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Quantitative determination of marine toxins associated with diarrhetic shellfish poisoning by liquid chromatography coupled with mass spectrometry

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Abstract

Quantitative determination by liquid chromatography (LC) coupled with mass spectrometry (MS) was achieved for the following 10 toxins found in association with diarrhetic shellfish poisoning: okadaic acid (OA), dinophysistoxin-1 (DTX1), 7-*O*-palmitoylokadaic acid (palOA), 7-*O*-palmitoyldinophysistoxin-1 (palDTX1), pectenotoxin-1 (PTX1), pectenotoxin-2 (PTX2), pectenotoxin-2 seco acid (PTX2SA), pectenotoxin-6 (PTX6), yessotoxin (YTX), and 45-hydroxyyessotoxin (YTXOH). Toxins in 2 g of the adductor muscle or the digestive glands of scallops, *Patinopecten yessoensis*, were extracted with 18 ml of methanol–water (9:1, v/v), freed of polar contaminants by partition between chloroform and water, and treated by solid-phase extraction on a silica cartridge column. Samples containing YTXOH were purified separately on a buffered reversed-phase column. Chromatographic separation was achieved by the following combinations of columns and mobile phases: a Symmetry C_{18} column with acetonitrile–0.05% acetic acid (7:3, v/v) for OA, DTX1, PTX6 and PTX2SA; a Develosil ODS column with the same mobile phase for PTX1 and PTX2; a Capcellpak column with methanol–2.5% acetic acid (98:2, v/v) for palOA and palDTX1; and an Inertsil ODS column with methanol–0.2 *M* ammonium acetate (8:2, v/v) for YTX and YTXOH. Carboxylic acid toxins were selectively monitored on $[M-H]^-$ ions, sulfated toxins on $[M-Na]^-$ ions, and neutral toxins on $[M+NH_4]^+$ ions. Average recoveries of the toxins spiked to tissue homogenates ranged from 70 to 134%. Detection limits in the muscle ranged from 5 to 40 ng/g and those in the digestive glands from 10 to 80 ng/g.

Keywords: Shellfish poisoning; Okadaic acid; Dinophysistoxin-1; 7-*O*-Palmytoylokadaic acid; 7-*O*-Palmytoyldinophysistoxin-1; Pectenotoxin-1; Pectenotoxin-2; Pectenotoxin-6; Pectenotoxin seco acid; Yessotoxin; 45-Hydroxyyessotoxin; Toxins

1. Introduction

Diarrhetic shellfish poisoning (DSP) first reported in Japan in 1978 [1] is now known to occur worldwide [2–7]. In order to prevent human intoxication, monitoring of shellfish toxicity is being implemented in many countries by mouse bioassays [8,9]. As the assays nonspecifically detect toxins, a number of toxins thus detected have been associated with DSP. Structurally, these toxins are classified into three groups (Fig. 1). The first group consists of carboxylic acid toxins sharing the same or similar

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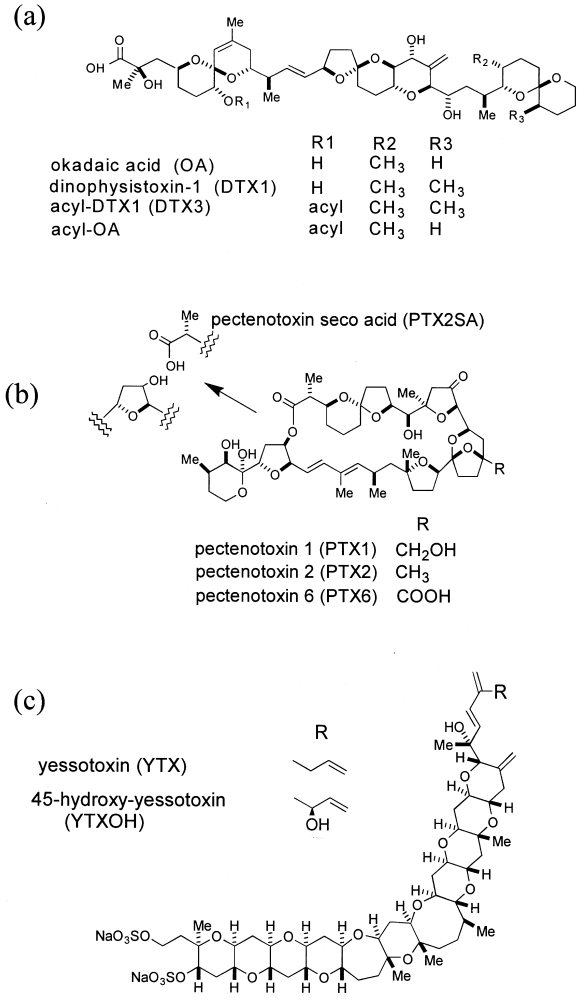

