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Short communication

Liquid chromatography/quadrupole time-of-flight mass spectrometry for determination of saxitoxin and decarbamoylsaxitoxin in shellfish

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Abstract

Saxitoxin (STX) and decarbamoylsaxitoxin (dcSTX) were determined by liquid chromatography with quadrupole time-of-flight mass spectrometry (Q-TOF MS). A shellfish tissue was extracted with 0.1 mol/l HCl under ultrasonication, and cleanup of extract was accomplished by solid-phase extraction with a C_{18} cartridge. Chromatographic separation was carried out on a C_{18} column (150 mm × 2.1 mm, 3.5 μm) with gradient elution of MeOH–H_2O (20:80) containing 0.05% heptafluorobutyric acid and MeOH–H_2O (15:85) containing 0.05% acetic acid. The protonated molecule $[M + H]^+$ ions at *m/z* 257 for dcSTX and 300 for STX were selected in precursor ion scanning for Q-TOF MS in the positive electrospray ionizaion mode. Average recoveries and relative standard deviations, by analyzing samples spiked at a level of 0.1, 0.8 or 1.6 μg/g, were 84–92 and 8–14%, respectively. Identification of the presence of the toxins in shellfish tissues was based on the structural information offered by Q-TOF MS.

Keywords: Shellfish poisoning; Saxitoxin; Decarbamoylsaxitoxin

1. Introduction

Paralytic shellfish poisoning (PSP) toxins, or 'saxitoxins', are caused by a group of toxins elaborated by planktonic algae upon which the shellfish feed, and generally associated with mussels, clams, cockles, and scallops. In addition, findings of PSP toxins in the viscera of mackerel, lobsters and crabs were also reported. Ingestion of contaminated shellfish may result in a wide variety of symptoms such as tingling, drowsiness, respiratory paralysis, and even death in severe cases. The occurrence of PSP toxins is widespread in the world, especially along the coast of Alaska, where many of the marine organisms that produce PSP are present. In China, the occurrence of PSP is sporadic and continuous in all waters close to land, particularly around the industrial cities.

Most shellfish contain a mixture of saxitoxins, depending on the species of algae, geographical region and organism. Further, they are altered in molecular structure within the shellfish. Saxitoxin (STX) and decarbamoylsaxitoxin (dcSTX) are the final degradation products of the various C-toxins and gonyautoxins, and are the most potent toxins known [1]. In addition, STX is also a chemical weapon compound (accessible at http://www.opcw.org). The structures of STX and dcSTX are shown in Fig. 1. Because of its high polarity and non-volatility, liquid chromatograph (LC) is the method of preference. Several detection techniques have been developed for LC, such as florescence detection following derivatization [2–4] and electrochemical detection [5]. Although they have been shown to be useful for certain applications, these methods cannot provide the structural information needed for confirmation of positive results. Mass spectrometry (MS) as an on-line detector for LC has become an important tool for the analysis of saxitoxins [6–12]. Quadrupole time-of-flight mass spectrometry (Q-TOF MS) not only can afford recording of selected precursor ions, but also can offer high mass resolution and accuracy. In this paper, we describe a method for the detection and identification of STX and dcSTX in shellfish tissues using LC with positive-ion electrospray Q-TOF MS.

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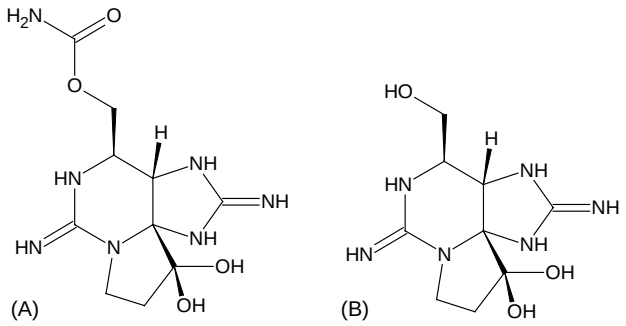

Fig. 1. Structures of: (A) STX and (B) dcSTX.

2. Experimental

2.1. Materials

Certified standards of STX·2HAc and dcSTX·2HAc were purchased from the Institute for Marine Bioscience (Halifax, Canada). Individual stock solution containing 10 μg/ml of STX and dcSTX was prepared in 30 mmol/l acetic acid, and was kept at 4 °C in the dark. Methanol was of HPLC grade from Fisher and heptafluorobutyric acid (HFBA) from Merck. Other chemicals were of analytical reagent grade. Water used was supplied by a Milli-Q purification system (Millipore Corp., Molsheim, France). The C_{18} SPE cartridge (3 ml) was obtained from Supelco (Bellefonte, PA, USA), the centrifuge spin filter Micorcon YM-3 (regenerated cellulose with 3 kDa cut-off) from Millipore (Bedford, MA, USA).

