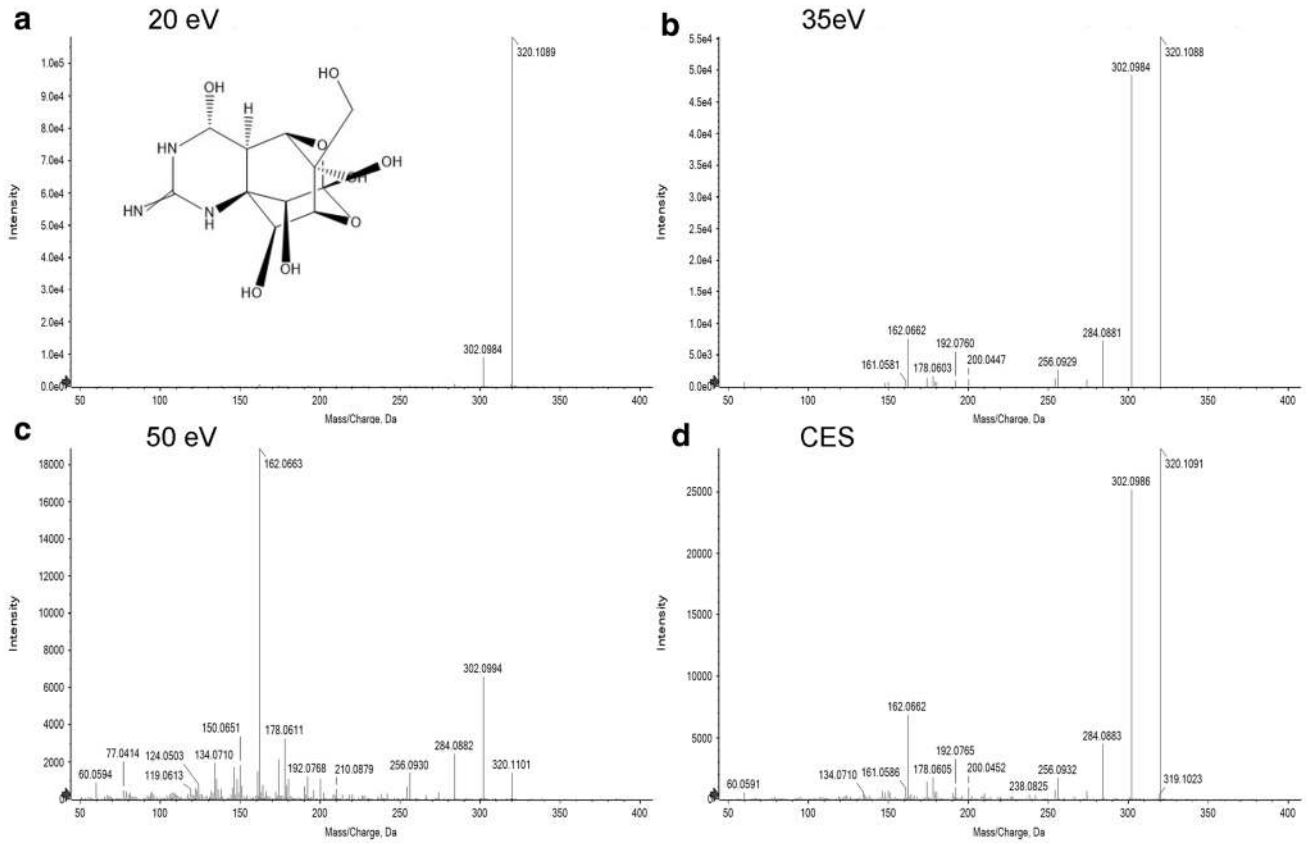

Fig. 2 Product ion spectra for tetrodotoxin at collision energy of **a** 20 eV, **b** 35 eV, **c** 50 eV, or **d** collision energy spread (CES), obtained by LC–QTOF-MS/MS