Fig. 1. Structures of the toxins tested in this study.

polycyclic-ether skeleton of okadaic acid (OA) [10]: e.g., OA, dinophysistoxin-1 (DTX1), dinophysistoxin-2 (DTX2), palmitoylokadaic acid (palOA), and palmitoyldinophysistoxin-1 (palDTX1) [11]. The second group consists of polycyclic-ether macrolides named pectenotoxin-1 (PTX1), pectenotoxin-2 (PTX2), pectenotoxin-6 (PTX6) and pectenotoxin-2 seco acid (PTX2SA) [12–14]. Other pectenotoxin congeners are known but they are of rare occurrence or artifacts. In the third group yessotoxin (YTX) and 45-hydroxyyesotoxin (YTXOH) are the major toxins to occur in shellfish [15–18]. These three groups of toxins differ toxicologically: the OA group causes diarrhea and tumor promotion [19]; the PTXs injure the liver [20]; and the YTXs cause myocardial injuries at high doses [21,22]. Etiology of these toxins is also variant, making toxin profiles in shellfish extremely variable. For assessment of health risks, therefore, determining accurate toxin profiles is of critical importance. Liquid chromatography (LC) with fluorimetric detection is applicable to some, but not all, toxins [23,24]. Recently, OA, DTX1, DTX2 and PTX2 in shellfish or plankton samples were effectively detected by LC coupled with mass spectrometry (MS), but other toxins remained untested [25–31]. To use LC–MS data for imposing regulatory actions, testing all the representative toxins is imperative. Establishing proper clean-up procedures is also necessary to eliminate substances which interfere with ionization of analytes or cause deterioration of columns upon repeated injection. In the present study we spiked shellfish tissues with 10 representative toxins and rigorously tested various columns and mobile phases to achieve best separation and quantitative recoveries.

2. Experimental

2.1. Scallop samples

Scallops, *Patinopecten yessoensis*, used as the negative control and for spiking were purchased from the Iwate Federation of Fisheries Union in June 1995 or from the fish market in Tama City, Tokyo, Japan in November 1997. The adductor muscle and digestive glands were separated from other tissues, homogenized, and kept frozen at −20°C until used.

2.2. Reagents

OA, DTX1, palDTX1 and PTX6 were purchased from Wako (Osaka, Japan). PTX1, PTX2, PTX2SA, YTX and YTXOH were purified from toxic digestive glands of scallops or mussels according to previous reports [12–16]. PalOA was synthesized from OA [32]. Acetonitrile and methanol of LC grade were purchased from Wako. Other reagents used for the study were of analytical grade. Tap water was deionized with ion-exchange resin, followed by

purification through a Milli-Q water purification system (Japan Millipore, Tokyo, Japan). Sep-Pak silica plus and Sep-Pak C_{18} plus cartridge columns were purchased from Waters (Milford, MA, USA).

2.3. Sample extraction and solvent partition

A 2-g amount of digestive glands or adductor muscle was homogenized with 18 ml of methanol–water (9:1, v/v) with a Physcotron homogenizer (Nichi-on, Tokyo, Japan) for 1 min at ambient temperature. The homogenates was centrifuged at 2500 rpm for 10 min. To 5 ml of the supernatant from the digestive glands was added 2 ml of 0.5% acetic acid, and to that from the muscle was added 2 ml of 0.5% acetic acid containing 2% (w/v) sodium chloride. The acidified solution was extracted twice with 7 ml of chloroform. The chloroform layers were combined and made up to 20 ml with chloroform. A 10-ml portion of the chloroform solution was evaporated and the residue was further dried under a nitrogen stream (defined as chloroform extract). The procedure is schematically shown in Fig. 2.

