

Table 1

Composite B-type brevetoxins by ELISA and the sum of brevetoxin biomarkers and BTX-3 by LC-MS in samples collected from the area where the outbreak implicated gastropods were collected.

Sample #	Common Name	Tissue	Weight (g)	ELISA ($\mu\text{g/g}$)	LC-MS ($\mu\text{g/g}$)
1	Banded tulip	Muscle	10.3	0.36	0.30
		Viscera	5.3	19.15	10.33
2	Banded tulip	Muscle	8.9	0.47	0.22
		Viscera	10.1	197.55	58.15
3	Pear whelk	Muscle	17.2	0.26	0.24
		Viscera	11.2	6.35	5.37
4	Pear whelk	Muscle	15.8	0.18	0.16
		Viscera	18.9	6.55	3.97
5	Sunray venus clam	Whole		42.13	13.88
6	Fighting conch	Muscle	6.8	0.35	0.09
		Viscera	7.0	1.02	0.22
7	Fighting conch	Muscle	13.5	0.37	trace
		Viscera	14.5	0.74	0.39
8	Fighting conch	Muscle	9.3	0.31	trace
		Viscera	14.3	1.1	0.41
9	Lightning whelk	Muscle	13.5	0.71	0.57
		Viscera	10.6	109.23	25.98
10	Lightning whelk	Muscle	70.4	0.16	trace
		Viscera	80.9	12.32	5.23
11	Horse conch	Muscle	467.9	0.05	0.03
		Viscera	311.6	2.14	1.77

reported previously in urine of NSP patients (Abraham et al., 2008) from the fragmentation pattern. Peak II was the smallest of the three ELISA peaks. The product ion spectrum of peak II corresponding metabolite MH^+ : m/z 913 (Fig. 5) was found to be in agreement with that of the previously identified urine metabolite 27-epoxy-BTX-3 (Abraham et al., 2008).

3.2. Field-collected specimens

Horse conch and lightning whelk were the largest of the specimens collected, with a total weight (without shell) of 780 g and 150 g, respectively (Table 1). The weights of gastropod sample viscera ranged from 5.3 to 312 g and those of the muscle ranged from 6.8 to 468 g.

There were no meal remnants available for this outbreak investigation; however, the patients identified the species consumed as horse conch based on a photo of the field-collected specimen and the characteristic orange color of the meat.

We examined the gastropod muscle and viscera extracts and the clam extract by ELISA and LC-MS/MS. All tested positive for B-type brevetoxins by ELISA. Composite B-type brevetoxin concentrations in the gastropods (muscle + viscera) collected from the harvest area of the NSP implicated gastropods ranged from 1.1 to 198 μg BTX-3 equiv./g by ELISA (Table 1). A high level of brevetoxin contamination was detected in the sunray venus clam (42.1 μg BTX-3 equiv./g) collected from the area. Brevetoxin levels in gastropod muscle tissues were much lower than in the viscera. The composite B-type brevetoxin concentrations ranged from 0.05 to 0.7 $\mu\text{g/g}$ and 0.7–197.5 $\mu\text{g/g}$ in gastropod muscle and viscera, respectively. Even though brevetoxin concentrations in the muscle tissues of some gastropods are below those thought to cause NSP, and may, in part, have resulted from contamination by toxins in the viscera during sample processing, the combined brevetoxin levels of the muscle and viscera of all the animals contained levels high enough to cause NSP in humans. Therefore, the meal preparation of the gastropod is an important element to consider when addressing NSP through gastropod consumption.

Brevetoxins were also confirmed in all gastropod samples and sunray venus clam by LC-MS/MS. Major B-type brevetoxin metabolites previously described in eastern oyster and clams were detected in gastropods, including S-desoxy BTX-B2 (MH^+ : m/z 1018), BTX-B2 (MH^+ : m/z 1034), BTX-B5 (MH^+ : m/z 911), BTX-B1 (MH^+ : m/z 1018) and BTX-3 (MH^+ : m/z 897).