2.2. LC/Q-TOF MS

Chromatographic separation was performed on a Waters 2695 LC system (Waters Corp., USA) with a Zorbax XDB-C18 column (150 mm × 2.1 mm, 3.5 μm) from Agilent. Eluent A was methanol–water (20:80, v/v) containing 0.05% heptafluorobutyric acid, and eluent B was methanol–water (15:85, v/v) containing 0.05% acetic acid. A gradient elution was as follows: eluent A in the first 1.5 min, then B for the next 10 min, and finally back to A to equilibrium for 10 min. The flow rate was 0.2 ml/min. A sample injection volume of 20 μl was used in most cases.

Mass spectra were obtained on a QUltima Q-TOF MS (Micromass, UK). The instrument was equipped with an atmospheric pressure ionization source which was used in positive electrospray mode. The electrospray conditions were as follows: capillary and cone voltages, 3.0 kV and 50 V; cone and desolvation temperatures, 110 and 300 °C; cone and desolvation gas flows (nitrogen), 90 and 480 l/h, respectively. The collision gas was argon and the collision energy was of 15 V. Both the high and low resolution for mass filter was set at 10 V. The pressure in the TOF cell was lower than 3.0×10^{-7} Torr (1 Torr = 133.322 Pa). In the Q-TOF MS analysis, the protonated molecule $[M + H]^+$ ions at *m/z* 257 (dcSTX) and 300 (STX) were selected by quadrupole MS, and full-scan spectra from *m/z* 160 to 330 was done by TOF MS, to measure fragment ions that are characteristic of the selected precursor ions.

2.3. Sample preparation

Shellfish tissue (approximately 20–30 g wet weight) was homogenized and analyzed fresh, or stored at −20 °C until analysis. The sample extraction was carried out as described elsewhere [13,14] with minor modifications. Briefly, a 1.0 g portion of homogenized tissue was weighed into a 10 ml centrifuge tube, mixed well with 2.0 ml of 0.1 mol/l HCl, sonicated for 15 min at 90 °C in sonication bath, and finally centrifuged at 3500 rpm for 10 min. The C_{18} SPE cartridge was pre-treated with 6 ml methanol and 6 ml of 0.1 mol/l HCl, and 1.5 ml supernatant (equivalent to 0.5 g sample) was filtered through the cartridge, then 2.0 ml of water was added. The filtrate (ca. 3.5 ml) was collected and concentrated to 1.0 ml at 90 °C under nitrogen stream, and finally passed through a centrifuge spin filter at 13,000 × *g* for 15 min until a sufficient volume had been obtained for analysis.

3. Results and discussion

3.1. Chromatographic separation

Polymeric stationary phases with eluents containing heptafluorobutyric acid as volatile ion-pairing reagent were successfully used to separate saxitoxins in the LC with MS detection [6,9]. In this paper, STX and dcSTX were performed on a C_{18} column with gradient elution of MeOH–H_2O (20:80) containing 0.05% heptafluorobutyric acid and MeOH–H_2O (15:85) containing 0.05% acetic acid. Fig. 2 illustrated the typical mass chromatograms of a blank mussel sample spiked with STX and dcSTX. Sharp peaks and good detector responses of dcSTX and STX were obtained. Although STX and dcSTX are coeluted under such conditions, the Q-TOF MS is capable of specifically differentiating between them because the protonated molecules each have a different mass.

3.2. Q-TOF MS

The effect of different mass spectrometric parameters, such as cone, capillary and collision voltage, was investigated by injecting standard solution (1.0 μg/ml) of STX and dcSTX. The results obtained showed that cone voltage in the range of 35–120 V had little influence on the detector response of STX and dcSTX, whereas capillary voltage greatly affected response. The response of STX and dcSTX were significantly increased with capillary voltage varying from 1 to 3 kV, then followed by tending to stable state, indicating dcSTX and STX were almost completely ionized at 3 kV, so a 3 kV of capillary voltage was chosen to give