2.4. Clean-up on a silica gel cartridge column

The chloroform extract was loaded onto a Sep-Pak plus silica cartridge column with 3 ml of chloroform in three portions. The column was washed with 1.5 ml of acetone and the washing was discarded. Then PTX1 and PTX2 were eluted with another 1.5 ml of acetone (fraction A). The column was again washed with 7 ml of acetone and the washing was discarded. Finally, the remaining toxins were eluted with 10 ml of acetone–methanol (3:7, v/v) (fraction B). The residue from fraction A was dissolved in 1 ml of methanol–water (8:2, v/v), removed of low polar lipids by washing twice with 1 ml of hexane, and evaporated. The residue was re-dissolved in 0.5 ml of methanol, and 1 μl of the solution was used for LC–MS analysis of PTX1 and PTX2. The residue in fraction B was dissolved in 0.5 ml of methanol and 1 μl of the solution was used for LC–MS analysis of

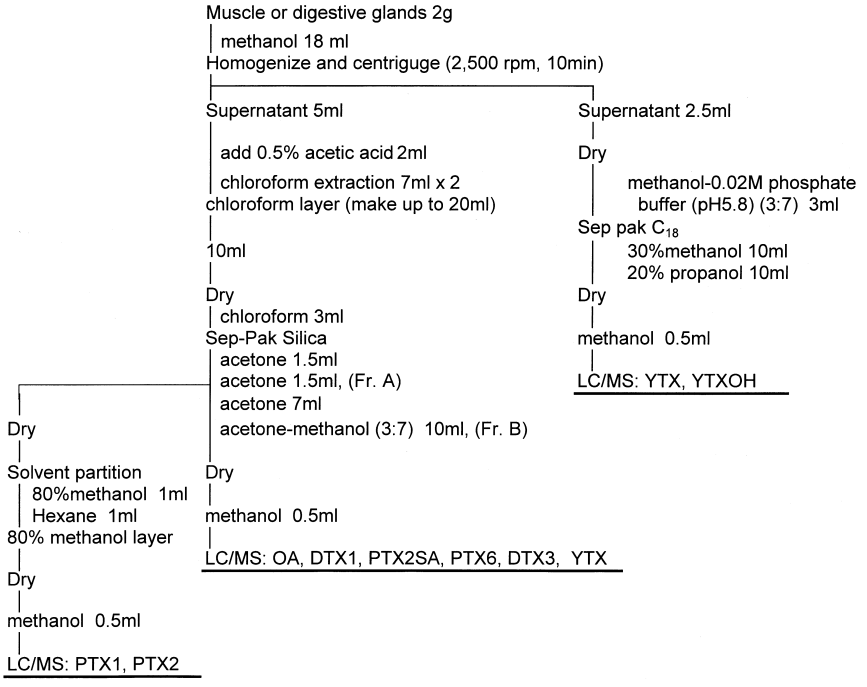

Fig. 2. Scheme for preparation of test solutions for LC–MS analysis.

OA, DTX1, DTX3, PTX6 and PTX2SA. The procedure is illustrated in Fig. 2.

2.5. Clean-up of YTXs on a C_{18} cartridge column

A 2.5-ml portion of the aqueous methanol extract from the tissue was evaporated. The residue was dissolved in 3 ml of 20 m*M* phosphate buffer (pH 5.8)–methanol (7:3, v/v), and the solution was loaded on a Sep-Pak C_{18} plus cartridge column equilibrated with the same buffer. The column was washed with 10 ml of methanol–water (3:7, v/v) and the toxins were eluted with 10 ml of propanol–water (2:8, v/v) (Fig. 2). The residue in the eluate was re-dissolved in 0.5 ml of methanol, and 1 μl of the solution was injected into the LC–MS system.

2.6. Liquid chromatography–mass spectrometry

Mass spectrometric measurements were performed with a Quattro II triple stage quadrupole mass spectrometer (Micromass UK, Cheshire, UK) equipped with an atmospheric pressure ionization source and an electrospray ionization (ESI) interface. ESI was effected by a spray voltage of 3.0 kV under capillary temperature of 120°C. LC was performed on a Nanospace system (Shiseido, Tokyo, Japan) or on a Gulliver system (Jasco, Tokyo, Japan).

Chromatographic separations were carried out under the following combinations of columns and mobile phases: Symmetry C_{18} (150×2.1 mm, 3.5 μm; Waters) with acetonitrile–0.05% acetic acid (7:3, v/v) for OA, DTX1, PTX6 and PTX2SA; Capcellpak C_{18} (250×1.5 mm, 5 μm; Shiseido) with methanol–2.5% acetic acid (98:2, v/v) for palOA and palDTX1; Inertsil ODS-3 (150×2.1 mm, 5 μm; GL Science, Tokyo, Japan) with methanol–0.2 *M* ammonium acetate (8:2, v/v) for YTX and YTXOH; Develosil ODS-MG-5 (150×2.0 mm, 5 μm; Nomura, Seto, Japan) with acetonitrile–0.05% acetic acid (7:3, v/v) for PTX1 and PTX2. The column temperature and flow-rate were kept at 40°C and 0.1 ml/min, respectively. Selected ion monitoring (SIM) for carboxylic acid toxins were performed on deprotonated $[M-H]^-$ ions: m/z 803 for OA, 817 for DTX1, 1041 for palOA, 1055 for palDTX1, 887 for PTX6, 875 for PTX2SA. For monitoring YTX and YTXOH, their $[M-Na]^-$ ions at m/z 1163 and 1147, respectively, were monitored. Monitoring of PTX1 and PTX2 were carried out on their $[M+NH_4]^+$ ions at m/z 892 and 876, respectively.