ion spectra of all substances were obtained by four different CE settings (see supplementary material Fig. S1). As an example the four spectra obtained for tetrodotoxin, which is one of the important toxins in food poisoning cases in Japan, are shown in Fig. 2. The $[M+H]^+$ (*m/z* 320.1088) was the most abundant ion at 20 eV (Fig. 2a), while it remarkably decreased at 35 eV (Fig. 2b) and became a very small peak at 50 eV (Fig. 2c), and the number and intensity of fragment ions increased instead (Fig. 2a–c). Product ion spectra obtained by CES mode showed both $[M+H]^+$ and fragment ions (Fig. 2d). CES mode can collect an average MS spectrum of different CE values in one single EPI scan, resulting in a full scan spectrum with both molecular and fragment ion information that can be used in library search-based identification with increased confidence. Digoxin, α-solanine, α-chaconine, digitoxin and dioscin provided $[M+HCOO]^-$, and amygdalin and cucurbitacin E showed $[M+NH_4]^+$ instead of $[M+H]^+$ (Table 1, supplementary material Fig. S1, nos. 36, 46, 47, 51, 57, 7, and 45, respectively); therefore, it is necessary to pay attention to this phenomenon.

Several precursor ions accompany unknown ions with strange mass defects. For example, the *m/z* of $[M+H]^+$ of strychnine (compounds no. 20) is 335.1754, but 6 ions were observed in the range of 335–337 (336.2 could be the isotopic ion) (supplementary material Fig. S1, no. 20). This is also observed in the spectra of berberine (no. 21), aflatoxin G1 (no. 27), colchicine (no. 32), veratramine (no. 33), jervine (no. 34), aflatoxin B1 (no. 37), cyclopamine (no. 39), and *Aconitum* alkaloids (nos. 9, 23, 26, 29, 38, and 40–42). Unfortunately, the sources of the ions are still unknown. In future, it is necessary to analyze the assignment of these ions.

With respect to the chromatographic separation, water/methanol both containing 10 mM ammonium formate was used. The used conditions provided RTs ranging from 1.9 min to 24.3 min (Table 1). Although some hydrophilic substances like ibotenic acid, musimol, and muscarine eluted quickly, it had no problems to detect them by high-resolution MS analysis.

Analysis of spiked samples

The present library was applied for the identification of lycorine and domoic acid spiked into blank blood plasma.

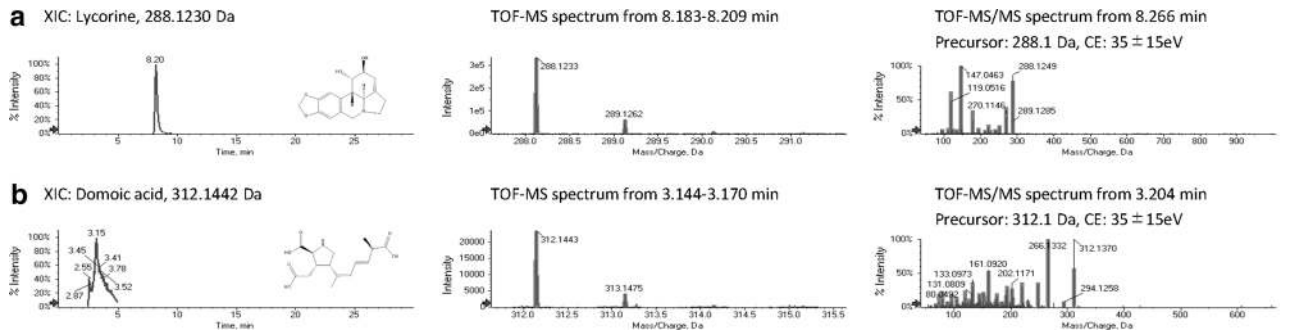

Fig. 3 Identification of lycorine (1 µg/mL) and domoic acid (10 µg/mL) spiked into blank blood plasma by LC–QTOF-MS/MS. XICs (left panels), TOF-MS spectra (100–1000 Da, middle panels) and TOF-MS/MS spectra (50–1000 Da, right panels) of **a** lycorine and **b** domoic acid