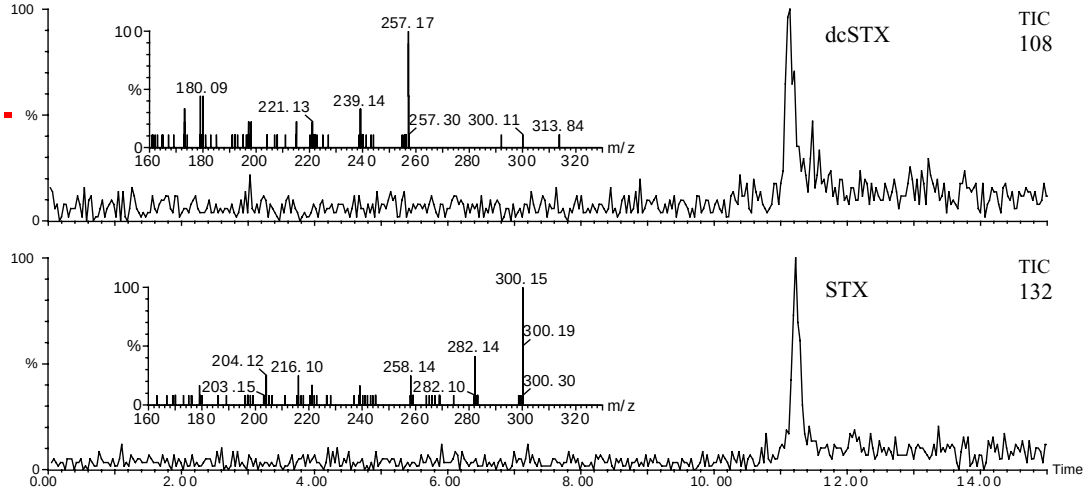

Fig. 2. Mass chromatograms of blank mussel spiked at 0.8 μg/g of dcSTX and STX.

high sensitivity. Fig. 3 showed the effect of collision energy on response of dcSTX and STX. It is seen from Fig. 3 that the total ion counts (TICs) of dcSTX and STX decreased significantly with increasing collision energy, while both the number and intensity of their fragment ions increased; therefore, the proper collision voltage was chosen to be beneficial to the selection of characteristic fragment ions. From the optimization experiments, it was found that 15 V collision voltage produced an acceptable compromise between sensitivity and specificity. Fig. 3 (15 V) revealed the mass spectra dominated by the protonated molecule ($[M + H]^+$) at *m/z* 257 for $[dcSTX + H]^+$ and 300 for $[STX + H]^+$, and the major fragment ions recorded at *m/z* 239 and 222 most probably for $[dcSTX + H–H_2O]^+$, $[dcSTX + H–H_2O–NH_3]^+$, and 282 for $[STX + H–H_2O]^+$, respectively, whereas what fragment ion at *m/z* 204 is not clearly known to us. The two key fragment ions plus their $[M + H]^+$ ions were used to identify the presence of dcSTX and STX.

3.3. Method validation

For shellfish samples, a matrix-related ion suppression was noted by LC/Q-TOF MS, leading to decrease in detector response, but which can be compensated for by using a matrix-matched standard. The matrix-matched calibration graphs for STX and dcSTX were prepared by injecting extracted blank spiked with increasing amounts of standard ranging from 0 to 2.0 μg/g, giving an acceptable linearity ($\gamma^2 = 0.996$) over the test range. The approach was validated by analyzing samples spiked at a level of 0.1, 0.8 or 1.6 μg/g. The overall results, summarized in Table 1, showed that the average recoveries were 84–92% and the relative standard deviations (R.S.D.s) were 8–14%. These results were in compliance with the method validation criteria [15]. The limit of quantification, defined as reliable measurement of the lowest concentration of analyte in samples, was 0.1 μg/g sample for dcSTX and STX.

3.4. Application

Fig. 4 showed a representative mass chromatogram of a mussel sample positive in the AOAC mouse bioassay [16]. The presence of STX in the sample was confirmed in terms of mass spectrum and retention time (11.2 min) compared to those of standard. The content (0.3 μg/g) was estimated by external standard method based on the matrix-matched calibration graphs.

Table 1
Results of dcSTX and STX Recoveries and R.S.D.s from shellfish ($n = 24$)

Shellfish	Spiked level (μg/g)	Number of experiments	Average recovery (%)		R.S.D. (%)	
			dcSTX	STX	dcSTX	STX
Mussel	0.1	4	91	88	14	12
Scallop	0.1	4	88	92	8	11
Mussel	0.8	4	84	89	10	8
Scallop	0.8	4	86	90	9	13
Mussel	1.6	4	93	92	12	14
Scallop	1.6	4	85	91	11	9