2.7. Dose responses and minimum detection level

By using the above LC and SIM conditions, the linearity of dose–responses was examined at four dose levels by injecting methanolic solution of each toxin. The average of triplicate measurements was used for plotting. Peak areas were used to express peak intensity. The minimum detection levels for matrix-free pure toxins were thus estimated from the data.

Based on the minimum detection levels acquired in the above experiment, appropriate amounts of individual toxins were calculated and added to tissue homogenates. The spiked tissue homogenates were extracted and treated on cartridge columns as depicted in Fig. 2. The minimum detection levels for the toxins in the tissues were determined in subsequent LC–MS analysis.

2.8. Recovery tests with spiked samples

To evaluate the method performance, homogenates of the muscle and digestive glands were spiked with the toxins. The dose levels of individual toxins used for spiking corresponded to 20 times the levels for minimum detection. The homogenates were extracted and the extracts were treated as described in previous sections and depicted in Fig. 2. Due to the limited amounts of the toxins for spiking, the number of repetition in the recovery tests was partly limited.

2.9. Quantification

Reference toxins were dissolved in methanol, respectively to give the following concentrations: 20 ng/ml for OA, 40 ng/ml for DTX1, 80 ng/ml for palOA and palDTX1, 160 ng/ml for PTX1, PTX2, PTX6, PTX2SA, YTX and YTXOH. The reference toxin solution (1 μl) was injected before and after sample analysis. The averaged peak area from the two injections was used for calibrating toxin amounts in tissues.

3. Results and discussion

3.1. Extraction and solvent partition

In preliminary tests with methanol, methanol–water (9:1, v/v) and acetone, the second solvent, methanol–water (9:1, v/v), gave the least residue and thus was chosen as extractant. Good recovery was secured by one extraction, when nine-times volume (v/w) of the extractant was used. Repetition of extraction did not improve the recovery. Comparison of chloroform and ethyl acetate for their efficiency to separate the toxins from polar contaminants by solvent partition, indicated poor recovery of YTX in ethyl acetate. Hence, chloroform was chosen for the partition.

3.2. Selected ion monitoring

Of the toxins tested only PTX1 and PTX2 are neutral compounds, and others are acidic compounds. Naturally, carboxylic acid toxins were effectively monitored on their deprotonated ions corresponding to $[M-H]^-$. In the case of YTX, ions corresponding, respectively, to $[M-Na]^-$, $[M-2Na+H]^-$ and $[M-Na-NaSO_3+H]^-$ were observed at m/z 1163, 1141 and 1061. As the de-sodiated ion $[M-Na]^-$ showed the highest intensity, the ion at m/z 1163 was selected for monitoring. Similarly, the de-sodiated ion at m/z 1179 was selected for monitoring YTXOH. Ionization of PTX1 and PTX2 was markedly suppressed by matrix in the negative mode, making negative ion monitoring unsuitable. Of the molecular-related positive ions, higher intensities were observed for $[M+NH_4]^+$ than for $[M+H]^+$ and $[M+Na]^+$. Thus, ammonium-adduct ions at m/z 892 and 876 were selected, respectively, for monitoring PTX1 and PTX2.

3.3. Clean-up on a Sep-Pak silica cartridge column

When the chloroform extracts from the digestive glands were spiked with toxins, the peak intensities of the toxins in LC–MS chromatograms corresponded to only 50% of the injected amounts, indicating significant suppression of ionization by contaminants. Treatment of the chloroform extracts on a silica cartridge column with chloroform–methanol did not improve the ionization rate. Therefore, using the chloroform extracts directly for injection or using chloroform–methanol for clean-up on a silica column, though convenient, was concluded to be inappropriate.

Treatment of extracts on silica cartridge columns as shown in Fig. 2 effectively removed contaminants that suppressed ionization. Losses of the toxins in the acetone washing were at tolerable levels (less than 10%), and elution of the toxins in acetone–methanol (3:7, v/v) was complete. PTX1 and PTX2 were effectively separated from contaminants by discarding the first 1.5 ml of acetone and collecting subsequent 1.5 ml. For further elimination of interfering substances, however, partition between methanol–water (8:2, v/v) and hexane was necessary.