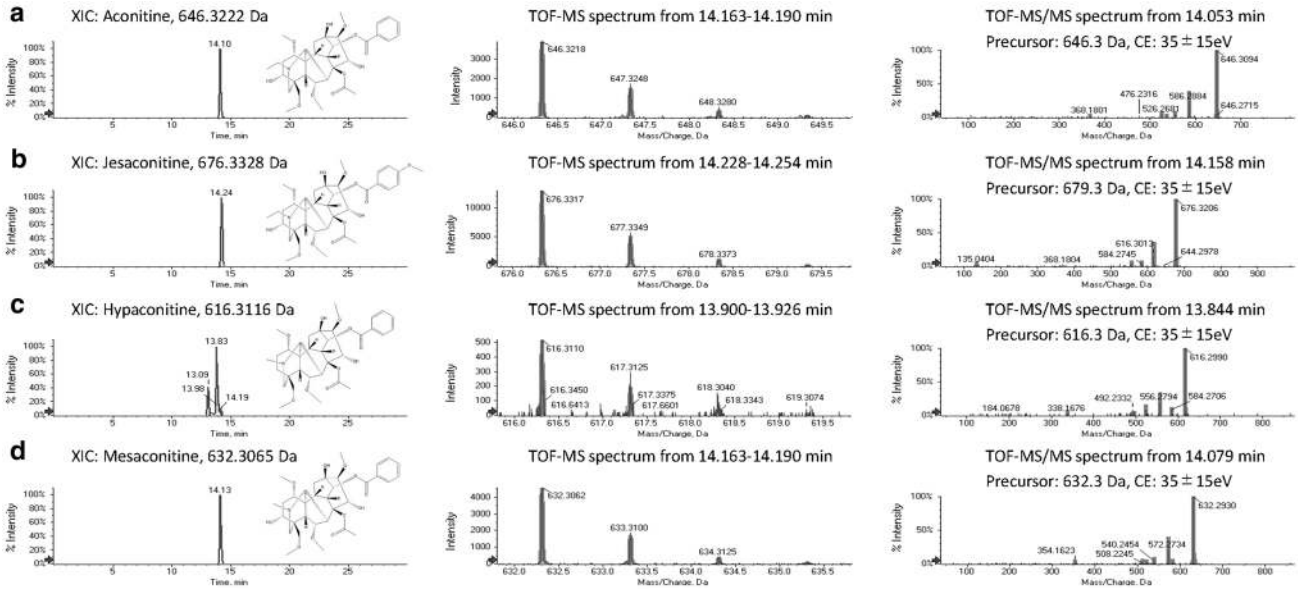

Fig. 4 Identification of aconitine, jesaconitine, hypaconitine, and mesaconitine in a forensic autopsy sample by LC–QTOF-MS/MS. XICs (left panels), TOF-MS spectra (100–1000 Da, middle panels), and TOF-MS/MS spectra (50–1000 Da, right panels) of **a** aconitine, **b** jesaconitine, **c** hypaconitine, and **d** mesaconitine

Figure 3 shows the results processed using the automatic extracted ion chromatograms (XICs) and spectra by TOF-MS and TOF-MS/MS. In the XICs on the left panels of Fig. 3a and b, the peaks corresponded to the target analytes. In the TOF-MS spectra on the middle panels, the measured masses were at *m/z* 288.1233 for lycorine and 312.1443 for domoic acid, which matched the theoretical masses with errors of 0.9 and 0.5 ppm, respectively. In the TOF-MS/MS spectra on the right panels, the masses of the fragment ions agreed very well with those of the registered MS/MS spectra. The purity scores were 79.1 and 67.2%, respectively.

Application to forensic autopsy samples

Femoral vein and right and left heart blood samples collected from a 45-year-old male at the forensic autopsy performed in our laboratory were analyzed using the present library. In all samples, aconitine, jesaconitine, hypaconitine, and mesaconitine were identified, and Fig. 4 shows representative results from the femoral vein sample. In the previous study, Niitsu et al. [22] reported the four poisons as major substances detected from blood in the cases of suicide by aconite poisoning. In the TOF-MS spectra, the measured masses matched the registered masses with mass errors of 0.5–1.0 ppm. In the TOF-MS/MS spectra, the masses of the fragment ions agree very well with the registered MS/MS spectra. The purity score was 54.6–60.3%.