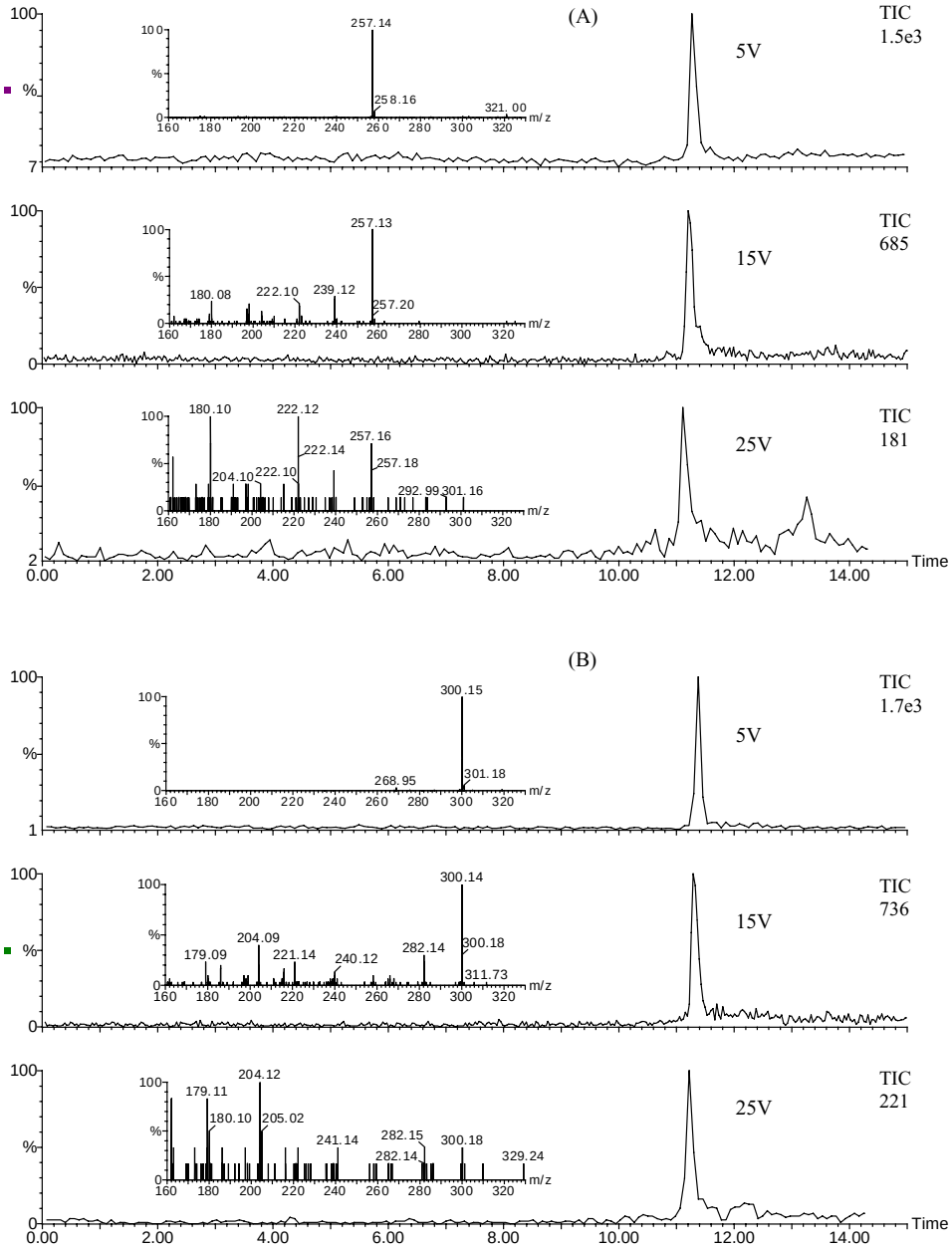

Fig. 3. Effect of collision voltage on response of: (A) dcSTX and (B) STX.

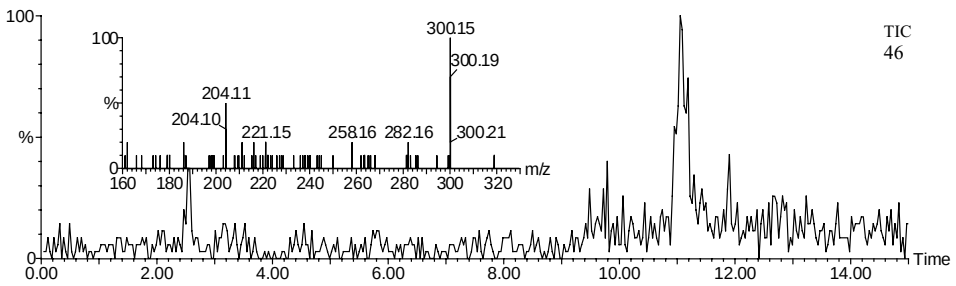

Fig. 4. Mass chromatograms of a mussel sample positive in mouse bioassay.

4. Conclusions

To our knowledge, this is the first report dealing with LC/Q-TOF MS for the analysis of dcSTX and STX toxins in shellfish samples. The detection of PSP toxins is simple and quick not involving additional sample preparation. The recoveries and R.S.D.s obtained are satisfactory, and the limit of quantification meets the requirements for regulation levels (0.8 μg/g). Additionally, the Q-TOF MS detection system employed yields full-scan mass spectrum about the structure of the molecule to enable unequivocal confirmation of the presence of the toxins.

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