Though in limited areas, YTXOH occurred in scallops together with YTX and had to be determined. Because of the poor extractability of YTXOH in chloroform and low recoveries from a silica cartridge column, clean-up of the two toxins had to be carried out on a reversed-phase cartridge column by slightly modifying the reported method [24]. About 6% of YTXOH was lost in methanol–water (3:7, v/v) washing, but the remaining was recovered with propanol–water (2:8, v/v).

3.4. Chromatographic separation

For simplicity and practicability of performance, isocratic separation was chosen. Although separating all toxins on a single column with the same mobile phase was desired, the widely varied polarities of the analytes posed an obstacle. Furthermore, occurrence of interfering contaminants did not allow us to achieve the desirable separation on a single column. Because adding another clean-up step to eliminate interfering substances was undesirable for handling many samples, our effort was directed to improving chromatographic conditions. For that purpose, changing the column was more effective than changing mobile phases. An example of dramatic change in elution pattern is shown in Fig. 3. On a Capcellpak C_{18} column, DTX1 and PTX6 were only narrowly separated, eluting at 13.0, and 13.3 min, respectively (Fig. 3a). On a Symmetry Pak C_{18} column, however, PTX6 was eluted at 7.6 min, while DTX1 remained

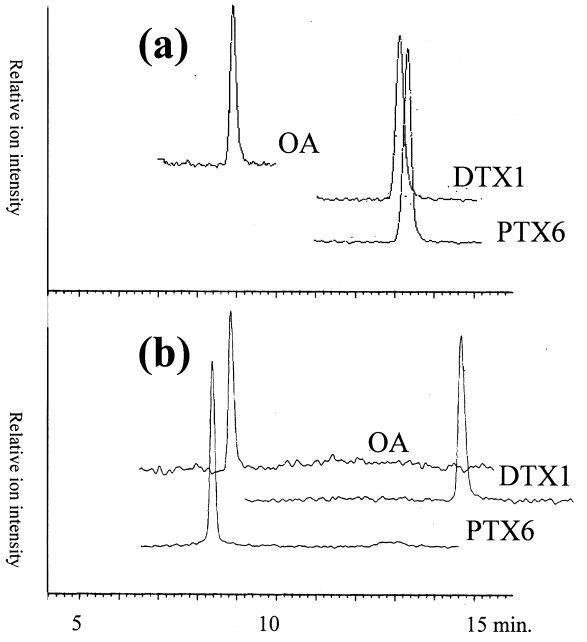

Fig. 3. Shift of PTX6 on different columns. (a) Capcellpak C_{18} column with acetonitrile–0.05% acetic acid (7:3, v/v); (b) Symmetry C_{18} column with acetonitrile–0.05% acetic acid (7:3, v/v).

unaffected eluting at 13.5 min (Fig. 3b). Occasional occurrence of background interference forced us to choose the Develosil ODS column for analysis of PTX1 and PTX2, otherwise the Symmetry C_{18} column could be used. Analysis of PalOA and palDTX1 could be also performed on the Symmetry column together with other carboxylic acid toxins. The long retention time for palDTX1 (40 min), however, made it necessary to change the column and mobile phase for analyzing many samples. The best columns and mobile phases for each toxin group were thus selected as described in Section 2.6.

3.5. Linearity of the dose responses

For all the toxins tested, good linearity ($r^2 > 0.997$) was observed between the amounts of injected toxins and the peak areas: from 5 to 200 pg for OA, from 10 to 400 pg for DTX1, from 20 to 800 pg for palOA and palDTX1, and from 40 to 1600 pg for PTX1, PTX2, PTX2SA, PTX6, YTX and YTXOH (data not shown).

3.6. Minimum detection levels

Fig. 4 shows the chromatograms of reference toxins dissolved in methanol, the Sep-Pak-treated extracts from the digestive glands spiked with low levels of toxins, and the extracts from non-spiked digestive glands. Similarly, the minimum detection levels for the toxins in the muscle were obtained. The detection limits in the muscle and digestive glands, respectively, were as follows: 5 and 10 ng/g for OA, 10 and 20 ng/g for DTX1, 20 and 40 ng/g for palOA and palDTX1, and 40 and 80 ng/g for PTX1, PTX2, PTX6, PTX2SA, YTX and YTXOH.