Discussion

In this article, we have created a forensic toxicological library including 56 natural toxic substances. The drugs of abuse originated from plants, such as Δ^9-tetrahydrocannabinol and cocaine have been excluded. To our knowledge, only one trial to construct libraries specific to natural toxic substances has been published [6]; but they used low-resolution LC–MS/MS instrument unable to make estimation of the molecular formulae, using its accurate mass numbers, which are very useful for tentative identification of an unknown substances. Recently, Wang et al. [23] reported high-throughput screening of more than 200 toxic substances including narcotic drugs, psychotropic drugs, pesticides, natural toxins, and other drugs; however, in their collection, only 3 substances were in common with those in our article. Moreover, they did not use a high-resolution MS instrument, but a low-resolution linear ion trap quadrupole MS coupled with a homemade extractive electrospray ionization. Broecker et al. [12] reported an article on development and application of a library for CID accurate mass spectra of more than 2500 toxic compounds by LC–QTOF-MS/MS. However, the readers cannot get access to the MS/MS spectra only with their paper. In the present article, the readers can readily gain access to the detailed high-resolution MS/MS spectra of 56 natural toxic substances located in the electronic supplementary material.

Martin et al. [24] compared the performance of three types of LC–QTOF-MS/MS platforms created by three different manufacturers including the Sciex Triple TOF 5600 system used in the present study. There are usually three parameters for compound identification by LC–QTOF-MS/MS: mass errors not greater than 4 or 5 ppm, RT differences with 0.2–0.5 min, and similarities of MS/MS spectrum profiles. The former two parameters were common to the three types of the instruments. For the similarity of the MS/MS profiles, one manufacturer did not incorporate such a parameter as of 2014. Another manufacturer provided MS/MS libraries at three collision energies for matching. The system of our instrument takes into consideration the presence/absence of all MS/MS spectral peaks and their relative abundance, which are compared to those of the MS/MS library record, calculating the purity score. In addition, the system also includes the CES mode, in which the CE ramp range is set to 35 ± 15 eV, in which a small parent peak and important small product ions are magnified automatically. Therefore, we presented three MS/MS spectra at CEs of 20, 35, and 50 eV, and one spectrum in the CES mode (Fig. 2, Fig. S1). Such algorithms adopted by Sciex for comparison of MS/MS spectrum profiles seem most sophisticated and thus reliable in current LC–QTOF-MS arena. Although some previous studies described identification of target compounds using the purity scores, their distinct criteria have not been established [16, 25–27]. According to our results on the spiked and forensic autopsies (Figs. 3, 4), the purity scores more than 50% seems to be acceptable prior to considering the matches of a mass error and RT.

When the present library of natural toxic substances by LC–QTOF-MS were created, low-resolution MS/MS spectra of 54 natural toxic substances were also recorded, except for picrotoxinin and diosgenin (unpublished observation). The low-resolution MS/MS spectra at CEs of 20, 35, and 50 eV, and one spectrum in the CES mode were acquired; the low-resolution MS/MS spectra were similar to the high-resolution MS/MS spectra in this study. Therefore, the detailed high-resolution MS/MS spectra of natural toxic substances located in the electronic supplementary material in the present article (Fig. 2, Fig. S1) seems to be also useful in routine forensic toxicological screening by low-resolution LC–MS/MS.

Conclusions

We have developed a forensic toxicological library for identification of 56 natural toxic substances by LC–QTOF-MS/MS. The applicability of the library was exemplified by identifying four plant toxins in blood samples collected from an autopsy. This library may be effective for the screening of natural toxic substances and can become a powerful tool for searching natural toxic substances in routine forensic toxicological analysis. To our knowledge, this is the first trial to develop a toxicological library for natural toxic substances using high-resolution LC–MS/MS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The analysis of blood samples from deceased subjects was requested by the judicial authorities.

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