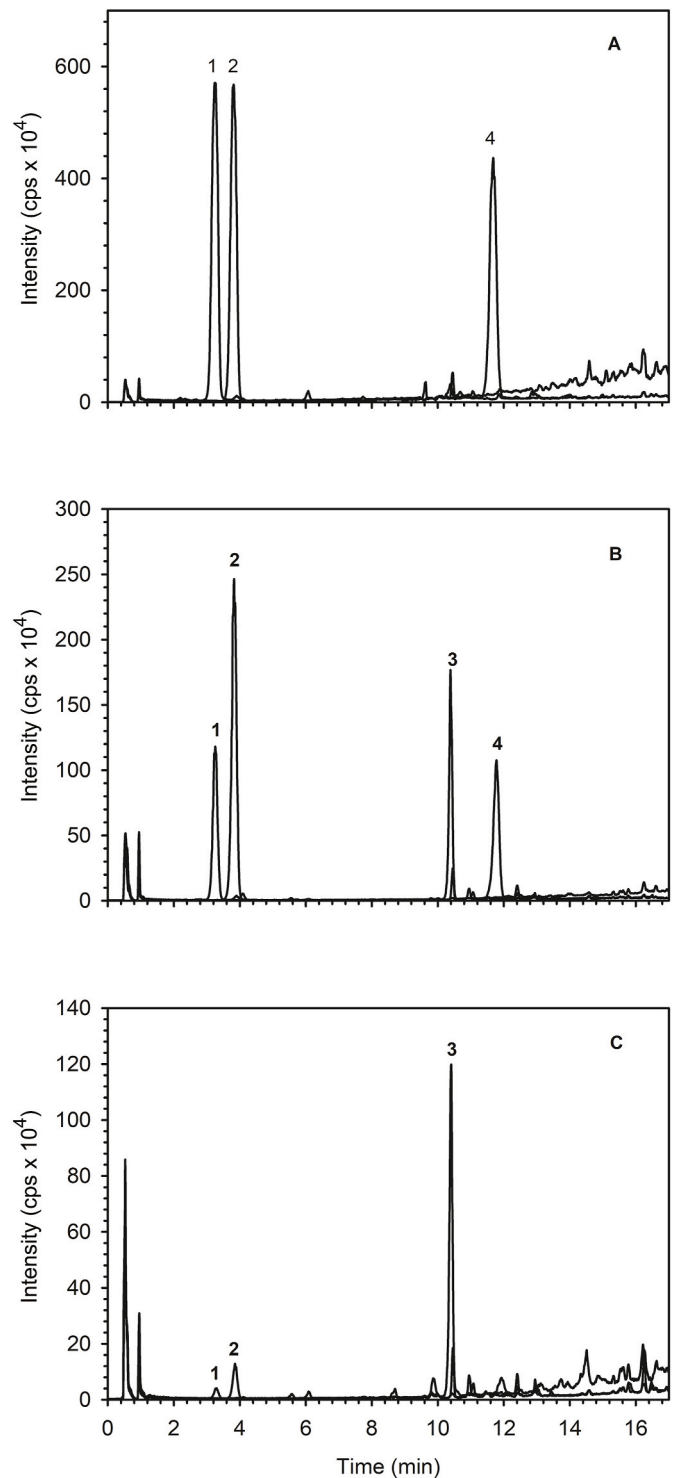


Fig. 6. LC-MS chromatograms of BTX-B2 (1), S-desoxy BTX-B2 (2), BTX-3 (3), and BTX-B1 (4) in Sunray venus clam (A) and Lightning whelk tissue extracts, viscera (B), and muscle (C).

The concentrations of brevetoxin biomarkers and BTX-3 (Fig. 6) determined by LC-MS/MS are presented in Table 1. BTX-3 was detected in muscle and viscera tissues of all the gastropods. Concentrations of BTX-3 ranged from trace to 0.53 $\mu\text{g/g}$ in gastropod muscle and 0.22–11.41 $\mu\text{g/g}$ in viscera. BTX-3 was not detected in sunray venus clam even though brevetoxin biomarkers were in high concentration (13.9 $\mu\text{g/g}$). Opened A-ring derivative of BTX-3 (m/z 915) was detected in muscle and viscera tissues of the gastropods. BTX-2, the abundant