3.7. Recovery

Recoveries of the acidic toxins extracted from tissue homogenates spiked with 20 times the levels for minimum detection and subsequently treated on a Sep-Pak silica or a Sep-Pak C_{18} column are shown in Table 1. Among the toxins treated on the silica column good recoveries (80–106%) were obtained except for those for PTX2SA and PTX6. The relatively low recoveries for these two toxins can be accounted for by their easiness to undergo epimerization in solution. The relative standard deviations (RSDs) were relatively high (8–22%), indicating the difficulty in eliminating the interference due to biomatrices. Recoveries for PTX1 and PTX2 treated on the silica column were significantly higher (128–134%) than the injected amounts. The background interference was even greater, when other columns were used for LC separation. On the contrary recoveries of sulfated toxins, YTX and YTXOH, were low (69–70%). As strong buffers, such as phosphate buffers, cannot be used in LC–MS to control the charge state of the sulfate moiety, broadening of peaks must have resulted in the relatively low recoveries. In the absence of appropriate inner standards or surrogates, however, the recoveries achieved in the present study prove the usefulness of LC–MS as an alternative to the conventional mouse bioassay. The toxin levels tested for recoveries were chosen to testify the practicability of the method for taking a regulatory action. As the method can detect toxins at far lower levels, routine monitoring by LC–MS is suited to give fishermen an early warning for shellfish toxicity. The

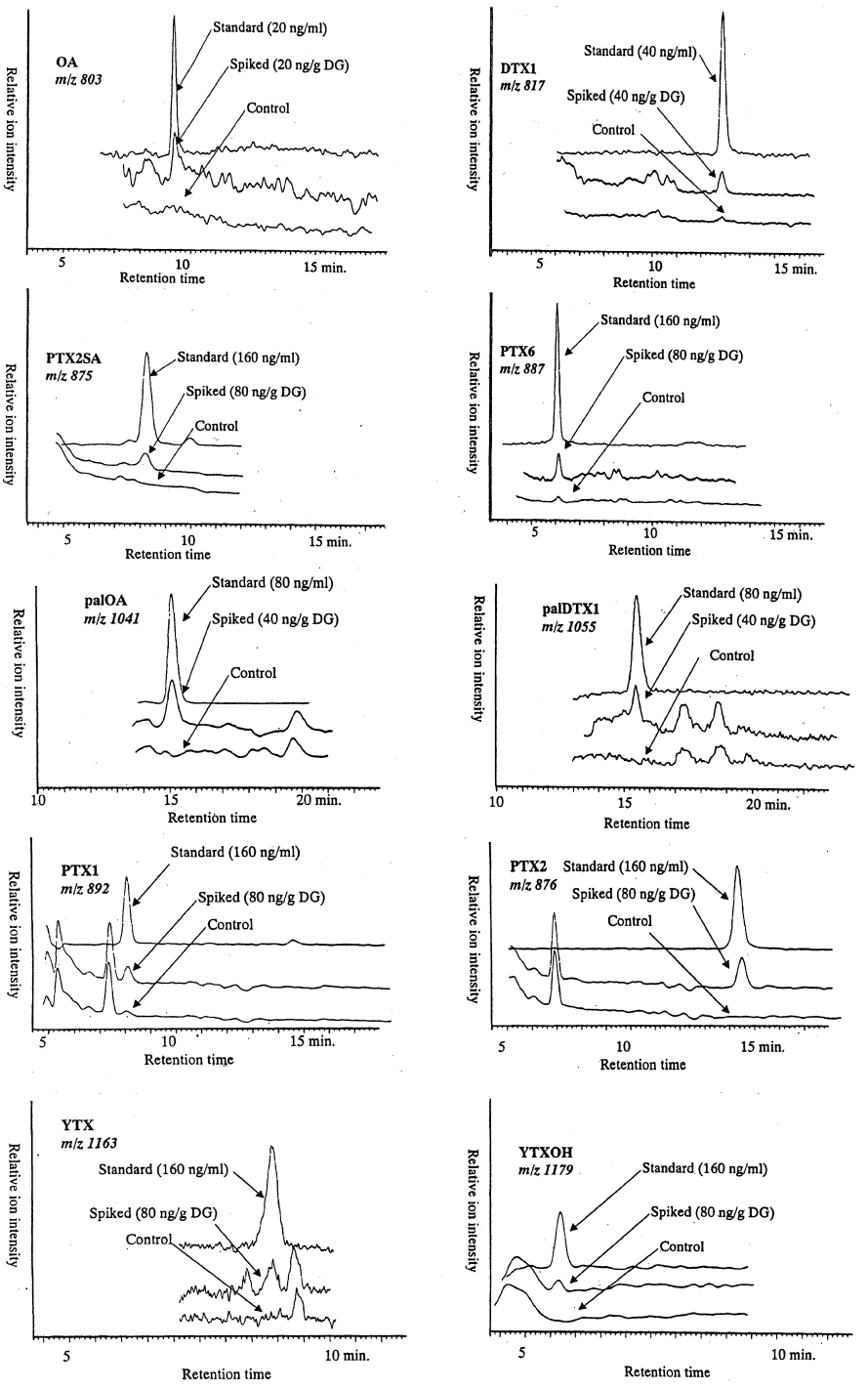

Fig. 4. Chromatograms of the toxins tested in this study. DG denotes digestive glands. The following columns and mobile phases were used: OA, DTX1, PTX2SA, and PTX6; Symmetry C_{18}, acetonitrile–0.05% acetic acid (7:3, v/v): PalOA and palDTX1; Capcellpak C_{18}, methanol–2.5% acetic acid (98:2, v/v): PTX1 and PTX2; Develosil ODS-MG-5, acetonitrile–0.05% acetic acid (7:3, v/v): YTX and YTXOH: Inertsil ODS-3, methanol–0.2 *M* ammonium acetate (9:1, v/v).

Table 1
Recoveries of toxins from muscle and digestive glands after clean-up on silica or reversed-phase cartridge

Tissue	Toxin[a]	Spiked level[e] (ppm)	Recovery (%) and repetition number					Mean (%)	RSD (%)
			1	2	3	4	5		
Muscle	OA	0.1	92	104	–	–	–	98	–
	palOA	0.2	101	78	–	–	–	90	–
	DTX1	0.2	91	110	–	–	–	101	–
	palDTX1	0.4	82	104	–	–	–	93	–
	PTX2SA	0.8	79	78	–	–	–	79	–
	PTX6	0.8	67	69	–	–	–	68	–
	YTX	0.8	86	95	–	–	–	90	–
Digestive glands	OA	0.2	88	94	104	108	99	99	8
	palOA	0.4	98	113	–	–	–	106	–
	DTX1	0.4	89	105	73	70	75	82	18
	palDTX1	0.8	75	78	89	76	86	81	8
	PTX2SA	1.6	63	85	–	–	–	74	–
	PTX6	1.6	66	57	94	79	96	78	22
	YTX[b]	1.6	80	90	77	77	74	80	8
	YTX[b,d]	1.6	79	59	–	–	–	69	–
	YTXOH[b,d]	1.6	74	65	–	–	–	70	–
	PTX1[c]	1.6	142	126	–	–	–	134	–
	PTX2[c]	1.6	144	111	–	–	–	128	–

[a] Toxins were monitored on the $[M-H]^-$ ion except for YTX, YTXOH, PTX1 and PTX2.
[b] YTX and YTXOH were monitored on the $[M-Na]^-$ ion.
[c] PTX1 and PTX2 were monitored on the $[M+NH_4]^+$ ion.
[d] These toxins were treated with the reversed-phase cartridge and all other toxins were treated with the silica cartridge.
[e] Spiked levels correspond to 20 times the detection limits.

present results are the first important step toward quantitative determination of the most of the DSP-associated toxins by LC–MS. For further improvement of the quantitative precision, preparation of appropriate internal standards, or surrogates, are desired.

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References

[1] T. Yasumoto, Y. Oshima, M. Yamaguchi, Bull. Jpn. Soc. Sci. Fish. 44 (1978) 1249.
[2] T. Yasumoto, Y. Oshima, W. Sugawara, Y. Fukuyo, H. Oguri, T. Igarashi, N. Fujita, Bull. Jpn. Soc. Sci. Fish. 46 (1980) 1405.
[3] M. Kat, in: D.M. Anderson, A.W. White, D.G. Baden (Eds.), Toxic Dinoflagellates Blooms, Elsevier, Amsterdam, 1985, p. 215.
[4] M. Kumagai, T. Yanagi, M. Murata, T. Yasumoto, M. Kat, P. Lassus, J.A. Rodriguez-Vazquez, Agric. Biol. Chem. 50 (1986) 1775.
[5] T. Hu, J. Doyle, D. Jackson, J. Marr, E. Nixon, S. Pleasance, M.A. Quilliam, J.A. Walter, J.L.C. Wright, J. Chem. Soc. Chem. Commun. (1992) 39.
[6] L. Boni, A. Poletti, M. Pompei, in: T.J. Smayda, Y. Shimizu (Eds.), Toxic Phytoplankton Blooms in the Sea, Elsevier, Amsterdam, 1993, p. 475.
[7] M.A. Quilliam, M.W. Gilgan, S. Pleasance, A.S.W. Defreitas, D. Douglas, L. Fritz, T. Hu, J.C. Marr, C. Smyth, J.L.C. Wright, in: T.J. Smayda, Y. Shimizu (Eds.), Toxic Phytoplankton Blooms in the Sea, Elsevier, Amsterdam, 1993, p. 547.
[8] T. Yasumoto, M. Murata, Y. Oshima, K. Matsumoto, in: E.P. Ragelis (Ed.), Seafood Toxins, ACS Symposium Series, No. 262, American Chemical Society, Washington, DC, 1984, p. 207.

[9] M.L. Fernandez, A. Miguez, E. Cacho, A. Martinez, in: T. Yasumoto, Y. Oshima, Y. Fukuyo (Eds.), Harmful Marine Algal Blooms, IOC UNESCO, Paris, 1996.
[10] K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D.V. Engen, J. Clardy, Y. Gopichand, J.J. Schmitz, J. Am. Chem. Soc. 103 (1981) 2469.
[11] M. Murata, M. Shimatani, H. Sugitani, Y. Oshima, T. Yasumoto, Bull. Jpn. Soc. Sci. Fish. 48 (1982) 549.
[12] T. Yasumoto, M. Murata, Y. Oshima, M. Sano, G.K. Matsumoto, J. Clardy, Tetrahedron 41 (1985) 1019.
[13] K. Sasaki, M. Satake, T. Yasumoto, Biosci. Biotech. Biochem. 61 (1997) 1783.
[14] M. Daiguji, M. Satake, K.J. James, A. Bishop, L. MacKenzie, H. Naoki, T. Yasumoto, Chem. Lett. (1998) 653.
[15] M. Murata, M. Kumagai, J.S. Lee, T. Yasumoto, Tetrahedron Lett. 28 (1987) 5689.
[16] M. Satake, K. Terasawa, Y. Kadowaki, T. Yasumoto, Tetrahedron Lett. 37 (1996) 5955.
[17] M. Satake, A. Tubaro, J.S. Lee, T. Yasumoto, Nat. Toxins 5 (1997) 255.
[18] P. Ciminiello, E. Fattorusso, M. Forino, S. Magno, R. Polleti, R. Viviani, Tetrahedron Lett. 39 (1998) 8897.
[19] M. Suganuma, H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada, Proc. Natl. Acad. Sci. 85 (1988) 1768.
[20] K. Terao, E. Ito, T. Yanagi, T. Yasumoto, Toxicon 24 (1986) 1141.
[21] H. Ogino, M. Kumagai, T. Yasumoto, Nat. Toxins 5 (1997) 255.
[22] K. Terao, E. Ito, M. Oarada, M. Murata, T. Yasumoto, Toxicon 28 (1990) 1095.
[23] J.S. Lee, T. Yanagi, R. Kemma, T. Yasumoto, Agric. Biol. Chem. 51 (1987) 877.
[24] T. Yasumoto, A. Takizawa, Biosci. Biotech. Biochem. 61 (1997) 1775.
[25] S. Pleasance, M.A. Quilliam, J.C. Marr, Rapid Commn. Mass Spectrom. 6 (1992) 121.
[26] R. Draisci, L. Lucentini, L. Giannetti, P. Boria, A. Stacchini, Toxicon 33 (1995) 1591.
[27] K.J. James, A.G. Bishop, M. Gillman, S.S. Kelly, R. Draisci, L. Lucentini, L. Giannetti, P. Boria, J. Chromatogr. A 777 (1997) 213.
[28] R. Draisci, L. Lucentini, L. Giannetti, P. Boria, K.J. James, A. Furey, M. Gillman, S.S. Kelly, J.AOAC Int. 81 (1998) 441.
[29] T. Suzuki, T. Mitsuya, H. Matsubara, M. Yamasaki, J. Chromatogr. A 815 (1998) 155.
[30] K.J. James, A.G. Bishop, R. Draisci, L. Palleschi, C. Marchiafava, E. Ferretti, M. Satake, T. Yasumoto, J. Chromatogr. A 844 (1999) 53.
[31] R. Draisci, L. Palleschi, L. Giannetti, L. Lucentini, K.J. James, A.G. Bishop, M. Satake, T. Yasumoto, J. Chromatogr. A 847 (1999) 213.
[32] T. Yanagi, M. Murata, K. Torigoe, T. Yasumoto, Agric. Biol. Chem. 53 (1989) 525.